

THESIS

PRE-WEANING PERFORMANCE IN LAMBS AFTER NUTRIENT SPECIFIC  
RESTRICTION IN GESTATION AND TRAINING COGNITIVE SKILLS TO IMPROVE  
INTEGRATION AND APPLICATION OF KNOWLEDGE IN ANIMAL PHYSIOLOGY

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## ABSTRACT

### PRE-WEANING PERFORMANCE IN LAMBS AFTER NUTRIENT SPECIFIC RESTRICTION IN GESTATION AND TRAINING COGNITIVE SKILLS TO IMPROVE INTEGRATION AND APPLICATION OF KNOWLEDGE IN ANIMAL PHYSIOLOGY

Due to seasonal availability and quality of range forage, extensively managed ewes often experience nutrient restriction during gestation. Neonatal lamb performance after severe experimental nutrient restriction during gestation has been well studied; however, there is a gap in understanding of how practical nutrient restriction experienced over the course of a production cycle impacts lamb success. Therefore, the objective of the first study was to investigate early life performance in lambs from ewes fed a diet from 30-125 days of gestation (dGA) that simulates winter forage or one that meets all nutritional requirements for gestation. Maternal BCS and weight losses were evident prior to the standard supplementation period suggesting impacts on the fetus could be happening due to nutrient restriction earlier in gestation. Lambs from dams who received the lower quality diet did not differ by bodyweight but were not able to thermoregulate as well as lambs whose dams had their requirements met during the first week and on specific weeks thereafter. Beginning at six weeks of life through weaning lambs from restricted dams had lower body weights compared to lambs from fully nourished dams. This data demonstrates that nutrient challenge during gestation, even when re-alimented in late gestation, decreases performance. The lamb data showed that while there are no apparent differences in growth during the first week of life, NR animals start to diverge later in life even when provided high quality nutrition. This

coupled with reduced thermoregulatory ability suggests tissue specific differences may underlie animal variation and warrants further investigation.

Case-based learning immerses students in real-world scenarios, prompting observation, action, and reflection to enhance cognitive skills. The open-ended nature of such learning can challenge students to reach higher levels of critical thinking, however, we have noticed that without guidance, students often do not know how to approach these types of questions. Therefore, the objective of study 2 was to investigate how two low-input interventions strategies may help train cognitive skills and improve student performance. Three semesters of an undergraduate physiology course employed a case-based assessment strategy. Semester 1 (S1) represented no intervention, Semester 2 (S2) included two dedicated class periods of instructor-led guidance on approaching case studies, and Semester 3 (S3) utilized an online peer evaluation platform, where students evaluated and provided feedback to each other. In S2 and S3 pre-surveys were administered to understand changes in students' perceptions of their own cognitive skills. The average grade on case studies after intervention was the highest in S3 where peer intervention was employed. When comparing changes in perceptions, student confidence in their cognitive skills generally increased in S2 and S3. Interestingly, while peer evaluation improved student performance, free response questions indicated that students did not enjoy peer evaluation and felt that it was not beneficial to their learning. Peer evaluation provides a unique opportunity for students to actively engage in the learning process and to practice cognitive skills. This process holds dual benefit as reviewing peers requires students to reflect, analyze, and evaluate, which are cognitive skills also needed to solve the case studies.

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## DEDICATION

I would like to dedicate this thesis to my family and friends who supported me throughout this journey. To my family, thank you for always supporting me in my endeavors and challenges without judgment. Your visits to Colorado during lonely and difficult times, as well as just to lend a friendly face for a few days, meant the world to me.

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## CHAPTER 1 - LITERATURE REVIEW

### *1.1 Sheep production in the United States*

United States (U.S.) sheep production contributes just over \$1.4 billion dollars in total economic value to the U.S. economy, with the largest contributors being slaughter lambs, wool, and labor (Marsh, 2023). However, the total number of sheep and lambs in the U.S. exhibited a constant decline throughout the 2000s (ASI, 2015). In 1990, the total sheep and lamb population in the U.S. was 11.4 million head, by 2023, this figure declined to 5.02 million head (NASS, 2023). The U.S sheep industry is primarily divided into two sectors: stock sheep and lamb feeders. Within these sectors, animals can be managed intensively, where there is a high level of input compared to land use, or extensively, lower-level input using large amounts of land (Redden and Thorne, 2020). Given the arid nature of the western states, two-thirds of U.S. sheep operations are concentrated in the Southern, Plains, Mountain, and Pacific regions (Hahn, 2020; Stillman et al., 1990). Stock sheep are primarily extensive operations, relying on range resources for production, often utilizing government lands such as the Bureau of Land Management or Forest Service Land. Feeder lambs are part of the stock sheep sector until they are placed in dry lots, under intensive management, and fed until slaughter (Stillman et al., 1990)

While overall lamb and mutton consumption has decreased among the general American population, there has been increased consumption within ethnic populations residing in large coastal metropolitan areas (Hahn, 2020). The domestic utilization of wool products has seen an increase driven by desire for the fabric's quality and its usage by the U.S. military, while 60 – 75% of domestic wool production is exported (ASI, 2020).

#### *1.1.1 Sheep production cycle*

The sheep production cycle, outlined in Figure 1.1, starts in the fall with the breeding season (Bradford et al., 2003). Sheep are seasonal breeders, and the short photoperiod of the fall and winter months stimulates the hypothalamic-pituitary-gonadal axis, allowing ovulation and receptivity to breeding (Weems et al., 2015). Most producers use one ram per 20-50 ewes, considering factors such as ram libido, age, and fertility (ASI, 2015; Bradford et al., 2003; Edmondson et al., 2012). Following breeding, pregnancy diagnosis by non-return to estrus, breeding marks, udder palpation, radiography, pregnancy hormone assay, and ultrasonography can be utilized to determine number of animals bred (Ganaie et al., 2009). Ewes experience an average gestation length of 148 days with a majority, as high as 85%, of lambs born during spring months (ASI, 2015; Redden and Thorne, 2020).

From early to late-gestation, ewes graze on winter ranges, typically in large plains or valleys (Bradford et al., 2003). Range lambing is most common in the Central and Western regions of the U.S., where 36.8% of lambs are born on open range (USDA APHIS, 2014). Often, flocks are brought closer to the farm on irrigated pasture during lambing to facilitate producer access and improve nutrition, either through better pasture quality or supplementation for the ewe during late gestation and lactation (Bradford et al., 2003; Redden and Thorne, 2020; Quintana et al., 2022). Producers aim for 150 - 200% lamb crop, but neonatal losses may occur due to dystocia, stillbirth, injury, hypothermia, predation, or starvation (Bradford et al., 2003; Refshauge et al., 2015). Management practices, such as vaccination, tail docking, and castration are typically performed in the first month and a half of a lamb's life (USDA APHIS, 2014; Tizard, 2021). After lambing, ewes nurse their lambs until they are weaned, a process that can occur when the lambs are anywhere from 30 to 90 days old, depending on the production system (Freitas-de-Melo et al., 2022; Redden and Thorne, 2020). Once weaned, lambs can be sold to

feedlots or directly to packers for slaughter (Bradford et al., 2003, ASI, 2015). Lambs are usually brought to a weight of 60-80lbs on forage before being sent to a feedlot for finishing before slaughter (Hahn, 2020). Ewe lambs and rams may either be sold for breeding purposes or retained in the flock as breeding replacement (ASI, 2015).

During the anestrus season, flocks typically graze on summer pasture, often at higher elevations on government or private land, until the breeding season starts again (ASI, 2015; Bradford et al., 2003). Shearing of wool breeds typically occurs during May and June to ensure that the weather is warm enough and will not adversely affect the newly shorn sheep. Some producers may shear prior to lambing to remove wool from around the hind end and udders (crutching), although it's important to note that the stress of shearing can lead to abortions during late gestation (Bradford et al., 2003; Cooper, 1933; Redden and Thorne, 2020). Before the return of breeding season in the fall, producers cull ewes, rams, and lambs, either for health, safety, or economic reasons (ASI, 2015).

### *1.2 Forage quality and availability in the mountain west*

The mountain west region encompasses a diverse landscape, featuring areas with mild arid to semiarid conditions, variable precipitation, and temperatures (Chambers and Pellant, 2008). In total, there are approximately 1.9 billion acres of rangeland in the continental U. S., and the Pacific Coast (WA, OR, CA) and Rocky Mountain (ID, MT, ND, SD, WY, NE, NV, UT, CO, KS, AZ, NM) regions together contain approximately 80% of the US rangelands (Mitchell, 2000). These rangelands play a vital role in the local economies of the western U.S., supporting livestock production with a low-cost feed source (ASI, 2015; Nelson et al., 2006; Quintana et al., 2022).

Rangelands in these regions are rich in a variety of grasses, browse, riparian area, woody vegetation, and forested areas (Chambers and Pellant, 2008; Mitchell, 2000). These grasslands are well-suited for ruminant livestock production because they are unsuitable for crop cultivation, and ruminants can effectively convert plant material into energy and, ultimately, protein for human consumption (Steinfeld et al., 2006).

The quantity and quality of forage varies throughout the year due to factors such as precipitation, temperature, and location (Quintana et al., 2022; Pieper, 2005). Forage availability is highest during spring and early summer but steadily declines, reaching its lowest point in winter (Adams et al., 1996). Seasonally, forage experiences changes in protein content, ranging from about 4-7%, and grassland growth peaks in the early summer before declining into winter (Adams et al., 1996). The digestibility of forage also decreases from spring to winter due to the increase in indigestible fiber as the plant matures (Adams et al., 1996; NRC 2007). It is important to note that due to changing climate patterns, these rangelands are undergoing transformations characterized by increased extreme weather events, reduced water availability, changing forage quality, increased fire incidents, and a decline in biodiversity (Chambers and Pellant, 2008; Gaughan, 2017).

### *1.3 Ewe nutritional requirements in gestation*

Gestation imposes an increased nutritional demand compared to other production phases due to the development and maintenance of the placenta and fetus, in addition to meeting the ewe's own metabolic needs (NRC, 2007; Rattray et al., 1974). Ewes should enter gestation with adequate body condition to ensure there is enough energy stores to carry themselves and the developing fetus, without metabolic ailments (Caldeira et al., 2007; Esmailzadeh et al., 2009). The ewe's nutritional requirements increase progressively throughout gestation, particularly if

she is carrying multiple fetuses or in her first pregnancy (ASI, 2015, Everett-Hincks et al., 2005; Rattray et al., 1974; Zhang et al. 2018).

For instance, a 200lb mature twin-bearing ewe during early gestation requires 4.41 lbs of dry matter intake (DMI) daily, comprised of 53% of total digestible nutrients (TDN) on a dry matter basis (DM) and 8.5 % crude protein (CP). In late gestation, the same ewe must increase her DMI to 5.90 lb/day and have 9% CP in order to maintain fetal growth and body functions (Cannas et al., 2004; NRC, 2007). The specific nutrient requirements vary based on factors such as number of lambs, exercise, stress, and age (Lassala et al., 2011; Dønnem et al., 2020; Everett-Hincks et al., 2005; Sejian et al., 2012; Zanon et al., 2022). For example, a 200lb mature ewe carrying a singleton in late gestation requires 4.74lb/day, with 53% TDN and 8.6% CP, while a triplet-bearing ewe's requirements increase to 5.37lb/day DMI, 66% TDN, and 10.7% CP (Cannas et al., 2004; NRC, 2007). Importantly, increases in fetal size reduce rumen capacity so ewes with multiples require more energy dense feed. On top of ewe intake, energy, and protein requirements, a variety of vitamins and minerals are needed to execute all maintenance and production requirements such as calcium for lactation and pregnancy and vitamin E for immune function (Braithwaite et al, 1970; Dønnem et al., 2015). Other minerals such as phosphorus, copper, selenium, zinc, manganese, chromium, and iron, support conception and maintenance of pregnancy (NRC, 2007; Kumar et al., 2011; LeKatz et al., 2011).

### *1.3.1 Supplementation in production*

Generally, forage from rangelands does not adequately meet the nutritional requirements of ewes throughout all stages of production (Grings et al., 1996; Quintana et al., 2022). In extensive settings, ewes will preferentially select certain forages based on preference, nutrition, and familiarity (Catanese et al., 2015; Parsons et al., 1994; Wild et al., 2023). Preferential

selection and competition for resources combined with the increased plant maturity, which results in increased indigestible fibers and decreased protein, in fall and winter seasons, can result in nutrient challenge for ewes (Heitschmidt et al., 1995; Wild et al., 2023). Consequently, ewes need supplementation of energy, protein, micronutrients, and amount of feed during crucial production phases to fulfill maintenance and production requirements (Braithwaite et al, 1970; Ojha et al., 2015; Radunz et al., 2011). One common practice is “flushing” which involves providing a diet with increased energy and protein to ewes three weeks before and after breeding. Flushing aims to help ewes reach a desired condition thus enhancing ovulation rates and diminishing early embryonic loss (Gunn et al., 1988; Teleni et al., 1989; Landau et al.; 1996). This can be achieved by moving ewes to a higher quality pasture or offering a daily grain of 0.3-1.0 lb per ewe (ASI, 2015).

When ewes are grazed on dormant, low-quality forage, producers supplement with energy and protein sources to ensure nutritional needs are met as gestational requirements increase (Quintana et al., 2022; Van Emon et al., 2015) (Figure 1.2). An energy supplementation, like corn, should be provided to sheep at rate of 0.25 to 0.50 percent of their body weight when grazing on low quality pastures (Caton and Dhuyvetter, 1997). Increasing protein intake in late gestation supports the maintenance of body weight, body condition, maternal body reserves, fetal development and pregnancy (Liamadis and Milis, 2007; Van Emon et al., 2014; Van Emon et al., 2015). Energy supplementation in late gestation is vital to support fetal development and ewe maintenance requirements (Campion et al., 2016; McGovern et al., 2015). Ewes in late gestation are also vulnerable to metabolic diseases, such as pregnancy toxemia, if nutrition is not carefully monitored and body condition maintained (Calderia et al., 2007; Sargison, 2007). Supplemental

feed may come in various forms, including pelleted feed, alfalfa hay, corn, barley, and dried distiller grains (Quintana et al., 2022; Taylor et al., 2002).

#### *1.4 Gestational milestones*

##### *1.4.1 Placental*

The placenta is a temporary organ that develops shortly after fertilization and attachment in mammals. It has multiple roles during gestation including nutrient exchange, waste removal, hormonal regulation, and buffering of the maternal immune response during gestation. The placenta will exchange oxygen, glucose, fatty acids, and amino acids between maternal and fetal blood supplies (Gude, et al., 2004; Regnault et al., 2005). In the ovine, the placenta is cotyledonary and has placentomes consisting of the maternal caruncle and fetal cotyledon, which serve as the site of nutrient exchange. Placental function will change with fetal demand, for example, the placenta mediates nutrient exchange and flow of glucose to match that of what the fetus requires to grow during that stage of gestation, in addition to increasing blood flow to achieve increased nutrient exchange (Fowden et al., 2010; Molina et al., 1991; Reynolds et al., 2005).

In sheep, placentation initiates within the first month after fertilization and implantation (Zhang et al., 2015). Trophoblast cells differentiate into maternofetal barriers and vascular structures as the conceptus grows, creating a highly vascularized organ, necessary for efficient nutrient exchange (Johnson et al., 2018). Early gestation sees increased uterine blood flow and maternal placental angiogenesis (Ahmed and Perkins, 2000; Reynolds et al., 2005), with a subsequent exponential rise in placental and umbilical blood flow and vascular remodeling, to accommodate fetal growth in the later stages of gestation (Gude et al., 2004; Reynolds et al., 2005; Reynolds and Redmer, 1995). Fetal and placental growth are linked in that as vasculature

and size of placenta increase, so does the fetus's ability to uptake nutrients and utilize them for growth and development. Placental and placentome weight increase till reaching a peak at mid-gestation where it is rather stagnant till parturition in the sheep (Zhang et al., 2015). Any increases in fetal demand after this time are met by reorganization of placental vasculature.

The placenta actively regulates hormones impacting pregnancy, metabolism, fetal growth, and parturition. The ewe will have a corpus luteum producing progesterone, but placental progesterone is the dominant source for most of gestation (Norman and Henry, 2014). The placenta produces sufficient progesterone to support gestation starting at day 55 of gestation, then increases till day 130 (Stabenfeldt et al., 1972; Weems et al., 1999). Additionally, the placenta produces or stimulates hormones such as placental growth hormone, placental lactogen, glucocorticoids, leptin, insulin-like growth factors, and estrogens, each influencing fetal, placental, or mammary growth and development throughout gestation (Gude et al., 2004; Norman and Henry, 2014; Zhang et al., 2015). The placenta itself is an organ that requires nutrient uptake (proteins, glucose, vitamins and minerals) to grow, complete metabolic reactions, undergo vascular changes, and hormone production (Dávila Ruiz et al., 2024; Ehrhardt and Bell, 1995; Hay, 1995). Additionally, a critical function of the placenta lies in establishing a barrier against maternal immune responses during pregnancy, preventing the passage of harmful substances like bacteria and viruses to the fetus, with pathogens rarely breaching this protective barrier (Gude et al., 2004; Delorme-Axford et al., 2014). To perform all of these functions, the placenta utilizes a large portion of nutrients before they reach the fetus.

#### *1.4.2 Mammary*

Mammary glands, essential for the provision of nutrient-rich milk and passive immunity to the neonate, are secretory glands arranged in a branching formation within round structures

known as alveoli (Paten et al., 2015). These alveoli house epithelial cells that utilize nutrients from the blood to synthesize and secrete milk into the luminal cavity (Collier, 1984).

Myoepithelial cells surround the alveolar epithelium to coordinate contractions of the milk out of the alveoli into the lactiferous ducts and glands cistern, where it is then ejected through the teat cistern upon suckling (McManaman and Neville, 2003; Yart et al., 2014). Neuroendocrine control, governed by hormones such as prolactin and oxytocin, regulates the mechanisms of milk synthesis and ejection, respectively (Anderson, 1975; McManaman and Neville, 2003; Tucker, 1981). The ovine has two udders with two teats typically, however, they can have more teats that may or may not be functional (Hughes, 2021).

Mammary glands undergo structural development during gestation, however, do not become functional till females become pregnant in adulthood. From birth to puberty, mammary tissue exhibits isometric growth with the rest of the body, transitioning to allometric growth at puberty governed by reproductive hormones (Yart et al., 2014). The majority of mammary gland growth, ranging from 60-94% depending on the species, occurs during the first gestation (Anderson, 1975; Hughes, 2021; Knight and Peaker, 1982).

In mid to late gestation, mammary tissues experience an increase in cell count and vascularity, with ductal and alveolar cells differentiating and enlarging as they progress to a secretory state (McManaman and Neville, 2003; Paten et al., 2015; Tucker, 1981). Metabolic demands prompt heightened vascularity to the udder, facilitating the provision of necessary nutrients for milk synthesis (Collier, 1984). Udder growth towards the end of gestation is significant, driven by increased synthesis (Hughes, 2021; Rattray et al., 1974). Milk, a vital source of nutrients including lactose, casein, fatty acids, minerals, and vitamins crucial for neonatal growth, also contributes essential immunoglobulins for the early immunocompetence of

offspring (McManaman and Neville, 2003; Paten et al., 2015; Hammer et al., 2011). By parturition, mammary gland growth and development in ewes is nearly complete, with only minor increases observed during early lactation (Hughes, 2021; Tucker, 1981). It is important to note that these milestones may be influenced by gestational nutrition.

#### *1.4.3 Fetal development*

The initial phase of gestation is marked by maternal recognition of pregnancy, attachment, and embryonic development (Bryden et al., 1972). In the first week post-fertilization, the zygote undergoes multiple cleavages, giving rise to a blastocyst with two distinct layers: the trophoblast, destined to form the placenta, and the inner cell mass, developing into the fetus (Watson et al., 2004). After attachment and recognition, the blastocyst will start gastrulation which is the movement of the cell layers to form three distinct layers called germinal layers (Muhr and Ackermen, 2020). Germinal layers will become different organs in the body and consist of the ectoderm, mesoderm, and endoderm. The ectoderm gives rise to the skin, nervous, and sensory organs; the mesoderm contributes to muscles, excretory organs, circulatory system, sex organs, and the skeleton; and the endoderm develops into digestive and respiratory organs (Bryden et al., 1972; Muhr and Ackerman, 2020). As gestation continues, these layers will continue to grow and develop the associated organs during a process called organogenesis.

Organogenesis occurs at the same time as peak placental growth by size. In ruminant fetuses, a heartbeat is established within a month of conception, and the first 50 days witness the formation of limbs, pancreas, liver, spleen, adrenals, lungs, thyroid, and kidneys, along with the development of the nervous system and muscle cells (Vonnahme, 2007; Hubbert et al., 1972). Mid-gestation involves waves of myogenesis, determining the total number of skeletal muscle cells in the body for life (Du et al., 2005; Reynolds et al., 2019). Organ development will cause

the fetus to grow mildly, while peak placental growth is being reached (Heasman et al., 1999). Importantly, peak placental growth occurs at mid-gestation, with increases in fetal demand accommodated by increased placental vascularity rather than size. Exponential fetal growth occurs in late gestation, with approximately 70% of fetal size achieved during the last 50 days of gestation (Robinson et al., 1977). Rapid fetal growth leads to functional maturity of organs, muscle hypertrophy, and increased adipogenesis (Du et al., 2005; Du et al., 2017; Zhang et al., 2015). It is important these events occur without error, as they will set the physiology of the animal for life (Figure 1.2).

### *1.5 Growth and development of the lamb*

#### *1.5.1 The early postnatal period*

Lamb birthweight and growth can depend on many factors such as breed, sex, progeny number, disease, and dam's nutrition (Gascoigne and Lovatt, 2015; Mellado et al., 2016). At birth, the abomasum is the largest chamber of the lambs stomach, with the reticulum and rumen being nonfunctional (Baldwin and Connor; 2017). Milk bypasses the reticulum and rumen through the esophageal groove and deposits directly into the abomasum for digestion (ASI, 2015; Baldwin and Connor; 2017) similar to a non-ruminant animal.

The first day of life it is crucial for lambs to bond with dams and ingest colostrum, as this will enable maternal care through weaning and increase health in a neonatal lamb through the presence of immunoglobulins, respectively (Freitas-de-Melo et al., 2022). The mothering of the dam by licking and drying the lamb provides stimulation and aids in thermoregulation (McCutcheon et al. 1983; McGlone and Stobart, 1986). The ewe's milk is rich in proteins, fats, and sugars required for rapid growth (Gascoigne and Lovatt, 2015; Mellor and Cockburn; 1986) and the neonatal lamb will obtain nearly all its energy nutrients from the dam's colostrum and

milk (Mellor and Cockburn; 1986). Sugars, mainly glucose, are the fundamental nutrient needed by the lamb in early life as it is the primary energy source for metabolism, which aids in thermoregulation (Plush et al., 2016; Thompson et al. 2006). Heat production and maintenance in early life is critical for lamb survival (Miller et al., 2010) and largely dependent on brown adipose tissue while the neonate starts to ingest more and more colostrum, thereby gaining nutrients for metabolism (Plush et al., 2016; Satterfield et al., 2013). Failure to ingest adequate colostrum and milk in the first week of life can result in a variety of challenges for lamb survival such as sickness, starvation, and hypothermia (Mellor and Cockburn, 1986; Miller et al., 2010). Milk will continue to be the primary source of nutrients during the pre-weaning period, however, increased ingestion of feedstuffs such as forage and concentrates will be instrumental in developing the rumen and increasing growth (Danso et al., 2014; Sun et al., 2021). Lambs will continue to nurse off their dams and begin ingesting solid feedstuffs until weaning.

As lambs start to nibble on forages, their rumen will be inoculated with microorganisms vital for rumen function and energy production (Ward, 2008). Lambs can have microbial activity starting at 2 weeks of age, and with the addition of concentrated feed, known as creep feed, lambs can be weaned early at 50- 60 days when their rumen has the capacity to support majority forage intake and minority milk (Gascoigne and Lovatt, 2015). Creep feeding lambs will increase their gastrointestinal development by increasing the production of volatile fatty acids (VFAs) and promoting rumen epithelial maturity (Sun et al., 2021; Wang et al., 2016). The reticulo-rumen continues to get larger up through 4 months of age. Prior to weaning lambs will have a linear trend in body weight with body structures showing a high growth period that starts to flatten around 6 weeks of age (ASI, 2015; Bhatt et al., 2009; Gibson et al., 2022). Lamb health and nutrition play a pivotal role in the growth and development trends in early life, which can

affect lambs' growth and performance later in the production cycle. (Gascoigne and Lovatt, 2015; Litherland and Lambert, 2000). Incidents of illness or injury will slow down their growth trajectory and the impacts can be seen at subsequent growth milestones.

#### *1.5.2 Post-weaning period*

Weaning typically occurs around 2-3 months of age, however, lambs can be weaned as late as 5 months of age. Weaning is a very stressful time for animals and should be accompanied by adequate nutrition to diminish other stressors (Freitas-de-Melo et al., 2022; Gascoigne and Lovatt, 2015). There are different production systems a lamb can enter once weaned, ranging from intensive to extensive or a combination (De Brito et al., 2017). Extensive operations are typically rangeland settings with low input by producers versus an intensive operation, such as a concentrated animal feeding operation, where lambs receive carefully designed diets to reach ideal weight and composition before slaughter (Kilgour et al., 2008). High quality forage with diverse plants, such as legumes, can be enough for growing lambs when pasture availability does not limit intake, as lambs need above maintenance requirements to achieve production goals (Freer and Dove, 2002; McGrath et al., 2015). While forage quality is usually high during weaning, to reach body and wool growth demands, lambs may need supplemental concentrate or protein in addition to grazing (Karim et al., 2007; Ramos et al., 2019).

Typically, lambs are ready for slaughter in the late spring or summer once they have reached market weight and ideal composition, however, depending on sex, timing of lambing, fat thickness, and breed, this timeline can vary (Levalley et al., 1991; Stanford et al., 1998; Whaley et al., 2020). Once ewe lambs reach 50-70% of mature weight they should reach puberty. The timeline in which ewe lambs can reach this goal is dependent on breed, season, and growth rate, especially fat deposition (Dickerson and Laster, 1975; Dýrmundsson, 1973a; Fogarty et al.,

2007; Rosales Nieto et al., 2015). Ram lambs attain puberty sooner than ewe lambs, typically at 35-65% of mature body weight, and the speed at which they reach sexual maturity is dependent on breed and growth rate (Dýrmundsson, 1973). Upon puberty attainment, ewe lambs or rams may be kept back for breeding in the current or next lambing cycle (Fogarty et al., 2007). While these lambs will go into the stock sheep production cycle and create offspring of their own, many extrinsic factors can affect mature sheep's ability to grow and reproduce successfully such as weather stress, disease, injury, and the plane of nutrition (Lima et al., 2020; Kleemann and Walker, 2005; Sejian et al., 2012).

### *1.6 Fetal programming*

When ewes are managed properly during gestation, placental, fetal, mammary, and dams nutrient requirements for homeostasis and gestation are met, resulting in a successful pregnancy and lambing (Fthenakis et al., 2012). However, inadequate maternal nutrition during pregnancy can program fetal development and affect lamb performance long term (Reynolds et al., 2017) (Figure 1.3). As gestation progresses, without proper nutrients, the ewes own metabolic needs are competing with a growing fetus, placenta, and mammary tissue. As the ewe enters mid-gestation, severe nutrient challenge can restrict placental growth, vascularity, and subsequently, nutrient supply, therefore restricting fetal growth (Bell et al., 2005). Decreased nutrient supply can alter fetal metabolism and growth by affecting skeletal muscle development, insulin action, and fat deposition (De Blasio et al., 2007a; Ford et al., 2007; Gauvin et al., 2020; Zhu et al., 2006). Lambs who experience severe nutrient restriction in mid or late gestation consistently show decreased birth weights and morphometrics, compared to lambs who were fully nourished during pregnancy (De Blasio et al., 2007b). In response to nutrient restriction, the fetus nutrient partitions, prioritizing essential organs such as the heart and brain compared to areas deemed less

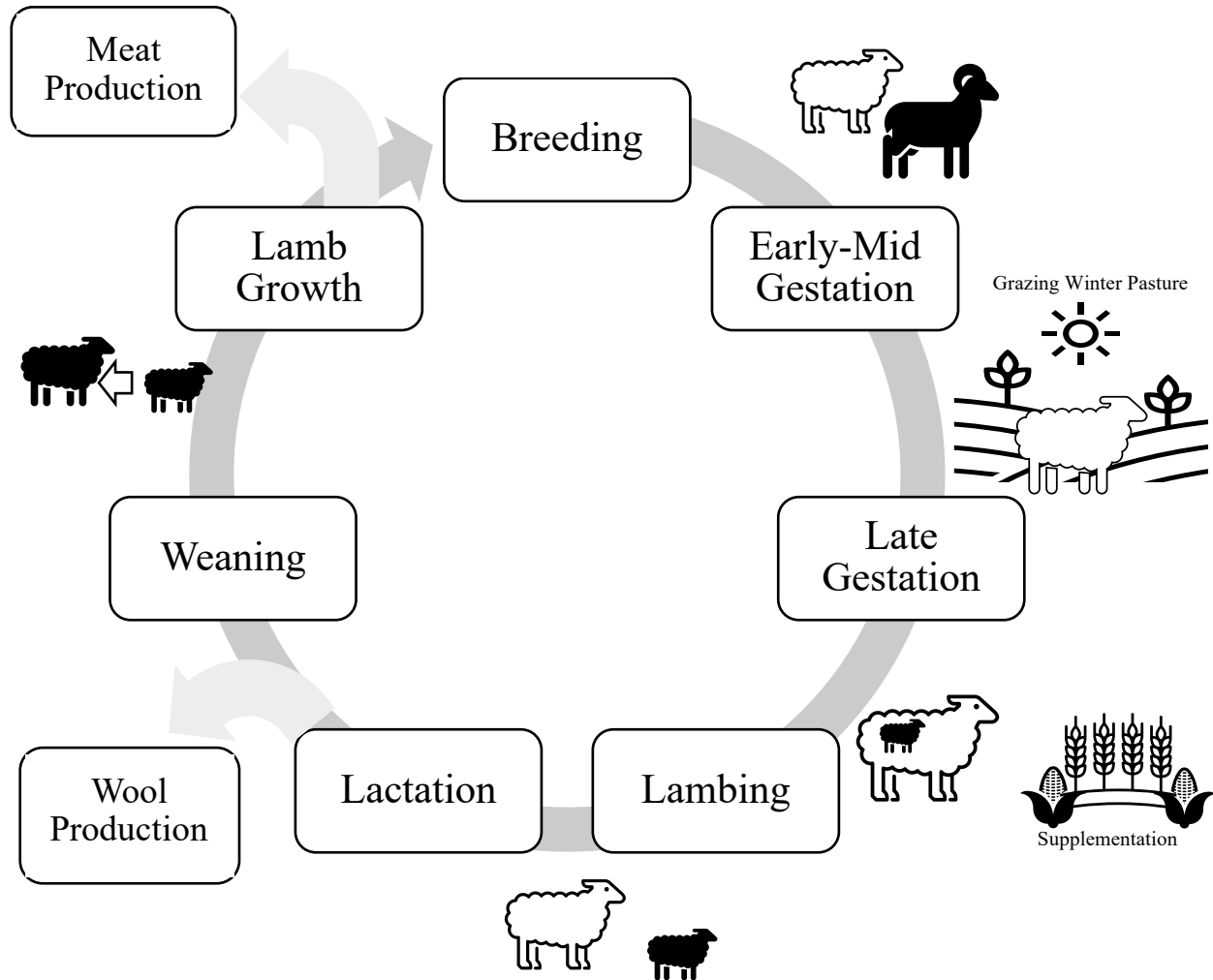
important such as skeletal muscle, reproductive organs, and wool (Abud et al., 2020; Barcellos et al., 2024; Hales and Barker, 2001). The development of some organs at the expense of others has lasting repercussions for lamb growth, metabolic, and endocrine functions (Khanal and Nielsen, 2017; Reynolds et al., 2019). The majority of current literature findings are a result of global restriction or severe nutrient restriction varying in timing or duration during gestation, limited research is available on how practical nutrient challenge, experienced in an extensive production cycle, affects gestational milestones and postnatal lamb performance.

### *1.7 Conclusion*

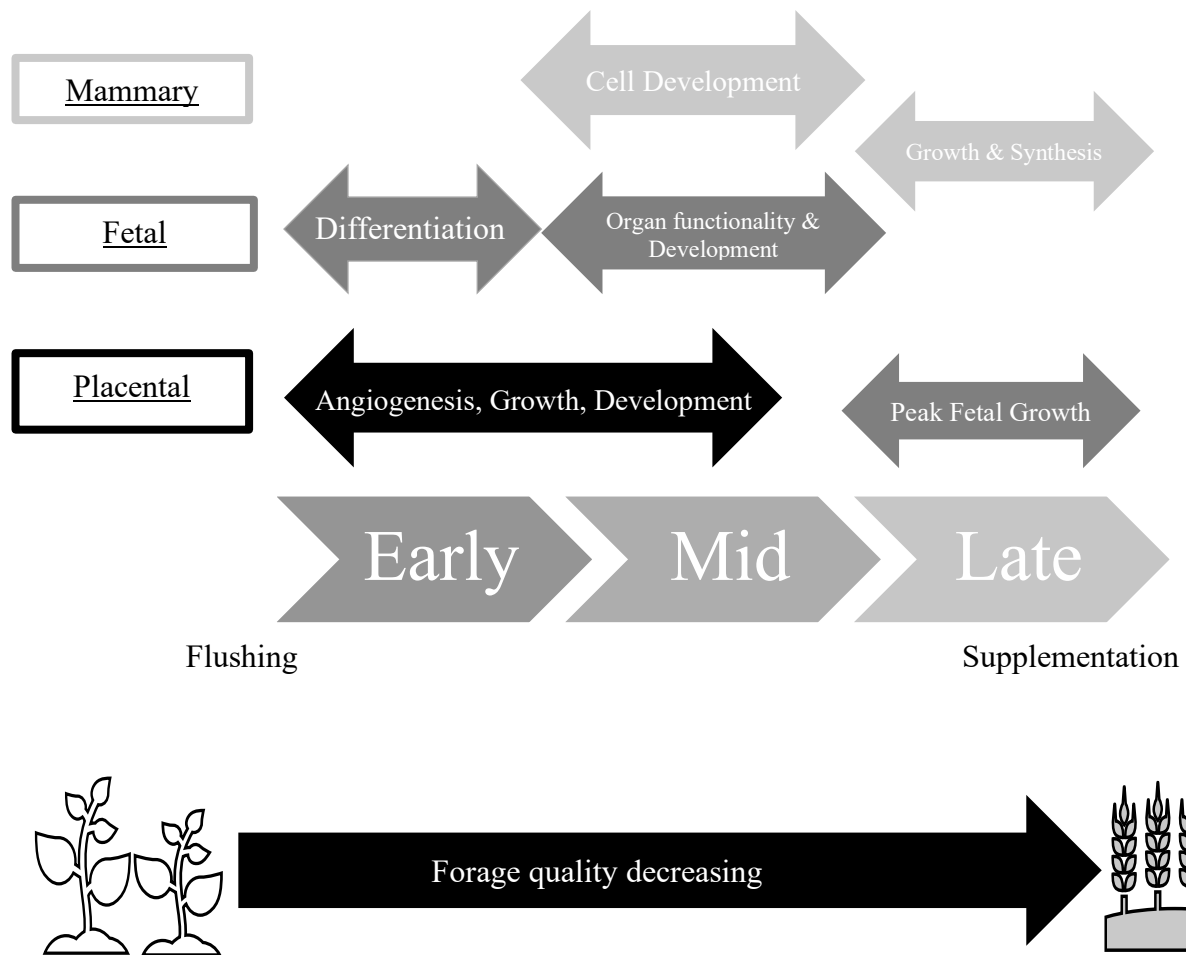
The role that maternal nutrition plays in placental and fetal growth has been important in understanding the physiology occurring during gestation, which may help explain postnatal performance. Sheep have often been used as a model for humans and other livestock species and these studies have aided in the understanding that mammalian physiology is programmed during gestation. The mechanisms of these programming events are beginning to be understood, which helps with the identification of potential solutions for how to mitigate diseases and growth insufficiencies. However, many studies are the result of global or severe experimental nutrient restriction with less research available on how more standard feeding strategies may impact fetal development. This also does not account for the multiple concurrent stressors, such as predators and climactic perturbation, which can compound nutritional stress and concurrent stressor impact still needs to be investigated. The adaptations a fetus must make in response to materno-fetal stress in gestation result in physiologic changes to tissues that have a large impact on drivers of profitability in the sheep industry. For example, fetal programming has been associated with impaired muscle development and aberrant metabolism, which persists postnatal, and results in animals with reduced growth, poor feed efficiency, and poor carcass characteristics. Less is

known about the impact on the other major driver of profitability, reproductive efficiency. It is important that we continue to investigate how current nutritional strategies may impact fetal development and postnatal performance to make better management decisions in our livestock species going forward.

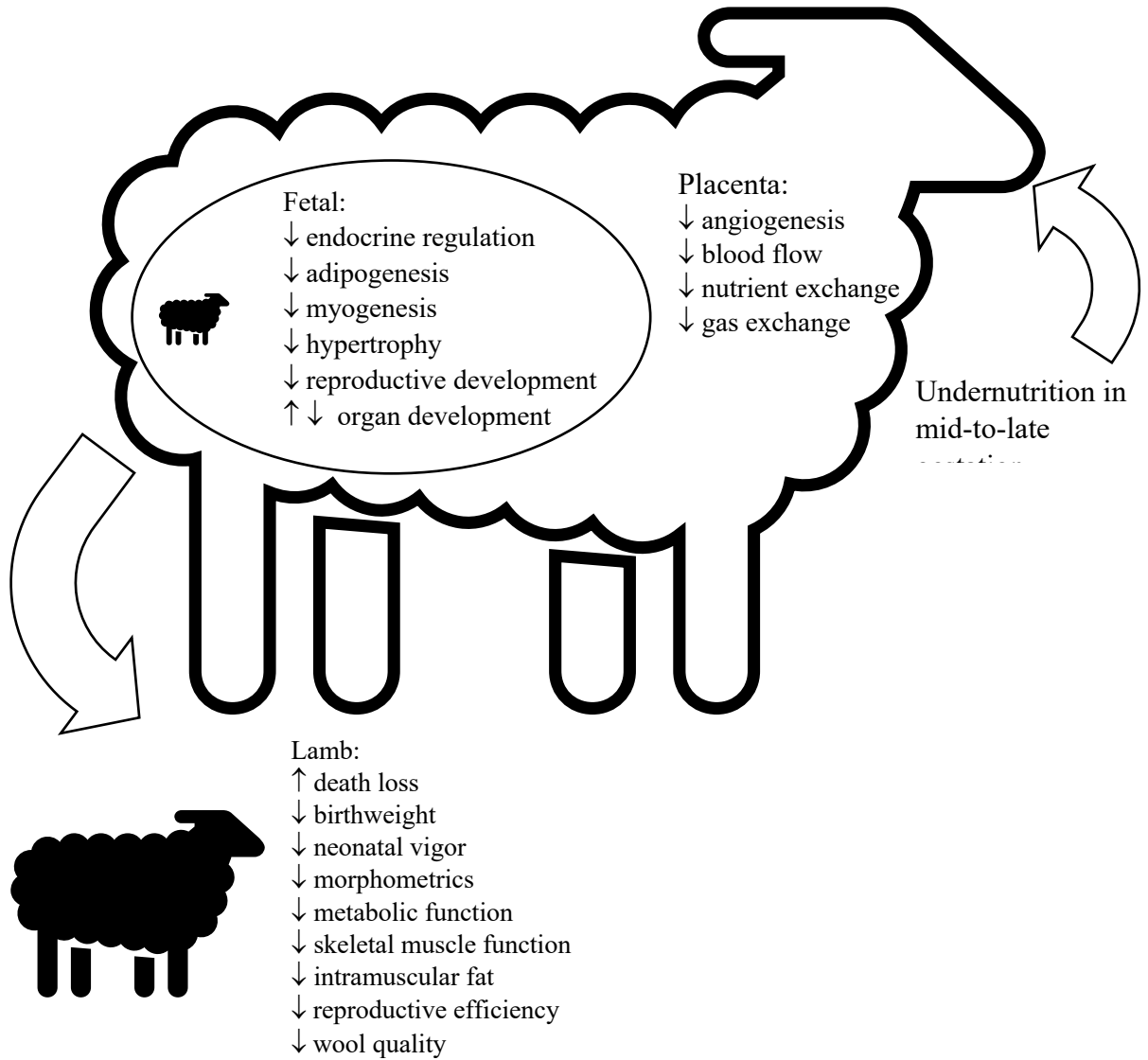
Figures



**Figure 1.1.** Overview of sheep production cycle from breeding to meat production.



**Figure 1.2.** Sheep gestational milestones overlaid with current feeding strategies.



**Figure 1.3.** Overview of dynamics between severe nutrient restriction in mid-to-late gestation and fetal programming outcomes in the lamb.

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## CHAPTER 2 - PRE-WEANING PERFORMANCE IN LAMBS AFTER NUTRIENT SPECIFIC RESTRICTION IN GESTATION

### *2.1 Introduction*

In an extensive sheep production system, ewes are bred in the fall and graze on rangeland throughout gestation (ASI, 2015; Bradford et al., 2003). This coordinates a period in which forage quality and quantity are at their lowest, when fetal development and organ functionality is occurring, and nutrient demands are at their highest (Julian et al., 2020; Gauvin et al., 2020; Heasman et al., 1999; Hubbert et al., 1972; Kamstra, 1973; Reynolds et al., 2019). As the season turns, range plants start to decrease in nutrient quality with lower protein and an increase in indigestible fibers, that without supplementation, is inadequate to meet ewes' nutritional needs for the entirety of gestation.

To account for this, during late gestation ewes are typically supplemented to try to meet nutrient demands for peak fetal growth and lactation. However, it is important to note that nutrient challenge can occur at any point of gestation (Campos et al., 2022; Freer and Dove, 2002; Quintana et al., 2022). Severe nutrient challenge in gestation has been well studied and consistently shows evidence of adaptive fetal programming (Reynolds et al., 2017). Adaptive fetal programming occurs when a fetus responds to malnutrition by nutrient-sparing and shunting nutrients to organs critical for survival at the expense of others deemed less essential (Abud et al., 2020; Barcellos et al., 2024; Hales and Barker, 2001). In this situation, cells and tissues must adapt their development to survive in a nutrient deficient environment, and when not corrected before birth, these adaptations are programmed for life. Lambs from adverse in-utero environments exhibit low birthweights, decreased skeletal muscle fibers, poor body condition,

decreased feed efficiency, and lower quality carcass traits (De Blasio et al., 2007; Ford et al., 2007; Gauvin et al., 2020; Zhu et al., 2006). For example, Tillquist et al. (2023) showed that ewes, when globally restricted to 60% of requirements from day 30 of gestation till parturition, produce lambs that are lighter and structurally smaller at birth and through the early growth period. Other studies looking at mid-to-late gestational restriction have reported similar findings including altered metabolic hormone function, altered lipid deposition, and abnormal feeding behavior in lambs (Gauvin et al., 2020; Pérez-Clariget et al., 2023; Smith et al., 2021; Soranno et al., 2022; Steinhauser et al., 2021), outcomes that affect valued production traits.

Previous research has focused on global restriction and shown adaptive fetal programming outcomes at birth and through life, but less is known about the effects of restriction at levels and qualities similar to the extensive rangeland cycle where nutrients vary on many facets simultaneously. Low birthweight pathologies cost the sheep industry 8-10% of annual product (APHIS, 2015; Wu et al., 2006); however, this number does not account for those animals that may experience fetal programming without obvious birthweight differences. We hypothesize that tissue specific differences exist in the absence of birthweight differences. Thus, we sought to investigate pre-weaning performance of lambs from ewes fed a diet that simulates the extensive production system.

## *2.2 Materials and methods*

### *2.2.1 Animals and experimental design*

This study was approved by the Colorado State University Institutional Animal Care and Use Committee. Three-year-old Merino X Rambouillet ewes, were synchronized using a twelve-day CIDR with Prostaglandin protocol. Upon CIDR removal, ewes were placed with a mature ram fitted with a marking harness, and dorsal marking date was recorded. Ewes were first

confirmed pregnant at ~25 days of gestation (dGA) by blood pregnancy test (BioPRYN, Moscow, ID). At ~60 dGA a flank ultrasound was performed to confirm pregnancy maintenance and determine progeny number. After pregnancy confirmation by blood, ewes were blocked by body weight, randomly assigned one of two diets, and placed in individual housing for the treatment period.

### *2.2.2 Diet composition and treatment allocation*

Two diets were formulated based on the Small Ruminant National Research Council (NRC, 2007) guidelines to either meet 100% of nutrient requirements for a mature medium-sized (200 lbs) twin-bearing ewe (fully-nourished) or a diet stimulating winter forage (restricted) formulated for a 50% reduction in crude protein (CP) and 30% reduction in total digestible nutrients (TDN). This study occurred over 2 continuous years to produce lambs from fully nourished (CONT; n=30 lambs from 17 ewes; 3 singletons, 21 twins, 6 triplets; 14 males and 16 female) and restricted (NR; n=28 lambs from 20 ewes; 5 singletons, 14 twins, 9 triplets; 19 males and 9 females) pregnancies. All animals had access to ad libitum water and mineral blocks in their individual pens. The fully nourished diet was composed of cracked corn (10.7%), corn silage (30%), grass hay (53%) and soybean meal (7.3%) on a dry matter basis. The restricted diet was composed of corn silage (40%) and wheat straw (60%) on a dry matter basis. Both diets were consistently sampled and analyzed for nutrient composition (Table 2.1).

### *2.2.3 Gestational management*

From 30-125 dGA, restricted ewes received the deficient diet and were pair-fed to fully nourished ewes of similar body weight to prevent intentional deficits in nutrient quality being overcome by increased intake. At 125 dGA restricted ewes were realimented to the fully nourished diet to simulate a normal late gestation supplementation period. Throughout the

treatment period, daily feed intake was collected and feed amounts were adjusted accordingly. For the entire study period, weekly maternal bodyweights (BW) and bimonthly (twice per month) body condition scores (BCS) were collected. After lambing, BW and BCS collection was collected monthly until weaning for future analysis.

#### *2.2.4 Lamb growth and development*

Ewes lambled independently in a group environment, unless intervention was needed, before being brought with their lambs to a jug. Placental weight and cotyledon number were collected after passage. At birth, progeny number, lamb rectal temperature (AmerisourceBergen, Conshohocken, PA), birthweight, and birth morphometrics (crown-rump length, CRL; crown circumference, CC; cannon-bone length, CBL; and abdominal circumference, AC) were collected. Weight and temperature collection continued daily for the first week of life. Ewes and lambs were monitored for health and vigor for 24-48 hours and then processed by ear tag and tail band placement before being released from the jug. If instances of hypothermia occurred, interventions were applied for animal care. Lamb BW was collected daily until 30 d and weekly thereafter. After the first week of life, temperature and morphometrics were collected weekly until weaning at ~70 d.

#### *2.2.5 Statistical analysis*

Data were analyzed using the mixed procedure of SAS 9.4 (SAS Institute; Cary, NC). Single observation measurements were analyzed by ANOVA for the fixed effects of treatment. Separate ANOVA analysis with repeated measures were performed on data related to growth and temperature to determine the effects of treatment, time, and the interaction. For all analyses, the fixed effects of progeny number, sex, and year were also included and where not significant, removed from the final model. Fisher's LSD test was used for mean separation. Individual lamb

was considered the experimental unit and significance was determined as a  $P$ -value of  $\leq 0.05$ , and tendencies were indicated by  $P$ -values of  $\leq 0.10$ . All data are presented as the mean  $\pm$  standard error.

## 2.3 Results

### 2.3.1 Maternal performance

A treatment by time interaction was observed for BW where restricted ewes weighed less ( $P < 0.0001$ ) than fully nourished ewes from weeks 9-17 of the treatment period, with a tendency to weigh less ( $P < 0.10$ ) at weeks 7 and 8 (Figure 2.1). There was also a progeny effect ( $P < 0.001$ ) in which ewes with twins were heavier, followed by triplets, then single-bearing ewes. Similarly, there was a treatment by time interaction for BCS where restricted ewes had lower ( $P < 0.001$ ) BCS scores than fully nourished ewes from weeks 10-16 of the trial period with a tendency to weigh less at week 6 (Figure 2.2). DMI decreased ( $P < 0.05$ ) across gestation but did not differ between treatment groups. Gestation length did not differ between fully nourished ewes ( $148.71 \pm 0.78$  d) and restricted ewes ( $149.03 \pm 0.69$  d). Similarly, there were no differences in cotyledon number ( $71.08 \pm 3.56$  vs  $73.20 \pm 3.46$ ) or placental weight ( $747.28 \pm 42.89$  g vs  $715.79 \pm 41.66$  g) between fully nourished and restricted ewes, respectively, but there was a progeny effect ( $P < 0.05$ ) for placental weight in which placentas for multiples were heavier.

### 2.3.2 Pre-weaning growth and biometrics

There were no differences in birthweight or birth morphometric ratios between CONT and NR lambs, however, there was a progeny effect ( $P < 0.05$ ) for birthweight with multiples being lighter individually. CB/birthweight was greater in males ( $P < 0.05$ ) and CC/birthweight tended ( $P < 0.10$ ) to be greater in male lambs compared to females, regardless of treatment.

NR lambs tended ( $P < 0.10$ ) to be heavier across the first week of life compared to CONT lambs when determined by bodyweight (Figure 2.3). However, beginning at 6 weeks of age through every week until weaning (~10 weeks) there was a treatment by time interaction for weekly BW, with NR lambs weighing less ( $P < 0.05$ ) than CONT lambs (Figure 2.4). CRL/BW was greater ( $P < 0.05$ ), and CC/BW, CB/BW, and AC/BW tended ( $P < 0.10$ ) to be greater in NR lambs compared to CONT, regardless of day. Additionally, CRL/BW was greater ( $P < 0.05$ ), and CC/BW and CB/BW tended ( $P < 0.10$ ) to be greater in female lambs, while no sex effects existed for AC/BW.

### *2.3.3 Lamb thermoregulation*

NR lambs had lower ( $P < 0.05$ ) rectal temperatures in the first week of life, regardless of time, compared to CONT lambs (Figure 2.5). A treatment by time interaction was observed in subsequent weekly temperatures with NR lambs having lower ( $P < 0.05$ ) average temperatures on weeks 1, 6, and 7 of life compared to CONT (Figure 2.6).

## *2.4 Discussion*

This study demonstrates that a nutrient challenge simulating what animals would encounter in a normal production system is significant enough to impact dam and progeny performance. Importantly, the diet formulation did not result in as restricted diet as anticipated, but evidence of impact is still present. Maternal weight and BCS declined well before the traditional supplementation period in late gestation, and the effects of this nutritional plane are seen in the lambs. Specifically, while not evident at birth, lambs from restricted ewes grow at a slower rate and remain 9% smaller at weaning. This coincides with differences in structural growth, indicated by morphometric measurements, that suggest reduced growth is not only due to differences in visceral tissue mass. Additionally, lambs from restricted ewes have lower

average body temperatures and anecdotally required more intervention due to thermoregulatory issues in the first week of life and beyond. This data supports our hypothesis that tissue specific differences are likely occurring in the absence of the obvious birthweight differences, but further research is needed to identify what specifically those differences are. Nevertheless, this data indicates that traditional supplementation strategies may not be sufficient to meet maternal and fetal demands for normal growth and development and should be reconsidered when making decisions for gestational animal management.

Late gestation represents the production period in which maternal nutrient demands are at the highest and thus, producers often choose to supplement dams at this time (Umberger, 2009); however, our findings show maternal BW and BCS are affected by poor nutrition prior to the traditional supplementation period. As ewes progress through gestation, a mid-gestational nutrient restriction with realimentation is not meeting their own nutritional requirements to maintain body reserves in addition to gestational demands, as shown by decreases in ewe BW and BCS (Pérez-Clariget et al., 2023; Vasquez-Hidalgo et al. 2023). During gestation there is a competition for nutrients between the ewe, placenta, and fetus (Rattray et al., 1974) and to meet all these demands with poor nutrition, the ewe must allocate nutrients away from her own reserves, resulting in decreased BW and BCS (Lemley et al.; 2012; Tillquist et al., 2023). Additional demands occur with common practices of breeding peripubertal ewes that have not yet met mature size, selection for lamb prolificacy, and mammary development and milk synthesis in late gestation (Gootwine et al., 2007; Hughes, 2021; Vautier and Cadaret, 2022). Previous studies have demonstrated that global restriction consistently reduces maternal BW and BCS, but less have looked at specific nutrient availability (Lemley et al.; 2012; Reed et al., 2007; Steinhauser et al., 2021; Vasquez-Hidalgo et al., 2022). Decreases in BW and BCS in mid-

gestation coincide with fetal organ development and may lead to adaptive fetal programming that persists postnatally (Abud et al., 2020). There were no differences between placental weights and cotyledon number, which suggests that impacts on the fetus could be a direct result of decreased nutrient availability rather than placental insufficiency (Lemly et al., 2012; Vasquez-Hidalgo et al., 2023). However, decreased nutrient exchange due to reduced blood flow could still be occurring as placental angiogenesis is dependent on maternal nutrition (Redmer et al., 2004; Vasquez-Hidalgo et al., 2023). With a more severe restriction in mid-gestation, umbilical blood flow is reduced and realimentation does not recover it, despite normal placental blood flow, weight, and cotyledon size near term (Vasquez-Hidalgo et al., 2023, Lemley et al., 2012). Similar effects may be occurring in this study, but more research is needed to confirm this.

Evidence of potential fetal programming is not apparent by birthweight differences or morphometrics, in fact, NR lambs tended to be heavier in their first week of life. While we did see an expected effect of progeny, with multiples being smaller, we did not see the sex effect that is common between male and female lambs. Sales et al., (2024), recently performed a study that determined differences in sex are lost when maternal undernutrition occurs during pregnancy and may help explain the findings of this study. Additionally, supplementation in late gestation when peak fetal growth by hypertrophy is occurring (Du et al., 2010), may be adequate to recover fetal size but not to undo adaptations in tissue development and functionality that occurred earlier in gestation (Pillai et al., 2017; Redmer et al., 2004). Low birthweights are seen after ewes are severely restricted through gestation due to placental stunting or restriction in late gestation which affect fetal growth by size (Hoffman et al., 2014; Wu et al., 2006). Lambs, as a result of prolonged and/or severe restriction, will have decreased skeletal muscle fibers, smaller bones, and lower adipose reserves making them smaller at birth (Ford et al., 2007; Gauvin et al., 2020;

Zhu et al., 2006). However, the supplementation in late gestation in this study may be preserving fetal size resulting, in no differences in birthweights or skeletal size (Greenwood and Thompson, 2007; Smith et al. 2021). While fetal size was not monitored throughout gestation in this study, we postulate that as the restricted fetus gets adequate nutrition in late gestation, it experiences a compensatory growth pattern to reach normal fetal size by birth. Other studies utilizing nutrient restriction as a maternal insult have found variable birthweights in both sheep and cattle (Long et al., 2009; Sandoval et al., 2020). When evaluated as separate groups, the nutrient restricted but normal size fetuses exhibited characteristics of both control and nutrient restricted groups, depending on the variable of interest, indicating that tissue specific differences may be occurring in the normally sized fetuses whose dams were nutrient restricted (Sandoval et al., 2020). This data supports our hypothesis in this study that programming effects may be in place without obvious birthweight differences and knowledge of this may aid in management strategies for producers who typically rely on phenotypic data for making decisions.

Differences in performance are evident as lambs grow older and closer to weaning. NR lambs had reduced growth beginning at six weeks of age which continued to delineate out until weaning. Skeletal muscle is one of the tissues most impacted by nutrient repartitioning due to its high glucose metabolic utilization. Other studies of nutrient restriction have demonstrated differences in growth and body composition due to reduced muscle mass, muscle fiber number, proportions of Type IIa oxidative fibers, GLUT 4 receptors, and increased fat mass (Du et al., 2010; Vautier and Cadaret 2022 ; Zhu et al., 2006). While not specifically evaluated in this study, it is likely that similar tissue specific changes are at least partially responsible for differences in lamb pre-weaning growth and development. In addition to poor body composition, asymmetric growth is a hallmark of fetal programming (Vautier and Cadaret, 2022) and was

evident in this study when considering ratios of structural versus tissue growth. Fetal adaptations result in differences in postnatal performance when animals are put into a nutrient rich environment, but mechanisms of metabolic thrift are already programmed and irreversible. Nutrient restricted lambs have shown feeding behaviors that prioritize higher energy feeds and eating for longer periods to try to overcome decreases in body reserves (Pérez-Clariget et al., 2023) due to aberrant growth in-utero and in the neonatal period. Nutrient restricted lambs are often smaller with poor feed efficiency, necessitating increased feed intake for the same amount of gain as their control counterparts. However, other studies have shown that these lambs may overcome weight differences with compensatory growth later in life (Tillquist et al., 2023). Compensatory growth is not evident in the postnatal animal thus far in this study and altered metabolic mechanisms such as lipid metabolism and neuroendocrine signaling (Smith et al., 2021; Soranno et al., 2022) could be underlying these differences but further research is needed. Nevertheless, if these animals stay on the same reduced growth trajectory, NR lambs will take longer to reach target growth goals for meat production and reproductive capability.

The most common losses in the first week of life are due to hypoglycemia and hypothermia in all lambs (dos Santos et al., 2023) and often are concurrent and compounding with hypoglycemia contributing to hypothermia. At birth, lambs need to be stimulated and dried by their dam, stand, and attempt to nurse within hours of birth, and the speed in which these occur is correlated to lamb survival (Dwyer et al., 2003). In all lamb births, lamb mortality is higher due to lack of mothering, low birthweight, larger litters, and environmental climate. Neonatal thermoregulation is accomplished by non-shivering thermogenesis (brown fat), shivering thermogenesis, colostrum intake, and metabolism (Dwyer et al., 2016). While nutrient restriction induced low birthweight is associated with increased hypothermia, the present study

demonstrated that NR lambs struggle to thermoregulate properly despite normal birthweights and progeny size. Maternal nutrient restriction has been associated with decreased brown adipose tissue in the near-term fetus (Satterfield et al., 2013; Ojha et al., 2013) and likely persists to the neonate contributing to decreased capacity for non-shivering thermogenesis and a predisposition to hypothermia. Indeed, our diet manipulation occurred concurrently with the timing of fetal brown adipose tissue deposition (70-120 dGA; Alexander, 1978) and may have limited development in these lambs. It should be noted that the temperatures exhibited in this study are not clinically hypothermic, however, these animals were intensively managed in a heated barn during lambing and any low temperatures were addressed promptly with interventions to restore normal body temperatures. These data demonstrate that lambs in a less controlled environment, such as extreme cold events experienced on rangelands, are at greater risk of becoming clinically hypothermic due to lower average body temperatures. Anecdotally, our NR lambs required intervention more often than CONT and were the only animals that needed resuscitative action. Importantly, these animals continued to have lower temperatures through the pre-weaning period, suggesting that thermostability in the first few hours-to-days of life may not correlate to lamb survival long-term. Lambing often occurs in the pasture or in a shed, pairs are penned together and/or monitored for ~24 h, and then released back into group housing in a dry lot or pasture. Lambs from animals that did not have all of their nutrient requirements met during gestation may experience more frequent complications in this type of production system and require increased intervention outside of traditional monitoring periods and input costs for success.

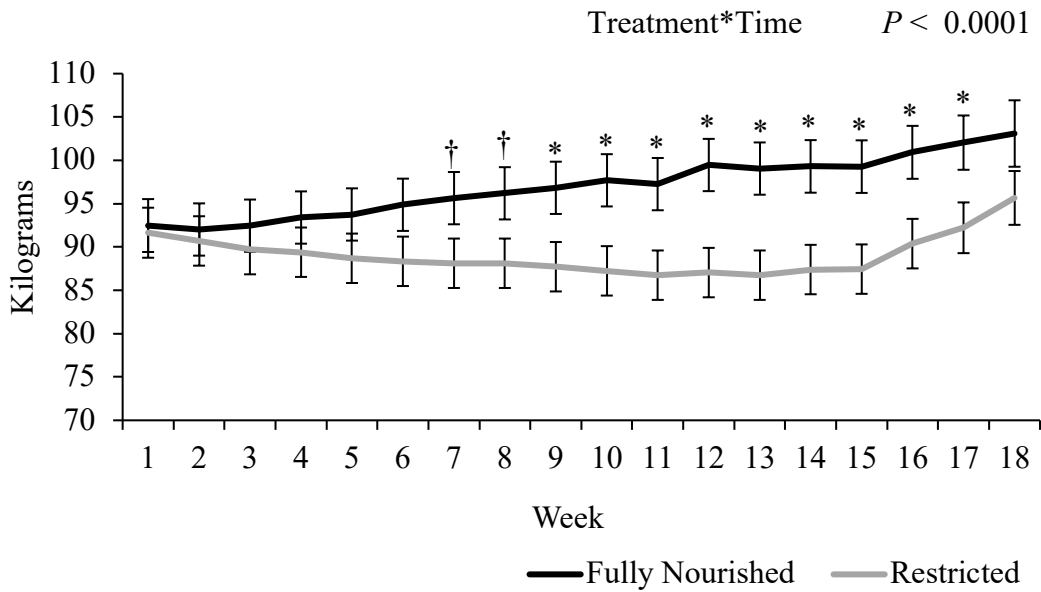
## *2.5 Conclusions and future directions*

This data builds a foundation for our hypothesis that tissue specific adaptations occur in the absence of birthweight differences as we are seeing performance differences in lambs reared identically. Differences between lambs was not evident at birth, however restricted lambs showed decreased body temperatures in early life and slower growth performance until weaning. These differences were likely due to adaptive metabolic, skeletal, adipose, or endocrine development during gestation due to nutrient repartitioning, however research including tissue sampling is needed to confirm this. While this study has provided important information about how standard production practices may be impacting lamb success, further research is needed to understand the mechanisms responsible for these differences. Additionally, animals should be followed through an entire production period to determine if these differences impact other aspects of the production cycle or if they can be rescued by strategic management. This would also identify any impacts on other traits of interest including reproductive competency and carcass merit. Lastly, studies investigating the economic tradeoff between improved supplementation strategies in extensive operations during gestation and lamb performance throughout life are needed to make informed management decisions.

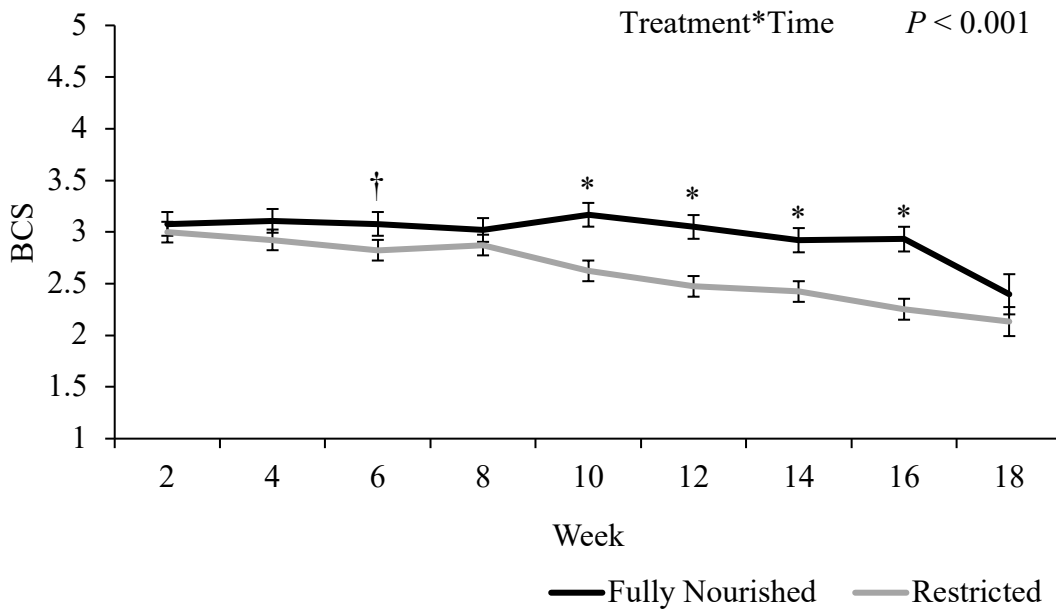
*Figures and tables*

**Table 2.1.** Nutrient analysis from feed sampling of each diet by year. Diets were formulated based on the Small Ruminant NRC (2007) guidelines to 100% nutrient requirements for a mature medium sized (200 lbs) twin bearing ewe (fully-nourished) or a diet stimulating winter forage (restricted) with 70% restricted TDN and 50% restricted CP of requirements. --- = data not available.

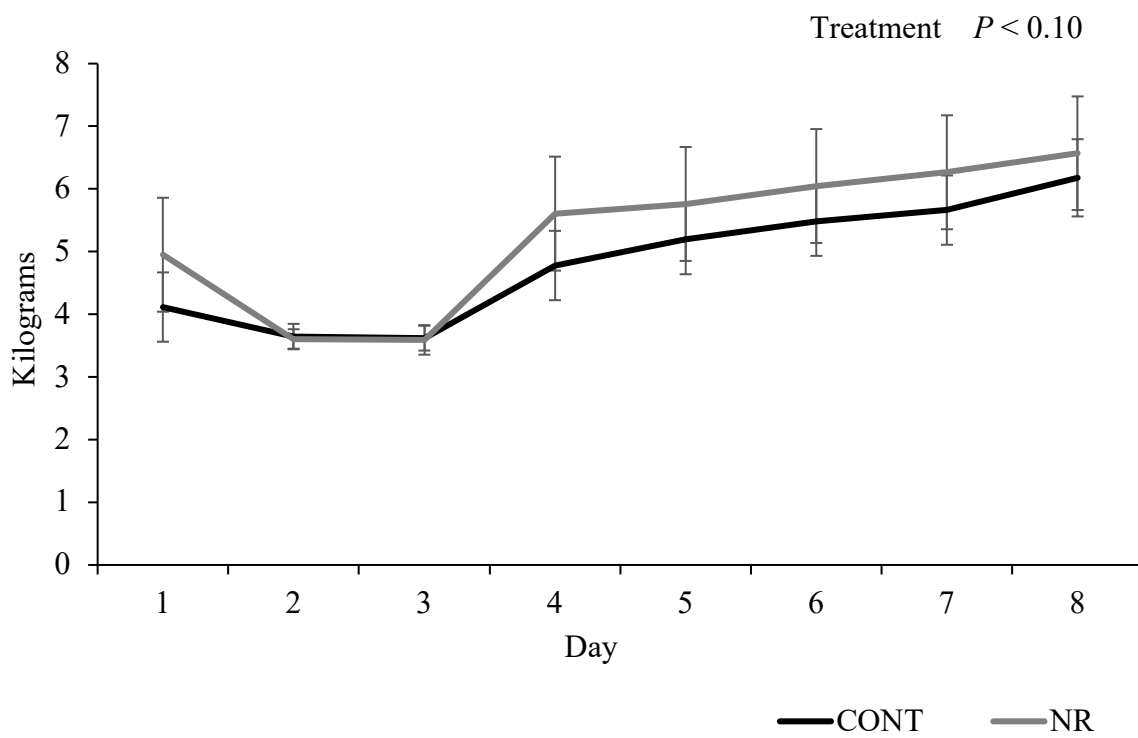
	Fully Nourished Diet		Restricted Diet	
	Year 1	Year 2	Year 1	Year 2
Moisture	38.24%	41.95%	22.73%	43.16%
Dry Matter	61.76%	58.05%	77.27%	53.51%
Protein, Crude (DM)	12.02%	10.05%	6.24%	6.79%
ADF (DM)	31.47%	32.39%	38.41%	40.25%
aNDF (DM)	46.68%	----	56.62%	----
TDN (DM)	63.44%	62.44%	57.46%	57.48%
DE (DM)	1.27 Mcal/lb	---	1.09 Mcal/lb	---
ME (DM)	1.05 Mcal/lb	---	0.90 Mcal/lb	---
Calcium (DM)	0.82 %	---	0.34%	---
Phosphorus (DM)	0.21%	---	0.09%	---
Potassium (DM)	2.24%	---	1.92%	---
Magnesium (DM)	0.23%	---	0.19%	---
Sodium (DM)	0.06%	---	0.03%	---
Sulfur (DM)	0.21 ppm	---	0.21 ppm	---
Aluminum (DM)	527 ppm	---	1259.50 ppm	---
Cobalt (DM)	0.49 ppm	---	1.16 ppm	---
Copper (DM)	7.09 ppm	---	6.80 ppm	---
Iron (DM)	479.5 ppm	---	942 ppm	---
Manganese (DM)	59.85 ppm	---	54.5 ppm	---
Molybdenum (DM)	1.74 ppm	---	2.36 ppm	---
Zinc (DM)	31.7 ppm	---	41.9 ppm	---



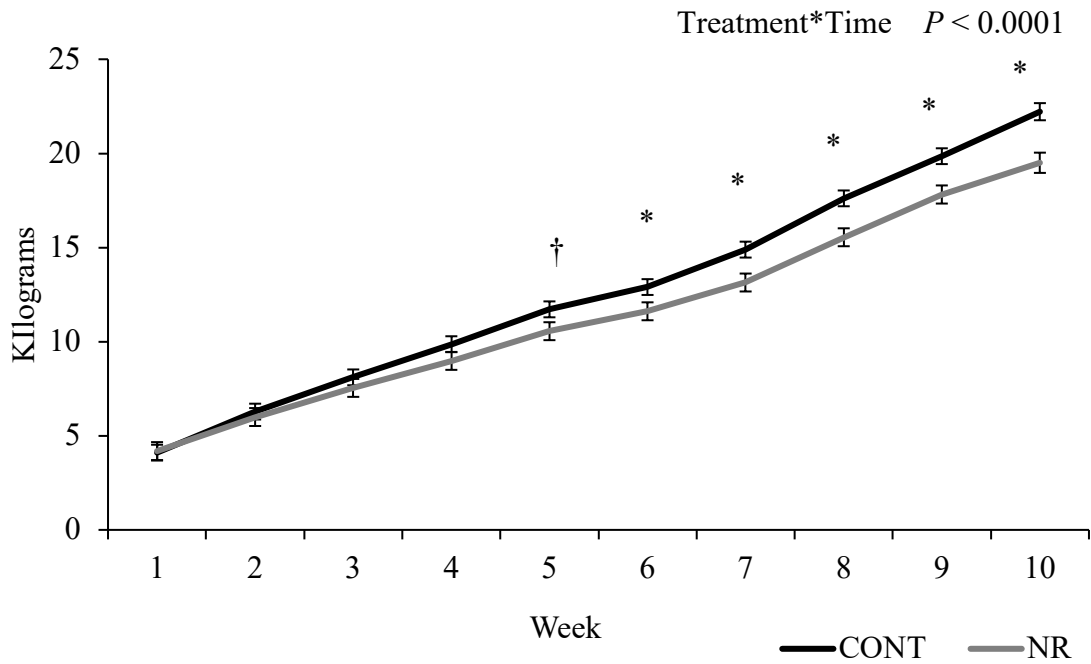
**Figure 2.1.** Weekly maternal bodyweight (kg) between fully nourished and restricted ewes during gestation (dGA 30 through lambing). \* means differ by  $P < 0.05$ , † means differ by  $P < 0.10$ .



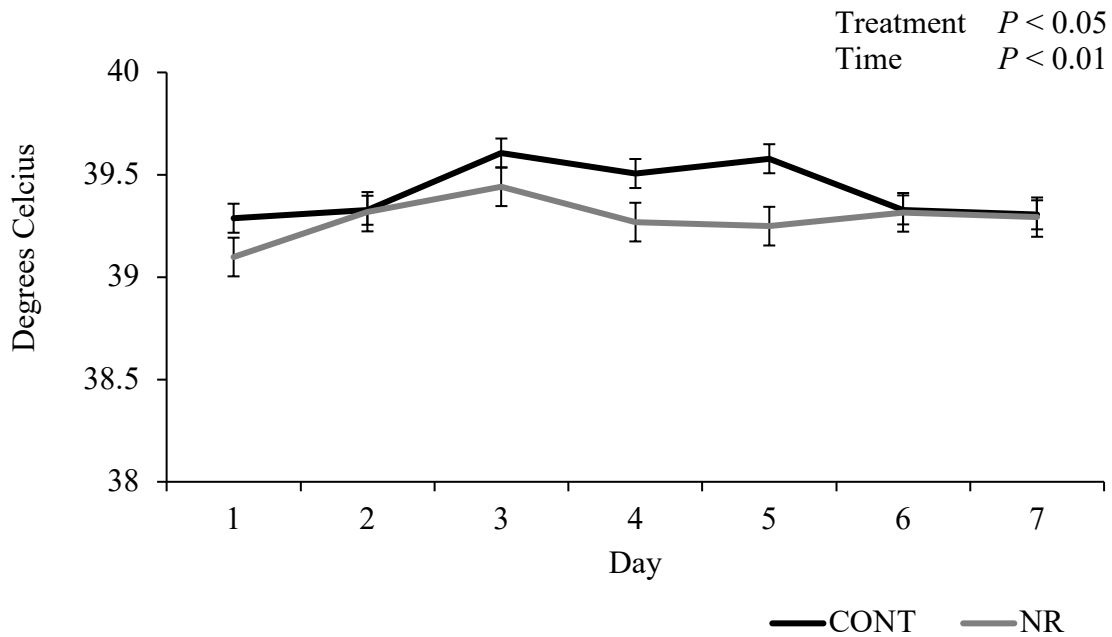
**Figure 2.2.** Bimonthly maternal body condition score between fully nourished and restricted ewes during gestation (dGA 30 through lambing). \* means differ by  $P < 0.05$ , † means differ by  $P < 0.10$



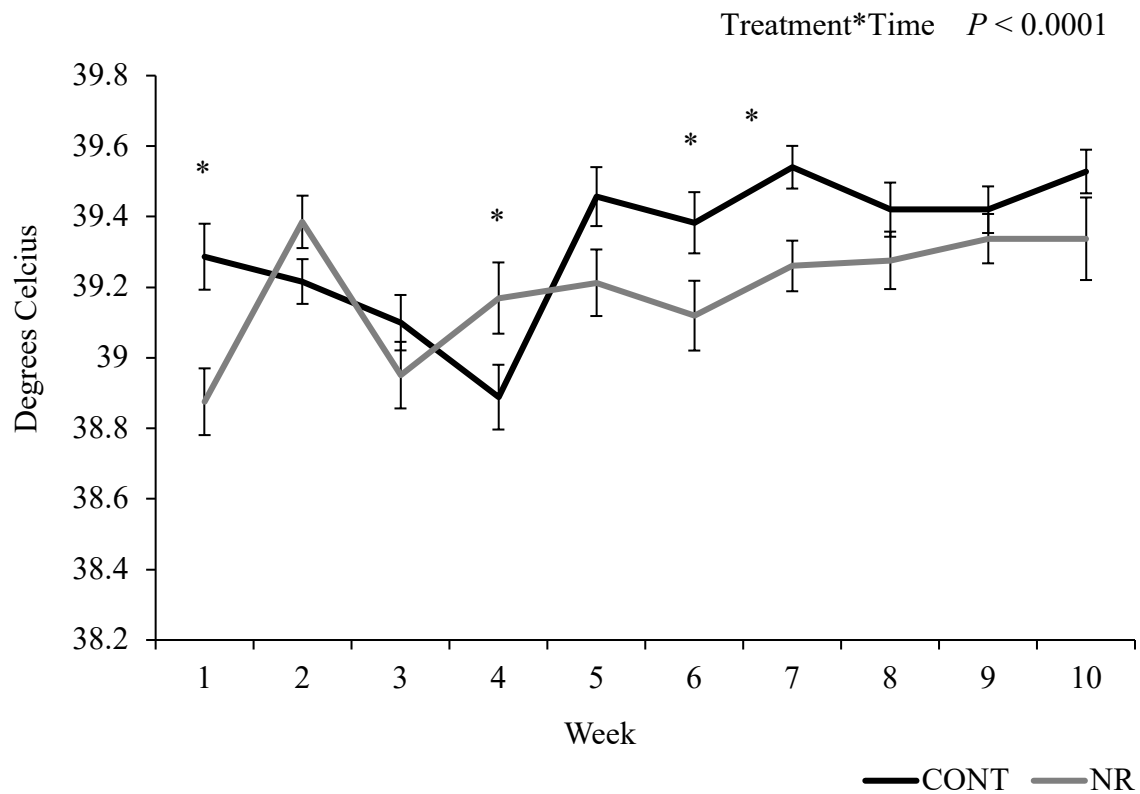
**Figure 2.3.** Body weight (kg) across the first week of life in lambs from dams who were fully nourished (CONT) and or whose dams received a restricted diet (NR) during gestation.



**Figure 2.4.** Weekly body weights (kg) from birth through weaning in lambs from dams who were fully nourished (CONT) and or whose dams received a restricted diet (NR) during gestation. \* means differ  $P < 0.05$ , † means differ  $P < 0.10$ .



**Figure 2.5.** Rectal temperatures in the first week of life from lambs whose dams were fully nourished (CONT) and whose dams received a restricted diet (NR) during gestation.



**Figure 2.6.** Weekly rectal temperatures from birth through weaning in lambs from dams who were fully nourished (CONT) and whose dams received a restricted diet (NR) during gestation. \* means differ  $P < 0.05$ .

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## CHAPTER 3 - TRAINING COGNITIVE SKILLS TO IMPROVE INTEGRATION AND APPLICATION OF KNOWLEDGE IN ANIMAL PHYSIOLOGY

### *3.1 Background*

Knowledge is the accumulation of experiences and ideas that form future thoughts and subsequently actions. By forming connections between ideas or experience we can acquire what we call knowledge in a constructive process (Wirth and Perkins, 2008). There are a series of events that happen to allow for the acquisition of knowledge and further application of it. Kolb's cycle of learning is a way to understand how knowledge is obtained. First it starts with the learner having an experience in the environment, then sensory information is integrated with previous experiences during a reflective observation. These are followed by abstract conceptualism where models and ideas are created from the intake of information then based off of this, new action is taken (active experimentation) (Kolb, 2014). This cycle continuously repeats and consists of both concrete and abstract dimensions of learning, in which some learning is observed or done (concrete) verses felt or thought about (abstract) (Kolb, 2014; Wirth and Perkins, 2008). As the brain continues this cycle, knowledge is organized into related networks where new knowledge can be added to existing frameworks. Neurologically, knowledge and learning is the formation of new synapses across neurons, strengthening and increasing complexity with other neurons from sensory experiences (Yang et al., 2019). Development of this schema (network of information) based on experiences, is how knowledge is expanded and built upon (Milligan, 1979; Wirth and Perkins, 2008).

Once there is an understanding of a topic, we can further build ideas or contribute to schema by expanding knowledge beyond acquisition of it. The stronger and larger the schema connections are, the more readily information can be recalled and used (Milligan, 1979).

Bloom's domains of learning provide a framework for the different areas where knowledge can be built. The three domains are cognitive, affective, and psychomotor. Cognitive being related to many types of thinking, affective which includes feelings, values, and motivation, and psychomotor which is physical and sensory skills (Hoque, 2016). Blooms taxonomy primarily relates to the cognitive domain and is a way to hierarchically organize the development of understanding, where the knowing of a fact is only the beginning of understanding (Hoque, 2016; Wirth and Perkins, 2008). As a learner continues to form knowledge through the other levels of thinking, they are moving from lower order cognitive skills to higher order cognitive skills, where factual knowledge accumulates and is used to see patterns (analysis), conceptualize new ideas (synthesis) or evaluate (Anderson, 1999; Bloom et al., 1956). Evaluation is the highest cognitive skill, and acquiring the ability to evaluate effectively takes time and effort outside of just accumulating knowledge (Anderson, 1999). Constructivism is a learning theory that aligns with the hierarchy of Bloom's taxonomy. Constructivism is when people learn something new and it is reflected on and connected to previous knowledge, resulting in the accumulation and questioning of previous understanding. Constructivist learning styles prioritize active learning where the student has to problem solve and reflect on understanding, thus utilizing higher order skills to achieve the task (Bada and Olusegun, 2015). There are a multitude of teaching or learning strategies that focus on a constructivist approach, such as active learning through problem based learning, and case base learning, which require reasoning through a problem or question to successfully answer it (Harris and Bacon, 2019; Hmelo-Silver, 2004).

Case based reasoning is a type of thinking that takes a real-world scenario and asks the learner to walk through that situation using previous cases or knowledge encountered, in order to consider all pathways of problem solution and decide on the most logical option (Kolodner, 2014). This approach in classrooms promotes student centered active learning, as students work through the process of recalling and combining information, often through socialization with peers or the instructor (Burgess et al., 2021). Through the process, learners are reviewing and reconstructing previous knowledge to form new connections that are required to understand and answer the scenario (Kolodner, 2014). Students have a high level of engagement and enthusiasm for case-based reasoning as there is preference for real world-based scenarios versus hypothetical ones (Burgess et al., 2021; McNaught et al., 2007; Raza et al., 2019). Case based reasoning as an assessment has shown increased performance or similar performance to that of traditional assessments; however, students feel that they have a better understanding of the material in case-based format, especially in the application of material (Cen et al., 2021; Grauer et al., 2008, Kaddoura, 2011). Case based reasoning is a learning strategy to help learners reach higher order skills and develop stronger knowledge connections between topics (Burgess et al., 2021; McNaught et al., 2007).

Case based reasoning involves learners reflecting on current and previous experiences to work through a scenario and elucidate a solution, therefore training their brain how to cognitively act in similar future scenarios. The process of reflection and cognitive reasoning seen in case-based reasoning provides problem solving, reasoning, and critical thinking skills that are valued in higher education contexts (Leake, 2017; Tawfik and Kolodner, 2016). The development of critical thinking skills can also be fostered through the implementation peer evaluation and metacognition training in higher education. Metacognition is the idea of thinking

about or understanding one's own thinking (Avargil et al., 2018). The process of learning about how one develops and build knowledge, aids in the utilization of higher-order thinking as the student has a vested interest in understanding the process versus acquiring facts, promoting self-regulated learning (Mahdavi, 2014; Pintrich, 2011). Metacognition promotes a lifelong learner; however, students may need support in transferring knowledge across disciplines and into different contexts (Avargil et al., 2018). Evaluation, especially peer evaluation, implemented in coursework also can promote critical thinking and self-regulated learning (Li et al., 2020). Peer evaluation is the process by which learners exchange information, such as writing excerpts, practice problems, or ideas, to give and receive constructive feedback or an initial evaluation of that information (Reese-Durham, 2005). This process makes students think critically about their peers work to evaluate it and provide feedback; however, in return students are simultaneously reflecting on their own work and content as well (Reese-Durham, 2005). This process of external and internal feedback develops higher-order cognitive skills outlined in Bloom's taxonomy (Iglesias Pérez et al., 2022; Issa, 2012). Both peer evaluation and metacognition improve students critical thinking skills by the successful use of higher-order cognitive skills (Li et al., 2020; Mahdavi, 2014). The main difference between peer evaluation and metacognition is that in metacognition learners are explicitly taught about the learning process, whereas in peer evaluation students practice it without details of the learning process (Li et al., 2020; Pintrich, 2011). These interventions in the learning process have their advantages and disadvantages depending on the context and content of the material or learning environment (Azevedo, 2020; Iglesias Pérez et al., 2022). Regardless, the use of higher order skills, whether through peer evaluation or metacognition, in academic settings can strengthen critical thinking skills that guide decision making in real-world scenarios (Franco et al., 2018; Renaud and Murray, 2003).

### *3.2 Introduction*

As there is a need for a workforce in the animal industry that can tackle big issues, there is an increasing demand for animal science students to enter the workforce with adequate hands-on experience and critical thinking skills. Experiential learning is a learning method for students to practice real-world scenarios in a controlled setting so they may apply their critical thinking skills (Kolb, 1984). Often, foundational animal science courses are lecture based with little experiential opportunities while also being students first exposure to rigorous college courses that require them to employ higher level thinking. Experiential learning classically looks like laboratories or field learning where students physically apply classroom skills. However, other instructional strategies such as case-based learning may be able to simulate a similar experience when out of classroom learning is not possible. The implementation of a case-based assessment strategy can be a useful tool for instructors to allow students to practice applying their knowledge and challenge them to use critical thinking skills more in the classroom.

The definition of critical thinking can vary depending on context and content, which is one of the reasons it is difficult to assess students' critical thinking performance (Moore, 2011; Gelder, 2005). Often, instructors want students to think more critically but find it difficult to identify effective training methods for critical thinking in the curriculum (Abrami, 2008; Halpern, 1993). Bloom's taxonomy is often utilized as a hierarchical method of classifying learning objectives into levels of complexity. Bloom's taxonomy has six levels starting with basic skills such as remember and understand, then builds up to higher levels such as apply, analyze, evaluate and create (Bloom et al., 1956; Adams, 2015). Bloom's taxonomy is a recommended framework for instructors to create objectives or test questions where students utilize multiple levels of thinking.

Traditional assessment strategies such as multiple-choice exams or quizzes often require students to remember and understand information, lower levels of cognitive domain, and are often implemented in STEM courses (Momsen et al., 2010; Abosalem, 2016). When courses employ higher-order exam questions to students, without training, students do not necessarily have or know how to access the cognitive skills needed to answer those questions (Zaidi et al. 2018). Although, students who practice higher-order questions do not necessarily perform better on long-term assessments than students who practice lower-order questions (Cadaret and Yates, 2021). There is difficulty in getting students to apply their higher-level cognitive skills in an assessment format without intervention and the benefit is lost if students are not successful in the practice period. Training of critical thinking skills in the classroom can expose students to the procedural knowledge of thinking critically and allow them to practice the skills, which in turn can benefit the students' performance (Holmes et al., 2015; Willingham, 2007).

Many different learning methods are designed to make students practice cognitive skills including problem-based learning, inquiry-based learning, flipped classrooms, and case-based learning (Harris and Bacon, 2019). These techniques are designed to help students reach higher level thinking. Case-based assessments present a problem to students and require them to not only recall information correctly but also apply, analyze, and evaluate how it can be part of creating a potential solution (McFee et al., 2018). This type of learning requires students to train and utilize higher-order cognitive skills and may better prepare them for subsequent courses and careers. Thus, the objective of this study was to evaluate two low input intervention strategies on their effectiveness in training students' cognitive skills and classroom performance. In addition, we sought to understand students' perceptions of their own cognitive skill development between strategies.

### *3.3 Materials and methods*

#### *3.3.1 Participation and inclusion*

All procedures for this study were approved for exempt status by the Human Subjects Institutional Review Board (IRB) at Colorado State University. This study was performed retrospectively with data from undergraduate students enrolled in the Functional Large Animal Physiology (ANEQ 305) course during the Spring 2022 (S1; n = 48), Spring 2023 (S2; n = 59), or the Fall 2023 (S3; n = 95) semesters. Students who dropped, withdrew, or did not complete the course with a C or better were not included in the performance portions of this study. Due to the administration of the surveys anonymously, students could not be removed post-hoc from survey data.

#### *3.3.2 Course format*

Functional Large Animal Physiology serves as the required introductory physiology course for undergraduate students majoring in Animal or Equine Sciences and an optional course for non-majors. The course is held over a 16-week period, meeting for three 1-hour lecture periods per week. The curriculum covers topics organized by body system, as well as fundamental physiological principles such as homeostasis and cell biology (Table 3.1). The course incorporates a blend of in-class activities, quizzes, and online homework assignments for practice and formative assessment. For the present study, in lieu of summative exams, student knowledge was assessed by case study.

#### *3.3.3 Pre- and post-test*

For each semester, students were assigned a bookending pre and post-test to gauge students' knowledge prior to starting the course and their acquisition of knowledge and retention by the end. The tests were assigned the first and final weeks of the semester via the Canvas learning

management system (Canvas LMS; Salt Lake City, UT). The identical tests contained 50 multiple choice, true/false, and matching style questions that covered all topics through the semester (~5 questions per topic). Students were allowed 50 minutes to complete the test with only one attempt allowed. All students, were encouraged not to study and regardless of test performance, received points for completion.

#### *3.3.4 Case study implementation*

Case studies 1-3 each covered ~2 body systems of material and were assigned via Canvas. For each case study, students were provided an introductory scenario and then tasked with answering 10-12 free response questions related to the physiologic concepts present in the scenario. Additionally, questions were designed according to Bloom's Taxonomy of cognitive domains to build from questions requiring lower order skills, such as recall and remember, up to those requiring higher order skills, such as differentiate, and formulate. Students were granted 2 weeks to complete their case studies outside of class, utilizing notes, lectures, and online sources. The final case study was cumulative and had free response questions related to every major physiologic system covered in the course previously. Students were allowed 2 weeks to complete the final case study and were expected to only use course materials and their own knowledge while completing their case study. Case studies were submitted electronically and analyzed by the Turnitin software (Oakland, CA) for plagiarism. In all semesters, case studies were graded using a standardized rubric and individualized feedback was provided by teaching assistants and/or the instructor.

#### *3.3.5 Cognitive training*

The three evaluated semesters represent periods of differing intervention strategies to train students in the cognitive skills needed to complete the case studies. In S1, students received no

intervention and attempted the case studies on their own, with only individualized feedback after submission. In S2, students completed the first case study on their own without intervention but, between case study one and two, an instructor unrelated to the course with expertise in education, provided two periods of training in cognitive processes. This training included presentation and active-learning activities that demonstrated how knowledge is built, stored, and utilized. Additionally, the instructor of the course walked through how to approach case studies from a procedural standpoint. On all subsequent case studies, no further intervention was implemented but individualized feedback was still employed after all submissions. In S3, the peer evaluation software Kritik (Toronto, ON), was used as an intervention. For case studies 1-3, students submitted their completed case study to the online program and were randomly assigned 5 of their peers to anonymously evaluate and provide feedback. Students were provided the standardized rubric and a key for grading. In addition, students were required to provide individual feedback based on their determination of their peer's performance. After peer feedback was provided, students could respond to comments and suggestions on the platform before the final submission. After all final submissions were made, the teaching assistants and instructors reviewed all submissions, corrected scores, and provided additional feedback as needed. Peer evaluation did not occur in the final case study, and grades were determined solely by the instructor and teaching assistants.

### *3.3.6 Perceptions surveys*

Throughout the course, three surveys gauged students' self-reported feelings on the format of the course, the discipline itself, and cognitive skills. Administered at the beginning (pre; excluded from S1), middle (mid), and end (post) of the semester, they were composed of 3-6 Likert scale questions, asking students to rank their agreeance based on a 5-point scale of Completely

Disagree, Somewhat Disagree, Neither Agree nor Disagree, Somewhat Agree and Completely Agree. There were also free response questions that delved into study tactics, thoughts on the course so far, and self-perceived cognitive skill improvements over the semester. Student responses were collected anonymously through Canvas and full credit was awarded for completion.

### *3.3.7 Statistical analysis*

All performance scores were analyzed using the mixed procedure of SAS (SAS Institute, Cary, NC) for the effects of semester, time, and the interaction, with case study score as a repeated measure. Surveys were combined by semester to illustrate perceptions of each intervention strategy. A thematic analysis of free response questions was completed to determine primary influences of the intervention or cognitive skill development that may affect student performance or perceptions. For Likert scale questions, agreeance was determined by responses of completely agree and somewhat agree. Similarly, disagreeance was determined by responses of completely disagree, and somewhat disagree. Summary statistics of Likert scale questions were performed in Excel. For all quantitative data, means were considered different at  $P < 0.05$  and a tendency at ( $P < 0.10$ ). All data are presented as mean  $\pm$  standard error.

## *3.4 Results*

### *3.4.1 Case study performance*

Between the first and second case study, grades decreased ( $P < 0.05$ ) in S2 and increased in S3 (Figure 3.1) and but did not differ in S1. The average grade on case studies after intervention (2-4) was higher ( $P < 0.05$ ) in S3 where peer intervention was employed, compared to S1 or S2, which did not differ (Figure 3.2).

No differences were found between pre-post test retention, first case study score, final case study score, or final grade. For the posttest alone, S3 tended to have higher ( $P < 0.10$ ) scores compared to S1 and S2.

#### *3.4.2 Likert-scale questions*

When comparing overall changes of student confidence in their cognitive skill, the most notable change was agreeance with the statement “I can take multiple ideas from different concepts and combine them to create new understanding.” In S2 and S3, there was a 6.9% and 16.5%, respectively, increase in agreeance between the pre-survey and the post-survey (Table 3.2). In S2, there was a 9.4% increase in agreeance with the statement “I can identify key concepts and vocabulary from bulk content.” The percentage of agreeance increased by 7.3% between pre- and post-surveys in S3 for the same statement. The other three Likert scale questions had smaller changes in the percentage of agreeance in S2 and S3 between pre- and post-surveys. Responses to “I am able to explain the meaning of key concepts and vocabulary I identify.” increased in agreeance in both S2 and S3, 1.9% and 4.3%, respectively. Agreeance with the statement “I am able to link similar concepts together” in S2 decreased by 0.5% and increased by 2.4% in S3. Agreeance decreased by 0.9% in S2 when prompted “I am able to take prior knowledge and apply it to new scenarios.” and in S3 increased by 2.4% between the pre- and post-surveys.

#### *3.4.3 Free response questions*

In the mid-survey students were asked “What feedback do you have about the course so far?” (Figure 3.3). In S1 major themes identified relating to interventions were difficulty interpreting questions asked, a preference for case studies over traditional assessments, and finding case studies challenging. Difficulty interpreting questions was mentioned 12 times out of all 60 responses, with responses such as, “*For the case studies, I also struggle to find the answers to what*

*we have learned in class until after the fact when we are shown the answers. The questions and the wording of the scenarios also really confuse me.”* Case study preference and challenge had less frequency at 9 and 4, respectively, mentions out of 60 students. In S2 major themes identified were challenge to understand content, case study preference, and challenges with course pace. Challenge to understand concept was the most mentioned theme with 21 out of 71 responses containing this theme. One response noted *“The focus on linking concepts and applying previous knowledge to new situations is challenge but is something I have always struggled with. I personally take a very long time to grasp concepts so sometimes catching things that are said in lectures is hard...”* Case study preference (14 mentions) and challenges with course pace (11 mentions) were mentioned less frequently out of the 71 responses. In S3 major themes were positive perception of case studies/assignments and negative perception of peer evaluation/Kritik. 29 out of 101 responses mentioned a positive perception of case studies/assignments, such as *“I appreciate the case studies because they allow me to deep dive into the material and really link the concepts learned in class together, in "real life" scenarios. I think the case studies are more productive in terms of actually learning rather than exams...”* 21 out of 101 responses mentioned a negative perception of peer evaluation/Kritik. One student responded *“Some students don’t understand critique vs criticism. When it come to the case studies, Kritik feedback has decreased my motivation to learn.”*

In the post-survey students were asked “Did your personal evaluation of your cognitive skills change throughout the semester? If so, in what way?” major themes identified across S2 & S3 were similar such as yes, increased confidence in cognitive skills; yes, improved study skills; unsure if skills changed/struggling with skills; no, cognitive skills stayed the same; or decreased confidence in cognitive skills (Figure 3.4). In S2, 33 out of 64 responses mentioned increased

confidence in skills and 59 out of 99 responses in S3, which is over half of all responses for both semesters. One student wrote, *“Yes, I believe they did. This is because when doing the case studies, I truly had to apply my learning and think about how the concepts related to each other. I was also encouraged to explain everything and explain why everything happened and how every step in the process came to be.”* In S2, 16 out of 64 students mentioned they were unsure if cognitive skills changed or that they stayed the same, *“I have been struggling with writing the case studies and still find it difficult to find a starting point still because it all seems so complicated in my mind.”* Decreasing in frequency of mentions, students mentioned change in cognitive skills in relation to improving study skills (6/64 mentions) and a decreased confidence in cognitive skills (4/64). One S2 student said, *“At the start of the semester I was very confident in my cognitive skills, specifically my ability to identify key concepts and my ability to take multiple ideas to create new understandings. I feel that I have this capability in some areas still, but I don't think that I had it in this physiology class. I struggled a lot to connect ideas to form new understandings, and my confidence in my cognitive skills throughout this course has actually decreased.”* More students (25/99) in S3 noted a change in cognitive skills through improved study skills, like *“... The focused efforts in my studies enabled me to better remember names, and functions, and understand the various physiological processes. Overall, the semester has been instrumental in enhancing my cognitive abilities and deepening my comprehension of different physiological endeavors.”* A decrease/stayed the same in cognitive skills confidence and unsure about/struggling with cognitive skills appeared at a close frequency (10/99 and 9/99, respectively) in all S3 responses.

### 3.5 Discussion

Our results show that both instructor-led and peer evaluation interventions in case-based assessments improve students' confidence in their cognitive skills evidenced by their increase in

agreeance in Likert scale questions between the beginning and end of the semester, and free response answers. Students performed better on case studies with peer evaluation as an intervention, however, students showed a strong dislike for peer evaluation in the free response. Simulating real-world scenarios in the lecture format through case-based assessments, peer evaluation, or other strategies can be a valuable way to train student cognitive skills, improve assessment performance, and prepare students to enter the workforce.

Overall, students in both intervention strategies had increased confidence in their ability to perform cognitive skills by the end of the semester, with the most confidence in their ability to combine multiple ideas from different concepts to create new understanding. In both semesters, students reported their cognitive skills improved by the end of the semester in over half of the total free responses. This was also reflected in overall increases in agreeance on Likert scale questions from the pre- to post-survey in both S2 and S3. Increases in confidence are likely a result of the repeated practice, or reconstruction, of those skills by building and using them in new scenarios (Holmes et al., 2015; Schwartz, 2009). As students are shown what critical thinking and cognitive skills look like, especially in a hierarchical format, they can sooner apply those skills within tasks (Resnick, 1975). This contributes to more successful implementation of higher-order skills, increased critical thinking and ability to answer complex case-based questions, and increased confidence in completing those skills again. Biwer et al. (2020) also saw an increase in student self-reported confidence in cognitive skills after teaching meta-cognition and critical thinking skills throughout three teaching sessions. This data suggests that interventions in the classroom that highlight the importance of critical thinking and promote strategies to improve student confidence in those skills are beneficial (Bean, 2021). The S2 intervention employed metacognitive teachings but we did not see improvement in student performance, despite increased

confidence in cognitive skills. However, the timing, type of intervention, teacher-student interaction, and course structure itself may also influence student confidence or training of cognitive skills (reviewed in Alsaleh, 2020; Kim and Sax, 2011; Salari et al., 2018) and further research is needed to understand how these variables play a role in training cognitive skills in case-based learning.

Students who participated in peer evaluation performed better compared to students with no intervention or instructor-led intervention on case-based assessments. No differences were observed in final course grade between interventions, indicating that improvements in performance were due to interventions rather than a cohort of higher performing students. The training of cognitive skills through any intervention in lecture-based courses helps improve students' ability to apply those skills to assessments (Salari et al. 2018; Biwer et al. 2020). However, it is important to note the type of intervention may influence the effectiveness of application. Evaluation is considered a higher-order skill and having students practice it repeatedly in class can prepare them to use higher-order skills more often, such as answering the case study questions (Schwartz, 2009). Peer evaluation has been successful in improving student writing through the process of receiving feedback from others and relating the feedback they are giving to their own writing (Huisman et al., 2018; Nicol and Macfarlane-Dick, 2006). As students practice critically reading, critiquing, and communicating their decisions, they are practicing higher-order skills and self-regulated learning (Liu and Carless, 2007). This process results in repetition and training of these skills by seeing it in their peers, which in turn results in increase use and awareness of critical thinking during students' own writing or assessments. Transfer of knowledge is promoted when students are in a social setting (Billing, 2007) and peer feedback can be an exercise to promote that

socialization and student dialogue in a lecture-based course, potentially improving student performance.

While students in the peer evaluation intervention performed better, they had a negative perception of completing peer evaluation. While the negative perception of peer feedback was not the most frequent theme mentioned in S3, it was a theme that stood out compared to others due to the direct mention of the intervention strategy implemented. Students in other semesters mentioned general feelings about case studies and cognitive skills, however, in S3 20.8 % of responses specifically mentioned negative feelings around completing peer evaluation or the Kritik component. The instructor-led intervention in S2 was mentioned only once specifically out of 64 responses. When asked if their cognitive skills changed across the semester, one student responded with *“Yes, after the guest lecture since I have not really been taught how to study so she helped me learn how to gauge if my studying is working.”* The desirable difficulty that comes with completing evaluation, may have led to the decreased desire to participate in peer feedback. Additionally, the type of feedback received (positive or negative) can influence student’s cognitive load and subsequently motivation to participate in creative activities, such as writing (Redifer et al., 2021). The procedural knowledge that comes with peer evaluation does add exercises that can improve cognitive skill development, through self-regulated learning. Other studies have similarly noted that students typically have a negative perception of peer feedback even though it improves student performance during assessment (Aminu, 2021; reviewed in Tenório et al., 2016). In the peer evaluation portion, students received a participation grade for completing the feedback that was dependent on accuracy and effort. This grading could also contribute to some students’ personal motivation to complete it, as they may have felt doing the peer feedback was for a small portion of their grade and therefore did not put in adequate effort to critically evaluate peers’

papers. It is possible that a combination of instructor-led and peer intervention would promote the best outcomes in performance and perceptions, however, further research is needed to determine this.

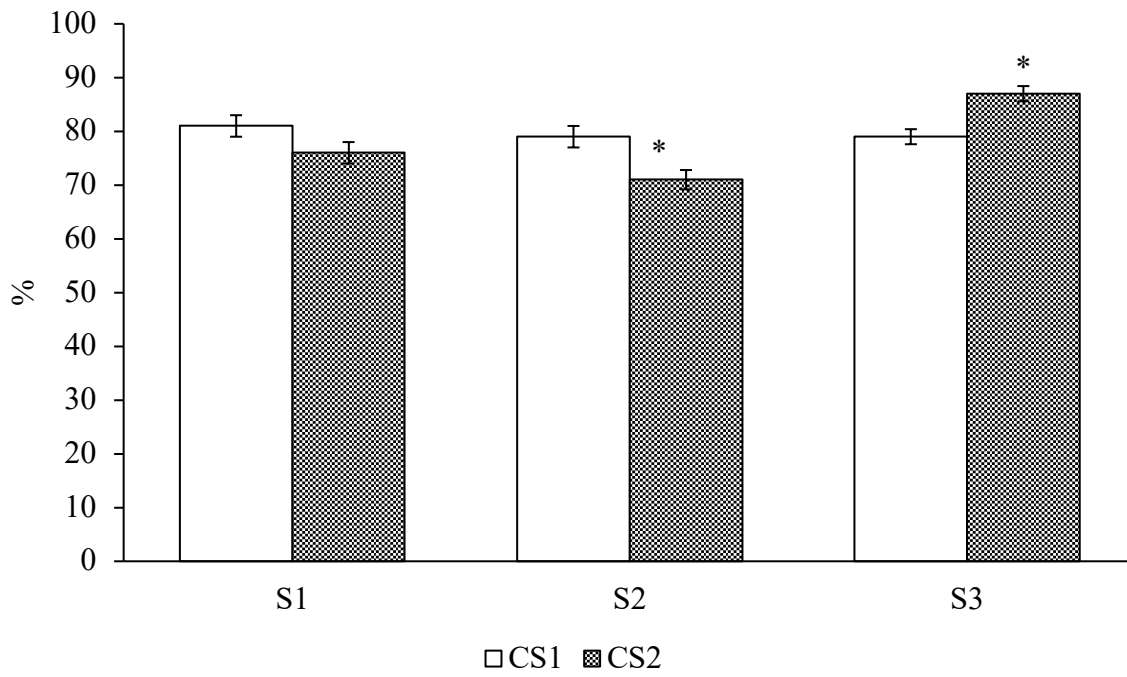
### *3.6 Conclusion*

The inclusion of intervention in case-based curriculum is advantageous to student's perception of their own cognitive skills and their performance on said case studies. This increased confidence may inherently promote students' desire to learn and promote transfer between contexts, such as different courses. Peer evaluation showed improvement in student performance in case-based assessments, however, students did not enjoy it or feel it was beneficial. Peer feedback requires higher-order cognitive skills and prepares students to enter the workforce with the ability to give and receive feedback professionally. Simulating real-world scenarios in the lecture format through case-based assessments, peer evaluation, or other strategies can be a valuable way to prepare animal scientists for the workforce. While this study focused on two low input intervention strategies, there are a multitude of strategies educators can use in the classroom to train cognitive skills, however, the context and content of the course should steer which interventions should be implemented.

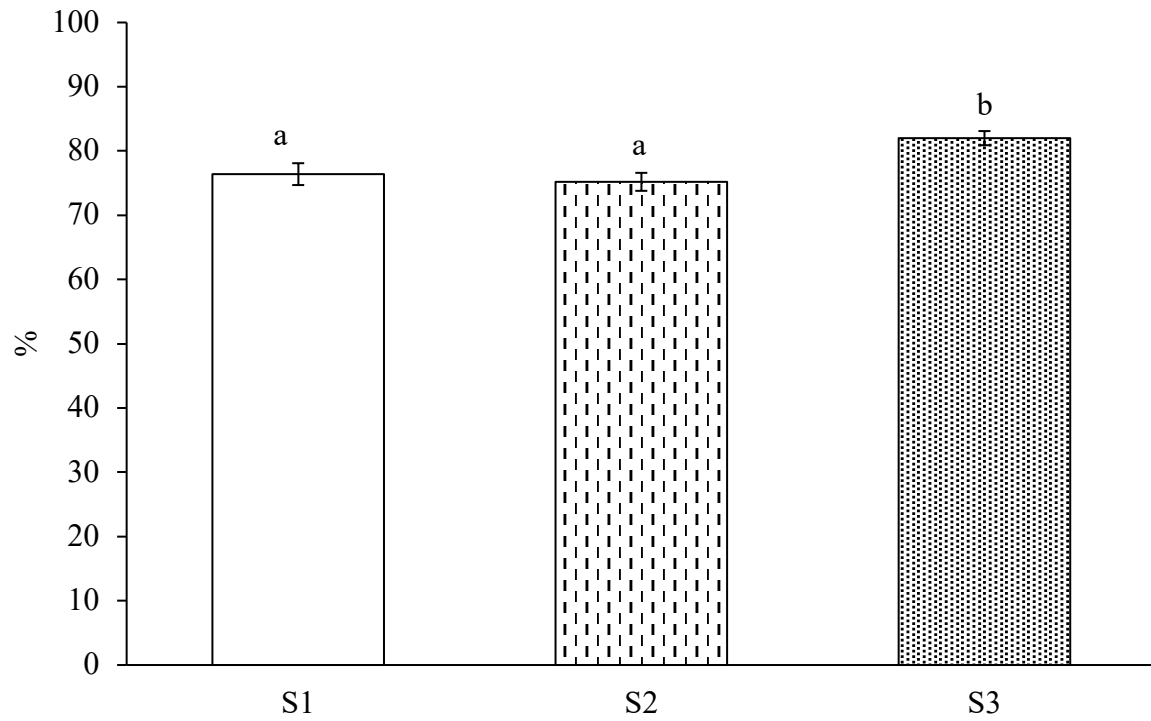
Figures and tables

**Table 3.1.** Introductory physiology course material and assessment timeline over the 16-week semester.

Week	Topic	Assignment		
		S1	S2	S3
1	Introduction & Homeostasis	Pre-test Pre-survey Quiz 1	Pre-test Pre-survey Quiz 1	Pre-test Pre-survey Intro Kritik Activity Quiz 1
2	Cell & Membrane Physiology	Quiz 2	Quiz 2	Quiz 2 Intro Kritik Activity
3	Nervous System	Quiz 3	Quiz 3	Quiz 3
4	Nervous System	Quiz 4 <b>Case Study 1</b>	Quiz 4 <b>Case Study 1</b>	Quiz 4 <b>Case Study 1</b>
5	Muscular System	Quiz 5	Quiz 5 <b>*S2 Intervention*</b> <b>Case Study 1</b> <b>Resubmission</b>	Quiz 5 <b>Peer Evaluation 1</b>
6	Endocrine System	Quiz 6	Quiz 6	Quiz 6
7	Endocrine System	Quiz 7 <b>Case Study 2</b>	Quiz 7 <b>Case Study 2</b>	Quiz 7 <b>Case Study 2</b>
8	Cardiovascular System	Quiz 8	Quiz 8	Quiz 8 <b>Peer Evaluation 2</b>
9	Cardiovascular/Respiratory System	Quiz 9 Mid-survey	Quiz 9 Mid survey	Quiz 9 Mid-survey
10	Respiratory System	Quiz 10	Quiz 10	Quiz 10
11	Immune System	Quiz 11 <b>Case Study 3</b>	Quiz 11 <b>Case Study 3</b>	Quiz 11 <b>Case Study 3</b>
12	Digestive System	Quiz 12	Quiz 12	Quiz 12 <b>Peer Evaluation 3</b>
13	Urinary System	Quiz 13	Quiz 13	Quiz 13
14	Lactation (Optional or condensed) <b>*Fall Break for S3*</b>	Quiz 14	No Quiz 14	Quiz 14
15	Finals Preparation or Lactation	Final Prep	Final Prep	Final Prep
16	Finals Week	Post-test Post-survey <b>Final Case Study</b>	Post-test Post-survey <b>Final Case Study</b>	Post-test Post-survey <b>Final Case Study</b>



**Figure 3.1.** Average case study scores between CS1 (no intervention) and CS2 (immediately after intervention) between semesters with no intervention (S1), instructor led (S2), and peer evaluation interventions. \* means differ  $P < 0.05$

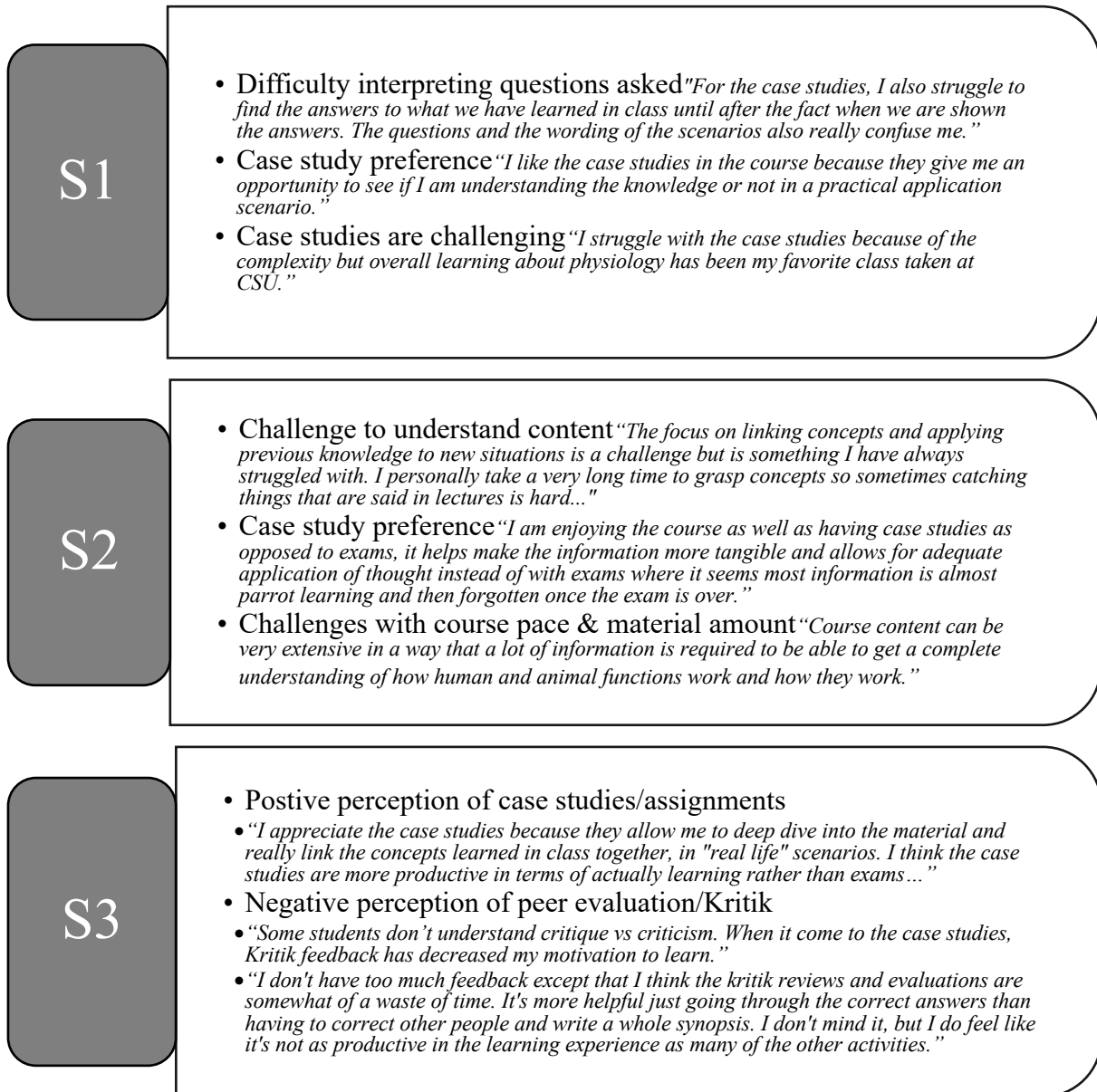


**Figure 3.2.** Average case study scores after intervention between semesters with no intervention (S1), instructor led (S2), and peer evaluation interventions. <sup>ab</sup> means with differing superscripts differ  $P < 0.05$

**Table 3.2.** Number of students who agreed to Likert Q1 through Q5 in the S2 and S3 pre-survey (n = 72 vs n = 104, respectively) and post-survey (n = 64 vs S3 n= 99, respectively).

Question	Semester	Agreeance Pre-survey	Agreeance Post-survey	% Change in Agreeance (Post – Pre)
Q1. I can identify key concepts and vocabulary from bulk content.	2	63/72 (87.5%)	62/64 (96.9 %)	9.4%
	3	88/104 (84.6%)	91/99 (91.9%)	7.3%
Q2. I am able to explain the meaning of key concepts and vocabulary I identify.	2	65/72 (90.3%)	59/64 (92.2%)	1.9%
	3	89/104 (85.6%)	89/99 (89.9%)	4.3%
Q3. I am able to link similar concepts together	2	69/72 (95.8%)	61/64 (95.3%)	-0.5%
	3	91/104 (87.5%)	89/99 (89.9%)	2.4%
Q4. I can take multiple ideas from different concepts and combine them to create new understanding.	2	58/72 (80.6%)	56/64 (87.5%)	6.9%
	3	70/104 (67.3%)	83/99 (83.8%)	16.5%
Q5. I am able to take prior knowledge and apply it to new scenarios.	2	67/72 (93.1%)	59/64 (92.2%)	-0.9%
	3	92/104 (88.5%)	90/99 (90.9%)	2.4%

Mid-survey: "What feedback do you have about the course so far?"



**Figure 3.3.** Major themes identified by semester in the mid-survey for S1 (n = 60), S2 (n = 71), and S3 (n = 101).

Post-survey: Did your personal evaluation of your cognitive skills change throughout the semester? If so, in what way?

S2

- **Yes, increased confidence in cognitive skills**
  - *"Towards the end of the semester I was more focused on understanding and being able to apply. I realized that I needed to be able to logically think, evaluate what I know and then go from there. But as I went through the semester I realized I needed to work on my cognitive skills so I tried to remember blooms taxonomy of learning pyramid and start at the bottom and go through each step."*
- **Unsure if skills changed/stayed the same**
  - *"I have been struggling with writing the case studies and still find it difficult to find a starting point still because it all seems so complicated in my mind."*
- **Yes, improved study skills**
  - *"I think my cognitive skills improved over the semester. I always have had a very front-to-back approach while doing homework/projects, and learning about metacognition showed me that there are other, more efficient ways to do things."*
- **Decreased confidence in cognitive skills**
  - *"At the start of the semester I was very confident in my cognitive skills, specifically my ability to identify key concepts and my ability to take multiple ideas to create new understandings. I feel that I have this capability in some areas still, but I don't think that I had it in this physiology class. I struggled a lot to connect ideas to form new understandings, and my confidence in my cognitive skills throughout this course has actually decreased."*

S3

- **Yes, increased confidence in cognitive skills**
  - *"My personal evaluation did change. Now I have a much higher evaluation in the way of understanding key terms and meaning as well as being able to extrapolate what could be going on based on evidence that is not explicit. I appreciated the context-based quizzes and case studies because I think I learned how to apply the knowledge, rather than just what the knowledge is."*
  - *"Yes, I believe they did. This is because when doing the case studies, I truly had to apply my learning and think about how the concepts related to each other. I was also encouraged to explain everything and explain why everything happened and how every step in the process came to be."*
- **Yes, improved study skills**
  - *"Yes, my evaluation of my cognitive skills did change throughout the semester, and it notably improved. The focused efforts in my studies enabled me to better remember names, and functions, and understand the various physiological processes. Overall, the semester has been instrumental in enhancing my cognitive abilities and deepening my comprehension of different physiological endeavors."*
- **No, decreased or the same confidence in cognitive skills**
  - *"I became less confident in my cognitive skills over the course of the semester."*
- **Unsure if skills changed/struggling with skills**
  - *"I think I am now better at identifying the important concepts and vocabulary from the bulk but I still struggle with linking concepts together."*

**Figure 3.4.** Major themes identified by semester in the post-survey for S2 (n = 64) and S3 (n = 99).

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