Perinatal innovations for calves and piglets

by

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BVMS, Federal University of Pampa, 2017
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AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

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Abstract

In animal production, the ultimate goal is to fulfill the pillars of sustainability to ensure that future generations will have access to natural resources and animal sources to meet their needs. The perinatal period offers many challenges for both dams and their offspring regardless of species. Alternatives and refined methods are needed to improve animal efficiency, care, and welfare. For this dissertation two high impact animal production systems were evaluated for perinatal methods: cow-calf and nursery swine. Twinning is a controversial topic among producers because even though the possibility of increasing weaned kg/cow seems promising, birth difficulties and perinatal mortality can threaten the achievement of this goal. To achieve twinning in an Angus-cross herd, a Fixed-Time Artificial Insemination (FTAI) protocol was used. Cows were inseminated with black Angus semen and 7 d (n = 75) after insemination an embryo transfer was performed to the contralateral uterine horn containing the corpus luteum (n=63). Cows that were inseminated and received an embryo achieved a pregnancy risk of 61.9%, with 42.9% carrying twins and 19% carrying singletons. Second born twins required some assistance during parturition. Survival proportions were not different among twins and singletons (81.6% and 80%, respectively; $P > 0.1$). Raising methods for twins and singletons were also discussed. When twins were born as twins, but one got grafted to another cow, they underperformed compared to singletons and twins raised both with their dam. The usage of the proposed twinning protocol successfully produced twins on commercial Angus herds. This is an opportunity for producers to increase the amount of weight weaned by cow (kg/cow) and improve sustainability. However, this protocol works better on a small herd or a subset herd that will be closely monitored for appropriate intake and parturition. Colostrum supplementation can be an important management tool when calving heifers and twins. Concern is raised due to lower

concentration of immunoglobulins and small volumes of first-calf heifers' colostrum. A randomized field trial was conducted to compare the supplementation of a commercial colostrum product (CS) and a control treatment (control). Immune measures, neonatal behaviors, and growth were assessed for both treatments. No significant differences in IgG concentrations or growth measures were found between treatments ($P > 0.1$). Latency of first nurse was found to be higher for calves on CS treatment ($P > 0.05$). Even though, immune measures and growth were not affected by colostrum supplementation, a delay was seen in nursing behaviors. This does not dismiss the usage of colostrum replacement products in challenging situations, such as cold stress and no colostrum available from dam. Precision livestock tools have been developed to assist management of animals. These tools are important especially during the neonatal periods to identify individual animals facing subclinical sickness that are not obvious to human observations. The nursery phase for piglets is very challenging due to many stressors (e.g., ear notching, ear tagging, tail docking, teeth clipping, and castration of males) that can implicate their naïve immune system. A study was conducted to use a precision livestock tool (NU*track*) *to* assess behavior of piglets either LPS-challenged (challenged) or non-challenged (saline injection; sham) and compare to human observations. The LPS challenge induced transient sickness in piglets. Receiver operating characteristics (ROC) curve and area under the curve (AUC) were used to analyze the data from human observation scores and NU*track*. Human observation presented a sensitivity of 88.5% and a specificity of 85% with an AUC of 0.871. While NU*track* evaluated pivot behaviors, presented a sensitivity of 96.8% and a specificity of 92.7% with an AUC of 0.999 in the day of the challenge. The sensitivity and specificity of human observations declined AUC < 0.6 within 2 d after challenge. NU*track* presented a good ability to identify challenged pigs and can aid caretakers to identify subtle behavior

modifications. Innovations for perinatal period in cattle and swine are indispensable to maintain the sustainability of animal productions. The management tools and protocols discussed aim to improve efficiency and animal welfare. Twining can be used to face sustainable challenges producers face in cow-calf operations, colostrum management is important to support passive immune transfer, and precision livestock technology can aid labor workers during management decision-making.

Key words: calf, neonatal, piglet, colostrum, twinning

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In animal production, the ultimate goal is to fulfill the pillars of sustainability to ensure that future generations will have access to natural resources and animal sources to meet their needs. The perinatal period offers many challenges for both dams and their offspring regardless of species. Alternatives and refined methods are needed to improve animal efficiency, care, and welfare. For this dissertation two high impact animal production systems were evaluated for perinatal methods: cow-calf and nursery swine. Twinning is a controversial topic among producers because even though the possibility of increasing weaned kg/cow seems promising, birth difficulties and perinatal mortality can threaten the achievement of this goal. To achieve twinning in an Angus-cross herd, a Fixed-Time Artificial Insemination (FTAI) protocol was used. Cows were inseminated with black Angus semen and 7 d (n = 75) after insemination an embryo transfer was performed to the contralateral uterine horn containing the corpus luteum (n=63). Cows that were inseminated and received an embryo achieved a pregnancy risk of 61.9%, with 42.9% carrying twins and 19% carrying singletons. Second born twins required some assistance during parturition. Survival proportions were not different among twins and singletons (81.6% and 80%, respectively; $P > 0.1$). Raising methods for twins and singletons were also discussed. When twins were born as twins, but one got grafted to another cow, they underperformed compared to singletons and twins raised both with their dam. The usage of the proposed twinning protocol successfully produced twins on commercial Angus herds. This is an opportunity for producers to increase the amount of weight weaned by cow (kg/cow) and improve sustainability. However, this protocol works better on a small herd or a subset herd that will be closely monitored for appropriate intake and parturition. Colostrum supplementation can be an important management tool when calving heifers and twins. Concern is raised due to lower

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Acknowledgements

"Nobody can do life on their own. There ain't nothing wrong with asking for help. As a matter of fact, a man told me one time that the best gift you can give someone is the opportunity to help you." – David Johnson.

It would be impossible for me to accomplish anything on my own. I have been blessed with amazing parents that always put my education first and supported my dreams through and through. It is not easy to be physically apart and miss many important dates with my family. But they never complained that I was here chasing my dreams. Mom and Dad, I hope I can always make you proud. I would like to acknowledge my husband Luís and my animals Rio, Roma, Tokyo, Melbourne, Maui, and Nala for making this foreign country feel like home. To my husband, who is my number one fan and the most supportive person, I want to say thank you. You have pushed me to finish this strong and accomplish our goal. I want to thank my brothers, Arthur and Felipe, for the visits, calls, and messages during these past five years. My utmost thank you to my advisors, Dr. Odde and Dr. Hulbert. You both trusted me way more than I ever trusted myself. You challenged me to write better, to research better, and to be better. You accepted me like family while my family was so far away, and for that I have not enough words to thank you.

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Dedication

I would like to dedicate to my parents, Adriane Mazzardo and Moacir Bortoluzzi. You guided me through life and offered me opportunities to accomplish my goals. Thank you!

Chapter 1 - Literature Review

A sustainable approach to the challenging neonatal period of swine and cattle

Sustainability

As the world population continues to rise, animal production faces the challenge of feeding the world sustainably. The term sustainability has been used since 1987 and can be defined as "meeting the needs of the present without compromising the ability of future generations to meet their own need" (Brundtland, 1987). In recent years, general public perception and opinion has demanded commitment from animal producers and government institutions to advance sustainable agriculture. Cow-calf operations in cattle, farrowing and nursery in swine, affords many opportunities to improve through management and precision animal technology. In addition, there is room to expand communication between scientists, farmers, and the general public. A better understanding of animal products can reduce the mistrust of the consumption of animal products. Variable tools can be used to measure sustainability within livestock production. The aspects of sustainability and its pillars will be further discussed, focusing on animal welfare, especially basic health and function through neonatal immunology.

Pillars of sustainability

Sustainability in agriculture has three classic domains: ecological/environmental sustainability, social sustainability, and economic sustainability (Latruffe et al., 2016). Within those domains are many pillars: environmental quality, animal welfare, food safety, workforce, and economics (Galloway et al., 2018). These pillars have been supported by Dr. Frank Mitloehner, leading researcher and communicator of livestock sustainability. The variables

within each pillar are, in many cases, not mutually exclusive, and aspects within them can and will affect each other.

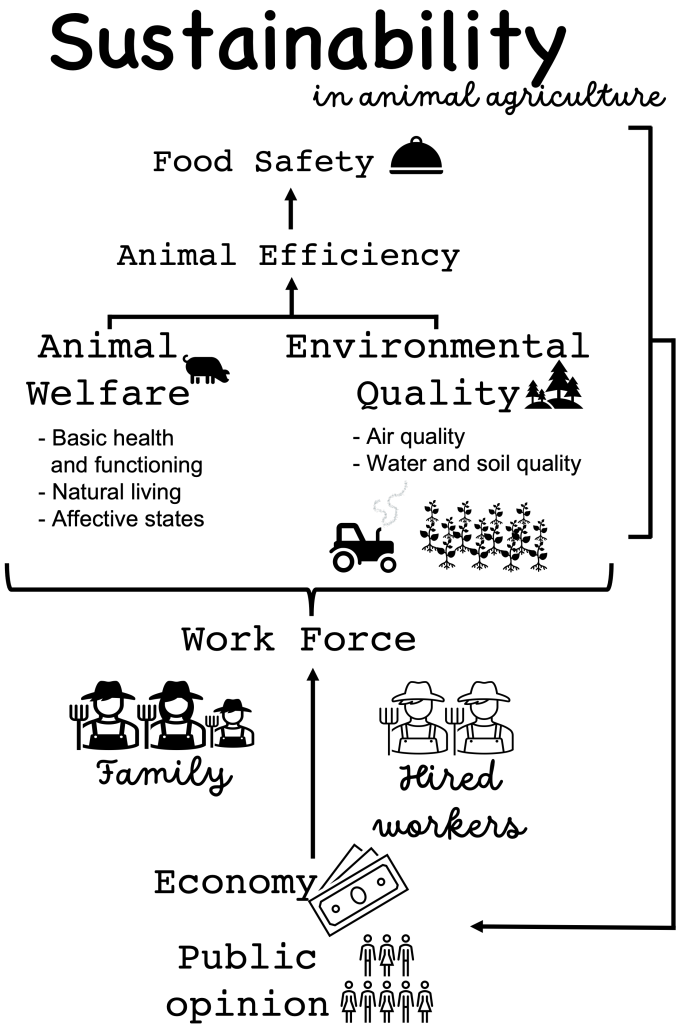

Figure 1.1– This flow chart represents the complexity of the animal production sustainability pillars. In the base of the chart is the economic and public opinion, which are variables driving the other pillars. With the economics are the work force that will likely depend on financial aspects and size of the production. There are two main groups: farmers and their families and hired workers. The workers are crucial to maintaining animal welfare and supporting management that will impact the environmental quality. In the middle portion of the chart, animal welfare and environmental quality are not only important to maintain the stability of a sustainable production, but it is also opinion drivers of the public opinion. Animal welfare assessment will be conducted by trained employees looking for health problems, adequate living conditions, and problems with animal behaviors. That will include the care for livestock animals as well as feeding and cleaning of the animals' housing. Environmental quality will be affected by the management of the farm with care for the air, water, and soil quality. Moving towards the

top is animal efficiency which reflects labor, genetics, feed quality, and animal welfare. Efficiency has a direct impact into environmental quality; however, efficiency is not always translated into a good animal welfare. At the top is food safety, the quality of animal products arriving the consumers households is dependent of domains and pillars already discussed with the addition of retail. The quality of the product can drive the prices increasing the revenues that are represented by the economics at the bottom of the chart.

Economic pillar

The economic pillar of sustainability is important for both producers and consumers. The cost of manufacture will likely affect the product's final cost that arrives at the consumers' table. Economic sustainability in animal production can be measured in profitability and productivity. Profitability refers to a comparison between revenue and costs, and their difference will be a positive income (Latruffe et al., 2016). One example of profitability in cow-calf operations would be the costs to produce a calf compared to the revenue generated when this calf is sold. Similarly, in swine it would be the costs to produce a pig litter compared to the revenue generated when piglets are sold or moved to the nursery. In addition, productivity can also be measured regarding the economic aspect of sustainability, including using natural resources and producing greenhouse gases (GHG). In swine systems, productivity can be measured by the number of pigs weaned per sow per year and growth. In cow-calf operations, productivity can be measured using reproductivity, calf mortality, and growth. Even though most economic evaluations are done because of profitability, macro-economic changes, future policies, and climatic and market hazards should also be considered (Doreau et al., 2013).

Workforce

Agriculture is highly dependent on labor quality. This sector became more efficient with increased technologies applied to crop and animal production, but human labor is still considered essential. Livestock careers require a certain intensity of work compared to conventional jobs, and animals will require care independently of weekends or holidays. Both cattle and swine can

be intensively managed to require a greater workforce. The U.S.'s agricultural workforce comprises self-employed farmers, their families, and hired workers (USDA, 2022). The labor workforce had a significant decrease in number during the 1990s when machinery and technology were added. However, the livestock sector had an 18% increase in job openings from 2010 to 2020 (USDA, 2022). Unfortunately, in 2020 the U.S. and the world faced the beginning of the COVID-19 pandemic, significantly affecting agricultural employees and causing significant job insecurity (Bochtis et al., 2020). Farm workers were considered “essential” and were working when COVID-19 exposure was at its highest (Charlton and Castillo, 2020). Even before 2020, there were indications of reduced labor supply directed to agriculture (Charlton et al., 2019). This reduction is attributed to immigrant workers preferring other jobs due to increased education (Charlton and Taylor, 2016). The pandemic affected the workforce even more with decreased seasonal migrant workers due to mobility restrictions (Bochtis et al., 2020). The shrinkage in the workforce affects all the other pillars of sustainability, impacting the correct application of animal welfare and management of environmental resources.

Animal welfare pillar

Initially, animal welfare was mainly focused on animal behavior (Gonyou, 1994; Marchant-Forde, 2015). Nowadays, it is considered a well-defined multidisciplinary area, encompassing animal health, immunology, endocrinology, neuroscience, and physiology, and it is affected by cultural and personal traits (Marchant-Forde, 2015; Buller et al., 2018). Studies and publications about animal welfare have had exponential growth in the past few years (Walker et al., 2014). Even though it is perceived differently among different scientists, conceptual frameworks can be used to assess the welfare of animals. One of the first frameworks introduced was the Five Freedoms developed by the Brambell Committee (FAWC, 1992),

recognized and adapted by the OIE (2014); 1-Freedom from hunger and thirst; 2- Freedom from discomfort; 3- Freedom from pain, injury, and disease; 4- Freedom to express their normal behavior; 5- Freedom from fear and distress. Then, Dr. David Fraser introduced the framework of the Three Circles: biological function and health, affective states, and natural living (Fraser, 2008). In addition, two more frameworks are internationally recognized; The Welfare Quality Network (2009) and The Four Guiding principles (Fraser, 2008). These frameworks are the foundation for accessing animal-, resource-, management-, and production-based welfare measures. Unfortunately, public perception of animal welfare differs from animal scientists' and veterinarians' perceptions in many cases. For neonatal piglets' and calves' welfare in the first couple of days of life it is extremely important to assess welfare measures since they can be indicators of survival outcomes. When considering the three circles (Fraser, 2008), we need to account for their naïve immune system at birth (basic health and functioning), neonatal behaviors to acquire colostrum and environment at birth (natural living), and the affective states of these neonatal animals.

It is impossible to discuss the welfare of neonatal animals without discussing their immunology and resilience at birth. In many eutherian mammals, including humans, the placenta is an organ that allows the passage of immunoglobulins from the dam to the fetus. This passage depends on the placenta's intimacy between the fetal and maternal portions. Swine and ruminant placentas have six layers and are the least intimate ones. The swine placenta is classified as epitheliochorial because the epithelium layer is intact on the maternal and fetal sides (Macdonald and Bosma, 1985). Likewise, ruminants have an epitheliochorial placenta, still in reason of its erosions and regrowth, maternal blood is exposed to the fetal chorion, and the placenta is known as syndesmochorial (Senger, 2012). The placentas of swine and cattle species have low

immunoglobulins' permeability due to the number of tissue layers between the maternal and fetal sides. That means the calf and piglet will be born without the correct tools to fight environmental microorganisms. Neonates of both species rely on the consumption of colostrum to achieve adequate passive immunity that will protect them against pathogens (Brambell, 1970; Pakkanen and Aalto, 1997; Foley and Otterby, 1978; Tizard, 2004; Stelwagen et al., 2009; Inoue and Tsukahara, 2021).

Immunological and behavioral traits of passive immune transfer

Colostrum

Colostrum is the first secretion of the mammary gland near parturition, rich in protein and poor in lactose and fat (Quesnel et al. 2012). A large portion of colostrum proteins comprises maternal immunoglobulins derived from the dam's adaptive immune system in maternal circulation (Barrigton et al., 2001). In both species, the most abundant isotype in colostrum is Immunoglobulin G (IgG), followed by immunoglobulin M (IgM) in cattle and immunoglobulin A (IgA) in swine (Klobasa et al., 1987; Tizard, 2017). In swine, these IgGs are transported to the mammary gland due to a relaxation of the tight junctions between mammary and epithelial cells (Quesnel and Farmer, 2019) or transported by an Fc receptor of the neonate (FcRn), similar to the process that occurs in cattle (Schnulle and Hurley, 2003; Ghetie and Ward, 2000; Barrigton et al., 2001). The FcRn presented in the cell surfaces binds to the IgG, forming an FcRn-IgG complex and delivered by endocytosis (Ghetie and Ward, 2000). The colostrogenesis process starts around ten days before farrowing and 3-4 weeks before calving (Huang et al., 1992; Brandon et al., 1971). The concentration of immunoglobulins decreases in colostrum with the increase in days post-partum (Foley and Otterby, 1978; Inoue and Tsukahara, 2021). Besides immunoglobulins, Inoue and Tsukahara (2021) reported that other colostrum glycoproteins,

including azurocidin and lactoferrin, have antimicrobial properties that assist in piglet gut health. Likewise, cattle colostrum contains antimicrobial proteins such as lactoferrin and lactoperoxidase (Hooijdonk et al., 2000). In addition, leukocytes present in colostrum can be transferred to the neonates' blood circulation and recirculate to their immune system organs (Duhamel et al., 1987; Williams, 1993). Maternal leukocytes were shown to improve immune cell maturation in calves in the first two weeks of life (Reber et al., 2008). Lymphocytes, most of which are T cells, are very concentrated in sows' colostrum.

Maternal immunoglobulins in the colostrum are absorbed by the intestinal epithelial cells by binding to an FcRn receptor. The highest permeability is during the first 6 hours of age, except that it can vary between species. This permeability has been associated with replacing intestinal epithelium cells with ones not expressing the FcRn. In piglets, the absorption is selective to IgG and IgM and can be retained for up to 4 days. At the same time, IgA will remain unabsorbed and circulating in piglets' intestines. In calves, all immunoglobulins will be absorbed through the intestinal epithelium without selectivity (Tizard, 2004). After 24 hours, absorption of these immunoglobulins will be significantly reduced. The dam's presence with her calf has been associated with an increased immunoglobulin absorption when colostrum was fed. The ethology of these neonatal animals will ensure an adequate passive transfer between the dam and the offspring.

Nursing behaviors and litter size effects

Colostrum intake by the neonatal piglet and calf is dependent on their innate behaviors of seeking, finding, and suckling. These precocious behaviors are imperative for neonatal passive immunity until their adaptive immune system can start producing memory cells. The factors

affecting immunoglobulin concentration in piglets and calves are similar, including the dam, environmental, and behavior factors.

Cattle are monotucous animals that commonly produce only one offspring per gestation, while swine are polytocous species producing a greater number of offspring. At parturition, calves rarely have a competition to acquire colostrum from their dam. Their acquisition depends more on calf vitality, environment, and the dam's maternal behavior. Once the calf is born, the cow will start licking the calf, which stimulates calf activity and dries the calf. This behavior is significant for establishing the cow-calf bond (Von Keserlingk and Weary, 2007). The stimulation of calf activity will motivate the calf to stand up, seek, and nurse. Beef calves were reported to have shorter latency to nurse than dairy cattle (Selman et al., 1970; Edwards and Broom, 1979; Bortoluzzi, 2019). The difference was attributed to beef calves being more precocious while dairy calves are more altricial (Selman et al., 1970; Edwards and Broom, 1979). In North America, dairy calves are rarely left to perform these neonatal behaviors and are fed the colostrum or colostrum replacer through an esophageal feeder or by nipple bottle. This management approach has been adopted because the delay in colostrum consumption by dairy calves can result in failure of passive transfer (FPT), leaving the calf more vulnerable to infections due to a decreased absorption of immunoglobulins (Besser and Gay, 1994; Godden et al., 2019).

Piglets face a different situation since litter size has increased through genetic selection in the past few years. The first piglets to be born are usually heavier, with increased vitality, and have almost no competition to acquire colostrum (Akdag et al., 2009). The last piglets to be born are usually lighter in weight, have lower vitality due to the amount of parturition time and hypoxia, and must compete to seek, find, and suckle colostrum from one of the 14-16 maternal

179 teats (Herpin et al., 1996; Olivieiro et al., 2019). Research has shown that an increase in litter
180 size can significantly affect birth weight, and a high variation in birth weight can increase the
181 variation of survival at weaning (Akdag et al., 2009). Colostrum production does not increase
182 with litter size, affecting their basic health and function and impacting their affective states early
183 in life (Devilles et al., 2007; Quesnel, 2011). In fact, Kielland et al. (2015) reported a linear
184 decrease of 0.4 g/l of IgG in piglet plasma with each born piglet. Lessard et al. (2018) reported
185 that in their study, the development of the innate and adaptative immune system in piglets was
186 proportional to the amount of growth during first two weeks of life. At the same time, growth
187 was mainly dependent on colostrum and milk consumption. This increase in litter size aimed to
188 increase swine production efficiency, requiring fewer sows to produce a larger number of piglets.
189 However, litter size has been negatively associated with perinatal mortality either by intrauterine
190 growth restriction, starvation, hypothermia, or piglet crushing by sow (Lund et al., 2002;
191 Edwards, 2002; Damm et al., 2005; Andersen et al., 2011). Edwards and Baxter (2015) pointed
192 out that crushing usually happens due to hypothermia and starvation resulting from a piglet
193 searching for heat by the sow and being too lethargic to move away. Neonatal mortality has a
194 significant economic impact on swine and beef cattle production. Total mortality in pigs ranges
195 from 16% to 20%, while in beef calves, 5.4% to 6.9% (USDA, 2010; Baxter and Edwards,
196 2018). In summary, large litter size is not always translated into efficiency in swine production.
197 The lack of appropriate colostrum and milk volumes to attend to all piglets can result in piglet
198 mortality, which is a welfare concern.

199 ***Neonatal cold stress***

200 For both species, environmental effects play a big role in thermoregulation. Piglets,
201 different from calves, are born with less hair coverage and no brown adipose tissue (Berthon et

al., 1993). However, in the process of parturition, neonates are born covered in fluids, and calves require a dam's licking behaviors to prevent evaporation and loss of temperature. On the other hand, piglets are born with little subcutaneous fat, relying on their moderate glycogen reserves to shiver and external heat sources to maintain their body temperature (Theil et al., 2011; Farmer and Edwards, 2020). Ingestion of colostrum will aid in energy to produce heat and will also serve as an internal heat source for calves and piglets (Herpin et al., 1994; Hardon et al., 1997). Lower body temperatures can decrease neonatal animals' rate of immunoglobulins absorption. Olson et al. (1980) subjected newborn calves to cold stress using cold-water immersion. The authors reported that the rate of immunoglobulins absorption was significantly decreased by cold stress.

Nonetheless, the net of immunoglobulin absorption was not affected. This was attributed to a short time in a hypothermic state. It was hypothesized that longer periods of hypothermia (body temperature below 34.4ºC) might decrease immunoglobulins net absorption by calves. When piglets got submitted to cold stress by intermittent exposure to cold air at birth, the colostral immunoglobulin absorption was reduced compared to piglets in the thermoneutral control treatment (Blecha and Kelley, 1981). These studies show that hypothermia can play a harmful role in the neonatal calves' immune defenses. A lower absorption rate of maternal immunoglobulins, usage of glycogen storage to maintain homeorhesis, and search for heated sources close to the dams could decrease neonates' essential health and function and possibly lead to high mortality percentages.

Management of cold stress

Producers can adopt a series of management procedures to minimize environmental effects on the neonatal calf and piglet. In swine production, heat lamps, heated pads, and controlled room temperature are used to maintain the thermoregulation of newborn piglets

(Robinson and Young, 1998; Lane, 2019). Calves born during winter can be moved to barns, dried using towels, put in heated boxes or warm baths (40.5°C), and provided an outside shelter with straw bedding to minimize the effects of cold stress (Butler et al., 2006). Similarly, dairy calves raised in individual hutches need attention to the effects of hypothermia. Management should consider closing rear ventilation doors of hutches and deep bedding to provide insulation during cold weather (Anderson and Bates, 1983).

In summary, animal behavior, physiology, and immunology will likely affect neonatal piglets' and calves' chances to survive, affecting the economy of cow-calf and swine productions and representing the ethical importance of ensuring animal welfare.

Environmental and Animal Efficiency

The environmental pillar tends to be the first one we think of when pondering sustainability. It pertains to the usage and management of natural resources, including air, soil, and water quality. The considerable attention to this pillar originated from the discussion around greenhouse gas (GHG) emissions and wastage generated by animal production. Public perception tends to hover around the negative environmental impacts (Van Eenennaam and Werth, 2021), while animal scientists see it as an opportunity to research strategies for mitigating GHG emissions and reducing wastage (Mitloehner, 2020; Place and Mitloehner, 2014).

GHG emissions (carbon dioxide [CO_2], methane [CH_4], nitrous oxide [N_2O]), and other air pollutants (NH_3 and particulate matter) affect air quality and increase heat retentions within their molecules, thus increasing global temperatures (Place and Mitloehner, 2014). Animals produce carbons such as CO_2 and CH_4 during respiration and in ruminants during enteric fermentation. The potential to retain heat is larger in CH_4 compared to CO_2; however, CH_4 has a 10-fold shorter life in the atmosphere than CO_2 (Howarth et al., 2011). In addition, if ruminant

248 herds are kept constant, grazing lands can act as carbon sinkers to help achieve carbon neutrality.
249 Furthermore, volatilized ammonia (NH_3) results from the mixture between urine and feces. The
250 most significant slice of CO_2 produced in livestock systems comes from the usage of fossil fuels
251 and not actually by animal respiration and fermentation (Steinfeld et al., 2006). In 1995,
252 concerns were already being expressed considering the heavy use of fossil fuels and their
253 correlation to higher agricultural productivity (Heitschmidt et al., 1995). Fortunately, efforts
254 toward renewable energy usage are already happening in animal production. The dairy industry
255 has successfully decreased the emission of CH_4 and CO_2 in California by using biogas produced
256 and trapped by covering their lagoons (Williams and Gould-Wells, 2003; Krich et al., 2005;
257 Montes et al., 2013). This strategy can be used for dairy, beef, and swine industries that use
258 lagoons to store manure. The anaerobic environment of lagoons creates methane that can be
259 trapped and used as energy, reducing the amount of fossil fuels needed. Land and water usage
260 are also a concern as we move forward with agriculture and animal production, as well as
261 reactive N loss.
262 When augmenting pastures or land for cropping, the removal or burning of natural
263 vegetation and forests result in increased CO_2 release in the atmosphere (Steinfeld et al., 2006).
264 Conversely, from the total pasture area in the U.S., 65% of the biomass produced is inedible to
265 humans but edible to ruminants that can convert it into animal products consumed by humans
266 (Place, 2018). Blue water (water for crop irrigation, drinking, and dust control) usage is primarily
267 used for crop irrigation, with high variation from location to location (Rotz et al., 2018). The
268 Midwest of the U.S. is dependent on groundwater irrigation from the Ogallala aquifer to
269 maintain or support agriculture (Deines et al., 2020). Sadly, this water source is finite, and
270 alternatives are already being researched to minimize water uptake and economic losses.

Recently efforts are being made to utilize crops for animal feed and biofuel that require less water, such as sorghum instead of corn (Roby et al., 2017).

Cow-calf environmental quality

In cow-calf operations, fuel usage is more than fourfold greater, and electricity is fivefold greater, than stocker or finishing phases (Rotz et al., 2019). In addition, most CH_4 is also attributed to this phase because it is a less efficient and extended phase of ruminants' lives. White et al. (2015) suggested that the efficiency of cow-calf production to improve sustainability would involve reproduction, genetic, and nutritional management. To evaluate efficiency, models were used to compare eight different scenarios with a control representing U.S. production. A baseline of 188 m^2 of land use, 712 L of blue water, and 21.9 kg/kg HCW beef was set. Once only nutrition management was improved, they demonstrated a reduction of 1.5% of the previously described metrics. This is supported by the research on CH_4 production and how it is dependent on the quality and quantity of diet, including forage, concentrate, and feed additives that affect the ruminal microbial population (Moss et al., 2000; Johnson and Johnson, 1995). Besides management, a decrease in CH_4 production was also noted during high environmental temperatures, decreasing feed intake and fermentation (Shibata and Terada, 2010). A reduction in calving interval could result in a 3.2% reduction and using twinning techniques could result in a reduction of up to 9.2% in blue water, land use, and GHG emissions (White et al., 2015). Additionally, the greater reductions were associated with genetic selection (12.4%) and use of expected progeny differences (EPDs) either in bull selection (11.1%) or A.I. (11.1%).

Swine production environmental quality

Some environmental sustainability impacts of swine production are related to feed production and waste management (Zira et al., 2021). As in cattle production, shifting their nutrition could possibly decrease the emission of CO_2 by fossil fuel usage issues; however, this is not done without trade-offs. When using feed available locally, ingredient options may decrease, leading to less efficient growth. Animals spend more time in the production chain to achieve desired weights translating into lower efficiency. Distilled-dried grains have been studied to substitute corn and soybean meal partially, reducing costs and environmental footprint (Haque et al., 2022). The waste by-products of swine production contain organic matter, nitrates, phosphates, salts, metals, minerals, and microorganisms that can contaminate water bodies when not managed properly (Lahav et al., 2013). The most common waste treatment in swine production is in anaerobic lagoons, commonly used to store waste with greater ammonia fluxes during summertime (Aneja et al., 2000). As previously mentioned, methane can be trapped from dairy lagoons into anaerobic digesters and used as a fuel source. However, nitrogen will still release ammonia into the water. To remove this ammonia, researchers proposed nitrification-denitrification means or electronically-regenerated ion exchange process (Rajagopal et al., 2011; Lahav et al., 2013).

The environmental pillar in both cow-calf and swine operations is constant research towards improvement. The following pillar to be discussed comprises an important aspect of sustainability that deserves adequate attention. Behavior and immunology are fundamental to quality of life, efficiency, and productivity for a positive revenue.

Food safety pillar

Food safety in livestock production embraces three main elements, chemical, microbiological, and physical aspects of food safety (Valeeva et al., 2004). To embrace these three elements, the OIE (2016) developed a farm management guidance focusing on animal health management, veterinary medicines and biologicals, animal feeding and watering, environment and infrastructure, and animal product handling to address food safety. Within this guidance, record keeping can be distinguished by ensuring product quality and traceability. In cattle and swine production, animal identification and traceability are imperative to secure the safety of animal products entering the food chain. Health records containing morbidity, mortality, treatment events, and drug usage will allow posterior record checks by farmers in case a disease outbreak surfaces.

Conclusion

Sustainability in animal production is dependent on many variables. During the perinatal and neonatal period, the management of calf and piglet is imperative for future efficiency and economic gain. The attention to environmental effects and passive immune transfers will ensure good welfare and health that will reflect growth and efficiency.

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Chapter 2 - Twinning in Beef Cattle

Usage of embryo transfer and artificial insemination to improve beef cow-calf efficiency, and effects of different twin calf-raising methods on neonatal behavior and growth

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Abstract

The objectives of these experiments were to produce twins using reproductive biotechnologies to increase pregnancy risk, calving risk, and efficiency of beef cows. In addition, to access the accuracy of ultrasonography diagnostic of twin pregnancies, embryo/fetal losses, evaluation of dystocia, progesterone concentration in late gestation, and effects of different twin-raising methods on neonatal behavior and growth. This project is divided into two experiments to account for measures of twinning technology and raising methods of twin calves. In experiment one, 77 multiparous Angus-cross cows from a commercial beef herd in northcentral South Dakota were estrous synchronized using a Fixed-Time Artificial Insemination (FTAI) protocol and artificially inseminated (AI) with black Angus semen. Seven days after AI, cows received an embryo transfer to the contralateral uterine horn to the ovary containing the CL. Cows were then assigned to two different treatments groups: only artificially inseminated (AI-only) and cows that received an embryo transfer following artificial insemination (ET+AI). Sensitivity and specificity were calculated for ultrasonography at d 45-53 gestational days when used to diagnose single and twin pregnancies. Pregnancy risk was on average 56% for ET+AI and AI

groups. Ultrasound twin detection achieved a sensitivity of 75%, specificity of 100% (Table 2), and presented an accuracy of 90.5%. Pregnancy loss from d 53 of gestation to term was 20.5%. Gestation length tended ($P = 0.06$) to be higher for twin pregnant cows, and twinning risk achieved 71.1%. The survival of all calves born during this experiment was 81.3%. In experiment two, 34 twin calves and 11 singleton calves were raised in different methods: 1) twin calves raised by their dam (Twin-Twin); 2) twin calves where one calf was grafted to another cow that lost her calf and one calf was left with their dam (Twin-Single); and 3) single born calves that were raised by their dams (Single-Single). Neonatal nursing behaviors and birth weights were recorded. Adjusted d 200 and d 280 were calculated as measures of vitality and growth. Calves' sera were used to quantify total serum protein, IgG1, and IgM as measures of passive transfer. Twin calves were born 20% lighter in weight than singletons; however, differences in weights were not significant at d 280 between twin-twin and single-single. Twin-single group had the shortest average latency to stand, but this did not translate into higher immunoglobulin concentrations. Twin-single calves had lower immunoglobulin concentrations between the three raising groups, while twin-twin and single-single did not significantly differ. Cows delivering and raising twins produced more kg than cows raising singletons. Using embryo transfer after artificial insemination proved to increase the twinning rate, and raising both twins with their dam did not decrease their growth. This twinning group was kept in the same pen and later same pasture, and cross-suckling happened equally among twins and singleton calves.

Keywords: Artificial insemination, beef production, cattle twinning, embryo transfer

Introduction

Sustainability in agriculture is a topic growing exponentially for both producers and consumers. The demands for more efficient animal production systems are necessary to satisfy the sustainability requirements. Beef production has a higher environmental footprint when compared to other animal-based proteins when considering a per-pound basis of crude protein (Poore and Nemecek, 2018). Cow-calf operations need the most improvement in efficiency based on the amount of greenhouse gas emissions. Rotz et al. (2019) attributed 77% of total cattle emissions to be associated with cow-calf production, as well as more significant fossil energy usage, blue water usage, and reactive N (total ammonia [NH_3], nitrous oxide [N_2O], nitrate [NO_3], and nitrogen oxides [NOx]) loss compared to stocker/background and finishing phases.

The reproductive rate has been previously associated with the efficiency of beef production (Davis et al., 1985). Discrete improvements in efficiency are related to reproductive traits that can increase economic outcomes (Van Tassel et al., 1998). An increase in twinning technologies increased production and economic efficiency by 24% when market at weaning (Guerra-Martinez et al., 1990). Even though twinning may result in low weaning weight per calf, an increase of 51% and 58.4% in weaning weight per cow has been previously reported (Davis et al., 1989; Gregory et al., 1996). Prior studies regarding increases in beef cattle fertility efficiency include genetic selection (Gregory et al., 1990a; Van Vleck et al., 1991; Gregory et al., 1997; Van Tassell et al., 1998), hormonal superovulation (Laster et al., 1971; Land and Hill, 1975), embryo transfer (Anderson et al., 1978; Davis et al., 1989), and artificial insemination followed by surgical (Sreenan and Diskin, 1989) or non-surgical embryo transfer (Tani et al., 2010; Dahlen et al., 2012). Conversely, twinning in cattle has raised some undesirable effects,

including dystocia, greater rebreeding interval, lower birth weights, and retained placenta (Çobanoglu, 2010; Echternkamp and Gregory, 1999; Gregory et al., 1996; Rutlegde, 1975). However, researchers reported an increased survival rate, gestation length, and birth weight when twins were gestated in bilateral uterine horns (Echternkamp et al., 2007a). Another study, however, found unilateral twins to be lighter in weight leading to less dystocia (Echternkamp et al., 2007b). Tani et al. (2010) attempted to produce twins in dairy cows using artificial insemination followed by embryo (produced *in vitro*) transfer in the uterine horn contralateral to the ovary containing the corpus luteum (CL). Dahlen et al. (2012) used the same technique but transferred an embryo (from donor cows) in the uterine horn ipsilateral to the ovary containing the CL. We hypothesize that transferring frozen and thawed high-grade embryos from donor cows to the contralateral uterine horn to the ovary containing the CL seven days after artificial insemination will increase beef cows' conception rate, calving rate, and efficiency. In addition, it is common practice among beef cattle producers to graft twin calves into other cows expecting that they will have more remarkable growth. However, there is not a lot of published data comparing behaviors and growth rates of twin calves compared to single calves based on their raising status. This experiment aimed to produce twin calves using artificial insemination followed by embryo transfer, increasing cow-calf efficiency, access the accuracy of twinning diagnosis using ultrasound, embryo/fetal losses, and evaluate of dystocia in twin pregnancies. As well as to compare twin calves rearing status on their neonatal behavior and growth rate.

Materials and methods

Cattle management for the duration of this experiment was approved by the Kansas State University's Institutional Animal Care and Use Committee and followed the guidelines of the

Guide for the Care and Use of Agricultural Animals in Agriculture Research and Teaching (IACUC #4282). The entire experiment took place between June 2019 and September 2020.

Experiment 1

Animals and treatments

Seventy-seven multiparous Angus-cross cows and their calves from a commercial beef herd in northcentral South Dakota were enrolled in this experiment. During the fertility treatment and gestation, cows were on pasture composed mainly of native grasslands with mineral supplementation, and water was provided *ad libitum*. Cattle were herded to the handling area with a squeeze chute (Daniels Manufacturing Co., Ainsworth, NE) for all procedures, including handling, sample collection, hormone injections, AI, embryo transfer, and ultrasonography.

Six weeks before their due date, cows were moved into a 0.4 km^2 corn-stock pasture with windbreaks and easy access to a maternity barn. Cows were provided with a nutritional feed ration to meet or exceed NRC (2016) requirements for pregnant beef cows (offered once daily). Freshwater was provided *ad libitum*. Cows were constantly checked for signs of parturition, and rounds occurred hourly or every other hour, depending on the amount of calving activity. As cows presented signs of parturition (stage I and II of parturition), they were moved to the maternity barn and housed in individual pens. All pens were bedded with 10 cm-deep straw and re-bedded daily. Twenty-four hours after parturition, cow-calf pairs were moved to an outside pen (twin pen), a common area for cows, and a calf nesting area with straw bedding and windbreaks. Calves could easily access the calf nesting area by using calf gates (e.g., shorter) that prevented the entrance of cows to prevent crowding and injuries.

783 ***Synchronization initiation***

784 All cows' estrous cycles were synchronized using a 7-day standardized protocol (CO-
785 Synch + CIDR®; Lamb et al., 2001). Synchronization was initiated with an intramuscular
786 injection of 100 μg of GnRH (Fertagyl®, Merck Animal Health, Madison, NJ). Then, an
787 intravaginal progesterone insert (CIDR®; 1.38 g of progesterone; Eazi-Breed™ CIDR Cattle
788 Insert; Zoetis Animal Health, Kalamazoo, MI) was applied. After 7 days, the CIDR was
789 removed. Then, cows received a 25 mg intramuscular injection of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$;
790 Lutelyse®; Zoetis Animal Health, Kalamazoo, MI). Finally, a breeding indicator patch was
791 placed halfway between the hip and the tail head (Estrotect™, Estrotect Breeding Indicators,
792 Spring Valley, WI). The breeding patch was activated when a cow was motivated to stay
793 immobilized and allow mounting by a groupmate. Breeding patches were checked 66 h after
794 placement, and activation of the patch was documented for each cow. Cows with less than 50%
795 of surface ink removed were considered inactivated and therefore did not likely display standstill
796 (standing to be breed) behavior. Cows that had inactivated patches at the 66-h check received an
797 additional intramuscular injection of 100 μg of GnRH (Fertagyl®, Merck Animal Health,
798 Madison, NJ). The proportion of cows presenting estrus was recorded.

799 ***Artificial insemination***

800 All cows received one dose of frozen-thawed black Angus semen at the end of the
801 synchronization protocol. Frozen semen doses from Connealy Uptown Bull were purchased from
802 Select Sires, Inc., Plain City, Ohio.

803 ***Corpus luteum detection***

804 Seven days after artificial insemination, the presence or absence of a corpus luteum on
805 both ovaries was detected using transrectal ultrasonography (5-10 MHz linear array transducer;

Sonosite M-Turbo, Fujifilm Sonosite, Bothell, WA). A fertility specialist veterinarian used both a qualitative assessment and the corpus luteum size to place each positive-corpus luteum cow into 1 of 3 categories: Excellent - palpable and firm, with >10 mm diameter; Good - palpable and moderately firm, with >10 mm diameter; or Poor - palpable but soft, with ≤10 mm diameter. Cows in the poor category were not eligible to receive an embryo transfer seven days after the AI. Cows in the excellent to good category were eligible for embryo transfer in addition to AI.

Embryo transfer

At the time of corpus luteum assessment, cows underwent their final fertility treatments. The ineligible cows did not receive embryo transfer (AI-only; n = 12); while the eligible cows received a 7-day frozen/thawed embryo (39 Red-Angus embryos and 24 Black-Angus embryos; Cross Country Genetics, Westmoreland, KS) via non-surgical embryo transfer to the contralateral uterine horn to the ovary with a present CL (ET+AI; n = 63). The goal of the embryo transfer seven days after artificial insemination was to create two calves for one cow by producing one embryo via artificial insemination and adding a 7-day embryo through embryo transfer.

Pregnancy and fetus number detection

Between 45-53 d after AI and embryo transfer, pregnancy risk and embryo counts were determined by transrectal ultrasonography of the uterus (4.0 MHz convex transducer; ReproScan XTC (VGA), Winterset, IA). Pregnancy was considered positive when one or more embryos were visible. Then, the number of embryos detected was recorded for each cow. Pregnancy risk was defined as the proportion of cows with at least one visible embryo via ultrasonography divided by all the cows that received just a dose of semen and a dose of semen and an embryo.

828 ***Peripartum data***

829 At calving time, data collection included gestation length, number of calves born per
830 cow, birth order for twin calves (1=first calf; 2=second calf), sex, birth weight, calf color (black
831 or red), calving ease scores for each calf on a scale from 1 to 5 (1 = unassisted calving; 2 = some
832 assistance; 3 = mechanical assistance; 4 = cesarian section; and 5 = abnormal presentation). Time
833 of birth - calf on the ground; first stand - all four limbs upright; and first suckle - mouth contact
834 with teat, were recorded for each calf.

835 ***Statistical analysis***

836 Statistical analyses were completed in SAS® software version 9.3 and SAS® Studio (SAS
837 Institute, Cary, NC, USA). Categorical data were arranged in tables with their frequencies, and
838 the FREQ procedure was used to analyze proportion differences. Differences in pregnancy risk,
839 fetal pregnancy loss, and calving risk were calculated for ET+AI and AI-only groups. All non-
840 categorical data were first tested for normality using the UNIVARIATE procedure and then
841 analyzed by a t-test Satterthwaite assuming unequal variances. Differences were tested for
842 gestation length among ET+AI and AI-only treatments. Data are expressed as mean ± standard
843 deviation where $P < 0.05$ was considered significant and $P > 0.05 < 0.1$ was considered
844 tendencies. The sensitivity, specificity, and accuracy of ultrasonography to detect twin
845 pregnancies were tested using the FREQ and NLMIXED procedures of SAS. Results are
846 expressed in percentage for sensitivity, specificity, positive predictive value, negative predictive
847 value, and accuracy with their respective 95% confidence intervals.
848
849
850

Experiment 2

Animals and treatments

Calves born to cows enrolled in Experiment 1 (twins, n=28; singletons n=11) and natural twin calves (calves born as twins to cows not previously enrolled in experiment one, n=6) were assigned to one of the three following raising groups: 1) twin calves raised by their dam (Twin-Twin); 2) twin calves where one calf was grafted to another cow that lost her calf and one calf was left with their dam (Twin-Single); and 3) single born calves that were raised by their dams (Single-Single). Calves grafted on twin-single groups were selected by the commercial herd management to better mimic standard beef production management. Times of birth (calf on the ground); first stand (all four limbs upright); first suckle (mouth contact with teat), and side of the first suckle were recorded for each calf as described by Bortoluzzi (2019). Calculations were made to achieve latencies relative to birth time: latency to stand and latency to first suckle. Twenty-four hours after parturition, cow-calf pairs were moved to an outside pen (twin pen), a common area for cows, and a calf nesting area with straw bedding and windbreakers. Calves could easily access the calf nesting area by using calf gates (e.g., shorter) that prevented the entrance of cows to avoid crowding and injuries.

Calf weight and blood collection

Before moving calves outside at 24 h of age, birth weights (BW) and blood samples were collected. Calves were weighed using a digital crane scale (Rural365, Sioux Falls, SD) at 24 h post-calving, and weights were recorded as birth weights. Calves were gently handled and placed in lateral recumbency for blood collection. A total of 10 mL of blood was collected from each calf via jugular venipuncture using a BD vacutainer serum blood collection tube (Becton, Dickinson and Company, Franklin Lakes, NJ). Whole blood was centrifuged, and supernatant

serum was harvested and frozen. Calves were gathered and weighed at approximately 6 months and 9 months of age with a squeeze chute containing a scale (Daniels Manufacturing Co., Ainsworth, NE). Since calves had different birthdays, a 200-d adjusted weight was calculated using the following equation: [(ADG x 200 d) + BW=A200dW] for the ~6 months weight, and a 280-d adjusted weight was calculated using the following equation: [(ADG x 280 d) + BW=A200dW] for the ~9 months weight

Nursing behaviors

Once cows and calves were moved outside, suckling behaviors were observed and recorded on 4 different days during a 3-hour period (13:30 – 16:30). Two observers familiar with the animals stood inside the pen and recorded the following suckling information: cow ID, calf ID, time of suckling start, and time of suckling stop. Calculations were made to achieve the total time of suckling behavior for each calf, time of non-dam nursing (suckling on a cow that was not its own dam), time of own dam nursing, and the number of bouts (a short period of intense suckling; > 1 minute). For accurate measures of suckling behaviors, twin cows that lost one of their calves (calf died or was grafted to a different cow) were considered as cows nursing a single calf. Only cows with two viable calves were considered as cows nursing a pair of twins.

Immune measures

Calves' sera were thawed in a refrigerator overnight and analyzed for total serum protein and immunoglobulins (IgG1 and IgM). Serum total protein was measured using a digital handheld refractometer (MISCO, Solon, OH). IgG1 and IgM were measured using commercially available ELISA kits (Bethyl Laboratories Inc., Montgomery, TX) using suggested dilutions. All sera samples were analyzed on one 96-well plate for each assay. Samples were randomly assigned to duplicate wells within a plate. A microplate reader was used at a wavelength of

450nm to measure optical densities, which were converted into sample concentrations using a standard curve. The intra-assay coefficient of variation was 8.64% for IgG1 and 8.65% for IgM.

Statistical analysis

Statistical analyses were completed in SAS software version 9.3 (SAS Institute, Cary, NC, USA). The procedure GLIMMIX was used to analyze non-categorical data. Dependent variables of BW, A200dW, A280dW, behavior measures, and measures of passive immune transfer were analyzed within a model that included the fixed effects of raising groups (twin born-twin raised, TT; twin born-single raised, TS; and single born-single raised; S), birth order (first and second born), and interaction between raising groups and birth order. Pearson correlations were calculated using the CORR procedure to determine relationships among BW, A200dW, A280dW, behavior measures, and measures of passive immune transfer. Differences of $P < 0.05$ were considered significant and when $P > 0.05 < 0.1$ were considered tendencies.

Results

Experiment 1

The total pregnancy risk for both treatment groups was 56% (42/75). The proportions of pregnancy risk differed among the two groups (P-value = 0.026; Table 1). The ET+AI achieved a pregnancy risk of 61.9%, where 42.9% (27/63) of the cows were pregnant with twins, and 19% (12/63) were pregnant with a singleton. The AI group achieved a pregnancy risk of 25% (3/12), and all three were pregnant with singletons.

When the ultrasound was used between 40 to 53 gestational days, the sensitivity to detect twin pregnancies was 75%, while the specificity was 100%. Only one cow from the AI group was misdiagnosed as open once pregnant. The accuracy of ultrasonography examination in the detection of twins was 90.5% (CI of 83.2%, 97.7%).

Pregnancy loss from d 53 to term was 20.5% for cows within the ET+AI group. The three cows in the AI-only group did not have any losses. When considering both cohorts, the overall pregnancy loss was 19.05%. Gestation length tended ($P = 0.06$) to be 3 d longer for cows carrying singletons than cows carrying twins (d 279 $\pm$ 5 vs. d 276 $\pm$ 2, respectively). Two abortions and a stillborn parturition were removed from the data analysis to provide a better overview of gestation length. A total of 53 calves were delivered at term, and this accounts for calves born as singletons or twins in both raising groups. The calving risk for this experiment was 70.6% (53 calves / 75 cows; Table 3). When cows were submitted to additional embryo transfers 7 d post-AI, the risk of calves being born as twins was 76%, and the risk of calves being born as singletons was 24%. All three calves born to the AI-only cows were singletons. The twinning risk for all cows was 71.7 %, and its proportions significantly differ among treatments ($P = 0.01$).

Calves born as second twin calf tended to require more assistance at delivery compared to the twin calf born first (85.7% and 14.3%, respectively; $P = 0.09$). Only one calf born first required minor assistance, while second-born calves required some assistance, mechanical assistance, and had an abnormal presentation (n=4, 1, and 1, respectively). Abnormal position is represented by cow 2029 (black calf) and other presentations, positions, and postures are depicted in Figure 2.1.

Survival proportions during the neonatal period (first 24 d of life) for calves born as twins or singleton were not different (81.6 % and 80%, respectively; $P > 0.1$). Of the total calves born during this experiment, 81.1% survived the neonatal period, and this accounts for 3 stillborn and 4 deaths of calves born as twins and 3 deaths of calves born as singletons.

943 **Experiment 2**

944 The least-square means and their standard error of growth, neonatal behaviors, and
945 measures of passive immune transfer are depicted in Table 4 for the three raising groups and
946 twin birth order. As expected, calves born as twins at birth were 20% lighter in weight than
947 calves born as singletons (29.3 kg and 36.6 kg, SEM = 1.79, respectively; $P < 0.001$). Heifer
948 calves were lighter in weight than bull calves (27.4 kg and 32.8 kg, SEM = 1.34, respectively; P
949 < 0.001). Adjusted 200-d weights were greater ($P < 0.05$) for singletons than calves born as
950 twins and raised as singletons. Weight differences were not significant at 280 days for twin-twin
951 first and second born, twin-single firstborn, and single-single calves. Second-born calves (n=5)
952 were more likely to be grafted to another cow than firstborn calves (n=1) for the twin-single
953 treatments. However, no significant differences were found within the twin-single group for BW,
954 A200dW, and A280dW for grafted calves and non-grafted calves.

955 Overall, the average latency to stand was 69 minutes, with firstborns in the twin-single
956 group having the shortest average latency (34.9 min; Table 4) and second-born in the twin-twin
957 having the longest average latency (97.4 min; Table 4). The average latency to the first nurse was
958 around 120 min, and latencies were not significantly different among raising groups. During the
959 12 hours observations of nursing behaviors, all calves spent more time nursing from their own
960 dam (58.4 min; Table 4) than nursing another cow (5.36 min; Table 4). Average dam and non-
961 dam nursing times were not significantly different among raising groups ($P > 0.1$). Twin-single
962 calves had almost 50% lower ($P < 0.05$; Table 4) serum IgG1 concentrations compared to
963 singleton calves. However, IgG1 concentrations were not significantly different between twin-
964 twin and twin-single calves. Cows that gestated twins and raised both calves produced 185 kg
965 more weaning weight at d 200 and 306.4 kg more at d 280 than cows that gestated and raised

singletons. In addition, there was a positive correlation between BW and A200dW (r = 0.32; $P < 0.05$; Figure 2.2), but no significant correlations were found between BW and A280dW. Birth weight was also positively correlated with serum IgG1 concentrations (r = 0.44; $P < 0.05$; Figure 2.2).

Discussion

Experiment 1

The pregnancy risk achieved in ET+AI group agrees with the risk of 56% reported by Sreenan and Diskin (1989) for a group of mature cows that were AI and then non-surgically embryo transferred to either ipsilateral or contralateral uterine horn. Dahlen et al. (2012) conducted a study with purebred Angus comparing AI, ET, and ET+AI, with the embryo placed in the ipsilateral horn to the ovary containing the CL. Their ET+AI conception rate was slightly lower (48.5%) on d 30-35 than what we achieved. Tani et al. (2009) used a smaller sample size of Japanese dairy cows and transferred the embryo into the contralateral uterine horn containing the CL, achieving a conception rate of 30.4%. The prohect occurred during the summer months when the conception rate was diminished due to heat stress. Discrepancies in conception rates between these studies are most likely due to breed aptitude and heat stress. Lactating dairy cows have been reported to have lower conception rates due to their great metabolic demand for high milk yield (Nebel and McGillard, 1993; Chagas et al., 2007). The lower conception rate for the AI group was expected based on their CL poor scores, which was reflected in the significant difference among groups.

Ultrasonography examinations were previously suggested as a diagnostic tool to identify twin gestations at late embryonic or early fetal stages, which resulted in better management decisions regarding cows carrying twins (Davis and Haibel, 1993; Fricke, 2002; Szelényi et al.,

989 2015). The usage of ultrasound between 40-53 gestational days in this experiment proved to be
990 an important diagnostic tool, even though the specificity was higher than the sensitivity. In
991 addition, since it was known that this cow population was submitted to fertility treatment, a
992 careful scan of both uterine horns for more than one embryo was performed, resulting in high
993 diagnostic accuracy.
994 Pregnancy loss has been previously attributed to reproductive inefficiency (Fricke, 2002).
995 Twin pregnancy losses occur early in gestation rather than late, and pregnancy is carried to term
996 once established (Sreenan and Beehan, 1976). The pregnancy loss percentage in this experiment
997 was higher than values published previously for heifers and multiparous beef cows (15.1% and
998 8-11%, respectively; Sreenan and Diskin, 1989; Guerra-Martinez et al., 1990). In contrast, a 60%
999 pregnancy loss was reported when a similar twinning technique was used in dairy cows, and its
1000 losses were attributed to twin pregnancy being more risky and prone to abortions (Tani et al.,
1001 2009). Based on our findings and previous studies that reported considerably lower losses, other
1002 factors might be associated with embryonic/fetal losses, such as breed, semen quality, and heat
1003 stress, than the twin pregnancy alone. Twin pregnancies were previously reported as having, on
1004 average, a shorter gestation length of 5-7 days compared to singleton pregnancies (Turman e al.,
1005 1971; Bellows et al., 1974; Anderson et al., 1982; Gregory et al., 1990; Echternkamp and
1006 Gregory, 1999). For this experiment, there was only a tendency for twin pregnancies to be
1007 shorter in length than singleton pregnancies. On average, twin pregnancies were 3 days shorter,
1008 with abortions records being excluded from the analysis. Many factors can affect gestation
1009 length, including breed differences, sex of the calf, dam weight, parity number, and sire
1010 (Andersen and Plum, 1965; Foote, 1981). One hypothesis of the similar gestation length between
1011 the two groups could be attributed to bilateral pregnancies, where each calf develops on one side

of the uterus, diminishing uterine crowding seen early in unilateral twin pregnancies (Echternkamp et al., 2007a). Uterine crowding has previously been attributed as a stimulus that causes secretion of the adrenal corticotropic hormone by the fetus' pituitary gland, which increases concentration of fetal cortisol, initiating the cascade of endocrine events that culminates in parturition (Senger, 2012). Parturition assistance occurred more often for twin calves compared to singles, especially for the second twin as anticipated. The majority of assistances were easy pulls, and managers could attribute this to the fact that cows were known to be bearing twins. Managers did not allow much time to pass after the first calf was born to provide assistance, aiming to decrease morbidity and mortality risk for the second born twin. Only one twin calf was in abnormal presentation and required repositioning. Retrospective studies suggested that twin calves are more likely to present abnormal position at birth (Echternkamp and Gregory, 1999). Elevated incidences of dystocia were reported when heifers were submitted to twinning compared to cows (Anderson et al., 1982). Even though the frequency of dystocia was higher for twin calves in our experiment, we believe that our technique produced bilateral twins. The development of each fetus in one of the uterine horns was published as a factor decreasing abnormal presentation incidence compared with unilateral twinning (Echternkamp and Gregory, 2002). Survival data of twin and singleton calves had been documented by Owens et al. (1984), and the survival proportion at weaning for twin calves was lower (62%) compared to singleton calves (73.6%). Higher survival proportions were reported for twins (76.2%) and singletons (88.6%) at d 200 (Echterkamp and Gregory, 2002). In the current experiment, both twins and singleton calves had similar survival proportions (~80%); however, our data represent the neonatal period.

Experiment 2

Neonatal behaviors are an important tool to access calf vigor in the first hours of life. Stand, seek, and nurse colostrum are essential behaviors for calf health and resilience. Latency to stand and nurse among the different raising groups are in agreement with literature for twin and singleton calves (Adams et al., 1993). Ewbank (1967) investigated natural twin and artificially paired twin calves' behaviors once they were turned out to pasture after being kept in a calf pen and concluded that twin behavior is probably mostly associated with the system of rearing than genetics itself. Price et al. (1981) noted that cows nursing twins in larger enclosures did pay less attention or did not nurse one or both twin calves compared to cows in smaller enclosures. Cows and calves in the current experiment were kept in the same herd, and this rearing system may have encouraged nursing behaviors that were not different between raising groups. It was possible to observe that cows rearing twins are used to having more than one calf nursing them and are less prone to notice calves that are "stealing" milk. A higher proportion of cows rearing twins (47% vs 13%, respectively; cows rearing twins and cows rearing singletons) that allowed cross suckle behaviors were previously reported in literature (Price et al., 1981).

Lower birth weights for twin calves were expected due to limited uterine space (Echternkamp et al., 2007a). Nevertheless, the weights in of this experiment were similar to the ones described in the literature that compared twins and singletons (Davis et al., 1989; Tagawa et al., 2008). Lower birth weights for heifers were also previously reported in the literature, and they agree with our findings (Davis et al., 1989; Odde, 1988). Weight differences among twin-single and single-single calves were still apparent at d 200 of age. These data are similar to growth rates reported by others (Diskin et al., 1990; Echternkamp and Gregory, 2002). However, both studies compared twin and singleton calves that nursed their dams until weaning without

1058 grafting one of the twin calves to another dam. In Echternkmap and Gregory's (2002) study, only
1059 triplets were grafted. Their growth was credited to their dam, and no comparisons were made
1060 between grafted- and nursed-dam calves. Lower weight gains were observed for twin-twin calves
1061 compared with twin-single calves and were reported by Gregory et al. (1990b). The opposite
1062 happened in the current experiemnt, where twin-twin calves were heavier at d 200. By d 280 of
1063 age, there were no significant differences. Other researchers demonstrated that nutrition
1064 utilization by calves that had their growth restricted in uterus or from birth to weaning did not
1065 appear to impact the nutrient utilization later in life (Greenwood and Cafe, 2007). Nevertheless,
1066 their yield grade could be reduced compared to calves with normal growth. Yield grade data was
1067 not collected nor are available because calves in the current experiment were sold at d 200-280.
1068 However, Echternkamp and Gregory (2002) reported that more than three-fourths of carcasses
1069 from their twin population were graded USDA grade Choice or above, which leads us to believe
1070 that restricted growth in uterus does not negatively affect beef quality. Future research is needed
1071 to follow twin calves in different raising systems to be followed to slaughter to analyze carcass
1072 data.

1073 As expected, twin calves had lower immunoglobulin concentrations, probably due to
1074 both calves having nursed in one cow, which leads to lower intake of colostrum volume by each
1075 calf (Odde, 1988) compared to a calf nursing alone. This trend is consistent with results
1076 published by Adams et al. (1993).

1077 Weaning weights are important a measurement in beef production due to their association
1078 with cow-calf operations efficiency (Odde and Field, 1987). Cows delivering and raising twin
1079 calves can produce between 48-60% more total weaning weight compared to cows delivering
1080 and raising singles (Wyatt et al., 1977; Davis et al., 1989; Echternkamp and Gregory, 2002). Our

results show an increase of 71% in total when comparing calves that were twins and raised as twins and calves born singletons and raised as singletons.

Conclusion

Embryo transfer seven days after artificial insemination is a successful technique in producing twin calves in beef cattle. Sensitivity and specificity of ultrasound exam to detect twins are appropriate to assist producers in decision making on management of cows pregnant with twins (e.g., keep cows close to barn or maternity chute, more frequent calving checks, etc.). Although twin calves are lightweight at birth compared to singletons, cow producing twins wean greater amount of calf weight (kg/cow). This increases cow-calf efficiency at weaning time. The potential for reducing number of cows needed to produce same amount of calf weight could increase sustainability of cow-calf production.

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Table 2.1. Pregnancy risk proportions of cows submitted to two different fertility protocols.

			Pregnancy risk		
Treatment	**AI (n)**	**ET (n)**	**Group[3]**	**Total[4]**	***P*-value[5]**
AI-only[1]	12	-	25.0%	4%	0.026
ET+AI[2]	63	63	61.9%	52%	
Total	75[*]	63	-	56%	

[1]Group of cows submitted to a 7-day CO-Synch + CIDR protocol and artificially inseminated using fixed timed (AI-only); [2]Group of cows submitted to a 7-day CO-Synch protocol, artificially inseminated using fixed timed AI, and 7 days after AI, received an embryo transfer (ET+AI); [3]Proportion of pregnancy risk for cows within each treatment group; [4]Proportion of pregnancy risk for cows in both treatment groups combined; [5]Fisher's exact test *P*-value; [*]Two cows were removed from the experiment (7016 – fail to remove CIDR; 7057 – presented a possible uterine infection at ultrasound examination).

Table 2.2. Sensitivity and specificity values for ultrasonography diagnostic of cows carrying twin calves.

Statistic	**Estimate**	**SE**	**95% Confidence Intervals**	
Sensitivity[1]	75%	8.84%	58.7%	92.3%
Specificity[2]	100%	-	100%	100%
Accuracy[3]	90.5%	-	-	-

[1]Proportion of cows correctly identified as pregnant with twins (true positives) using ultrasound as diagnostic test in the twin cows pregnant population; [2]Proportion of cows correctly identified as pregnant with singletons (true negatives) using ultrasound as diagnostic test in the singleton cows population. [3]Proportion of cows correctly identified as carrying twins of singles (true positives and true negatives) in the pregnant cow population.

Table 2.3. Number of calves born for AI-only and ET+AI cows.

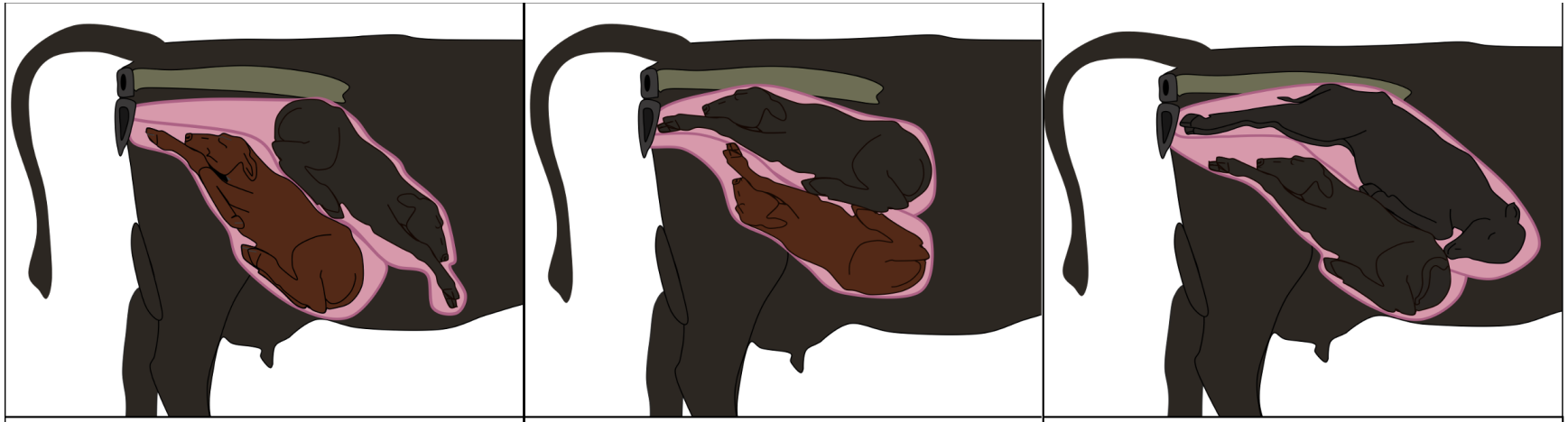
	Number of calves born		
Treatment	**Singleton**	**Twins**	***P*-value[3]**
AI-only[1]	3	--	0.01
ET+AI[2]	12	38	
Total	15	38	53

[1]Group of cows submitted to a 7-day CO-Synch + CIDR protocol and artificially inseminated using fixed timed AI-only; [2]Group of cows submitted to a 7-day CO-Synch protocol, artificially inseminated using fixed timed AI, and 7 d after AI, received an embryo transfer; [3]Fisher's exact test *P*-value.

Table 2.4. Performance, behavioral observations, and measures of passive immunity for different groups of raising twin beef calves.

		Raising groups[5]					
	N	Twin-Twin		Twin-Single		Single-Single	SEM
Birth order	-	1	2	1	2	-	-
Weight, kg							
Birth	39	27.8[a]	29.4[a]	31.5[a]	28.5[a]	36.3[b]	1.79
Adjusted 200-d	39	215.7[a]	226.6[ab]	209.7[a]	216.4[a]	257.3[b]	16.09
Adjusted 280-d	31	310.9[ab]	318.5[ab]	280.3[ab]	273.3[a]	323.0[b]	18.41
Behavior							
Latency to stand, min[1]	33	73.2 [ab]	97.4 [a]	34.9 [b]	80.2 [ab]	63.0 [ab]	23.52
Latency to nurse, min[2]	33	111.2	163.0	92.8	96.0	135.9	30.82
Duration of dam nursing, min/12h[3]	28	67.2	61.0	72.0	46.2	46.0	12.35
Duration of non-dam nursing, min/12h[4]	24	10.4	5.0	4.0	3.8	3.6	3.68
Passive immune transfer							
Total serum protein, g/dL	37	7.1[a]	7.1[a]	5.7[ab]	5.3[b]	6.1[ab]	0.56
Serum IgG1, mg/mL	36	26.8[ab]	28.8[ab]	17.2[a]	16.2[a]	35.5[b]	7.83
Serum IgM, mg/mL	36	1.7	2.1	0.9	1.1	1.7	0.54

[ab]LS-means in a row without a common superscript differ $P < 0.05$; [1]Time between birth and first standing; [2]Time between birth and first nursing; [3]Time spent nursing own dam (min) in four 3-hour observation periods in the neonatal phase (first 24 d of life); [4]Time spent nursing a different dam (min) in four 3-hour observation periods in the neonatal phase (first 24 d of life).[5]Raising groups were selected by the commercial herd management to better mimic standard beef production management.



Cow 2029	Cow 9221	Cow 8216
* Red calf – Anterior presentation, dorsal position, left carpal flexure. * Black calf - Posterior presentation, dorsal position, bilateral hip flexion (true breech).	* Black calf - Anterior presentation, dorsal position, complete extension of head and fore limbs. * Red calf – Anterior presentation, ventral position, complete extension of head and fore limbs.	* Black calf A – Posterior presentation, dorsal position, complete extension of hind limbs. * Black calf B - Anterior presentation, dorsal position, complete extension of head and fore limbs.

Figure 2.1. Examples calf presentations, positions, and posture encountered during parturition of twin calves born from cows submitted to embryo transfer to the contralateral uterine horn containing the corpus luteum seven days after artificial insemination.

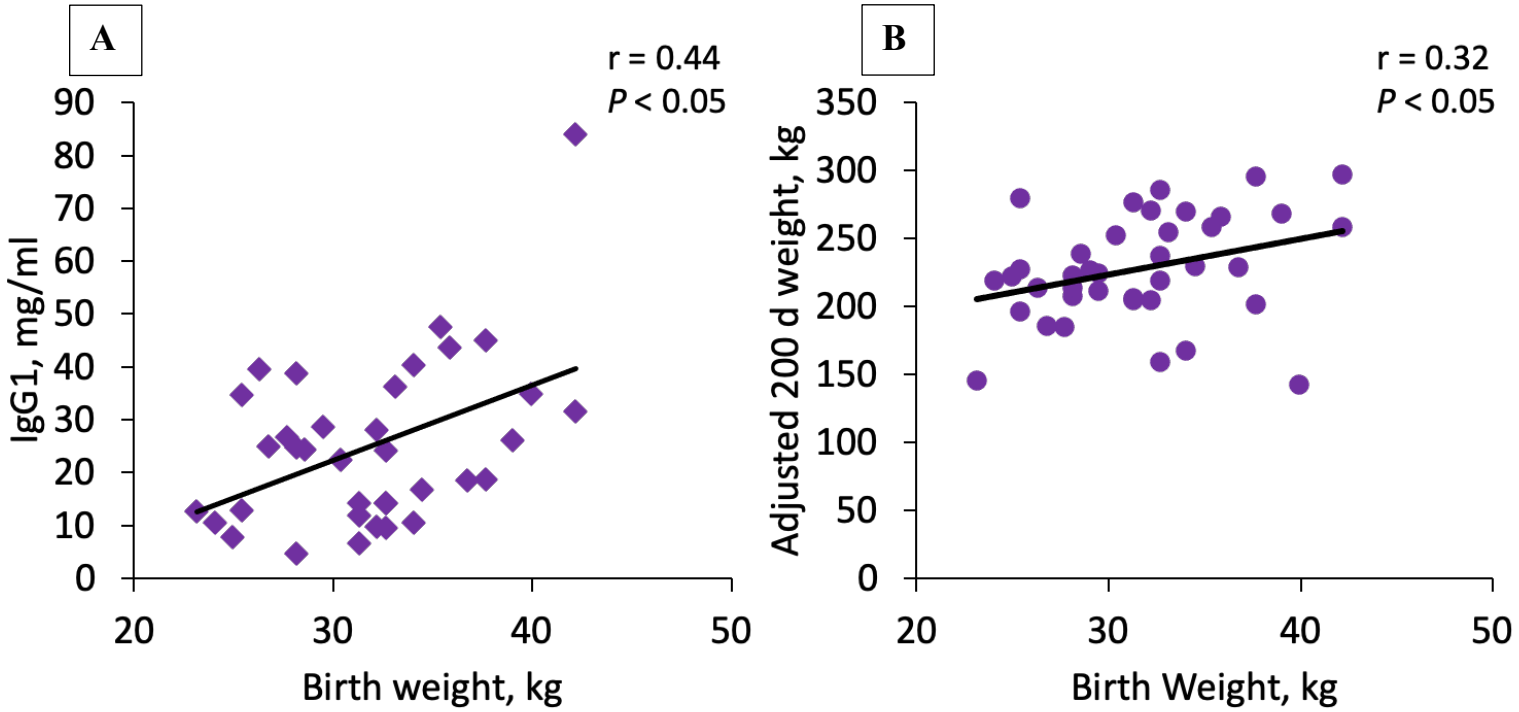


Figure 2.2. - A) Positive Pearson's correlation between birth weight (kg; at 24 h of age) and serum IgG1(mg/ml) representing the entire calf population enrolled in the experiment. B) Positive Pearson's correlation between birth weight (kg; at 24 h of age) and adjusted 200 d weight (kg). Weights ~ 6 months of age were adjusted using the following equation: [(ADG x 200 d) + BW = A200dW].

Chapter 3 - Colostrum Supplement Fed to Calves

Neonatal calf behaviors and measures of passive immune transfer of calves fed a commercial colostrum replacement as a supplement during the first two hours of life

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Abstract

The agamaglobulinaemic status of neonatal calves makes colostrum consumption in the first hours of life imperative for lower morbidity and mortality prevalence. Age of the dam is considered an important factor in the quality and quantity of colostrum production. Calves born to first-calf heifers have been reported to have lower serum IgG concentrations, predisposing them to neonatal infections. A randomized field trial was conducted in a commercial cow-calf operation in northcentral South Dakota. The goal of the study was to determine the effects of supplementing a commercial colostrum replacement product containing > 60 g of IgG for calves born to first-calf heifers in the first 2 hours of life. Sixty Angus-cross neonatal calves were randomly assigned one of the two treatments: colostrum supplement (CS; 1L with 60 g/L IgG) fed using an oro-esophageal tube feeder and a control (Control; n=30) treatment where calves had unlimited access to nurse their dams without supplementation. Colostrum supplement occurred within 120 minutes of birth. Neonatal calf behaviors, mothering scores, and calving ease were recorded by monitoring cow-calf pairs. Birth weights (BW) and blood samples were collected around 24 hours of age to access IgG concentrations. Growth was measured by

adjusted weights at 100 and 200 d (A100dW and A200dW) and their respective average daily gain (ADG). Growth, behavior, and passive immune transfer measures were tested using ANOVA and Pearson Correlations were used to draw associations. No differences were significant between treatments, sex, or interaction for percentage of serum protein, IgG serum concentrations, or growth measures ($P > 0.1$). Latency to first stand was not different for calves in both treatments ($P > 0.1$). Latency to first nurse was found to be significantly higher ($P = 0.05$) for calves receiving colostrum supplementation than control calves. Feeding colostrum supplement through an oro-esophageal tube in the first 2 hours of life did not significantly increase serum IgG. Nonetheless it has affected natural neonatal behaviors by delaying colostrum nursing.

Key words: colostrum, colostrum replacement, first-calf heifers, IgG, neonatal behaviors

Introduction

The ingestion and absorption of immunoglobulins present in colostrum is imperative for ruminants' passive immunity because of their agammaglobulinemic condition at birth. Intake of decreased volume of colostrum can be associated with failure of passive transfer (FPT), resulting in less circulating immunoglobulins and increased susceptibility to infections. Calves that are born to nulliparous cows were reported to have lower serum immunoglobulin concentrations when compared to calves born to multiparous cows, and this condition may be associated with less mammary development, reduced IgG transport to the mammary gland, and consequentially lower colostrum volume being produce (Devery-Pocius and Larson, 1983;Odde, 1988). To avoid failure of passive transfer, the dairy industry has increased measurement of fresh colostrum density using a colostrometer or a refractometer (%Brix) to ensure appropriate immunoglobulins concentration. When the maternal colostrum has lower concentration of immunoglobulins (<21% Brix) or its volume is inadequate, frozen colostrum and colostrum replacement are fed as a strategy to secure an adequate passive immune transfer (Quigley et al., 2013; Cabral et al., 2013). This strategy is not common among beef producers. Conversely to dairy management procedures, beef calves are left to nurse maternal colostrum from their dam (Earley et al., 2000; McGee and Earley, 2018). The calf must be able to stand, find the teat, and suckle within 2 hours of calving while the cow should stand and bond with the calf. Any delays in this process can affect colostrum intake and immunoglobulin absorption (Larson et al., 2004). Disruption of nursing behaviors and cow-calf bond are a concern when feeding colostrum replacement as supplement to neonatal calves because they will be briefly separated from the cow. We hypothesized that early supplementation of a commercially available colostrum replacement fed via an oro-esophageal tube feeder can increase immunoglobulin G concentration in beef calves

born to nulliparous cows without disruption of the cow-calf bond and their sub-sequential nursing behaviors. The objective of the present study was to determine the effects of supplementing a commercial colostrum replacement product containing > 60 g of IgG for calves born to first-calf heifers in the first 2 hours of life.

Materials and methods

The protocols for this experiment were approved by the Kansas State University's Institutional Animal Care and Use Committee and followed the guidelines of the Guide for the Care and Use of Agricultural Animals in Agriculture Research and Teaching (IACUC #4521).

Animals and housing

First-calf heifers (n=60) and their neonatal Angus-cross calves (n=60; BW=31.7 kg, SD=4.24 kg) from a commercial beef herd in northcentral South Dakota were enrolled in this study. The experiment took place between March and April 2021. Heifers were housed in a corn stock pasture two weeks prior to their due date and were fed a ration to meet or exceed NRC (2016) requirements once a day. Freshwater was provided *ad libitum*. The pasture was monitored every 2 hours and at first signs of parturition (stages I and II of parturition), heifers were moved to individual maternity pens indoors. The maternity barn consisted in 11 individual pens, and a maternity chute bedded with 10 cm-deep straw. Soiled straw was removed daily and replaced with clean straw. Heifers and their calves were moved to an outside pen 12-24 hours after parturition, depending on weather conditions. The outside pen was located closely to the maternity barn and consisted of a common area for cows and a calf nesting area with straw bedding and windbreaks. The strategy of monitoring heifers every two hours and moving them indoors were to ensure cow-calf bonding and assist heifers that were having difficulties at

parturition. Then, cow-calf pairs were moved to native grasslands heifer pastures every other day.

Treatments

Calves were allotted to one of the two treatments in a completely randomized design. The treatments consisted of a colostrum supplement treatment (CS, n=30) where a commercially available colostrum replacement used as colostrum supplement, was fed using an oro-esophageal tube feeder within 2 hours after birth with subsequently unlimited access to the dam; and a control (Control; n=30) treatment where calves had unlimited access to nurse on their dams without colostrum replacement supplementation. The colostrum replacement of election was the Bovine IgG Calf's Choice Total Gold (Saskatoon Colostrum Company, Saskatoon, SK, Canada) that contains > 60 g of IgG and it is a maternal colostrum-only product. The colostrum replacement powder was mixed in warm water at 37ºC according to label directions to a final volume of approximately 1 liter. Calves on CS treatment were cleaned and groomed by their dams prior to the CS feeding to ensure cow-calf bonding, around 30 minutes before calf got the supplementation. These calves were then briefly removed from the pen and supplemented with colostrum replacement using an oro-esophageal tube feeder. Calves were immediately placed back in the pens in sternal recumbency position where they had unlimited access to their dam.

Dystocia and mothering scores

Calving ease was recorded for each calf on a scale from 1 to 5 (1 = unassisted calving; 2 = some assistance; 3 = mechanical assistance; 4 = cesarean section; and 5 = abnormal presentation). And mothering scores as previously published by Mihura et al. (1995) (1 = accepted the calf; 2 = hesitated to accept the calf; 3 = physically abused calf) with mothering descriptions were also recorded.

Behavior measures

Calves were closely monitored by three trained observers (inter-observer reliability > 95%). Two video cameras were placed inside the barn (Eversecu 1080P 320 Pan 90 Tilt 2-way audio, night and day, Aurora, Ontario, Canada) to minimize disruption of the cow and calf. Neonatal behaviors were recorded for all calves such as time of birth (calf hit the ground), time of first stand (four limbs upright for at least 5 seconds), and time of first suckle (first mouth contact with any teat). For calves on CS treatment, time of colostrum replacement supplementation and time of suckle after supplementation were also recorded. If calves did not suckle within 3 hours after birth or after colostrum replacement supplement, personnel manually assisted nursing process using the maternity pen.

Measures of passive immune transfer

Blood samples were collected via jugular venipuncture at 18-30 hours of age. Plain glass containing no clot activators, anticoagulants, preservatives, or separator materials tubes (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) were used to collect 10 mL of blood from each calf. Samples were allowed to clot at room temperature from 15-30 minutes, and then centrifuged at 2000 x g for 10 minutes. The supernatant serum was harvested and transferred to two aliquoted tubes, labeled with calf ID and frozen at -20^{o}C. Samples were properly stored and shipped on ice to the SCCL Quality Assurance Laboratory (Saskatoon, SK, Canada) where IgG concentrations were measured by radial immunodiffusion assay. The laboratory staff were blinded to treatment.

Weights

Calves were weighted at 18-30 hours (BW) of age using a digital crane scale (Rural365, Sioux Falls, SD). Around 100 days and 200 days of age, calves were gathered from the heifer

pasture and weighted with a squeeze chute containing a scale (Daniels Manufacturing Co., Ainsworth, NE). Since birth dates were variable, the days were adjusted to 100 days (A100dW) using the following equation: [(ADG x 100 d) + BW=A100dW] and to 200 days (A200dW) [(ADG x 200 d) + BW=A200dW]. At 100 days, four calves had lost their ear tags, preventing their identification, and their weights were not recorded. At 200 days weight, nine calves had been already sold or had lost their tags and their weights were not recorded.

Statistical Analysis

All statistical analyses were performed in SAS® software version 9.3 (SAS institute, Cary, NC). Continuous data of behavior, calf performance and immune measures were tested for normality by evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure. Then, data were analyzed by ANOVA using GLM procedure of SAS, with fixed effects of treatment, sex, and interaction between treatment and sex. Scheffe's procedure was used to control EER. Calving ease and mothering scores were not significant due to lack of statistical power. Pair-wise comparisons were made among the two treatments (CS and Control), sex (bull and heifer), and the interactions between treatment and sex. Least square means (± SEM) are reported throughout. Differences of $P < 0.05$ were considered significant and $P < 0.10$ were considered tendencies when biologically appropriated. Correlations between calf performance, immune measures, and neonatal behaviors were performed using Pearson's correlation for each variable using the CORR procedure. Differences of $P < 0.05$ were considered significant and $P < 0.10$ were considered tendencies when biologically appropriated. Calving ease and mothering scores were computed in probabilities within each treatment using the FREQ procedure of SAS.

Results

Data analyzed for this study accounts for 28 calves enrolled on CS treatment (bulls = 6; heifers = 22) and 30 calves enrolled on Control treatment (bulls = 9; heifers = 21). Two calves from the colostrum treatment (heifer, n=1; bull, n=1) were removed from the analysis. The heifer calf was a twin that was paired with the wrong dam and the bull calf presented neurological clinical signs (rhythmically head tremors and difficulty in standing).

Growth

Birth weights were homogenous between treatments and sexes. Bull calves in both treatments tented to be heavier at A100dW compared to heifers ($P = 0.07$; Table 3.1). Their average daily gain tended to be higher than heifer calves during the same time period. Those differences were not found to be significant for A200dW or ADG at 200 days. As expected, birth weights were positively correlated with A100dW and A200dW ($r = 0.50$, $P \leq 0.001$; $r = 0.46$, $P \leq 0.01$, respectively).

Passive immune transfer

Most colostrum supplementations occurred before calves nursed on their dam (76.6%). No differences were significant between treatments, sex, or interaction for percentage of serum protein or IgG serum concentrations ($P > 0.1$; Table 3.2). Serum protein averaged 7% for all calves enrolled in the study. Distributions of IgG serum concentrations for each treatment are shown by histograms (Figure 3.1). Only one calf on Control treatment failed transfer of passive immunity with IgG concentrations <10 g/L. Two calves (Control = 1; CS = 1) had inadequate transfer of passive immunity (ITPI) with IgG concentrations < 24 g/L. All remaining calves achieved adequate transfer of passive immunity with serum IgG concentrations > 24 g/L.

Neonatal behaviors

Latency to first stand were not different for calves in both treatments ($P = 0.20$). All calves took on average 51 minutes to first stand, with bull calves on CS having the longest ($\mu =$ 63 min) and heifer calves on control having the shortest latency ($\mu = 46$ min). Calves on CS treatment were fed colostrum supplement within their first two hours of life ($\mu = 60$ min; 95% CI [45, 65]). Latency to first nurse was found to be significantly higher ($P = 0.05$; Table 3.3) for calves receiving colostrum supplementation (108 ± 14.14 min) than control calves (69 ± 12.23 min). After being fed colostrum, calves on CS treatment took on average 67 minutes to start nursing on their dams. Birth weights were positively correlated with latency to stand (r =0.31, $P \leq 0.01$; Table 3.4). Latency to first nurse was negatively correlated with serum protein (r = -0.25; $P \leq 0.05$; Table 3.4), but not with serum IgG concentrations (r = -0.18; $P > 0.05$; Table 3.4). In addition, latency from birth to colostrum supplementation tended to be negatively correlated with serum protein (r = -0.36; $P = 0.06$; Table 3.4) and negatively correlated with serum IgG concentrations (r = -0.41, $P \leq 0.05$).

Dystocia and mothering scores

Dystocia accounted for 12.28% of parturition in this study. On the CS group, 10.71% (3/28) required some assistance, and 3.57% (1/28) required mechanical assistance to be born. On the Control group, 6.9% (2/30) required some assistance, and 3.45% (1/30) required mechanical assistance to be born. Most of the first-calf heifers presented good maternal abilities, and 84.21 % of the cows cleaned their calves and allowed them to nurse without assistance. On CS and Control treatments, 14.29% (4/28) and 10.34% (3/30) of dams, respectively, took some time to start cleaning the calf but did not require human assistance to get calf nursing. However, only two dams (CS, n = 1; Control, n =1) required assistance to clean and get calf to nurse.

Discussion

Colostrum supplementation using a commercial colostrum replacement product was investigated in calves born to first-calf heifers. Birth weights are seen as an important measure for the beef industry, either by being used as an initial reference point for subsequent development, as well as a welfare trait for both calf and cow concerning dystocia and calf survival (Holland and Odde, 1992; Andersen, 1993). As expected, birth weights were not different between treatments. Holland and Odde (1992) reported that calf sex is a factor that influences birth weight, with an advantage for male over female calves. In this study calf sex did not seem to statistically affect calf birth weights. However, it is important to note that there were more females than males enrolled in this study. Males and females in the control group had a birth weight difference of 2.6 kg, which is in agreement with accepted differences between sexes (BIF, 2010). In addition to BW, ADG is an important trait to be measured in order to estimate beef calves' efficiency pre- and post-weaning. Previous publication measuring ADG on calves fed different colostrum supplements showed significant differences at 60 d and a tendency to be different at 250 d of age (Mihura et al., 1995). Treatments of that study varied from dairy colostrum, two commercial colostrum substitutes, and a control (calf left to nurse only on dam), with dairy colostrum having the highest ADGs. An Irish study using weights from 1,511 beef calves at birth and weaning (200 d) found a slightly greater ADG (0.92 kg/d) than compared to ADG in our study (0.86 kg/d) (Dewell et al., 2006). However, they had a greater variation ranging from 0.42 to 1.26 kg/d, while ours ranged 0.71 to 1.05 kg/d. Their study comprised purebred Charolais and Belgian Blue-cross calves born to 4-year-old dams, whereas calves on our study were Angus-cross born to 2-year-old first-calf heifers. The enrollment of only Angus-cross calves might have granted a more homogenous birth weight outcome. Providing colostrum

supplement or not to these calves did not seem to affect their ADG or the adjusted weights at 100 or 200 d of age. Nevertheless, ADG and adjusted weights at 100 d of age tended to be greater for male calves.

The average IgG serum concentration for calves enrolled in both treatments was greater than a previous study done within the same herd (25.2 g/L) by Ruiz (2019) and recent published studies (33.2 g/L, Gamsjäger et al., 2021; 12.0 g/L, Todd et al., 2018; 35.9 g/L, Pearson et al., 2019). It is worth noting that Ruiz (2019) measured IgG1 and Todd et al. (2018) measured IgG, where both used ELISAs. IgG quantification method could be the reason of greater IgG concentrations in our study. Radial immunodiffusion and ELISA have a strong positive association with 1.8 fold difference between values, and thus they can provide variation of IgG absolute concentration values (Dunn et al., 2017). The small volume used to supplement IgG (60g of IgG in 1 L) in our study did not seem to significantly impact the IgG serum concentrations between treatment, sexes, or their interaction. However, when visualizing the distribution of calves based on IgG concentrations, the CS treatment presented a more symmetrical bell-shaped curve than Control treatment. Only one calf in the Control treatment failed the TPI with IgG concentration <10 g/L suggested by Godden et al. (2019). This calf stood in 26 minutes and had its first nurse in 65 minutes. The only factor that could contribute to FTPI was the mothering score of 2 because dam hesitated in accepting the calf. In addition, two calves, one in each treatment, had ITPI with serum IgG concentrations <24 g/L. Dewell et al. (2006) reported that beef calves with serum IgG1 concentrations < 24 g/L were almost two-fold more likely to be sick before weaning. Besides, male calves are known to present slightly lower serum IgG concentrations than female calves as described in literature (Bragg et al., 2020), which is in agreement with results in our study, however, with no statistical significance. Differences in IgG

between calf sexes has been described by Odde (1988) and thought to be attributed to higher BW and consequently increased calving difficulties. A recent study found a positive correlation among serum protein and BW (Turini et al., 2020). Although our results were not statistically significant, the association between serum protein and IgG concentrations were negative. Further research is needed to confirm this negative association based on the higher serum dilution of IgG in heavier calves. The small number of calves assisted during calving in our study was not sufficient to show associations with poor colostral status like seen in other studies (Bragg et al., 2010).

Latency to stand ranged from 7 to 165 minutes for both treatments. Only 3 calves took longer latencies to stand (>2 h) than standard latencies previously described in the literature (McGee and Earley, 2018; Gamsjäger et al., 2021). When data from these calves were evaluated independently, two calves were enrolled in the CR and one in the Control treatment. A common factor among them was their dam presenting a mothering score of 2. Nonetheless, their serum IgG concentration was <30 g/L and they were considered to have adequate TPI. Colostrum supplement was provided via esophageal tube feeder within 2 h after birth and CS calves had greater latency to nurse their dam than Control calves. One hypothesis for the increased latency to nurse is that the use of an oro-esophageal tube can cause irritation or trauma thus modifying the subsequent nursing behaviors of the neonatal calf (Chigerwe et al., 2012; Bonk et al., 2016). Calves receiving a colostrum product or bottle refusal trough an oro-esophageal tube were reported to present longer latencies to stand and to nurse than calves nursing only from nipple bottles (Gamsjäger et al., 2021). Even though, oro-esophageal tube feeders were purchased specifically for this study, revised before and after each feeding, and cleaned thoroughly, it is still possible that a foreign object can cause irritation of the esophageal mucosa. Another

hypothesis brought to discussion by Gamsjäger et al (2021) is that the motor mechanism of suckling to obtain colostrum from the nipple bottle causes stimulation and benefits to the respiratory system and to calves' metabolism. The prompt human intervention to provide colostrum supplement to neonatal calves was correlated with decrease serum IgG concentrations. Human interventions such as calving assistance and colostrum feeding assistance were previously considered risk factors with FPTI (Bragg et al., 2020).

Conclusions

This study indicated that supplementing calves born to first-calf heifer with colostrum replacement (60 g of IgG) did not increase serum IgG concentrations at 24 hours of life and subsequent growth compared to calves not being supplemented. In addition, it is evident that removing the calf from cow to fed colostrum supplementation using an oro-esophageal tube decreased expression of normal neonatal calf behaviors. However, colostrum replacement has practical management strategies in beef cattle, especially when fed to calves in high-risk conditions.

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Table 3.1 - Growth data of bulls and heifers beef calves either supplemented (CS) or not (Control) with a commercial colostrum replacement product in the first 2 hours of life.

		Treatment					*P*-value		
	n	CS[1]		Control		SEM	Treatment	Sex	Treatment x Sex
Sex		Bull	Heifer	Bull	Heifer				
BW, kg[2]	58	30.7	31.5	33.9	31.3	1.73	0.23	0.51	0.18
A100dW, kg[3]	54	127.5	118.1	126.5	121.4	4.95	0.75	0.07	0.58
ADG 100d, kg/d[4]	54	0.96	0.86	0.91	0.90	0.04	0.78	0.08	0.22
A200dW, kg[5]	45	208.5	200.3	212.9	203.2	8.98	0.58	0.17	0.90
ADG 200d, kg/d[6]	45	0.89	0.84	0.89	0.85	0.04	0.87	0.17	0.83

[1]Colostrum Supplement treatment; [2]Birth weight; [3]Average 100 d weight: [(ADG x 100 d) + BW=A100dW]; [4]Average daily gain at 100 d; [5]Average 200 d weight: [(ADG x 200 d) + BW=A200dW]; [6]Average daily gain at 200 d.

Table 3.2 - Measures of passive immune transfer of bulls and heifers beef calves either supplemented (CS) or not (Control) with a commercial colostrum replacement product in the first 2 hours of life.

		Treatment					*P*-value		
	n	CS[1]		Control		SEM	Treatment	Sex	Treatment x Sex
Sex		Bull	Heifer	Bull	Heifer				
Serum Protein, g/dL	58	6.9	6.9	6.9	7.1	0.35	0.59	0.55	0.69
IgG, g/L[2]	58	49.2	51.0	47.6	54.3	6.18	0.85	0.36	0.60

[1]Colostrum Supplement; [2]Immunoglobulin G serum concentration.

Table 3.3 - Neonatal behaviors of bulls and heifers beef calves either supplemented (CS) or not (Control) with a commercial colostrum replacement product in the first 2 hours of life.

		Treatment					*P*-value		
	n	CS[1]		Control		SEM	Treatment	Sex	Treatment x Sex
Sex		Bull	Heifer	Bull	Heifer				
Latency to stand, min[2]	58	63.1	53.4	41.7	45.8	15.23	0.20	0.80	0.54
Latency to first nurse, min[3]	58	117	98.7	62.4	75.3	25.07	0.04	0.88	0.40
Latency birth to CS, min[4]	28	52	57.8	-	-	8.53	-	0.55	-
Latency CS to nurse, min[5]	28	81.3	54	-	-	25.19	-	0.34	-

[1]Colostrum Supplement treatment; [2]Interval from time of birth to first stand; [3]Interval from time of birth to first nurse; [4]Interval from birth to colostrum supplement feeding; [5]Interval from colostrum supplement feeding to nurse on dam.

Table 3.4 - Correlation coefficients of performance, immunological, and behavior measures of beef calves either supplemented (CS) or not (Control) with a commercial colostrum replacement product in the first 2 hours of life.

Variable	1	2	3	4	5	6	7	8	9	10	11
1 BW, kg[1]		0.50***	0.17	0.46**	0.23	-0.02	0.12	0.31**	0.16	0.43*	0.48*
2 A100dW, kg[2]			0.93***	0.65***	0.56***	0.10	0.01	0.18	0.07	0.04	0.40*
3 ADG 100d, kg[3]				0.55***	0.55***	0.14	0.09	0.08	0.02	-0.06	0.27
4 A200dW, kg[4]					0.97***	-0.03	-0.2	0.02	-0.04	0.09	0.03
5 ADG 200d, kg[5]						-0.04	-0.18	-0.04	-0.05	-0.12	-0.03
6 Serum Protein, g/dL							0.86***	-0.10	-0.25*	-0.36†	-0.17
7 IgG, g/L								-0.03	-0.18	-0.41*	-0.17
8 Latency to stand, min									0.79***	0.50**	0.74***
9 Latency to first nurse, min										0.59**	0.85***
10 Latency birth to CS, min											0.46*
11 Latency CS to nurse, min											

[1]Birth weight; [2]Average 100 d weight: [(ADG x 100 d) + BW=A100dW]; [3]Average daily gain at 100 d; [4]Average 200 d weight: [(ADG x 200 d) + BW=A200dW]; [5]Average daily gain at 200 d. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; † $P > 0.05 < 0.1$;

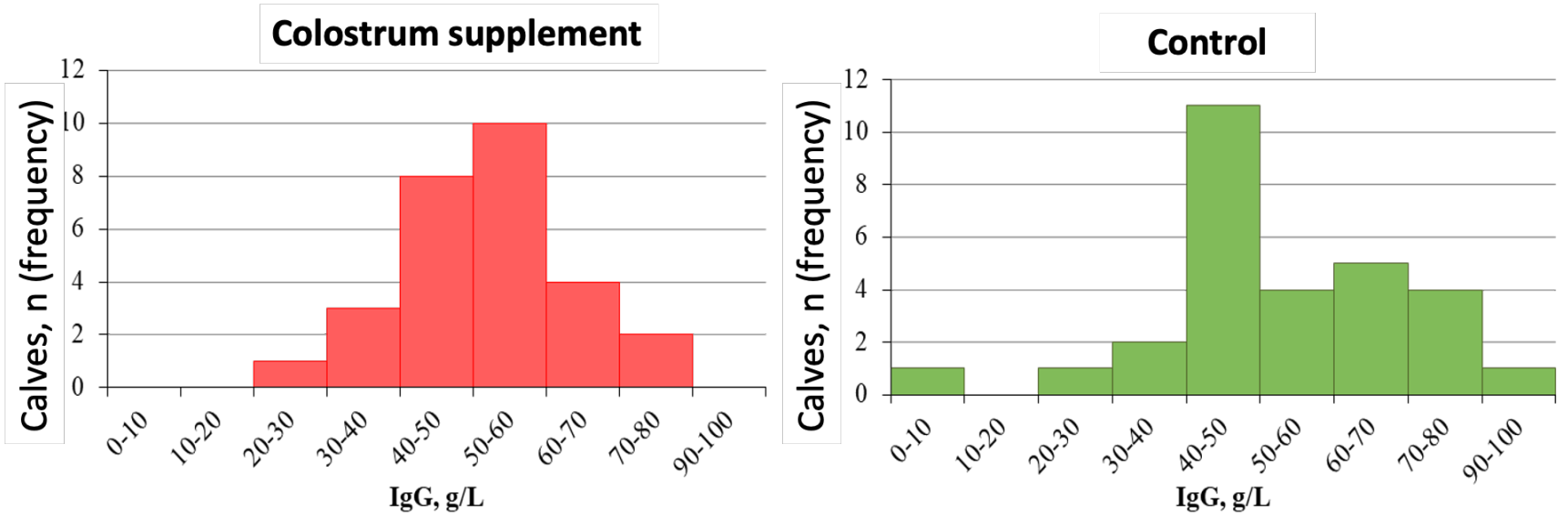

Figure 3.1- Histograms of beef calves' distribution according to their IgG serum concentrations for Colostrum Supplement (red) and Control (green) treatments.

Chapter 4 - Precision livestock technology

Determination of cutoff behavior values from a precision livestock technology to aid the identification of nursery piglets challenged with LPS

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Abstract

A precision livestock technology developed to track swine individual behavior was used to identify behavior markers indicative of sickness in 192 newly weaned piglets. Induction of subclinical and clinical signs were done through a LPS challenge. The objectives of this study were to compare sickness scores used by trained caretakers with NU*track* behaviors indicative of LPS challenge, find their cutoff values, and evaluate sensitivity and specificity of NU*track* tracking piglets displaying subclinical and clinical signs. Piglets were randomly assigned to pen and pens were randomly assigned to treatment groups: Control (**100SHAM**) no immune challenge; LPS (**100CHALLENGED**) immune challenge, and mixed pen where half of pigs were challenged, and half were not challenged (**50CHALLENGED**). Cutoff values for structural and spatial behaviors were calculated using the ROC function of RStudio. When cutoffs were applied, NU*track* demonstrated a better ability to identify piglets treated with LPS. All behaviors collected by NU*track* were able to indicate sick and non-sick piglets (AUC > 0.70) within an adequate sensitivity and specificity up to 72 hours after challenge. The Nu*track* system has potential to aid caretakers on decision making thus improving overall farm sustainability.

Key words: LPS, NU*track*, precision livestock technology, swine

Introduction

In animal production, caretakers are responsible to identify signs of illness, document them, and care for the animals. Changes in behavior have been used as indications of sickness and were well documented throughout the years. In swine production, the nursery phase brings many challenges to recently weaned piglets. The process of weaning is stressful due to piglet-sow separation, exposure to a novel environment, transportation, handling, and comingling with unfamiliar pigs from different litters (Dybkjær, 1992; Roldan-Santiago et al., 2013; Campbell et al., 2013). Stress upon piglets influences physiological processes within the body, affecting health status (De Jonge et al., 1996; Johnson et al., 1992). Psychosocial stressors disrupting social hierarchy are potent activators of the hypothalamic-pituitary-adrenal (HPA) axis culminating in a complex hormonal cascade affecting their already naïve immune system (Dickerson and Kemeny, 2004). A disruption of the homeostasis and the attempt to regulate the concentration of stress hormones can cause impairments of the immunological system and raise percentages of morbidity and mortality (Johnson et al., 1992). Respiratory and gastrointestinal disorders are leading causes of morbidity and mortality in post-weaning piglets (USDA, 2016). Early identification, segregation, and treatment of piglets can decrease morbidity and prevent mortality. Identification of sickly pigs can be difficult, especially during the subclinical stages. Human observations are used in swine production in order to identify pigs requiring attention (Friendship, 2005). The procedure of animal observation is called “walking pens” and requires significant training for caretakers to identify subtle abnormal behaviors correctly. Moreover, this procedure is a snapshot in time that does not translate into overall daily piglet behavior and is prone to human error.

Collection of samples such as blood collection to measure subtle deviations of normal physiology are deemed invasive and can be stressful. With advances in technology, precision livestock technology has been applied to identify sickly pigs via audio, video, or wearable devices to assist caretakers and overcome the limitation of human observation (Benjamin and Yik, 2019; Yin et al., 2021; Pandey et al., 2021). NU*track* was developed as an identification tool and tracking system for swine production. The automated monitoring video system is capable of tracking swine individual behavior (Psota et al., 2019; Psota et al., 2020; Schmidt et al., 2022). We hypothesize that NU*track* behavioral data will have the ability to identify recently weaned nursery piglets challenged by Lipopolysaccharide from *E. coli* (LPS) due to modification of their normal behavior for longer and with greater accuracy than human scores. The objectives of this study are to identify behaviors indicative of LPS challenge, establish cutoff values, and evaluate the sensitivity and specificity of NU*track* tracking piglets displaying subclinical and clinical signs. In addition, human observations will provide a comparison to NU*track,* and the possibility of this precision livestock technology aiding sickness identification will be evaluated.

Materials and Methods

Animals and housing

Piglet management for the duration of this experiment was approved by the University of Nebraska – Lincoln Institutional Animal Care and Use Committee and followed the Guide for the Care and Use of Agricultural Animals in Agriculture Research and Teaching (IACUC #1409).

One hundred and ninety-two newly weaned piglets were sourced from the University of Nebraska – Lincoln swine unit and housed in 12 nursery pens (16 pigs/pen) within the Animal Science Complex's Swine Nursery Facility. *Ad libitum* water was provided through water

nipples and feed was offered through a 3-hole feeder. Diet was formulated to meet the NRC requirements for nursery piglets (NRC, 2012). Ten days before treatment, all piglets were weighed and received a unique ear tag with a pattern combination of color, letters, and numbers to identify the animal within a nursery pen (Schmidt et al., 2022). A day before treatment (d -1), all piglets were weighed to appropriately calculate LPS dosage prior to treatment day. A timeline of the nursery period is depicted in Figure 4.1. Piglets were randomly assigned to pen, then pen was randomly assigned to one of the three treatment groups: Control (**100SHAM**) no immune challenge for 100% of piglets in the pen; LPS immune challenge for 100% of piglets in the pen (**100CHALLENGED**) and mixed pen with 50% of the piglets within pen challenged and 50% of piglets within pen non-challenged (**50CHALLENGED**). All piglets in the 100CHALLENGED and half of the piglets in the 50CHALLENGED pens were subcutaneously injected in the left medial inguinal area with LPS (300 μg/kg BW of *E. coli* O111:B4). Piglets on 100SHAM and half of the piglets in the 50CHALLENGED pens were sham-handled and received a subcutaneous injection in the left medial inguinal area of saline solution.

NU*track* precision monitoring system

The NU*track* system was installed above the 12 nursery pens before the placement of piglets. The system is composed of a depth-enabled camera (Kinect v2™, Microsoft, Redmond, WA) that is connected to a mini-PC (NUC, Intel, Santa Clara, CA) and a four-terabyte Fantom hard drive (Fantom Drive™, Torrance, CA). Specifications of the system methods were previously published (Psota et al., 2019; Psota et al., 2020a; Psota et al., 2020b; Schmidt et al., 2022). The monitoring system continuously captured behavioral data for the entire nursery phase; however, this experiment focused on LPS challenge day (0 d), and d 1 to d 7 post-challenge (Figure 1). The precision livestock technology developed for the NU*track* system

calculates distance walked (m/d), pivot behavior (angle of turn, radius), and durations of structural behaviors such as eat (sec/d) and total lying behavior (sec/d). An ethogram with behavior measures, descriptions, and visuals is depicted in Figure 4.2.

Sickness and hide score

Trained observers (interobserver reliability > 95%) stood in front of the pen and classified the animals according to a visual sickness and hide score for 3 minutes. The sickness score ranged from 0 to 2 (0 = normal and alert; 1 = sleepy or drowsy, responsive to stimuli; 2 = clinical sickness – lateral lying, open mouth, non-responsive to stimuli; Figure 4.3); and the hide scores ranged from 0 to 2 (0 = clean, free of fecal matter; 1 = dried fecal matter on body; 2 = wet fecal matter on body; Figure 4.4). Emesis events were also recorded (0 or 1).

Rescue interventions

Piglets were observed and rescue interventions were performed when piglets were unresponsive to external stimuli. The veterinarian in charge of IACUC provided a protocol of epinephrine (0.5 mL/piglet) and dexamethasone (0.5 mL/piglet) intramuscularly to improve cardio-respiratory function.

Statistical analysis

The behavioral data originated from the NU*track* precision monitoring system were analyzed using the receiver operator characteristic (ROC) curve analysis on RStudio (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA) for the entire population (all 12 pens) and for the mixed pens subset population (50CHALLENGED). From these analyses, the area under the curve (AUC) provided the accuracy measures for each factor computed by NU*track*, with a 0 to 1 range (0.5 = no diagnostic value; 1 = perfect accuracy). The sickness scores were used as a contrast reference. The behaviors were fitted to a linear regression

equation, using the binomial family option to predict the known outcome of sick (received LPS) or not sick (did not receive LPS) individuals. ROC curves were plotted, and AUC values for the regression, pivot behavior, lying behavior, distance walked, and human sick scores were calculated starting on treatment day (d 0) and moving individually to each day post-treatment (d 1 to d 7). The cutoff values were calculated on RStudio based on the Youden's index, maximizing the sum of sensitivity (Se) and specificity (Sp) (Youden, 1950; Zweig and Campbell, 1993; Greiner et al., 2000). Hide scores were not included in the analysis as post LPS treatment and the initial human hide scores, wet fecal matter (hide score 2) was present in non-challenged piglets due to their proximity with the challenged ones. Emesis events were also not included in the analysis due to piglet vomit ingestion prior to data collection.

Results

Nine piglets died from the LPS challenge despite the use of the rescue protocol intervention, translating into a case-fatality of 9.37%. Piglet's initial sickness scores and behavior data collected by NU*track* were included in the dataset for treatment day (d 0).

Both NU*track* and the human sickness scores were tested for the validity (sensitivity and specificity) of screening pens to find sickness in piglets. Observers used a scoring system on an interval scale ranging from 0 (not sick) to 2 (very sick) to identify clinical signs, while NU*track* collected continuous variables based on the piglets' behaviors. Human observation presented a high true positive and low false positive rate (Se = 88.5%; Sp = 85.4%) with a significant ability to diagnose LPS-challenged piglets on day of treatment (d 0, AUC = 0.871; Table 4.1) and on the day post-treatment (d 1, AUC = 0.849; Table 4.1). When applying the cutoff values (Table 4.1) to collected behaviors, NU*track* showed a better ability to identify piglets treated with LPS. All behaviors collected by NU*track* were able to distinguish between sick and non-sick piglets (AUC

> 0.70). However, pivot behavior (AUC = 0.999) presented the greatest ability to identify sick piglets based on a cutoff of < 2668 radius per day on d 0. Using a cutoff value of < 365.7 m/day, distance walked had the greatest ability to identify sick piglets on d 1 (AUC = 0.999). On d 4, the ability of NU*track* to identify piglets treated with LPS decreased. When evaluating only the mixed pens where half of the piglets received LPS and half were SHAM treated, the human observation presented lower ability to identify true positives and true negatives (Se = 75%; Sp = 65.6%; Figure 4.2). However, behaviors collected by NUtrack maintained high sensitivity and specificity demonstrating an ability to identify sick piglets.

Human observations sensitivity and specificity fell under 60% 2 d after treatment (d 2), increasing the false negative and false positive rate. The following days (d 5, d 6, d7) present a higher chance of NU*track* and human observation to incorrectly identify piglets that were sham-challenged.

Discussion

One of the goals of precision livestock technology is to improve the efficiency of animal production by increasing animal health and welfare and providing support to workforce. Because behavior has been used as an indication of sickness, we have selected structural behaviors (total lying time and pivot) and spatial behaviors (distance walked and eat) to identify challenged pigs presenting subclinical or clinical signs of sickness. Behaviors that are valid in indicating illness are those with high sensitivity and high specificity when compared to a "golden standard" (Weary et al., 2009). Measures of sensitivity and specificity have been previously used to assess the validity of precision livestock technology in diverse animal production systems (Mertens et al., 2010; Rojo-Gimeno et al., 2019; Garcia et al., 2020). The behaviors selected in this study

were measured as continued variables, so cutoff values were necessary to maximize sensitivity and specificity (Gordis, 2013).

The ability of a human to identify a challenged piglet on d 0 was adequate; however, all behaviors collected through NU*track* had higher sensitivities and specificities. That is important because when using human observations, more false positives and negatives will happen. False positive piglets in commercial swine production may be treated without the need, and false negative piglets might not get the adequate treatment, resulting in decreased efficiency and increased cost. The lower accuracy of sickness scores when only mixed pens are considered, demonstrates a challenge for humans to identify sick piglets when pens have non-sick and sick piglets housed together.

The ability of NU*track* to correctly identify piglets based on their behavior reflects their physiology. In general, sick animals decrease their intake behaviors (water and feed) and increase time resting to conserve energy while the immune system fights the infection (Hart, 1988). On d 1, human observations resulted in lower sensitivity and increased specificity, with more false-negative piglets. We hypothesize that challenged pigs were showing subtle clinical signs that were hard to identify with a snapshot in time. While behaviors captured by NU*track* represent an entire 24-hour evaluation resulting in an increased ability to correctly identify challenged and non-challenged piglets. Even though distance walked and total lying maintain the AUC better than eat and pivot we must consider that lying behaviors can be affected by many variables, including temperature and humidity (Ekkel et al., 2003; Hillmann et al., 2005). While intake can also fluctuate within different temperature and humidity, Weary et al. (2009) reported that sick animals can be identified using feed intake. Even though eat behaviors reported by NU*track* represent the sum of time within the feeder area instead of actual feeding behavior, we

can extrapolate those animals had the intention to eat. Similar to piglets in our study, once challenged with LPS, dairy cows decrease their feed intake (Waldron et al., 2006).

On the other hand, pivot behavior represents social interactions either by play or agonistic behaviors (Newberry et al., 1988; Luo, 2017). When considering an ethopyramid that depicts behaviors essential for survival, reproductive and social behaviors are at the top, which means they are the least requirements to maintain integral positive affective states during illness (Luo, 2017). That explains pivot behavior captured by NU*track* having good sensitivity and specificity to detect challenged piglets. Nordgreen et al. (2018) studied the effects of LPS on the behavior and physiology of pigs individually housed. They found through video observations that behaviors went back to baseline within 3 d post-challenge. Their findings align with the present study's decrease in sensitivity and specificity of behaviors and human scores.

The usage of precision livestock technology does not substitute work force for swine operations. Instead, it serves as an additional tool to help decision-making. Presence of caretakers to check piglets' health is necessary because it causes a moderate disruption of behaviors, providing piglets with a chance of showing exploratory behaviors that will facilitate the identification of sick individuals by NU*track*. This study's cutoff values for maintenance and social behaviors can probably be extrapolated to identify nursery piglets with different conditions and disorders. However, caution must be taken when applying cutoffs for different age groups and environments due to changes in physiology. One of the limitations of the present study is the possible variation of immunological response when challenging animals with LPS (Weary et al., 2009).

Conclusions

NU*track* presents a high ability to identify challenged pigs within the first 3 days post LPS. The NU*track* system has high AUC, sensitivity, and specificity when compared to human observations, which can help to identify subtle modifications in piglet behavior. NU*track* will aid caretakers during their health and management decisions for piglets. The correct identification of sick piglets can improve sustainability within swine production by improving economics, the labor force, and animal welfare.

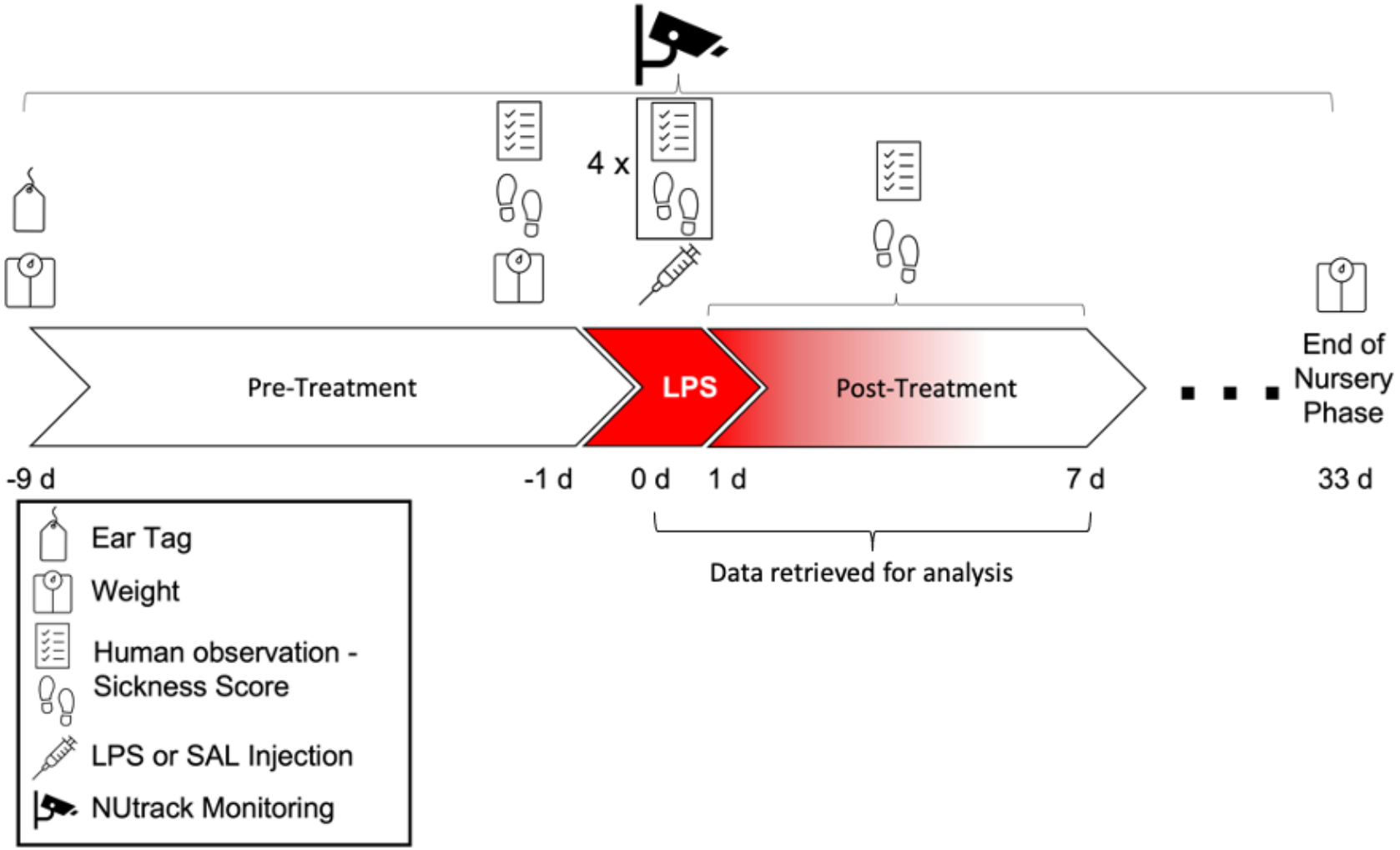


Figure 4.1 – Timeline of piglets enrolled in the study with procedures performed in each day. Ear tag, weights, human observation scores, LPS challenge or SHAM treatment (saline) test, and NU*track* monitoring

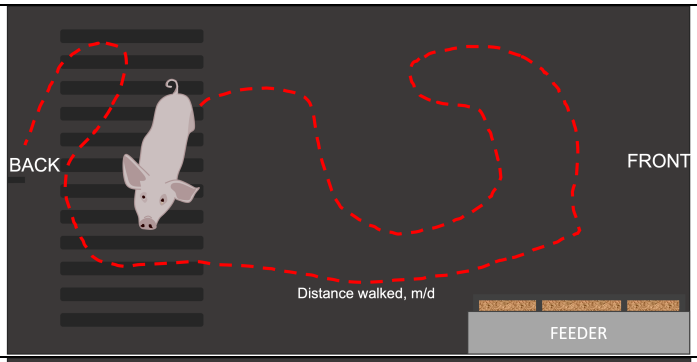
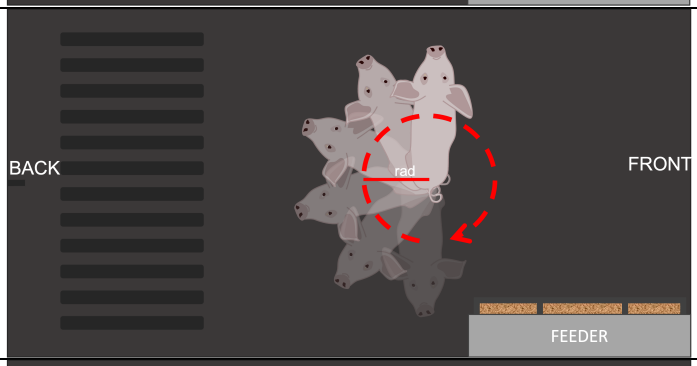
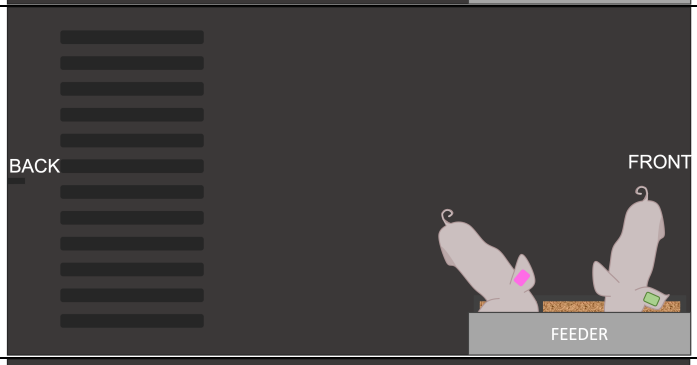
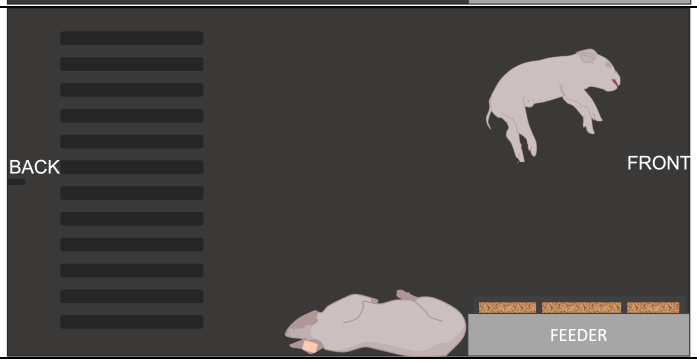
Behavior	Measure	Description	Visual
Distance walked	m/d	Quantification of movement in meters inside the pen	
Pivot behavior	rad/d	Movement of front limbs while back limbs are still (Lu, 2017)	
Feed	s/d	Head over feeder area (Hurnick et al., 1995; Rudine et al., 2007)	
Total lying	s/d	Body in contact with the flooring. Legs straight, bent, or tucked under piglet's body (Schmidt et al., 2022)	

Figure 4.2 - Ethogram used by NU*track* system to collect and record behavioral data, with measures, descriptions, and visuals.

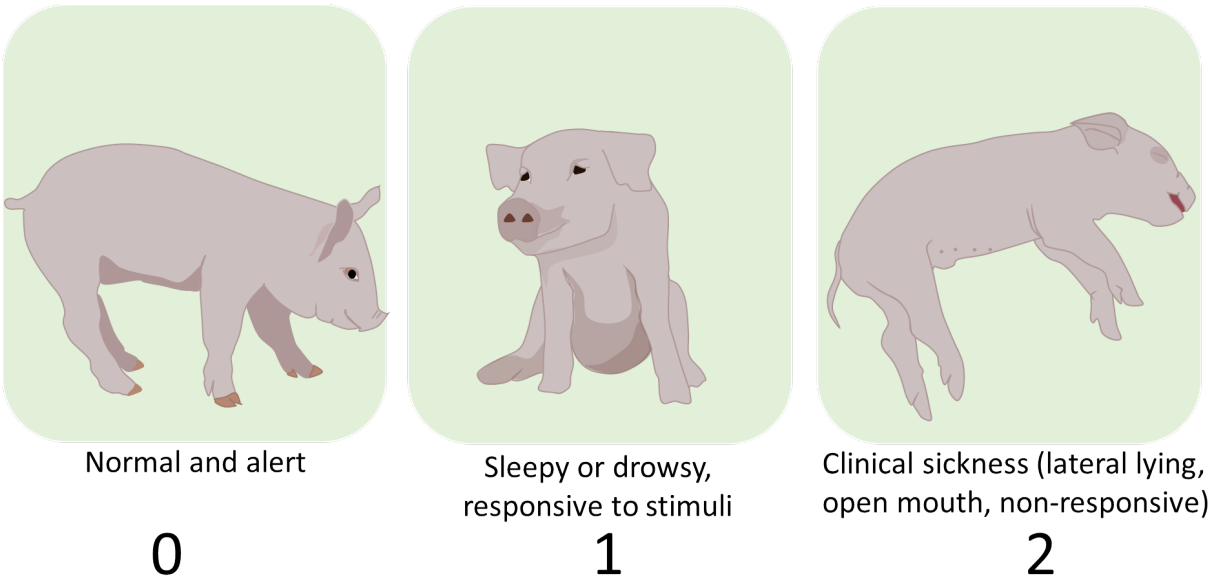


Figure 4.3 - Sickness score used by observers to classify piglets according to their clinical signs.

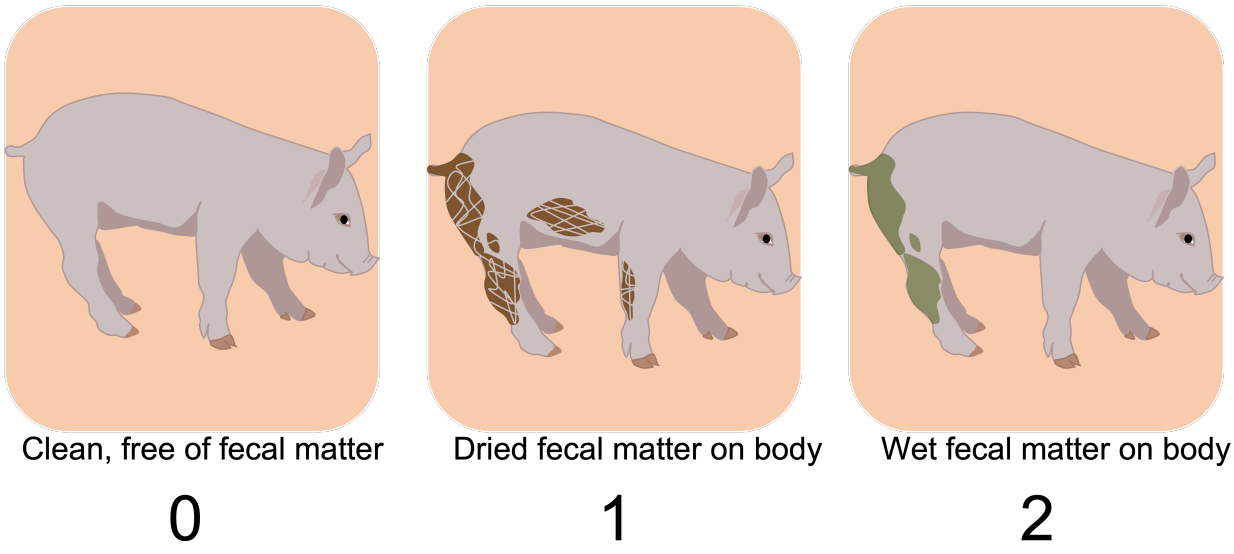


Figure 4.4 - Hide score used by observers to classify piglets according to their clinical signs.

Table 4.1 - Cutoff values, the area under the curve (AUC), sensitivity, and specificity for human observations (sickness scores), distance walked, pivot behavior, eat, and total lie behavior collected by NU*track* for newly-weaned piglets challenged by E. coli lipopolysaccharide (LPS) and injected with saline solution (100CHALLENGED, 100SHAM, and 50CHALLENGED) on the day of treatment (d 0) and subsequent week (d 1 – d 7).

	Day							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Human observations								
Sick Score								
Cutoff	-	-	-	-	-	-	-	-
AUC	0.871	0.849	0.614	-	0.570	0.503	0.510	-
Se (%)	88.5	70.7	53.4	-	84.4	83.3	68.8	-
Sp (%)	85.4	97.7	68.8	-	28.7	17.2	33.3	-
Distance walked								
Cutoff, m/day	784.6	365.7	958.7	1177.8	1132.1	1078.2	1008.4	1870.0
AUC	0.981	0.999	0.929	0.846	0.686	0.542	0.481	0.391
Se (%)	94.7	98.9	79.1	81.2	72.9	89.5	94.7	12.2
Sp (%)	91.6	98.8	96.2	76.7	60.9	21.8	12.6	93.1
Pivot behavior								
Cutoff, rad/d	2668	1384	3317	4151	3859	3761	4073	7583
AUC	0.999	0.998	0.935	0.798	0.627	0.479	0.440	0.324
Se (%)	96.8	98.9	82.2	83.3	80.2	86.4	73.3	10.0
Sp (%)	92.7	97.7	95.4	70.9	49.4	24.1	28.7	10.0
Eat								
Cutoff, sec/d	7681	6885	10922	9148	11376	11489	9029	18136
AUC	0.992	0.987	0.787	0.695	0.628	0.588	0.490	0.428
Se (%)	95.8	96.8	73.9	92.7	48.9	51.0	84.3	4.1
Sp (%)	93.7	94.3	70.4	39.5	74.7	64.3	20.6	9.8
Total lie								
Cutoff, sec/d	69390	74387	67042	65262	67750	68346	66379	56058
AUC	0.993	0.996	0.903	0.800	0.715	0.518	0.487	0.381
Se (%)	97.9	96.5	90.9	81.3	57.7	19.5	33.1	10.0
Sp (%)	94.8	97.9	77.0	69.7	80.2	88.5	75.0	20.8

Area under the curve (AUC) was calculated using the linear regression model and provides the validity measures for each behavior computed by NU*track*, with a 0 to 1 range (0.5 = no diagnostic value; 1 = perfect accuracy); Cutoffs were based on Youden's index, maximizing the sum of Sensitivity and Specificity (Youden, 1950; Greiner et al., 2000; Zweig and Campbell 1993).

Table 4.2 - Cutoff values, the area under the curve (AUC), sensitivity, and specificity for human observations (sick scores), distance walked, pivot behavior, eat, and total lie behavior collected by NUtrack for piglets housed in pens where half piglets were challenged with newly-weaned E. coli lipopolysaccharide (LPS) and the other half was sham treated with saline (50CHALLENGED) on d 0 and subsequent week (d 1 – d 7).

	Day							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Human observations								
Sick Score								
Cutoff	-	-	-	-	-	-	-	-
AUC	0.703	0.738	0.569	-	0.662	0.525	0.513	-
Sensitivity, %	75.0	92.9	50.0	-	46.4	14.3	21.4	-
Specificity, %	65.6	53.1	62.5	-	84.4	90.6	81.2	-
Distance walked								
Cutoff, m/day	784.6	304.3	803.3	1191.1	1251.1	1449.5	1478.0	1578.7
AUC	0.970	0.992	0.799	0.729	0.710	0.680	0.648	0.580
Sensitivity, %	90.6	100.0	81.2	75.0	65.6	59.3	50.0	43.7
Specificity, %	96.8	96.4	75.0	67.8	75.0	82.1	78.5	78.5
Pivot behavior								
Cutoff, rad/d	2863	1489	2891	4610	4199	4578	4341	5572
AUC	0.988	0.985	0.792	0.676	0.663	0.629	0.612	0.517
Sensitivity, %	90.6	96.9	84.3	59.3	71.8	65.6	75.0	25.0
Specificity, %	100	92.8	75.0	82.1	57.1	67.8	50.0	89.2
Eat								
Cutoff, sec/d	7856	5554	10922	9358	8667	10405	11955	18136
AUC	0.976	0.967	0.685	0.643	0.641	0.612	0.557	0.422
Sensitivity, %	87.5	100.0	71.8	93.7	90.6	81.2	53.1	62.5
Specificity, %	96.8	89.2	64.2	46.6	42.8	42.8	64.2	100.0
Total lie								
Cutoff, sec/d	67660	74387	68184	65262	67442	65125	65280	64283
AUC	0.988	0.980	0.787	0.762	0.775	0.703	0.665	0.487
Sensitivity, %	100.0	89.2	75.0	78.5	67.8	64.2	60.7	50.0
Specificity, %	87.5	96.8	75.0	78.1	87.5	75.0	81.2	59.9

Area under the curve (AUC) was calculated using the linear regression model and provides the validity measures for each behavior computed by NU*track*, with a 0 to 1 range (0.5 = no diagnostic value; 1 = perfect accuracy); Cutoffs were based on Youden's index, maximizing the sum of Sensitivity and Specificity (Youden, 1950; Greiner et al., 2000; Zweig and Campbell 1993).

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