

THE EFFECT OF LATE LACTATION AND PERICONCEPTION NUTRITION ON
REPRODUCTIVE OUTCOMES IN AN ACCELERATED LAMB PRODUCTION SYSTEM

By

Jordan Moody

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Animal Science – Master of Science

2020

ABSTRACT

THE EFFECT OF LATE LACTATION AND PERICONCEPTION NUTRITION ON REPRODUCTIVE OUTCOMES IN AN ACCELERATED LAMB PRODUCTION SYSTEM

By

Jordan Moody

Accelerated lamb production systems enable aseasonal production but are hampered by poor reproductive performance in the long day season. Preliminary data suggest peri-conceptual nutrition affects reproductive outcomes. To evaluate this, we randomly assigned Polypay x Dorset ewes to nutritional treatments (5) over 2 periods: the last 28-d of lactation, followed by flushing (21-d pre-breeding and 34-d breeding period). During lactation, ewes were fed 100% (C), 70% (L), or 150% (H) of lactation energy requirements by rearing status. During flushing, L or H ewes were then fed 70% (L) or 160% (H) of energy requirements for maintenance. This was repeated over two seasons: Short (n=117) and Long (n=108) at 42.73°N and 84.5°W. Ewes were exposed to rams with a ram:ewe ratio of 1:19 to 1:25. Conception rate, litter size, and incidence of fetal loss were examined by ultrasound. Dietary treatments produced changes in BW and BCS consistent between seasons. Litter size was higher in Short ($P < 0.05$), while fetal loss tended to be higher in Long ($P = 0.06$). Treatments had no effect on reproductive outcomes. There was a significant regression relationship between BCS change during lactation on litter size ($P < 0.05$). Gestational length differed between seasons (145 ± 0.2 -d in Long vs. 147 ± 0.18 -d in Short; $P < 0.05$) as did lamb birth weight (5.1 ± 0.09 kg in Short, $4.6 \text{ kg} \pm 0.05 \text{ kg}$ in Long; $P < 0.01$). These results indicate that high conception rates in the long day season are attainable with natural mating and that NRC feeding standards are not sufficient to both allow for the recovery of body condition and evoke a flushing response in an accelerated lamb production system.

ACKNOWLEDGEMENTS

Firstly, I would like to thank Dr. Ehrhardt. He has provided me with irreplaceable learning experiences throughout my graduate program. I am thankful for the opportunity to learn about and gain practical experience in the field of extension, as well as the opportunity to travel to various industry and academic conferences to broaden the scope of my education. Next, I would like to thank Dr. Veiga-Lopez for her thoughtful guidance throughout my program, and for pushing me to hold myself to higher standards. Additionally, I would like to thank Drs. Zhou and Stewart for their guidance in the trajectory of my graduate program.

I would like to thank Dr. Recktenwald and Barb Makela for their assistance in my research project, from milking ewes to designing PUN assays and everything in between, these women were irreplaceable. I would also like to thank Lacey Quail for the care and keeping of the sheep on my study, as well as for the countless hours of companionship, support, and Quest Pizza when I needed it the most.

I would like to thank my family for encouraging me to pursue my degree, and for always being my biggest cheerleaders, no matter what. I would be remiss not to acknowledge the amount of late-night phone calls, missed holidays, and unwavering support that they provided me during the past two years. Finally, I would like to thank Eric for his patience, kindness, and support throughout my graduate program.

TABLE OF CONTENTS

LIST OF TABLES.....	v
LIST OF FIGURES	vi
KEY TO ABBREVIATIONS.....	vii
CHAPTER 1: REVIEW OF LITERATURE.....	1
CONSTRAINTS TO NORTH AMERICAN LAMB PRODUCTION.....	1
ACCELERATED LAMB PRODUCTION SYSTEMS.....	2
MANAGEMENT STRATEGIES FOR ACCELERATED LAMB PRODUCTION SYSTEMS.....	5
BIOLOGY OF FEMALE REPRODUCTION IN THE SHEEP.....	10
NUTRITIONAL CONTROL OF SHEEP REPRODUCTION.....	16
CONCLUSIONS.....	24
REFERENCES.....	26
CHAPTER 2: THE EFFECT OF LATE LACTATION AND PRE-BREEDING NUTRITION ON REPRODUCTIVE OUTCOMES IN AN ACCELERATED LAMB PRODUCTION SYSTEM.....	36
INTRODUCTION.....	36
MATERIALS AND METHODS.....	37
RESULTS AND ANALYSES.....	42
DISCUSSION.....	47
IMPLICATIONS AND FURTHER DIRECTIONS.....	64
APPENDIX.....	67
REFERENCES.....	84

LIST OF TABLES

Table 1. Ingredient and Nutrient Composition of treatment diets for lactation and flushing....	69
Table 2. Least Square Means of body weight, body condition score, and back fat depth for ewes fed C, L or H diets during the last 28 days of lactation.....	72
Table 3. Least Square Means of BW, BCS, and BF during flushing and cumulatively during lactation and flushing.....	75
Table 4. Effect of dietary treatment and season on reproductive outcomes in an accelerated lamb production system.....	78

LIST OF FIGURES

Figure 1. Experimental design.....	68
Figure 2. Metabolizable energy intake during lactation compared to NRC 1985 and 2007.....	70
Figure 3. Calculated protein intake during lactation compared to NRC 1985 and 2007.....	71
Figure 4. Effect of dietary treatment, rearing status, and season on BW, BCS, and BF during lactation.....	73
Figure 5. Metabolizable energy intake of C, L, and H diets during the flushing period.....	74
Figure 6. Effect of dietary treatment, rearing status, and season on BW, BCS, and BF change during flushing.....	76
Figure 7. Effect of dietary treatment, rearing status, and season on BW, BCS, and BF change during lactation and flushing.....	77
Figure 8. Effect of dietary treatment and season on conception rate.....	79
Figure 9. Effect of dietary treatment and season on litter size.....	80
Figure 10. Effect of season of conception on incidence of fetal loss	81
Figure 11. Effect of dietary treatment and season of conception on gestational length.....	82
Figure 12. Effect of dietary treatment, season, and litter size on birth weight.....	83

KEY TO ABBREVIATIONS

BF	Back Fat
BCS	Body Condition Score
BW	Body Weight
CAMAL	Cornell Alternate Month Lambing System
CIDR	Controlled Internal Drug Release
CP	Crude Protein
D	Day
DM	Dry Matter
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
LH	Luteinizing Hormone
ME	Metabolizable Energy
NDF	Neutral Detergent Fiber
NEFA	Non-Esterified Fatty Acids
NRC	National Research Council
PC	Post-Coitus
PMSG	Pregnant Mare Serum Gonadotropin
PUN	Plasma Urea Nitrogen
TMR	Total Mixed Ration
TRUS	Transrectal Ultrasonography
TAUS	Transabdominal Ultrasonography

CHAPTER 1: REVIEW OF LITERATURE

CONSTRAINTS TO NORTH AMERICAN LAMB PRODUCTION

Seasonality

The vast majority of sheep in North America are raised in a production system that allows for one birth period per year. Because sheep are seasonal breeders and entrained to express estrus during short day periods, the majority of ewes in the North America are bred between August and January (USDA NASS, 2020) and consequently, based in a 2011 survey, 88.3% of lambs are born between January and June. This seasonal nature of lamb production impacts most of the sheep meat value chain, including producers, feeders, and packers. In the United States, most lambs are weaned in the fall and finished in commercial feedlots (Miller et al., 2016). Feedlot operators often must stretch this large intake of inventory over several months in order to provide a steady supply of live, semi-uniform lambs to packers throughout the year (Redden et al., 2018). This often results in lambs being kept in feedlots longer than necessary, leading to large, over-finished, overly mature lambs (Miller et al., 2016). This poses economic challenges, as feeding these overfat lambs beyond their optimal harvest date increases the cost of gain dramatically. At packing houses, these overfat lambs increase the amount of lower quality lamb in the supply chain, with an estimated 67.5% of carcasses exceeding industry standard for 12th rib fat thickness in the summer slaughter season (Whaley et al., 2019). Packing houses are also affected by this seasonality of lamb supply, struggling to fill the floor at certain points of the year, causing an inefficient use of facilities and labor during these slow times, and leaving American consumers with access to only a seasonal supply of fresh domestic lamb (USDA APHIS, 2011; Miller et al., 2016)

Productivity

In addition to seasonal constraints on North American lamb production, the American Sheep Industry Association has identified low national flock productivity as a major barrier to industry growth (Miller et al., 2016). Census data gathered by the USDA indicate that there is an average of 1.07 lambs born per ewe per year (USDA NASS, 2020). This average, however, differs by geographic area and production system ranging from 0.56 for Arizona to 1.44 in Iowa, with a higher number of lambs born per ewe in intensively managed sheep production systems such as found in the Upper Midwest and East regions of the United States (USDA NASS, 2020). In order for the domestic industry to be competitive against imports from countries with lower cost of production, such as Australia and New Zealand, producers must examine their input costs versus outputs in productivity. The feed cost structure of imported lamb tends to be lower than domestic lamb due primarily to the cost of winter feeding which is greater in the USA due mostly to the longer winter period (Muhammad et al., 2007). Given the constraint of greater winter feed cost, producers must consider options to increase flock productivity as a means of improving the overall efficiency of production (Miller et al., 2016).

ACCELERATED LAMB PRODUCTION SYSTEMS

Accelerated lamb production is a practice that reduces the interbreeding interval to less than 12 months, which is the standard interval in commercial production in the United States. Accelerated lamb production gives each ewe the opportunity to lamb more than once per year and with a birth interval of less than a year it also simultaneously improves seasonal supply with the opportunity to create a consistent year-round supply of lambs. Therefore, accelerated production is a system that has the potential to address the two primary constraints in USA lamb

production: low productivity and seasonality. Accelerated lambing is accomplished by altering the seasons in which ewes are exposed to rams and by decreasing the interbreeding interval. The constraint of seasonal breeding in sheep can be overcome by use of sheep genetics that are less constrained by season in reproduction (Hogue, 1987; Lewis et al., 1996; Notter, 1981), and/or by altering photoperiod (Cameron et al., 2010), and/or by use of exogenous hormone therapies (Wheaton et al., 1992; Knights et al., 2003). In a national flock survey only 38% of respondents who utilized out of season breeding indicated that they were satisfied with their flock performance in the suboptimal breeding season (Miller et al., 2016).

Several studies have investigated the performance of ewes managed on an accelerated lambing system (Iniguez et al., 1986; Lewis et al., 1996; Notter, 1981). Notter and Copenhaver, (1981) investigated the reproductive performance of $\frac{1}{2}$ Finn x $\frac{1}{2}$ Rambouillet, $\frac{1}{2}$ Suffolk x $\frac{1}{2}$ Rambouillet, and $\frac{1}{4}$ Finn x $\frac{3}{4}$ Rambouillet cross ewes on an eight month breeding interval, with birth periods in January, April, and September (8-month system). This system gives each ewe the opportunity to lamb three times in two years (1.5 times per year). In this study, each ewe averaged 1.27 births per year over a five-year study period. The highest conception rate was observed in ewes bred in August (90%), followed by November (79%), and the lowest in April (53%). Conception rates in April were significantly higher for $\frac{1}{2}$ Finn x $\frac{1}{2}$ Rambouillet ewes than the other two crossbred groups. The study also found that conception rates were reduced by 5% in ewes that had lambed 2-4 months prior to breeding, in the previous 8-month interval. Litter sizes also differed among breeding seasons, with the highest in November (2.46), then August (2.21), and the lowest in April (1.84).

Two other accelerated lambing systems, Morlam and CAMAL, were evaluated by Iniguez et al (1986) at Cornell University. The Morlam system was developed by scientists at the

USDA Beltsville Agriculture Research Center using the Morlam breed. It is a continuous ram exposure system, where rams are only removed from ewes for a short period, post-lambing. Morlam sheep are a composite breed, consisting of crosses between Merino, Dorset, Columbia, Hampshire, and Suffolk breeds. The average interbreeding interval for the Morlam system was 293 days, or 1.27 births per ewe per year. The Cornell Alternate Month Accelerated Lambing, or CAMAL system was developed with Dorset ewes at Cornell University. This system divides ewes into four breeding groups that are exposed to rams on an alternate month basis, which allows for ewes to lamb at either a 6, 8, or 10-month interval. Ewes on the CAMAL system averaged 1.21 births per ewe per year, with a 303-day interbreeding interval. Both CAMAL and Morlam systems were successful in increasing the number of birth periods per ewe per year when compared to an annual production system.

The Cornell STAR system was developed 1981 at Cornell University (Lewis et al., 1996). It has five distinct 73-day production intervals per year. At the commencement of each 73-day interval, nonpregnant ewes are given the opportunity to be bred which is simultaneous with a birth period for those ewes near term. After lambing, ewes are given the remainder of the 73-d period to lactate, lambs are then weaned, and ewes are given the opportunity to re-breed. At any given time in this system, there are three categories of ewes: pregnant, lactating, or open. Each ewe has the opportunity to lamb five times in three years in this system, with the optimum interbreeding interval of 7.2 months. The STAR system has the structure to be more intensive than the CAMAL, Morlam, or 8-month system with the potential for a shorter interbreeding interval (7.2 months) and therefore potentially greater average birth frequency (1.67 births per ewe per year vs 1.21, 1.27 and 1.5 for CAMAL, Morlam and 8 month system, respectively). Reproductive performance in the STAR system over a period of five years was analyzed by

Lewis and colleagues in 1996, and they found that reproductive performance of ewes in the STAR system varied by season, with the natural mating season (August to January) seeing higher conception rates, as well as a tendency toward larger litter sizes. In addition, it was found that in the optimal season, ewes conceived earlier in the 73-day cycle, thus weaning heavier lambs than ewes bred in suboptimal seasons. Regardless of breeding interval, mean conception rates for ewes in unfavorable seasons for the duration of the study was 20% versus mean conception rate of optimal seasons, 51%. Despite the opportunity for a shortened interbreeding interval in the STAR systems, performance of ewes on this system averaged one birth per year for the duration of the five-year study. This study was performed with purebred Dorset ewes that were relatively low in prolificacy (1.44 lambs/ewe), which may explain the relatively low productivity of this system (Cochran et al., 1984). Field evidence suggests that use of breeds with greater prolificacy would generate much greater productivity on the STAR management system (M.L. Thonney, Cornell University, personal communication).

MANAGEMENT STRATEGIES FOR ACCELERATED LAMB PRODUCTION SYSTEMS

Exogenous hormone therapy

Several studies have utilized exogenous hormone therapies to increase efficiency of accelerated lamb production systems in order to overcome the long day seasonal anestrous period common to most sheep of temperate origin. Wheaton et al. (1992) investigated the use of progesterone administered intravaginally by controlled internal drug release devices (CIDRs) for a 12-day period, to induce estrus and reduce the interbreeding interval in Finn cross and Columbia ewes over two breeding seasons: July/August and March/April. CIDR treated ewes and a control group were exposed to rams and their interbreeding interval and conception rate

were compared. The majority of CIDR treated ewes conceived within the first estrus cycle post-ram introduction, whereas the vast majority of control ewes conceived in the following cycle. CIDR treatment effectively advanced the breeding season and reduced the interbreeding interval compared to controls. Conception rate and litter size did not differ however, between CIDR treated and control ewes in all seasons evaluated. All groups exhibited a high conception rate (range of 85-95%) regardless of season. Litter size was significantly reduced (1.5 vs. 2.4) for late spring breeding when compared to fall breeding, regardless of treatment. Overall, the use of the ram effect in combination with CIDR treatment advanced the breeding season and synchronized estrus relative to control ewes experiencing the ram effect alone. The ram effect with or without CIDRs was highly effective in inducing estrus, however neither were able to overcome seasonal depression in litter size found in the spring mating.

Later studies by Knights and colleagues investigated the use of FSH to circumvent the reduced litter size found in the suboptimal breeding season. In a 2003 study, ewes were dosed with 0, 42, or 68 mg of FSH at either 12 or 36-h prior to CIDR removal and fertile ram introduction. They found no significant effects of FSH on litter size, but despite this, found an increase in ovulation rate when FSH was provided 12 but not 36 h before fertile ram introduction. Despite this finding, the total number of lambs born per ewe did not differ among treatments (Knights et al., 2003). In 2007, this same group investigated the efficacy of GnRH supplementation on improving anestrous ewe response to the ram effect. This study found that GnRH injection (100 µg) either 2, 4, or 7 days after progesterone treatment and introduction of fertile rams did not affect conception rate or number of corpora lutea (CL) formed in Suffolk, Dorset or Katahdin ewes during the suboptimal breeding season (Jordan et al., 2007).

Male biostimulation or the ram effect

A well-documented strategy to induce ovulation and synchronize estrus in the sub-optimal breeding season is the use of male biostimulation, or commonly referred to in sheep as the ram effect (Martin & Scaramuzzi, 1983; Rosa, Juniper, & Bryant, 2000; Schinckel, 1954; Ungerfeld et al., 2002). The ram effect is a powerful management strategy that can be employed by producers to hasten the onset of reproductive cyclicity and synchronize ewes in both the long and short (spring and fall) day breeding season. Isolation of ewes from the sight or smell of rams and ram lambs for six weeks prior to the breeding season is necessary to induce the ram effect. The ram effect induces ovulation by increasing tonic luteinizing hormone (LH) secretion, and subsequently inducing a preovulatory LH surge (Ungerfeld et al., 2002). Olfactory and behavioral cues are sufficient to evoke the ram effect (Watson and Radford, 1960). A sudden introduction of rams to ewes during the anestrus period induces ovulation, though the first ovulation often occurs with a silent estrus, due to the fact that there is no corpus luteum, and thus no progesterone priming effect (Martin & Scaramuzzi, 1983). After this silent estrus, in roughly half the ewes, a short cycle occurs and the corpus luteum regresses, then approximately 8 days later, another ovulation occurs without behavioral estrus (Martin et al., 1981). In the remaining portion of ewes, ovulation is spontaneously induced at the time of ram introduction, and subsequently one full cycle (17 days) later. It is thought that the differing response to the ram effect may be influenced by the variation in stage of the reproductive cycle at the time of ram introduction (Fabre-Nys et al., 2016). This explains a variable response in the timing of ovulation induced by the ram effect, and implies that the subsequent lambing should follow a bimodal pattern, with approximately half the ewes lambing 164 days post ram introduction and the remainder 172 days post ram introduction (Fabre-Nys et al., 2015) The arrival of

laparoscopy technology enabled evaluation of ovary characteristics and differentiation of cycling and non-cycling ewes, and thus allowed for the identification of differential response to the ram effect (Oldham et al., 1979). Laparoscopic techniques allowed for the identification of the timing of ovulation relative to ram introduction, as well as differentiation between ewes exhibiting a silent estrus and those that did not respond to stimulation by the ram effect.

Schinkel et al., (1954) noted that in a flock of Merino ewes, one group teased by vasectomized rams 16 days prior to breeding and the other not, resulted in an advancement of lambing date by approximately 16 days, with a higher proportion of ewes displaying estrus behavior by day 6 after fertile ram introduction from the teased group than the control. Further replications of this study conducted later in the breeding season failed to show such uniform synchronization, which indicated that the ram effect is more powerful when ewes are at the transition period between anestrus and estrus. Responsiveness to ram stimulus varies among breeds (Marshall, 1903), as well as season and depth of anestrus (Martin et al., 1985). Ewes that have a shorter anestrus period and a greater propensity to breed outside of the natural mating period show a more pronounced response to the ram effect than breeds whose patterns of reproductive cyclicity are more seasonal (Nugent et al., 1988). Breed of ram also influences the success of the ram effect. When compared to Suffolk rams, Dorset rams proved to be more effective in synchronizing estrus and inducing ovulation in the sub-optimal breeding season (Nugent et al., 1988). The ram effect is more successful when rams are introduced in morning hours than evening hours, and morning introduction of rams has been shown to increase ovulation rate (Martin et al., 1985). The ram effect is a useful tool for the management of an accelerated lamb production system in that it induces ovulation in the sub-optimal breeding season and synchronizes ewes prior to breeding such that the birth periods are shorter and more

uniform. It is not known whether male stimulation is effective in inducing ovulation of ewes deep in anestrus, such as in breeds that display more rigid seasonal patterns of reproductive cyclicity. Shortened birth periods allow for longer lactation periods, and a longer interval from birth to the subsequent re-breeding period.

Photoperiod manipulation

In addition to the use of exogenous hormone therapies, photoperiod manipulation has proven to be a successful management tool to decrease the interbreeding interval and induce estrus in accelerated lamb production systems (see *seasonal control of reproduction in sheep* for mechanistic details). The use of alternating four-month sequences of long days (16-h light, 8-h dark) and short days (8-h light, 16-h dark) to obtain 8-month breeding intervals was evaluated by Cameron and colleagues (2010). Four groups of light-treated ewes, staggered by two months, were studied against control ewes treated with intravaginal progesterone sponges and pregnant mare's serum gonadotropin (PMSG) for three consecutive breeding seasons. The study found that ewes treated with the alternate long and short-day light protocol conceived 9.4 days post-ram introduction. Analysis of plasma progesterone at the time of ram introduction concluded that 30% of ewes were cycling prior to ram introduction. This indicates that light protocols are effective in inducing ovulation in ewes, sans the presence of rams. Overall conception rate over the course of the experiment was 91.6% for light treated ewes and 76.3% for control ewes, and litter size was increased in light-treated ewes (2.81) over control (2.27). Light-treated ewes produced a remarkable 3.78 lambs per ewe per year compared to 2.79 lambs born per ewe treated with progesterone treatment.

Both exogenous hormone therapies and photoperiod manipulation show promise in increasing the reproductive efficiency of accelerated lamb production systems by inducing

ovulation and synchronizing ewes to reduce the interbreeding interval. While both strategies are effective in inducing ovulation and increasing conception rate in the sub-optimal breeding season, exogenous hormone therapies do not prevent the seasonal reduction in litter size that is found in the literature. Light protocols are able increase reproductive efficiency by both increasing conception rate and litter size in the sub-optimal season. Despite these promising technologies, inputs to these systems (labor and facilities) are significant, necessitating that the returns in productivity be proportionally greater in ensure profitability.

Barriers to adoption of accelerated systems

Accelerated lamb production systems present an opportunity to increase the reproductive rate of ewes while producing a consistent supply of weaned lambs throughout the year. Reduced conception rate and prolificacy in the long day breeding seasons are the major constraints to adoption of this system (Hogue, 1987; Iniguez et al., 1986; Notter, 1981; Wheaton et al., 1992). Increasing reproductive efficiency without the need for exogenous hormone therapies or expensive light manipulation protocols will make accelerated lamb production systems more accessible and practical for commercial producers, as well as lowering the risk for decreased consumer acceptance of their product. It is not known whether nutritional management strategies are an effective way to increase reproductive efficiency in accelerated lamb production systems.

BIOLOGY OF FEMALE REPRODUCTION IN THE SHEEP

Estrous cycle

The seasonal nature of sheep reproductive activity is well documented in the literature and has been reviewed extensively (Ortavant et al., 1988; Rosa & Bryant, 2003; Scaramuzzi & Martin, 2008; Ungerfeld & Bielli, 2012). The estrous cycle of the ewe is 16 to 17 days in length.

The follicular phase is 3 days in length and the luteal phase is 14 days in length (Bartlewski et al., 2011). Length of the estrous cycle varies little between breeds. Reproductive events are regulated by hormones secreted by the hypothalamus, pituitary gland, pineal gland, adipose tissue, ovary, and uterus. This hormone secretion is further modulated by environmental factors including nutrition, male pheromones, and photoperiod. The secretion of gonadotropins from the anterior pituitary, follicle stimulating hormone (FSH), and luteinizing hormone (LH), are regulated by secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus. GnRH secretion is stimulated by decreased progesterone concentration and increased estradiol secretion, as well as kisspeptins, and other neuropeptides such as neurokinin B and dynorphin (Nestor et al., 2018).

Folliculogenesis occurs in a wavelike pattern, ranging from 3 to 4 waves per cycle. Folliculogenesis in the ewe is driven by increased secretion of FSH, while the final stages of follicular development and ovulation are driven primarily by estradiol secretion. (Bartlewski et al., 1998). In particularly prolific breeds known for higher ovulation rates, antral follicles from the final two follicular waves have been shown to ovulate simultaneously (Bartlewski et al., 1998). It is suggested that the ability of these breeds to rescue follicles from atresia is attributed to increased FSH concentrations, and that ovine follicles are less likely to tend toward dominance that prevents a subsequent follicular wave (Scaramuzzi & Martin, 2008). Furthermore, highly prolific breeds achieve smaller antral follicle diameters when compared to less prolific breeds (Souza et al., 2014). FSH secretion is suppressed by increased estradiol secretion from the preovulatory follicle and inhibin secretion from large antral follicles (Rosa & Bryant, 2003). Estradiol secretion is mediated by LH secretion, while inhibin works independent of LH (Baird et al., 1991). The LH surge and subsequent ovulation occur on day 0 of the estrous

cycle. Formation of corpora lutea result in increased progesterone secretion, with the (CL) reaching its maximal size at 6 days post-ovulation. Luteal regression occurs between days 12-15 of the estrous cycle, and is triggered by Prostaglandin $F_{2\alpha}$, which is secreted by uterine tissues in the absence of pregnancy (Knickerbocker et al., 1988).

Gestational length

Gestational length is influenced by the genetics of both the dam and fetus. Tilton (1964) noted that breeds raised primarily for meat production generally have shorter gestation lengths than that of breeds raised primarily for fine wool characteristics. Results from McKenzie and Phillips in 1932 revealed that the gestation length of Hampshire, Shropshire, and Southdown ewes ranged from 143.7 to 144.6 days, whereas Quinlan and Mare noted that duration of pregnancy in Merino ewes averaged 149 days. Other investigations into breed influence on gestational length have revealed that larger mature sized, faster growing sheep breeds tended to have shorter gestation length than smaller mature size or fine wool breeds (Forbes, 1967; Johnson, 1943; Tilton, 1964). Breed of sire has also been demonstrated to influence gestational length. When Rambouillet ewes were serviced by either Rambouillet or Hampshire sires, gestational length was two days greater in that of Rambouillet x Rambouillet matings (Kelley, 1943). Later studies such as the one conducted at University of California at Davis by Anderson and colleagues investigated the gestational length of Finnish Landrace ewes, Targhee ewes, and that of reciprocal embryonic transfers between these breeds. They reported shorter (144.9 days) gestations for Finnish Landrace than Targhee (150.4 days), and that breed of lamb was a strong driver in length of gestation, with Targhee offspring contributing to a 2.10 day gain in gestational length in Finnish Landrace recipient ewes, whereas Finnish Landrace offspring reduced length of gestation in Targhee dams by an average of 2.99 days (Anderson et al., 1981).

One factor that influences the length of gestation is age of dam at parturition. Terrill and Hazel found that as ewes age, there is a 0.27-day increase in gestational length per year of age. Tilton (1964) found that mean length of gestation for ewes at one year of age was 143.8 whereas means for ewes at ages 7 and 8 were 148.09 and 148.05 days, respectively, and additionally found an annual increase in gestation length of 0.56 days per year of age.

The effect of litter size on gestation length has been well documented in sheep. Kelley (1943) and Terrill & Hazel (1947) both found no significant difference in gestational length, however single lambs tended to have incrementally longer gestation. Later, Forbes (1967) found that twin bearing Scottish Halfbred (Cheviot x Border Leicester cross) ewes had significantly shorter gestation than single bearing ewes of the same breed. When compared to Speckled Welsh ewes, Scottish Halfbred ewes had longer gestational lengths (147.6 days *versus* 146.3 days). Forbes also reported that lambs that spent more time in utero were heavier at birth. Substantiating Forbes's data, Anderson et al. (1981) found single lambs to be in utero 0.6 days longer than twins, and twins 0.73 days less than triplets. It has been established that sex of lamb does not have a significant impact on gestational length in sheep (McCandish, 1922; Terrill & Hazel, 1947; McDowel et al., 1959). The prolific Polypay breed is documented to have between 144 and 150 day gestational length (Hulet et al., 1984).

Seasonal control of the reproductive cycle in sheep

In breeds of temperate origin, ewes are seasonally polyestrous, exhibiting a fertile (estrous) period during the fall and winter, and an infertile (anestrous) period in the spring and summer as days increase in length. This transition from estrous to anestrous allows for the alignment of times of ample feed resources with reproductive events such as gestation and lactation with increased energetic demands. Various investigations into the regulation of this

pattern of seasonal reproductive cyclicity have helped to elucidate the complex underlying mechanisms of seasonal reproduction (Chemineau et al., 1992; Karsch, et al., 1980; Legan et al., 1977; Malpaux et al., 2001). The primary modulator of seasonality in the ewe is regulation of melatonin secretion by the pineal gland (Rosa & Bryant Review, 2003). Photoperiodic information is gathered by the retina and transmitted to the superchiasmatic nuclei, located in the hypothalamus. This signal is then directed to the pineal gland by way of neurons in the paraventricular nuclei, intermediolateral spinal cord, and superior cervical ganglion. The pineal gland is responsible for secreting melatonin. During the long day season, less melatonin is secreted, and conversely, more melatonin is secreted as day length decreases. As day length decreases in the autumn, increased melatonin secretion is associated with decreased negative feedback by estradiol on GnRH secretion. Because GnRH secretion is reduced by estradiol in the long day season, gonadotropin secretion is reduced. Prevailing hypotheses suggest that modulation of estradiol feedback on GnRH secretion is controlled by morphological changes in GnRH neurons by season which are regulated by thyroid hormones. Nevertheless, this model does not fully explain the transition from estrous to anestrus in the ewe (Adams et al., 2006). Despite this reduction in GnRH secretion, follicular waves continue to occur during anestrus, however LH pulse frequency is reduced by 4-8 hours, and ovulation does not occur when compared to the natural mating season. Without the presence of ovulation and subsequent formation of the corpus luteum, plasma progesterone concentrations remain very low during anestrus (Karsch et al., 1980).

Seasonal effects on conception rate, litter size and gestational length

Within the context of an accelerated lamb production system, there are clear reductions in conception rate and litter size in the suboptimal breeding season. The primary barrier to

conception rate in the suboptimal breeding season is the genetic predisposition for out of season reproductive cyclicity, and in turn, the ability to respond to external (ram effect) and internal (hormone therapies) stimuli to induce ovulation (Notter, 2012a). Rambouillet, Dorset, Merino and high prolificacy breeds such as Finn and Romanov sheep have been identified as breeds with extended periods of reproductive cyclicity, and thus, increased conception rate in the suboptimal breeding season (Ungerfeld & Bielli, 2012). Sheep breeds originating from equatorial zones also have a longer breeding season, and are more able to breed out of season (Notter, 2012b).

Reduction in litter size in the suboptimal, or long day breeding season is well documented in the literature (Iniguez et al., 1986a; Lewis, Notter, Hogue, & Magee, 1996b; Notter, 1981a; Wheaton et al., 1992). Notter and colleagues reported an estimated 30% reduction in litter size in the long day season compared to the short-day season. Photoperiodic control of reproduction, and dampened GnRH secretion in the suboptimal breeding season may contribute to reduced litter sizes. Forcada et al., (1995) found that in ewes supplemented with 18 mg of melatonin showed increased ovulation rates in the long day season when compared to control. This study also concluded that there was a positive interaction between melatonin supplementation and increased plane of energy nutrition prior to breeding on ovulation rates in Romanov cross ewes. This same group found that in superovulated ewes during the long day season, melatonin implants increased the ovulation rate and embryo viability in Rasa Aragonesa ewes (Forcada et al., 2006). The underlying genetic determinants of ovulation rate persist regardless of season, implying that relative patterns of prolificacy among breeds will be similar in both the long and short day, despite overall reduction in ovulation rate and number of viable embryos in the long day season (Webb et al., 1992).

Seasonal variations in gestation length were noted in several explorations into suboptimal season lamb production systems (Gardner et al. , 2007a; Gootwine & Rozov, 2006; Wheaton et al., 1992). Gestation length has been found to vary by 1.5 to 3 days across season in repeated experiments, with ewes mated in the suboptimal season having shorter mean gestation lengths than those mated during the natural mating season. Clues to this variation in gestation length were found when variations in lamb birth weight was investigated in Romney ewes by Jenkinson et al., (1995). Ewes were mated in either the optimal or suboptimal breeding season, and ewes were slaughtered at 140 d of gestation. It was found that ewes mated in the natural mating season had larger mean placental weights, a higher number of placentomes, and a higher ratio of occupied to unoccupied caruncles. Lambs born to ewes mated in the suboptimal season were smaller. These differences in placentation across season may impact gestational length and/or size at birth however the mechanistic basis for how season might impact placentation remains unknown.

NUTRITIONAL CONTROL OF SHEEP REPRODUCTION

Nutritional management of sheep can have profound effects on reproductive performance. Nutritional regulation of reproduction is associated with an evolutionary mechanism to align season of reproduction with adequate food supply, in an effort to match periods of highest nutrient demand with periods of high forage availability. Powerful regulatory mechanisms are in place to ensure that adequate resources for reproduction are available. Nutrition, particularly energy intake impacts ability to conceive, ovulation rate, and embryonic and fetal loss in sheep.

Nutritional impacts on conception rate

Ruminant animals are well equipped with homeorhetic mechanisms to direct and partition energy to a particular production state, such as pregnancy or lactation (Bauman & Currie, 1980). They also have adaptive mechanism to allow protect against periods of inadequate energy intake provided that the ewe has body fat reserves to tap in to for energy (Chilliard et al., 1998). Severe long-term undernutrition in sheep can cause anorexia nervosa and can lead to inhibition of GnRH pulsatility, resulting in anovulation as well as anestrous, however the extent and duration of undernutrition necessary to impede reproduction is not well documented (Kendall et al., 2004). Although undernutrition can inhibit reproduction, Edwards & McMillen found that underfeeding Merino cross ewes at 70% of maintenance requirements for 60 days prior to breeding in an annual system did not negatively affect conception rate compared to those fed 100% maintenance (Edwards & McMillen, 2002). In addition to nutrition prior to mating, adequate nutrition during the conception period is necessary to ensure successful implantation (Parr 1987; Annett & Carson, 2006).

The effects of undernutrition prior to and during the conception period have been evaluated to determine the extent and duration of under nutrition necessary to hamper conception. Macias-Cruz (2017) and colleagues found that in hair sheep in the tropics, maintained on an estrus synchronization protocol, feeding at 60% predicted maintenance energy requirements for 30 days pre-conception, 50 days post-conception, or cumulatively 80 days (30 days pre and 50 days post-conception), conception rate and fetal loss were unaffected. Though this study was conducted with a sample size of 48 ewes, it suggests that more severe undernutrition is necessary to inhibit conception. A review published by Abecia et al (2006) described inconsistent findings by several studies providing 50% maintenance requirements prior to breeding on oocyte quality, number,

and embryo survivability. Much of this variability is due to the duration of underfeeding, and that most experimental models were examining super-ovulated ewes. Parr (1987) found that feeding 25% and 100% predicted energy requirements for 14 days post-conception yielded similar conception rates, however intakes of 200% maintenance energy decreased pregnancy rate (67% vs 48%). This study also found that the decreased pregnancy rates in overfed ewes was able to be rescued by progesterone supplementation. Later investigations (Annett & Carson, 2006) found that conception rate in mature ewes was unaffected with under (60%) and over (200%) nutrition for 30 days post-mating after the induction of ovulation by the use of a combination of progesterone pessary followed by injection of a gonadotropin analogue. Conversely, in adolescent (7-8 months of age) ewes subjected to the same mating protocol, both intakes of 100% and 200% predicted maintenance energy requirements had lower conception rate when compared to underfed ewe lambs (60% vs 81%). It is unclear in this study if adolescent ewes were fed to meet their predicted requirements for growth as well as maintenance. The prevailing hypothesis is that increased energy intake peri-conceptionally reduces circulating progesterone concentrations, while undernutrition either has no effect or increases progesterone concentrations, thereby affecting conception rates.

Nutritional Flushing: Increasing nutrition prior to breeding to increase ovulation rate

Nutritional management prior to breeding is important to ensure reproductive success. The practice of intentionally increasing plane of energy or increasing body weight and body condition score prior to breeding is called flushing. Flushing of ewes increases the ovulation rate, and thus, potentially litter size of ewes. Flushing is an inexpensive way to dramatically increase production in a commercial sheep flock. Numerous descriptive studies have quantified the effects of flushing in different breeds, seasons, and production systems, and have reported up

to a 35% increase in ovulation rate (Nottle et al., 1997). Despite this large body of descriptive work, the physiological mechanisms responsible for the increase in ovulation rate in sheep are not well understood. There is experimental evidence suggesting that leptin (Kendall et al., 2004) and Insulin-like Growth Factor-1 (IGF-1) (Scaramuzzi et al., 2006) may mediate aspects of nutritional effects on ovulation rate in sheep, however, an in depth understanding of the underlying mechanisms remains poorly understood.

Nutrient requirements for folliculogenesis are relatively low, when compared to other reproductive events such as pregnancy and lactation. Because of this, it is thought that mechanisms involved in regulating folliculogenesis are not out of nutritional necessity, but instead are responsible for environmental sensing prior to the large energetic investment in reproduction (Scaramuzzi & Martin, 2008). Circulating blood metabolites such as glucose and hormones such as insulin seem to have some nutrient sensing effect on the ovary directly, however, direct effects on the ovary cannot solely explain an increased ovulation rate in response to increased plane of nutrition (Gutierrez et al., 2011). Instead, a coordinated effort of metabolic and reproductive hormone signaling is responsible for the flushing effect through primarily through central mechanisms. Rather than solely by direct action of metabolic hormones on the follicle or ovary, these metabolites and hormones act in an orchestral manner on the hypothalamic-pituitary-gonadal axis (HPG axis) to indicate the nutritional status of the ewe, which cues changes in the sensitivity of the GnRH pulse generator, thus determining ovulation rate (Lenz Souza et al., 2014; Scaramuzzi et al., 2006). The complex underlying nutrient sensing pathways within the brain and the ovary involved are not well understood.

Positive energy balance occurs when a ewe is fed in excess of her metabolic requirements. This inevitably leads to storage of excess energy in hepatic and skeletal muscle glycogen and adipose

tissue, which can be measured in gain in weight and body condition. Positive energy balance in the ewe leads to increased plasma insulin and leptin concentrations, as well as an increase in insulin dependent glucose uptake by the ovary mediated by the facilitative glucose transport molecule isoform GLUT4 (Scaramuzzi et al., 2006) . While prevailing hypotheses point to central control of ovulation by circulating metabolites and reproductive hormones, these responses to increased nutrition may also involve direct action on the ovary, allowing an integration of signals at different levels of the hypothalamic-pituitary-gonadal axis to impact folliculogenesis, follicular recruitment, and ovulation rate (Scaramuzzi et al., 2006). In addition, positive energy balance is associated with an increased rate of steroid hormone clearance (Parr et al., 1987) decreasing estradiol concentrations, thereby increasing FSH. Furthermore, nutritional stimulation, either by the intravenous infusion of glucose, or the supplementary feeding of concentrates decreases estradiol secretion during the follicular phase of the reproductive cycle (Scaramuzzi et al., 2006).

There are three stages of positive energy balance. Firstly, acute positive energy balance refers to an increase in available energy, leading to rapid increases in circulating glucose, insulin, and IGF-1, without an increase in body weight or body condition. Dynamic positive energy balance refers to a state in which a ewe is actively gaining body weight and body condition in response to increased energy intake. Finally, static positive energy balance refers to the point in which a stasis is reached as body weight and body condition have effectively plateaued in response to elevated dietary energy. Additionally, this static level of positive energy balance eventually leads to metabolic disorder, including hyperinsulinemia. During static obesity, higher dietary energy per kg of lean body mass is required to maintain weight in obese sheep than lean sheep (McCann et al., 1992). It is thought that a nutritional flushing response is evoked when ewes are

in acute or dynamic positive energy balance, which could be anywhere from a four day to three week window (Viñoles et al., 2010).

Scaramuzzi et al., (2006) proposed a model for the nutritional stimulation of folliculogenesis based on the inhibition of follicular estradiol secretion by the insulin-glucose, IGF-1, and leptin systems in the acute stage of positive energy balance. This reduction in estradiol leads to an increase in FSH secretion, and increased growth of FSH-responsive follicles. They also suggest that the leptin system is responsible for the modulation of reproduction in the dynamic and static stages of positive energy balance. Negative energy balance in the ewe decreases the concentration of IGF-1 and increases plasma Growth Hormone (GH) which leads to the inhibition of GnRH pulsatility (Llewellyn et al., 2007). A study conducted by Llewellyn and colleagues found that during periods of severe negative energy balance in dairy cows, bioavailability of IGF-1 is decreased due to reduced expression of IGF binding protein 2, which may affect follicular recruitment (Llewellyn et al., 2007).

Capacity to respond to nutritional flushing differs among sheep of different genetic background, as well as by body condition prior to flushing, with both ewes that are under and over-conditioned not responding to nutritional supplementation (Scaramuzzi et al., 2006). In addition, breeds that are known for higher prolificacy show a more dramatic increase in ovulation rate than breeds whose capacity for high ovulation rate is lower (Gutierrez et al., 2011; Lassoued et al., 2004). Genetic predisposition for very large litters, such as those found in the Romanov and Finn breeds are thought to be affected by clusters of genes involving gonadotropin secretion and signaling that are not found in breeds of low fecundity, such as Texel (Xu et al., 2018). Despite this characterization, little is understood about the physiological mechanisms that dictate this ovulatory response.

The energy intake requirements of sheep have been assessed by the National Research Council in order to optimize feeding strategies to maximize the flushing response. Two iterations (1985 and 2007) of these recommendations are commonly used as feeding standards for both experimentation and commercial industry. The 1985 recommendations are based, largely, on the requirements of larger mature size range sheep, whereas the 2007 recommendations are based on a model described in by Cannas (Cannas et al., 2004) primarily to maximize milk production in sheep dairying systems. The recommendations pertinent to this thesis are those for lactation and flushing. Maintenance requirements between the two iterations for mature ewes do not differ. The 2007 requirements are markedly lower for late lactation than 1985. The 2007 NRC requirements for late lactation suggest roughly 36% lower energy intakes for twin-rearing ewes and 40% lower intakes for single-rearing ewes. This is particularly important in the context of an accelerated lamb production system, as nutrient requirements may be elevated due to limited time spent in maintenance, as well as a shortened interval between late lactation and the ensuing breeding period. This shortened interval may not allow for recovery of body condition lost in the metabolic investment of lactation. The predicted energy requirements for flushing are well described in the 1985 NRC recommendations, and call for an elevation in energy intake of 1.6 times maintenance for 2 weeks prior to and 2-4 weeks after mating. Drastically different recommendations are made in NRC 2007, which recommends an energy intake increase of only 10% over maintenance for two weeks prior and three weeks post ram introduction. While a few studies have shown that modest increases in pre-mating nutrition, such as supplementation of lupin grain for 6-14 days prior to mating, can increase ovulation rate (Nottle et al., 1997; Stewart & Oldham, 1986), the bulk of the literature indicates that greater elevation in energy intake prior to mating is required in order to evoke a flushing response (Knight et al., 1975; Downing &

Scaramuzzi 1991, Lassoued et al., 2004). Finally, both iterations of nutrient recommendations are designed for an annual lamb production system, thus it is important to evaluate both the 1985 and 2007 NRC requirements in the context of an accelerated lamb production system due to the decreased interbreeding interval, and thus less time to recover body condition between lactation and the ensuing breeding period.

Embryonic survival

Increased litter size is not only influenced by ovulation rate of the ewe, but survival of fertilized embryos beyond implantation. It is estimated that 20-30% of all fertilized ova perish in the first several weeks of pregnancy (Bolet, 1986). The majority of embryonic loss occurs before day 18 of gestation, and 1-5% of ewes experience embryonic or fetal loss from day 30-term (Quinlivan et al., 1966). Breeds with higher ovulation rate experience higher incidence of embryonic loss than breeds with lower ovulation rates (Knights et al., 2003). Nutrition post-conception, especially pre-implantation (day 0-14) and implantation (day 14-28) is critical for embryonic survival (Guillomot et al., 1981, Rickard et al., 2017). Parr et al., (1987) proposed that both over nutrition and undernutrition during this window are detrimental to the embryo. Parr et al (1992) found that ewes fed to 200% of maintenance requirements from day 8-14 post-conception exhibited a higher rate of embryonic mortality than did ewes fed at 100% or 25% of maintenance requirements. This was attributed to lower progesterone levels in overfed ewes, caused by an increased metabolic clearance of steroid hormones in overfed ewes. In early pregnancy, embryonic survival is largely dependent on progesterone concentrations (Parr, 1992), which could be affected by season of mating as well. Despite feeding 25% maintenance requirements, no negative impacts on conception rate or embryonic survival were noted in this study. Dixon and colleagues measured patterns of embryonic and fetal loss via ultrasonography from day 25-

85 of gestation. This study found that higher incidence of loss after day 25 of gestation was directly correlated with lower maternal serum progesterone concentrations at day 25 (Dixon et al., 2007). It is not known whether these nutritional effects on patterns of embryonic loss are further impacted by season.

CONCLUSIONS

Accelerated lamb production systems offer a unique solution to both low productivity and seasonality of lamb production, two problems faced by the US sheep industry. Successful adoption of accelerated production systems has been limited by reduced reproductive performance in the suboptimal, or long day breeding season. There are several management strategies available to increase conception rate (exogenous hormone therapies, the ram effect) and litter size (light manipulation protocols) in the long day breeding season. These strategies are often intensive in nature, requiring more labor and financial inputs than natural mating. Additionally, with consumer acceptance of exogenous hormone therapies on the decline, it is important to consider alternate means to increase reproductive efficiency in accelerated lamb production systems. Nutrition prior to mating can affect conception rate, ovulation rate, and incidence of embryonic loss in sheep. It is not known whether these effects persist across season. Despite this knowledge, there are no published studies investigating the nutritional management of accelerated production systems. Accelerated lamb production systems have a decreased interbreeding interval, necessitating that ewes quickly recover body condition lost in lactation in preparation for the ensuing breeding period and it is not clear that current feeding standards are sufficient to allow for this recovery. Finally, evidence in rams (Fabre-Nys et al., 2016) indicates that improved nutrition in the suboptimal breeding season can improve reproductive performance in moderately aseasonal breeds. It remains unknown if this nutrition

by season interaction with reproduction might apply to ewes in the context of accelerated production.

REFERENCES

REFERENCES

- Abecia, J. A., Forcada, F., Palacín, I., Sánchez-Prieto, L., Sosa, C., Fernández-Foren, A., & Meikle, A. (2015). Undernutrition affects embryo quality of superovulated ewes. *Zygote*, 23(1), 116.
- Adams, V. L., Goodman, R. L., Salm, A. K., Coolen, L. M., Karsch, F. J., & Lehman, M. N. (2006). Morphological plasticity in the neural circuitry responsible for seasonal breeding in the ewe. *Endocrinology*, 147(10), 4843–4851. <https://doi.org/10.1210/en.2006-0408>
- Anderson, G. B., Bradford, G. E., & Cupps, P. T. (1981). Length of gestation in ewes carrying lambs of two different breeds. *Theriogenology*, 16(1), 119–129. [https://doi.org/10.1016/0093-691X\(81\)90120-5](https://doi.org/10.1016/0093-691X(81)90120-5)
- Annett, R. W., & Carson, A. F. (2006). Effects of plane of nutrition during the first month of pregnancy on conception rate, foetal development and lamb output of mature and adolescent ewes. *Animal Science*, 82(6), 947–954. <https://doi.org/10.1017/ASC2006111>
- Bartlewski, P. M., Beard, A. P., Cook, S. J., & Rawlings, N. C. (1998). Ovarian follicular dynamics during anoestrus in ewes. *Reproduction*, 113(2), 275–285. <https://doi.org/10.1530/jrf.0.1130275>
- Bartlewski, Pawel M., Baby, T. E., & Giffin, J. L. (2011). Reproductive cycles in sheep. *Animal Reproduction Science*. <https://doi.org/10.1016/j.anireprosci.2011.02.024>
- Bauman, D. E., & Bruce Currie, W. (1980). Partitioning of Nutrients During Pregnancy and Lactation: A Review of Mechanisms Involving Homeostasis and Homeorhesis. *Journal of Dairy Science*, 63(9). [https://doi.org/10.3168/jds.S0022-0302\(80\)83111-0](https://doi.org/10.3168/jds.S0022-0302(80)83111-0)
- Blache, D., Zhang, S., & Martin, G. B. (2006). Dynamic and integrative aspects of the regulation of reproduction by metabolic status in male sheep. *Reproduction Nutrition Development*, 46(4), 379–390. <https://doi.org/10.1051/rnd:2006019>
- Cannas, A., & Atzori, A. S. (2004). Development and evaluation of a model to predict sheep nutrient requirements and feed utilisation. *Italian Journal of Animal Science*, 4(SUPPL. 1). <https://doi.org/10.4081/ijas.2005.1s.15>
- Chemineau, P., Malpoux, B., Delgadillo, J. A., Guérin, Y., Ravault, J. P., Thimonier, J., & Pelletier, J. (1992). Control of sheep and goat reproduction: Use of light and melatonin. *Animal Reproduction Science*, 30(1–3), 157–184. <https://doi.org/10.1016/0378->

- Chilliard, Y., Bocquier, F., & Doreau, M. (1998). Digestive and metabolic adaptations of ruminants to undernutrition, and consequences on reproduction. *Reproduction Nutrition Development*, 38(2), 131–152. <https://doi.org/10.1051/rnd:19980201>
- Clarke, I. J., & Arbabi, L. (2016). New concepts of the central control of reproduction, integrating influence of stress, metabolic state, and season. *Domestic Animal Endocrinology*, Vol. 56, pp. S165–S179. <https://doi.org/10.1016/j.domaniend.2016.03.001>
- Cochran, K. P., Notter, D. R., & McClaugherty, F. S. (1984). A comparison of Dorset and Finnish Landrace crossbred ewes. *Journal of Animal Science*, 59(2), 329–337. <https://doi.org/10.2527/jas1984.592329x>
- Delgadillo, J. A., Gelez, H., Ungerfeld, R., Hawken, P. A. R., & Martin, G. B. (2009). The “male effect” in sheep and goats-Revisiting the dogmas. *Behavioural Brain Research*, 200(2), 304–314. <https://doi.org/10.1016/j.bbr.2009.02.004>
- Dixon, A. B., Knights, M., Winkler, J. L., Marsh, D. J., Pate, J. L., Wilson, M. E., ... Inskip, E. K. (2007). Patterns of late embryonic and fetal mortality and association with several factors in sheep. *Journal of Animal Science*, 85(5), 1274–1284. <https://doi.org/10.2527/jas.2006-129>
- Downing, J., & Scaramuzzi, R. (1991). Nutrient effects on ovulation rate, ovarian function and the secretion of gonadotrophic and metabolic hormones in sheep. *Reproduction, Fertility and Development*. <https://doi.org/10.1530/biosciproc.2.017>
- Edwards, L. J., & McMillen, I. C. (2002). Impact of Maternal Undernutrition During the Periconceptional Period, Fetal Number, and Fetal Sex on the Development of the Hypothalamo-Pituitary Adrenal Axis in Sheep During Late Gestation. *Biology of Reproduction*, 66(5), 1562–1569. <https://doi.org/10.1095/biolreprod66.5.1562>
- El-Saied, U. M., de la Fuente, L. F., & San Primitivo, F. (2006). Lifetime traits comparison between annual and accelerated lambing systems for dairy ewes. *Livestock Science*, 101(1–3). <https://doi.org/10.1016/j.livsci.2005.11.003>
- Fabre-Nys, C., Chanvallon, A., Dupont, J., Lardic, L., Lomet, D., Martinet, S., & Scaramuzzi, R. J. (2016). The “ram effect”: A “non-classical” mechanism for inducing LH surges in sheep. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0158530>

- Fabre-Nys, C., Kendrick, K. M., & Scaramuzzi, R. J. (2015). The “ram effect”: New insights into neural modulation of the gonadotropic axis by male odors and socio-sexual interactions. *Frontiers in Neuroscience*. <https://doi.org/10.3389/fnins.2015.00111>
- Forbes, J. M. (1967). Factors affecting the gestation length in sheep. *The Journal of Agricultural Science*, 68(2), 191–194. <https://doi.org/10.1017/S0021859600016221>
- Forcada, F., Zarazaga, L., Abecia, J. (1995). Effect of exogenous melatonin and plane of nutrition after weaning on estrous activity, endocrine status and ovulation rate in salz ewes lambing in the seasonal anestrus. *Theriogenology*, 43.
- Forcada, F., Abecia, J. A., Cebrián-Pérez, J. A., Muño-Blanco, T., Valares, J. A., Palacín, I., & Casao, A. (2006). The effect of melatonin implants during the seasonal anestrus on embryo production after superovulation in aged high-prolificacy Rasa Aragonesa ewes. *Theriogenology*, 65(2), 356–365. <https://doi.org/10.1016/j.theriogenology.2005.05.038>
- Gardner, D. S., Buttery, P. J., Daniel, Z., & Symonds, M. E. (2007a). Factors affecting birth weight in sheep: Maternal environment. *Reproduction*. <https://doi.org/10.1530/REP-06-0042>
- Gardner, D. S., Buttery, P. J., Daniel, Z., & Symonds, M. E. (2007b). Factors affecting birth weight in sheep: Maternal environment. *Reproduction*, 133(1), 297–307. <https://doi.org/10.1530/REP-06-0042>
- Gootwine, E., & Rozov, A. (2006). Seasonal effects on birth weight of lambs born to prolific ewes maintained under intensive management. *Livestock Science*, 105(1–3), 277–283. <https://doi.org/10.1016/j.livsci.2006.06.018>
- Gutierrez, C. G., Ferraro, S., Martinez, V., Saharrea, A., Cortez, C., Lassala, A., ... Hernandez, J. (2011). Increasing ovulation quota: More than a matter of energy. *Acta Scientiae Veterinariae*, 39(SUPPL. 1).
- Hogue, D. E. (1987). Frequent lambing systems. *New Techniques in Sheep Production* (pp. 57–63). <https://doi.org/10.1016/b978-0-408-10134-9.50011-x>
- Hulet, C. V., Ercanbrack, S. K., & Knight, A. D. (1984). Development of the Polypay breed of sheep. *Journal of Animal Science*. <https://doi.org/10.2527/jas1984.58115x>

- Iniguez, L. C., Quaas, R. L., & Van Vleck, L. D. (1986). Lambing performance of Morlam and Dorset ewes under accelerated lambing systems. *Journal of Animal Science*, 63(6), 1769–1778. <https://doi.org/10.2527/jas1986.6361769x>
- Jenkinson, C. M. C., Peterson, S. W., Mackenzie, D. D. S., McDonald, M. F., & McCutcheon, S. N. (1995). Seasonal effects on birth weight in sheep are associated with changes in placental development. *New Zealand Journal of Agricultural Research*, 38(3), 337–345. <https://doi.org/10.1080/00288233.1995.9513135>
- Johnson, L. E. (1943). Differences in Lengths of Gestation Periods of Breeds of Beef Cattle, Swine, and Sheep. *Academy of Science*.
- Jordan, R. M., & Hanke, H. E. (1977). Effect of Level of Grain Fed Ewes During Late Lactation on Lamb Production. *Journal of Animal Science*, 45(5), 945–947. <https://doi.org/10.2527/jas1977.455945x>
- Karsch, F. J., Legan, S. J., Ryan, K. D., & Foster, D. L. (1980). Importance of Estradiol and Progesterone in Regulating LH Secretion and Estrous Behavior During the Sheep Estrous Cycle1. *Biology of Reproduction*. <https://doi.org/10.1095/biolreprod23.2.404>
- Kelley, R. (1943). The Length of Gestation Periods Preceding Single and Multiple Births in Sheep. *Australian Veterinary Journal*, 19, 43–45.
- Kendall, N. R., Gutierrez, C. G., Scaramuzzi, R. J., Baird, D. T., Webb, R., & Campbell, B. K. (2004). Direct in vivo effects of leptin on ovarian steroidogenesis in sheep. *Reproduction*, 128(6), 757–765. <https://doi.org/10.1530/rep.1.00256>
- Kenyon, P. R., Maloney, S. K., & Blache, D. (2014). Review of sheep body condition score in relation to production characteristics. *New Zealand Journal of Agricultural Research*, 57(1), 38–64. <https://doi.org/10.1080/00288233.2013.857698>
- Knickerbocker, J. J., Wiltbank, M. C., & Niswender, G. D. (1988). Mechanisms of luteolysis in domestic livestock. *Domestic Animal Endocrinology*, 5(2), 91–107. [https://doi.org/10.1016/0739-7240\(88\)90011-2](https://doi.org/10.1016/0739-7240(88)90011-2)
- Knights, M., Baptiste, Q. S., Dixon, A. B., Pate, J. L., Marsh, D. J., Inskeep, E. K., & Lewis, P. E. (2003). Effects of dosage of FSH, vehicle and time of treatment on ovulation rate and prolificacy in ewes during the anestrous season. *Small Ruminant Research*, 50(1–2), 1–9. [https://doi.org/10.1016/S0921-4488\(03\)00111-1](https://doi.org/10.1016/S0921-4488(03)00111-1)

- Lassoued, N., Rekik, M., Mahouachi, M., & Ben Hamouda, M. (2004). The effect of nutrition prior to and during mating on ovulation rate, reproductive wastage, and lambing rate in three sheep breeds. *Small Ruminant Research*, 52(1–2), 117–125. [https://doi.org/10.1016/S0921-4488\(03\)00250-5](https://doi.org/10.1016/S0921-4488(03)00250-5)
- Legan, S. J., Karsch, F. J., & Foster, D. L. (1977). The endocrine control of seasonal reproductive function in the ewe: A marked change in response to the negative feedback action of estradiol on luteinizing hormone secretion. *Endocrinology*, 101(3), 818–824. <https://doi.org/10.1210/endo-101-3-818>
- Lenz Souza, M., Leandro Gressler, M., & Uribe Velásquez, L. (2014). Interrelationships of nutrition, metabolic hormones and reproduction of female sheep. *Revista CES Medicina Veterinaria y Zootecnia*, 9(2). <https://doi.org/10.21615/3148>
- Lewis, R. M., Notter, D. R., Hogue, D. E., & Magee, B. H. (1996). Ewe Fertility in the STAR Accelerated Lambing System. *Journal of Animal Science*. <https://doi.org/10.2527/1996.7471511x>
- Ley, W. B., Sprecher, D. J., Thatcher, C. D., Pelzer, K. D., & Umberger, S. H. (1990). Use of the point-score system for breeding soundness examination in yearling Dorset, Hampshire and Suffolk rams. *Theriogenology*, 34(4), 721–733. [https://doi.org/10.1016/0093-691X\(90\)90027-Q](https://doi.org/10.1016/0093-691X(90)90027-Q)
- Llewellyn, S., Fitzpatrick, R., Kenny, D. A., Murphy, J. J., Scaramuzzi, R. J., & Wathes, D. C. (2007). Effect of negative energy balance on the insulin-like growth factor system in pre-recruitment ovarian follicles of post partum dairy cows. *Reproduction*, 133(3), 627–639. <https://doi.org/10.1530/REP-06-0122>
- Macías-Cruz, U., Vicente-Pérez, R., Correa-Calderón, A., Mellado, M., Meza-Herrera, C. A., & Avendaño-Reyes, L. (2017). Undernutrition pre- and post-mating affects serum levels of glucose, cholesterol and progesterone, but not the reproductive efficiency of crossbred hair ewes synchronized for estrus. *Livestock Science*, 205, 64–69. <https://doi.org/10.1016/j.livsci.2017.09.016>
- Malpaux, B., Migaud, M., Tricoire, H., & Chemineau, P. (2001). Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. *Journal of Biological Rhythms*, 16(4), 336–347. <https://doi.org/10.1177/074873001129002051>
- Martin, G. B., Cognie, Y., Schirar, A., Nunes-Ribeiro, A., Fabre-Nys, C., & Thiéry, J. C. (1985).

- Diurnal variation in the response of anoestrous ewes to the ram effect. *Journal of Reproduction and Fertility*, 75(1), 275–284. <https://doi.org/10.1530/jrf.0.0750275>
- Martin, G.B., & Scaramuzzi, R. J. (1983). The induction of oestrus and ovulation in seasonally anovular ewes by exposure to rams. *Journal of Steroid Biochemistry*, 19(1 PART 3), 869–875. [https://doi.org/10.1016/0022-4731\(83\)90026-2](https://doi.org/10.1016/0022-4731(83)90026-2)
- Martin, G. B., Scaramuzzi, R. J., & Lindsay, D. R. (1981). Induction of ovulation in seasonally anovular ewes by the introduction of rams: Effects of progesterone and active immunization against androstenedione. *Australian Journal of Biological Sciences*, 34(6), 569–576. <https://doi.org/10.1071/BI9810569>
- McCann, J. P., Bergman, E. N., & Beermann, D. H. (1992). Dynamic and static phases of severe dietary obesity in sheep: Food intakes, endocrinology and carcass and organ chemical composition. *Journal of Nutrition*, 122(3), 496–505. <https://doi.org/10.1093/jn/122.3.496>
- Miller, L. R., Shiflett, J. S., Marsh, D. J., & Rodgers, P. (2016). *U . S . Sheep Industry Research , Development , And Education Priorities*.
- Nestor, C. C., Bedenbaugh, M. N., Hileman, S. M., Coolen, L. M., Lehman, M. N., & Goodman, R. L. (2018). Regulation of GnRH pulsatility in ewes. *Reproduction*, 156(3), R83–R99. <https://doi.org/10.1530/REP-18-0127>
- Notter, D. R. (1981). Repeatability of Conception Rate and Litter Size for Ewes in an Accelerated Lambing System. *Journal of Animal Science*, 53(3), 643–650. <https://doi.org/10.2527/jas1981.533643x>
- Notter, D. R. (2012). Genetic improvement of reproductive efficiency of sheep and goats. *Animal Reproduction Science*, 130(3–4), 147–151. <https://doi.org/10.1016/j.anireprosci.2012.01.008>
- Nottle, M. B., Kleemann, D. O., Grosser, T. I., & Seamark, R. F. (1997). Evaluation of a nutritional strategy to increase ovulation rate in Merino ewes mated in late spring-early summer. *Animal Reproduction Science*, 47(4). [https://doi.org/10.1016/S0378-4320\(97\)00025-0](https://doi.org/10.1016/S0378-4320(97)00025-0)
- Nugent, R. A., Notter, D. R., & Beal, W. E. (1988). Effects of ewe breed and ram exposure on estrous behavior in May and June. *Journal of Animal Science*, 66(6), 1363–1370. <https://doi.org/10.2527/jas1988.6661363x>

- Nugent, R. A., Notter, D. R., & McClure, W. H. (1988). Effects of ram preexposure and ram breed on fertility of ewes in summer breeding. *Journal of Animal Science*, 66(7), 1622–1626. <https://doi.org/10.2527/jas1988.6671622x>
- Oldham, C., Martin, G. B., & Knight, T. (1979). Stimulation of seasonally anovular merino ewes by rams. *Animal Reproduction Science*, 1, 283–290.
- Ortavant, R., Bocquier, F., Pelletier, J., Ravault, J. P., Thimonier, J., & Volland-Nail, P. (1988). Seasonality of Reproduction in Sheep and its Control by Photoperiod. *Australian Journal of Biological Sciences*, 41(1), 69–86. <https://doi.org/10.1071/BI9880069>
- Parr, R. A. (1992). Nutrition-progesterone interactions during early pregnancy in sheep. *Reproduction, Fertility and Development*, 4(3), 297–300. <https://doi.org/10.1071/RD9920297>
- Parr, R. A., Davis, I. F., Fairclough, R. J., & Miles, M. A. (1987). Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. *Journal of Reproduction and Fertility*, 80(1), 317–320. <https://doi.org/10.1530/jrf.0.0800317>
- Quinlivan, T. D., Martin, C. A., Taylor, W. B., & Cairney, I. M. (1966). Estimates of pre- and perinatal mortality in the New Zealand Romney Marsh ewe. I. Pre- and perinatal mortality in those ewes that conceived to one service. *Journal of Reproduction and Fertility*, 11(3), 379–390. <https://doi.org/10.1530/jrf.0.0110379>
- Redden, R., Sanko, E., Ehrhardt, R. A., & Heimke, C. (2018). *Seasonality of the US Lamb Industry*.
- Rosa, H. J. D., & Bryant, M. J. (2003). Seasonality of reproduction in sheep. *Small Ruminant Research*, 48(3), 155–171. [https://doi.org/10.1016/S0921-4488\(03\)00038-5](https://doi.org/10.1016/S0921-4488(03)00038-5)
- Rosa, H. J. D., Juniper, D. T., & Bryant, M. J. (2000). The effect of exposure to oestrous ewes on rams' sexual behaviour, plasma testosterone concentration and ability to stimulate ovulation in seasonally anoestrous ewes. *Applied Animal Behaviour Science*, 67(4). [https://doi.org/10.1016/S0168-1591\(00\)00086-1](https://doi.org/10.1016/S0168-1591(00)00086-1)
- Scaramuzzi, R. J., Adams, N. R., Bairdc, D. T., Campbell, B. K., Downing, J. A., Findlay, J. K., ... Tsonis, C. G. (1993). A model for follicle selection and the determination of ovulation rate in the ewe. *Reproduction, Fertility and Development*, 5(5), 457–478. <https://doi.org/10.1071/RD9930459>

- Scaramuzzi, R. J., & Martin, G. B. (2008). The Importance of Interactions Among Nutrition, Seasonality and Socio-sexual Factors in the Development of Hormone-free Methods for Controlling Fertility. *Reproduction in Domestic Animals*, 43(SUPPL.2), 129–136. <https://doi.org/10.1111/j.1439-0531.2008.01152.x>
- Scaramuzzi, Rex J., Campbell, B. K., Downing, J. A., Kendall, N. R., Khalid, M., Muñoz-Gutiérrez, M., & Somchit, A. (2006). A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. *Reproduction Nutrition Development*, 46(4), 339–354. <https://doi.org/10.1051/rnd:2006016>
- Schinckel, P. G. (1954). the Effect of the Ram on the Incidence and Occurrence of Oestrus in Ewes. *Australian Veterinary Journal*, 30(7), 189–195. <https://doi.org/10.1111/j.1751-0813.1954.tb08198.x>
- Stewart, R., & Oldham, C. M. (1986). Feeding lupins to ewes for four days during the luteal phase can increase ovulation rate. *Proceedings of the Australian Society of Animal Production*, 16, 367–370.
- The role of nutrition on reproduction of sheep. (2014). *Acta Veterinaria Brasilica*, 8(supl.2). <https://doi.org/10.21708/avb.2014.8.0.3955>
- Tilton, J. E. (1964). *Factors influencing the gestation length in sheep*. [Master's Thesis, Illinois State Normal University].
- Ungerfeld, R., Carbajal, B., Rubianes, E., & Forsberg, M. (2005). Endocrine and ovarian changes in response to the ram effect in medroxyprogesterone acetate-primed corriedale ewes during the breeding and nonbreeding season. *Acta Veterinaria Scandinavica*, 46(1–2), 33–44. <https://doi.org/10.1186/1751-0147-46-33>
- Ungerfeld, R., Pinczak, A., Forsberg, M., & Rubianes, E. (2002). Ovarian responses of anestrous ewes to the “ram effect.” *Canadian Journal of Animal Science*, 82(4). <https://doi.org/10.4141/A02-005>
- Ungerfeld, Rodolfo. (2012). Sexual behavior of medium-ranked rams toward non-estrous ewes is stimulated by the presence of low-ranked rams. *Journal of Veterinary Behavior: Clinical Applications and Research*, 7(2). <https://doi.org/10.1016/j.jveb.2011.05.023>
- Ungerfeld, Rodolfo, & Bielli, A. (2012). Seasonal and social factors affecting reproduction. *Livestock Reproduction: Bovine, Swine, and Ruminants” Encyclopaedia of Life Support*

- USDA NASS. (2020). Sheep and Goats. *Critical Quarterly*, 2(1), 64–71.
<https://doi.org/10.1111/j.1467-8705.1960.tb00840.x>
- Viñoles, C., Paganoni, B., Glover, K. M. M., Milton, J. T. B., Blache, D., Blackberry, M. A., & Martin, G. B. (2010). The use of a “first-wave” model to study the effect of nutrition on ovarian follicular dynamics and ovulation rate in the sheep. *Reproduction*, 140(6).
<https://doi.org/10.1530/REP-10-0196>
- Webb, R., Baxter, G., McBride, D., Ritchie, M., & Springbett, A. J. (1992). Mechanism controlling ovulation rate in ewes in relation to seasonal anoestrus. *Journal of Reproduction and Fertility*, 94(1), 143–151. <https://doi.org/10.1530/jrf.0.0940143>
- Wheaton, J. E., Windels, H. F., & Johnston, L. J. (1992). Accelerated lambing using exogenous progesterone and the ram effect. *Journal of Animal Science*, 70(9), 2628–2635.
<https://doi.org/10.2527/1992.7092628x>
- Xu, S. S., Gao, L., Xie, X. L., Ren, Y. L., Shen, Z. Q., Wang, F., ... Li, M. H. (2018). Genome-wide association analyses highlight the potential for different genetic mechanisms for litter size among sheep breeds. *Frontiers in Genetics*, 9(APR), 1–14.
<https://doi.org/10.3389/fgene.2018.00118>

CHAPTER 2: THE EFFECT OF LATE LACTATION AND PRE-BREEDING NUTRITION ON REPRODUCTIVE OUTCOMES IN AN ACCELERATED LAMB PRODUCTION SYSTEM.

INTRODUCTION

Low national flock productivity and seasonal constraints on lamb supply are two challenges faced by the sheep industry. A potential unique solution to both challenges is accelerated lamb production. Accelerated lamb production systems reduce the interbreeding interval to less than one year, thereby potentially breaking the seasonality of lamb supply by creating multiple birth periods throughout the year (Iniguez et al., 1986; Lewis et al., 1996; Notter, 1981). Decreasing the interbreeding interval to 8 months presents an opportunity for each ewe to lamb 1.5 times per year, and reduces the proportion of time that a ewe spends in a non-productive state. Despite these promises, adoption of accelerated systems is hampered by poor reproductive performance in the sub-optimal (long day) breeding season (Iniguez et al., 1986; Lewis et al., 1996; Notter, 1981; Schoeman & Burger, 1992). Little research has been conducted on optimizing the reproductive performance nor on the nutritional requirements of accelerated lamb production systems. Field observations and preliminary findings (Rosales-Nieto, unpublished; Ehrhardt 2006, SARE report) suggest that dietary energy intake during the preconception period is an important factor influencing both litter size and conception rate in long day (spring and early summer) mating periods in accelerated lambing systems. This suggests that the nutritional status of the ewe prior to the pre-breeding period and during the previous lactation in an 8-month accelerated lambing system may play an important role in determining subsequent reproductive outcomes. Therefore the objective of this study is to better define the nutritional requirements of accelerated lambing systems in order to improve conception rate and litter size by examining the

impact of the plane of energy nutrition in both late lactation and during the ensuing period before and during ram exposure.

MATERIALS AND METHODS

Animals and study design

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Michigan State University (protocol # 03-18-038-00). Prolific, multiparous, Dorset and Polypay x Dorset ewes were studied in a design repeated over two seasons: optimal (Short, n=117) from November 1, 2018-January 26, 2019, and suboptimal (Long, n=108) from March 1 – May 28, 2018. The ewes housed at the Michigan State University Sheep Teaching and Research Unit (42.73°N and 84.5°W), were randomly assigned to one of five nutritional treatments at mid-lactation. Treatment groups were balanced for parity (parity 1 vs. parity 2 or more), body weight, body condition score (scale of 1 to 5, 1= thin, 5= fat; Russel et al. 1969), and breed (Purebred Dorset vs Dorset x Polypay). Two consecutive treatment periods ensued (Figure 1), defined as: the last 28 days of lactation (Lactation), followed by the flushing period consisting of the 21-day pre-breeding and the 34-day breeding period (Flushing; total 55 days). Ewes were allowed a 5-day dry-off period between Lactation and Flushing, where they were fed a common, low energy diet. Mean age of lambs at the commencement of the Lactation treatment period was 33 ± 0.7 days.

Lactation dietary treatments

During mid lactation (day 33 ± 0.7), ewes were assigned dietary treatments designed to provide either 70% (L), 100% (C), or 150% (H) of predicted metabolizable energy requirements according to rearing status (single or twin; three sets of triplets were recorded, 2 in Long, 1 in

Short but were not included in analysis), as defined by the NRC (1985) for this stage of lactation. The L diet was a total mixed ration (TMR) that consisted principally of cool-season grass silage (orchard grass, *Dactylis glomerata*) and soybean meal (Table 1) and each pen (single or twin) was fed once daily an amount of TMR to target 70% of daily metabolizable energy requirements according to ewe rearing status. The C diet was a TMR consisting principally of alfalfa (*Medicago sativa*) silage and corn (*Zea mays*) silage. Feed was administered once daily an amount of TMR to target the 1985 NRC requirement for the first 6-8 weeks of lactation according to rearing status (single or twin). The H diet was a TMR consisting primarily of alfalfa (*Medicago sativa*) silage and corn (*Zea mays*) silage but was further fortified with grain (dry corn grain and soybean meal) as indicated in Table 1. The H diet was fed once daily to the ewes rearing twins in an unlimited manner to allow for a minimum of 10% residual feed in order for this group to express its voluntary feed intake. The H diet was fed once daily to the ewes rearing single lambs at an amount 15% less than the weekly recorded intake calculated from the H twin rearing ewes' voluntary feed intake. The amount of feed offered to each pen was adjusted weekly based on the cumulative weight of each pen, measured weekly, as well as the ME density of the diet analyzed from a composite TMR sample taken over a 3-day period the previous week. Weekly composite samples taken over a 3-day period and were analyzed for nutritional composition. This analysis included measurement of dry matter, crude protein and additional fractions required to derive metabolizable energy concentration (non-fiber carbohydrates, ether extract, neutral detergent fiber) and was assessed by wet chemistry methodologies (Dairy One, Ithaca, New York, USA). Metabolizable energy was calculated from these fractions as described in NRC 2001.

All diets were designed to meet or exceed the protein requirements for lactation as defined by NRC 1985 and NRC 2007. At the conclusion of lactation, lambs were weaned from ewes and all ewes were placed on a common, low energy dry-off diet for a period of five days. The dry-off diet consisted of 30% dry straw, 17.5% grass silage, 17.5% alfalfa silage and 35% corn silage on a DM basis and was provided to all sheep at 2.5% of bodyweight on a DM basis. Lambs were weaned on the third day of the 5-day dry-off diet.

Pre-breeding and Breeding (Flushing) dietary treatments

At the commencement of Flushing, ewes previously on L or H treatment during Lactation were assigned to either 70% (L) or 160% (H) of energy requirements for maintenance as defined by NRC (1985). This resulted in five distinct treatment groups across season; those fed L during Lactation and Flushing (LL), L during Lactation and H during Flushing (LH), H during Lactation and L during Flushing (HL), and those fed H diet during both Lactation and Flushing (HH). C ewes were fed to 100% of predicted energy requirements for both treatment periods. The Flushing diet composition was the same for each dietary treatment group, and consisted of 25% alfalfa silage, 25% grass silage and 50% corn silage on a DM basis, as detailed in Table 1. This diet was fed at different amounts to achieve the desired target plane of energy nutrition (70%, 100% or 160% of predicted energy requirements for maintenance). The amount of feed offered each pen was adjusted weekly based on the cumulative weight of each pen recorded weekly as well as the ME density of the diet analyzed on a composite TMR sample taken over a 3 day period the previous week.

Breeding Management

On day seven of Flushing, vasectomized rams were introduced to ewes. To maintain a similar ram to ewe ratio during both non-fertile (vasectomized) and later during fertile ram exposure in both breeding seasons, ewes were grouped according to treatment in pens to create similar housing density and ewe number (5 pens total: 1 C group, 2 L groups, and 2 H groups with 19-25 ewes per pen). Vasectomized rams were rotated among pens once daily.. On day 21 of the flushing treatment, vasectomized rams were removed and replaced with fertile, mature purebred Dorset rams. Rams were rotated among pens every 12h. Fertility in breeding rams was documented by assessment of semen quality with a breeding soundness exam performed 5 days prior to mating in each season. All rams received a score of “satisfactory” which encompassed motility (>30%), morphology (>50%) , and scrotal palpation scores (Ley et al., 1990; Society for Theriogenology). Rams were fitted with marking harnesses to document mating activity. Additionally, ewes were observed for mating behavior (standing heat) for fifteen minutes immediately after introduction of rams performed every 12 h. All breeding marks and observed mating behaviors were recorded every 12 h over the 34-day fertile ram exposure period and used to calculate gestational length.

Animal Measurements

Pregnancy status, as well as litter size, and incidence of fetal loss were determined through weekly transabdominal ultrasound starting at 3 days post ram removal (37 days after initial ram introduction), and ending 42 days later. Fetal loss was measured by serial ultrasound measurements biweekly up to day 70 post-coitus (PC) and then by observations made at birth. Fetal loss, whether there was loss of single or multiples fetuses from a ewe, was counted as a

single incidence. Stillborn lambs were defined as those lambs born dead but estimated to have died within the last 24 hours of pregnancy. Fetal loss was recorded during 3 stages of pregnancy: early pregnancy day 20-69 PC, mid to late pregnancy day 69 to term, and during what was estimated to be the final day prior to birth. Cumulative fetal loss included all incidences of loss between day 20 and term, including stillborn lambs. Ewes were weighed 2 times per week at 3-4 day intervals. Blood samples were collected via jugular venipuncture twice per week for the duration of the treatment period and until the confirmation of pregnancy. Maternal body condition was measured by subjective assessment of body condition score (Russell et al., 1969) and by ultrasound measure of subcutaneous fat depth at a defined location (10 cm from midline at the 13th rib) taken using a 3.5 MHz convex transducer at the beginning and end of each treatment period.

Statistical Analysis

Data for conception rate were analyzed as binary data using a logistic regression model accounting for the fixed effects of dietary treatment and season, and the random effect of ewe (SAS 9.4, SAS Institute, Cary, NC, USA) Data for litter size was analyzed using a general linear mixed model:

$$Y_{ijk} = \mu + E_i + S_j + T_k + S_j \times T_k + e_{ijk}$$

where E_i = random effect of ewe, S_j = fixed effect of season ($j = 1$ to 2), T_k = fixed effect of treatment ($k = 1$ to 5), $S_j \times T_k$ = the interaction between season and treatment, and e_{ijk} = residual error term.

Gestation length and birth weight data was analyzed using a mixed model:

$$Y_{ijk} = \mu + E_i + S_j + T_k + R_l + R_l \times T_k + R_l \times S_j + S_j \times T_k + R_l \times S_j \times T_k + e_{ijkl}$$

where E_i = random effect of ewe, S_j = fixed effect of season ($j = 1$ to 2), T_k = fixed effect of treatment ($k = 1$ to 5), R_l = fixed effect of rearing status ($l = 1$ to 2), $S_j \times T_k$ = the interaction between season and treatment, $R_l \times T_k$ = interaction between rearing status and treatment, $R_l \times S_j$ = interaction between rearing status and season, $R_l \times S_j \times T_k$ = interaction among season, treatment, and rearing status, and e_{ijkl} = residual error term.

LS-Mean differences were separated using the post-hoc Tukey's Honestly Significant Difference. Data from ewes who were removed from the study, or who aborted prior to term, were not used for analysis on lambs born alive, gestation length, or lamb birth weight. Ewes that were removed from the study included 2 ewes whose lambs were supplemented with milk replacer by farm staff, five ewes who had mastitis, one ewe with chronic pneumonia, one ewe with BCS below 2, and three ewes died prior to the end of dietary treatment, one necropsy revealed hepatic lipidosis, and two from unknown causes.

RESULTS AND ANALYSES

Effect of Lactation treatment on body measurements

Dietary treatments were administered to target percentages of energy intake requirements for the first 6-8 weeks of lactation as defined by NRC 1985, which were calculated based on pen weights of ewes on the present study were 74 kcal ME and 85 kcal ME / kg bodyweight / day for singles and twins, respectively (2.76 and 3.14 kcal per kg of metabolic bodyweight). Specific

ingredients and composition of the diets fed are found in Table 1. The energy intake achieved across season with the C diet was close to target at 70 and 85 kcal of ME/kg BW/day for single and twin rearing ewes respectively. The energy intakes, calculated on a pen basis, achieved across season for the L diets were 46 and 53 kcal of ME/kg BW/day and for the H diets were 109 and 131 kcal of ME/kg BW/day for single and twin rearing ewes respectively. The daily energy intakes were all within 10% of target intake levels (Figure 2). Twin rearing ewes on the H diet were provided unlimited feed access allowing an estimate of voluntary dry matter intake during the lactation study period. Twin rearing ewes on the H diet had achieved an average voluntary feed intake (%BW on a DM basis) of 4.95 ± 0.06 during Long and 5.22 ± 0.07 during Short during the Lactation treatment period (day 34-52 of lactation).

These dietary treatments resulted in significant ($P < 0.01$) differences in maternal body weight change during Lactation across season, regardless of rearing status, with a loss of 7.8 kg for L, a loss of 2.5 kg for C and a gain of 7.5 kg for H treatment groups (Table 2). Consistent with these body weight (BW) changes, dietary treatments produced significant ($P < 0.01$) changes in both body condition score (BCS) and subcutaneous back fat depth (BF) during Lactation, with H ewes gaining weight and body condition, C ewes showing modest reduction in weight and condition, and L ewes losing weight and body condition. These body weight and body condition score changes differed subtly according to both rearing status and season (Table 2 and Figure 4). With H ewes in Short exhibiting slightly more body condition than in Long. Overall, within season, L groups lost greater BCS and BF than C groups and H groups exhibited the greatest gain in both BCS and BF across both seasons ($P < 0.05$ for all comparisons). There was however, a slight but significantly greater loss of both BCS and BF in lactation in both L and C groups regardless of rearing status in the Short as compared to the Long day breeding season ($P < 0.05$).

Effect of Flushing treatment on body measurements

Target energy intakes for C ewes (100% maintenance) was 31 kcal of ME/kgBW/day, calculated using pen weights and average pen intake data, measured weekly throughout the treatment period. An entirely forage-based silage diet was fed at different amounts to achieve the desired daily energy intakes targeted by the three dietary treatments (Table 1). The calculated energy intakes recorded were consistent between seasons (Figure 5) however, the energy intake for all groups exceeded the respective target levels at 80%, 110%, and 177% of maintenance for L, C and H, respectively.

The dietary treatments applied during the Flushing period resulted in consistent changes in bodyweight across season with the magnitude of response for the L and H diets dependent on the diet fed during the preceding lactation period (Figure 6). Maternal bodyweight change during the Flushing period was uniform across season, but differed substantially according to treatment ($P<0.01$) with C ewes losing 1.8 kg. Ewes fed 70% of maintenance requirement lost either 9.2 kg (LL) 15.8 kg (HL). Ewes fed 160% maintenance requirements gained either 7.4 kg (LH) or maintained weight, losing 0.1 kg (HH). Changes in BCS and BF during flushing, according to nutritional treatment, were consistent with clear nutritional treatment impacts ($P<0.01$). HL ewes exhibited the greatest loss in body condition and adiposity, followed by LL ewes. CC ewes exhibited a modest increase in BCS. LH ewes displayed the greatest increase in BF and BCS, and HH ewes showed a modest increase in both BCS and BF (Table 3; Figure 6). There was also a seasonal impact of dietary treatment on BCS and BF ($P<0.01$). LL and HL ewes lost more BCS and BF (Figure 6; Table 3) during the Long day season, while LH and HH ewes gained more BCS and BF during Long than in Short (Table 3).

Cumulative impact of diet during lactation and flushing on BW, BF, and BCS.

Examining the cumulative impact of the dietary treatments across both the lactation and flushing periods on bodyweight changes across season revealed that HH ewes gained 7.0 kg whereas all other treatments lost weight (C: -4.3 kg, LL: -16.6 kg, LH: -0.76 kg, HL: -7.73 kg; Table 3).

There were seasonal differences in cumulative dietary impact on bodyweight change evident as well (Figure 1.6), but these were quantitatively small, with LL and LH ewes losing more weight during Short ($P < 0.05$), HL ewes losing less weight and HH ewe gaining less weight than their respective treatment groups in Long ($P < 0.05$). Overall, the cumulative change in BCS and BF (Figure 1.6) mirrored each other according to treatment and season. Still, cumulative gains in BCS and BF were greater during Long season as compared to Short ($P < 0.05$).

Reproductive Outcomes

Conception Rate and Litter Size

A total of 226 (Short $n = 109$, Long $n = 117$) ewes were analyzed for reproductive outcomes.

Overall, conception rate was high in both seasons, with an average of 94.5% across season (Table 1.4). Conception rate did not differ significantly among treatment groups, nor between seasons and ranged from 86-100% for all treatment groups and seasons. (Figure 8).

Litter size (Figure 9) at 69.9 ± 0.4 days post-coitus did not differ according to nutritional treatment within season. There was, however, a large impact of season on litter size ($P < 0.01$), with litter size across treatments, averaging 1.72 lambs per ewe during Short and 1.46 lambs per ewe during Long. A linear regression analysis revealed a significant relationship between body condition during lactation on litter size, with litter size increasing with increased body condition score ($P < 0.05$). Similarly, the number of live lambs born per ewe was not impacted by

nutrition and was greater in Short (1.70 lambs) as compared to Long (1.40 lambs; $P<0.01$). Cumulative fetal loss recorded over the entire pregnancy showed a strong tendency ($P=0.06$) to be greater during Short (20 events per 109 ewes) as compared to Long (11 events per 117 ewes; Figure 10). From day 0-70 PC there were 6 incidences of loss in Short and 13 in Long. Few lambs were lost from day 70 to term (2 in Short vs. 2 in Long). There were 3 stillborn lambs in Short and 5 in Long.

Gestational length

Gestational length data are displayed in Figure 1.10. Gestational length was not impacted by nutritional treatment but did vary according to season ($P<0.01$). When controlled for litter size, ewes conceiving in Long had shorter pregnancies (145 ± 0.2 days) than those conceiving in Short (147 ± 0.18 days; $P < 0.05$). Twin pregnant ewes tended to have shorter gestational lengths (145.6 ± 0.2 days) than ewes pregnant with singles (146.2 ± 0.2 days; $P = 0.06$; data not shown).

Birth weight and lamb mortality

Birth weight data are displayed in Figure 12. Birth weight differed according to season ($P<0.01$) with lambs conceived in Short (5.1 ± 0.08 kg) weighing more than those conceived in Long (4.6 ± 0.07 kg), after accounting for the impact of season on litter size. As expected, birth weight was different according to sex ($P<0.01$) and litter size ($P<0.01$). Ewe lambs were smaller, (4.9 ± 0.07 kg), than ram lambs, (5.2 ± 0.08 kg, $P<0.01$). Lambs born as singles, (5.4 ± 0.09 kg), were larger than those born as twins, (4.6 ± 0.05 kg, $P<0.01$). Lamb mortality was recorded for the first two weeks postnatally and was less than 5% across season and did not differ between seasons. There were 5 mortalities out of 133 lambs born for Long and 4 mortalities out of 176

lambs born for Short over this 2-week period, with no differences noted according to season or nutritional treatment.

DISCUSSION

Accelerated lamb production systems pose a unique opportunity to both increase year-round lamb production as well as lambs born per ewe per year. The adoption of this production system in the sheep industry however has been hampered by low conception rates and reduced litter sizes in the suboptimal (late summer and fall) breeding seasons (El-Saied et al., 2006; Iniguez et al., 1986; Lewis et al., 1996; Notter, 1981). There were no significant effects of altered plane of energy nutrition during Lactation or Flushing on reproductive performance in an 8-month accelerated lamb production system. In contrast, there were significant seasonal differences in litter size, gestation length, and lamb birth weight.

Effects of dietary treatment on nutritional status

Lactation

Dietary treatments during lactation were designed to provide either under or overnutrition during day 34-52 of lactation, relative to established feeding standards (NRC 1985) with the C diet designed to match the energy intake recommendations. This period coincided with the last 28 days of lactation in accelerated production because the lactation period of accelerated production is generally shorter (6-10 weeks) as compared to annual production (10-14 weeks). The energy intake of the L nutritional treatments in this study within 2% of those recommended to meet the requirements of ewes during lactation according to rearing status (single or twin) by NRC 2007, and as such, can provide insights into the suitability of these requirements for sheep in an accelerated lamb production system. The C treatment ewes lost body weight and body condition

during lactation during both seasons. Predicted energy requirements for this period of lactation outlined by NRC (1985) predict that ewes suckling singles will lose 25 g per day, and after a 28 day period should have lost 0.7 kg, while those ewes raising twins are predicted to lose 60 g per day with a cumulative loss of 1.7 kg over this period. The single rearing ewes lost 2.0 kg and the twin rearing ewes lost 3.0 kg, which are slightly more than NRC (1985) predictions. This may be explained in part by a higher level of milk production by our study ewes compared to that modeled for NRC predictions. NRC estimates that a ewe suckling twins will produce 2.6 The NRC (1985) cites the work of Jordan and Hanke (1977), who fed approximately 85% and 120% of the predicted energy requirements for lactation during weeks 4-8 of lactation in an annual system. This study found that there were no significant repercussions on lamb growth by reducing maternal energy intake during this period, however both groups of ewes indeed lost body weight, the ewes fed lower energy lost 4 kg, while those fed 120% of their predicted energy requirements lost only 0.32 kg. This level and timing of feeding are similar to ewes in the present study, and the amount of body weight change was similar between the studies, however C ewes in the present study lost 2.5 kg, and L ewes lost 7.8 which is a slightly larger loss of body weight than those found by Jordan and Hanke. It is difficult to compare the effect of rearing status on patterns of body weight change between these studies, as Jordan and Hanke did not report changes based on rearing status, Despite the loss of body weight in lactation for C ewes, retention of BCS and BF was observed, with only very small decreases over the 28-day treatment period, suggesting that large deficits in energy balance were not observed. Conversely, a gain of 0.37 units of BCS and 0.11 mm of BF was found in H ewes, and a loss of 0.33 BCS units and 0.11 mm BF was found in L ewes. This indicates that a state of negative energy balance was induced by the L diet, and adipose tissue was mobilized to maintain milk production, whereas in

H ewes, adipose tissue accretion occurred, suggesting that the H diet induced a state of positive energy balance to sustain lactation energy requirements and gain body condition and adiposity.

The NRC denotes the use of 1.74 kg of milk per day for ewes suckling singles and 2.60 kg per day for ewes rearing twins. Preliminary findings from milking a subset of ewes on this study indicate that single rearing C ewes at mean day 34 of lactation produced, 2.32 kg per day while those rearing twins yielded 2.63 kg per day (data not shown). H ewes rearing singles gained 5.2 kg and while those rearing twins gained 7.8 kg which is a significant amount of weight gain for lactation, and indicative of a state of positive energy balance as further evidenced by positive gains in both body condition and the thickness of back fat in these ewes. Conversely, L ewes which were consumed a dietary energy intake nearly equivalent to that recommended by NRC 2007 lost 7.8 kg during this period along with a substantial loss of body condition and back fat indicating a period of significant negative energy balance. This indicates that recommendations for energy intake outlined in NRC 2007 fall short in meeting energy requirements for this period resulting in weight loss and one would predict depressed milk production.

Flushing Period

Ewes in an 8-month accelerated lamb production system with roughly a 60-day lactation period have approximately three weeks between weaning of lambs to the introduction of fertile rams, and the commencement of the breeding season. This three-week period may be critical for regaining body condition lost during lactation to enable a return to reproductive cyclicity, as well as to potentially increase ovulation rate and ensure embryonic survival. The C dietary treatments during the pre-breeding (21-day) and subsequent 34-day breeding season were designed to match maintenance energy intake requirements (NRC 1985 and 2007), which

suggest that ewes should maintain body weight with the exception of a small increase due to wool growth. C ewes in our study consumed energy is slight excess of maintenance requirements during Flushing yet lost a modest amount of weight (1.8 kg across season). This may be explained by the fact that ewes this accelerated production system had just completed a 54-day lactation period and had not fully reached a steady state of basal metabolism consistent with maintenance. This suggests ewes in accelerated production may need additional energy over that of ewes that have fully recovered from lactation in terms of energy needs during the pre-breeding period. HH ewes, fed over 50% greater than maintenance energy requirements during Flushing lost -0.1 kg during the last 8 weeks of this treatment period indicating that they had adapted to a high level of feeding and reached a static level of energy metabolism (McCann et al., 1992). According to the NRC (1985), ewes subjected to a flushing protocol should gain 100 g per day, and at the end of a 55-day treatment period, ewes should have gained 5.5 kg. Despite this, after the initial weight gain during the lactation treatment, HH ewes only maintained body weight during the flushing protocol, however ewes fed the L treatment during lactation followed by H treatment during flushing (LH) gained a 7.4 kg. This increase in body weight over that predicted by NRC (1985) for Flushing may be explained in part by the fact that our ewes consumed 1.73x maintenance which is approximately 10% more than recommended by NRC (1985). Ewes subjected to chronic undernutrition, such as those on LL, lost a significant amount of body weight (16 kg) and body condition (0.45 units) indicating that they were in a chronic state of energy insufficiency. In our trial, overall, ewes fed to the NRC (1985) requirements for lactation and maintenance lost 4.3 kg across season but largely maintained body condition score and backfat thickness. Collectively these data suggest that higher energy intake

than that suggested by NRC (2007) during lactation and flushing are required to replenish energy reserves prior to mating in an 8-month accelerated lamb production system.

Effect of nutritional status on reproductive outcomes

Conception Rate

In this study, there were no significant impacts of dietary energy intake on ability to conceive in either the long or short-day season. However, previous studies have reported that undernutrition in ewes prior to breeding can affect reproduction in both the long term and short term. For instance, severe long term undernutrition in sheep can cause anorexia nervosa and can lead to inhibition of GnRH pulsatility, resulting in anovulation as well as anestrus, however the extent and duration of undernutrition necessary to impede reproductive cyclicity is not well documented (Kendall et al., 2004). In contrast, Abecia et al (2006) detailed inconsistent findings by several studies providing 50% maintenance requirements prior to breeding on oocyte quality, number, and embryo survivability. Much of this variability may have been due to the duration of underfeeding, and that most experimental models were examining super-ovulated ewes rather than ewes in a natural mating system. Despite these inconsistent findings, underfeeding Merino cross ewes at 70% of predicted maintenance requirements for 60 days prior to breeding in an annual production system did not negatively affect conception rate compared to those fed 100% maintenance (Edwards & McMillen, 2002). The study conducted by Edwards & McMillen found that feeding 70% of predicted energy requirements resulted in 4% loss in bodyweight, however in the present study, feeding at 70% of predicted maintenance requirements periconceptionally resulted in body weight losses of 12% for LL ewes and 20% for HL ewes. These differences may be explained by the fact that the prior study did not involve lactation,

whereas the present study included the last 28 days of lactation, which demands much more energy than maintenance. In a more recent study, the level of nutrition prior to breeding, post breeding, and the additive effects of chronic underfeeding pre and periconceptionally was evaluated by Macias-Cruz et al. (2017) in 48 Katahdin cross ewes. Providing 60% of maintenance requirements either 30 days pre-conception, 50 days post-conception, or nutrient restriction during both periods had no effect on conception rate. However, due to the small sample size of 48 ewes, drastic effects on conception rate would have been necessary to detect a significant difference among treatment groups. Nevertheless, the results of the present study confirm these findings, in that conception rate was not negatively impacted by chronic underfeeding at 70% of predicted energy requirements for a period of 83 days. An innovative aspect of this study is that its design allowed us to look at the effect of both static (chronic) versus more acute and dynamic changes in energy status on reproductive outcomes. In the literature, static effects of body weight and body condition on conception rate have been found, and suggest that higher body weights are correlated with increased conception rate (reviewed by Kenyon et al., 2014). The present experiment affected nutritional status statically in sheep exposed to prolonged undernutrition (LL), and dynamically in treatment groups such as HL and LH with short term changes in nutrient intake just prior to mating. Despite creating marked changes in energy status both statically and dynamically, our treatments did not impact the ability to conceive in either the optimal or suboptimal mating season. Contrarily, Gunn et al. (1972) found reduced conception rate in ewes with lower BCS at mating, however, average BCS for these ewes was 1.5, whereas the average BCS of LL ewes on the present study was 2.6. In the present study, there were no effects of undernutrition on conception rate. This may be explained in part by the fact that ruminants are well equipped with homeostatic mechanisms to

cope with short term reductions in energy intake via alterations in insulin action that spare glucose for central metabolism from peripheral use and also by use of catabolic substrates such as amino acids and glycerol as gluconeogenic substrates. (Pettersson et al 1993; Chilliard et al., 1998)). These metabolic adaptations may explain why short periods of undernutrition have little consequence on conception rate in sheep (Clarke & Arbabi, 2016; Scaramuzzi & Martin, 2008, Downing and Scaramuzzi 1991). The capacity of the ewe to suffer little change in glycemia during periods of short term energy insufficiency may result in relatively unchanged signaling of energy status to the various elements of the hypothalamic-pituitary-gonadal axis that control conception rate (Scaramuzzi & Martin, 2008; Scaramuzzi et al., 2006; Scaramuzzi et al., 2004). Mobilization of subcutaneous adipose tissue stores, as seen in ewes in LH and HL treatments, could have compensated for underfeeding prior to breeding. Severe and prolonged undernutrition will create hypoglycemia and hypoinsulinemia, and depressed IGF-1 resulting in inhibitory effects on reproduction via the hypothalamic-pituitary-gonadal axis. Studies on the effect of IGF-1 on reproduction in the dairy cow have indicated that IGF-1 and its binding proteins are important for follicular growth and recruitment, and influences estradiol secretion by dominant follicles (Llewellyn et al., 2007) and thus, severe undernutrition prior to conception can have direct effects on reproduction. Severe undernutrition thus affects a ewe's ability to maintain normal patterns of follicular growth, estradiol production, and in some cases can lead to anovulation (Scaramuzzi et al., 2006). The intensity and duration of underfeeding in this experiment may not have been severe enough, however, to dampen reproductive success, regardless of day length. Further insight into this would be gained by an evaluation of the metabolic and endocrine status of these ewes gained by evaluation of circulating hormones such as progesterone, FSH, and estradiol as well as metabolites including glucose, insulin, and IGF-1.

Litter Size

A nutritional flushing response (increasing ovulation rate by increasing plane of nutrition prior to breeding) was not achieved in this experiment, despite exceeding flushing requirements defined by the NRC (1985) by 10%. Additionally, expected weight gains predicted by the NRC were also exceeded by those ewes on the LH treatment group, yet no flushing response was detected (100 g/d *versus* 135 g/d). Ewes fed the C diet consumed slightly higher than projected energy requirements for maintenance, and roughly 2 kcal/kgBW, or 5% higher than the flushing protocol outlined in NRC 2007 however, no flushing response was detected. Mechanisms of nutritional flushing are not well understood, though it is clear that both acute and dynamic changes in energy status play a role in this response. Infusions of intravenous glucose (50 mmol/h; Munoz-Gutierrez et al 2002) for five days in addition to a straw diet were proven to increase ovulation rate in ewes. Additionally, supplementation of 750 g lupin grain daily for a period of four days was found to increase the number of twin versus single ovulations in ewes by Stewart and Oldham (1986). Several models seeking to describe the underlying mechanisms of nutritional flushing suggest that ewes must in a state of positive energy balance during the flushing period. This includes ewes transitioning from either negative energy balance or maintenance prior to the flushing period just before mating (Downing & Scaramuzzi, 2019; Scaramuzzi et al., 1993; Scaramuzzi et al., 2006). A flushing effect can be evoked in either acute or dynamic positive energy balance. This was achieved in the LH treatment group, in which ewes gained 7.4 kg over the treatment period in this experiment, however despite this no increase in litter size was found. Studies into flushing in prolific breeds of sheep found that there was a significant increase in ovulation rate by 30% when ewes were fed 220% vs 160% of predicted energy requirements for maintenance for 6 weeks prior to mating (Lassoued et al., 2004). These

findings indicate that we may not have increased energy enough prior to mating to see a flushing response in the present study. Despite this, due to the extended period (49 days prior to and 34 days after fertile ram introduction) of overnutrition seen by ewes on the HH diet may have resulted in a point of static positive energy balance given the fact that these ewes lost 0.1 kg during the flushing treatment across season. Static positive energy balance is defined as elevated but static body weight in response to overnutrition, which could have prevented the physiological response necessary to see an increase in ovulation rate (and thus litter size). Though we achieved large differences in ME intake among treatment groups, we did not detect differences in litter size among treatment groups. Contrary to our results, early studies into the static effects of body condition score on litter size was reviewed by Kenyon and colleagues (2014). The authors suggest a positive curvilinear response in ovulation rate to BCS, and that there is an increase in ovulation rate with increased BCS. However, the marginal increase in ovulation rate decreases as ewes gain body condition, and it is suggested that at a certain BCS, ovulation rate can no longer be increased with elevated nutrition. Our study results were inconsistent with these findings as HH ewes did not have an increased litter size over ewes with lower BCS present in the study, such as the LL ewes, ~3.75 units vs. 2.75 units, respectively. A potential explanation for this inconsistency could be explained by the negative impact of overfeeding during the implantation period found by Parr (1987), Lassoued (2004), and Annett (2006). These studies suggest that overfeeding during the peri-implantation period decrease circulating progesterone concentrations by increased metabolic clearance of progesterone, negatively impacting fertility and prolificacy.

Flushing protocols are designed to elevate plane of nutrition above maintenance, just prior to breeding, however, in an accelerated system, there is a short window between weaning

and the subsequent breeding period, whereas in an annual system, there are typically several months in which ewes are kept at maintenance feeding during this period. Because of this short window in accelerated systems, ewes must transition to a period of neutral or negative energy balance during lactation to the breeding period in a short time period which may necessitate a larger increase in energy intake than indicated in standard flushing protocols to elicit a response. Further investigations into timing and duration of increased energy intake prior to mating are necessary to understand the flushing requirements of accelerated lamb production systems. The sensing of energy status by the brain and/or ovary via peripheral signals are thought to elicit a flushing response during periods of dynamic change in energy status. Candidate peripheral signals include circulating metabolites and hormones such as glucose, insulin, IGF-1, and leptin, (Kendall et al., 2000; Downing et al., 1999; Scaramuzzi et al., 2019; Dupont et al., 2014; Munoz-Gutierrez et al 2002). Evaluation of these hormones and metabolites in this study may provide additional insight into whether or not sufficient changes in these putative signals were evoked to elicit a flushing response. Additionally, the positive correlation between body condition during lactation, regardless of dietary treatment, on litter size suggests that body condition prior to weaning is important to ensure maximized genetic potential for prolificacy in an accelerated lamb production system.

Fetal Loss

Peri-implantation nutrition is important for embryonic survival (Guillomot et al 1981; Rickard et al 2017; Parr et al., 1987; Parr et al., 1992). The critical period for embryo survival in sheep is from day 0-14 PC (Dixon et al., 2007). An experiment conducted by Parr and colleagues (1987) provided either 25%, 100%, or 200% of maintenance requirements, and saw decreased conception rate and increased incidence of embryonic loss in the overfed ewes when compared

to maintenance and underfed ewes. Ewes in the present study were subjected to underfeeding consuming 82% of maintenance energy intake and overfeeding consuming 172% maintenance energy intake, yet no significant effect of nutritional treatment on fetal loss was detected.

Lassoued et al (2004) investigated the differential response to dietary energy intake on conception rate, ovulation rate, and fetal loss in three breeds of sheep. Three different breeds of varying levels of prolificacy were provided either 100%, 160%, or 220% maintenance for three weeks prior to and 6 weeks after mating and found that an increase in ovulation rate with 220% maintenance energy intake only occurred in breeds genetically predisposed to higher ovulation rates. Additionally, Lassoued and colleagues found that there was a higher incidence of embryonic loss in prolific ewes, and a tendency for more embryonic loss in ewes fed at 220% maintenance than those fed to maintenance requirements. There were no notable effects of dietary treatment on fetal loss in the present study, however analysis of plasma progesterone concentrations at the time of mating may provide additional insight to understand if dietary treatment may have had the potential to alter metabolic clearance of progesterone. A limitation of this study is the inability to detect incidences of embryonic loss prior to day 30 PC. The use of transrectal ultrasonography to count corpora lutea and incidences of early embryonic death may provide a clearer estimation of the effects of maternal dietary treatment.

Lamb birth weight

We found no significant effect of maternal dietary treatment pre-and periconception on lamb birth weight. In contrast to our findings, previous investigations into maternal nutrition on lamb birth weight have revealed minor effects of under and overnutrition in early pregnancy on birthweight (Roca Fraga et al 2018). A meta-analysis conducted by Roca Fraga and colleagues (2018) found that undernutrition in early pregnancy had very minor effects on birthweight, but

that those differences were reconciled if maternal nutrition was subsequently elevated to meet pregnancy requirements in mid and late gestation. This study failed to quantify the extent of maternal undernutrition necessary to impact lamb birthweight. Conversely, Gardner and colleagues noted that there were no significant effects on lamb birthweight due to maternal over or undernutrition. The results of the present study confirm these results, as there were no detectable effects of maternal diet on lamb birth weight, regardless of season.

In the present study male lambs were 6% larger than female lambs regardless of litter size, and that twin lambs were 10% smaller than single born lambs, regardless of sex. Differences in birth weight by sex and litter size are well documented in literature (Gardner et al 2007; Robinson et al 1977; de Zegher et al 1999). These studies have concluded that male lambs are born significantly heavier than female lambs, regardless of litter size, and that twin lambs are significantly smaller than single born lambs. Gardner (2007) found that male lambs were twin lambs were 10-22% of the size of single born lambs on average, and that males were 10-15% larger than female lambs, regardless of litter size. The sexual dimorphism in prenatal growth that we observed was less as was the difference in litter size. This may be explained in part by differences in genetics and feeding. In our study, ewes were fed carefully during late pregnancy according to litter size which may have minimized differences in prenatal nutrient supply between singles and twins.

Effect of Season on Reproductive outcomes

Conception Rate

Overall, conception rate was high in both seasons (mean 94.5%; range of 86-100%) especially within the context of an accelerated lamb production system utilizing exclusively natural reproduction. Conception rates were markedly higher than those reported by other investigations

into accelerated lamb production systems (Lewis et al., 1996; Notter and Copenhaver 1980, Wheaton et al., 1992), especially in the long day season. Notter and Copenhaver (1980) reported conception rates of 90% for short day and 53% for long day breeding season in Finn, Rambouillet, and Finn x Rambouillet cross ewes in an 8-month accelerated lamb production system over a 5-year period. Lewis and colleagues (1996) reported much lower conception rates in the long day season (21%) in the Cornell STAR accelerated lambing system in Dorset ewes. Notably, in this analysis, STAR system ewes only gave birth 0.98 times per year, despite the opportunity given for ewes to give birth 1.67 times per year. The results of the present study are comparable to those found in ewes managed within an 8-month accelerated lambing system under a defined photoperiod control regime or given exogenous hormone therapies (controlled internal drug release of progesterone plus PMSG) reported by Cameron et al (2010), Malpaux et al (1997). Cameron et al (2010) found that under intensively managed alternate 4-month long and short-day light protocols, that conception rates averaged just under 92% across season, and 76% for those not exposed to photoperiod manipulation protocols, whereas in the present study, mean conception rate across season and dietary treatment was 94.5%. Investigations by Wheaton et al., (1992) into the use of intravaginal progesterone CIDRs in combination with the ram effect in an 8-month lamb production system found that 91% of ewes conceived in the short day and 86% conceived in the long day season. Our results indicate that it is possible to achieve high conception rates regardless of breeding season, without the use of extensively managed lighting protocols or exogenous hormone therapies.

Therefore, the high conception rates in the long day season despite chronic underfeeding, found in these results could be attributed to intensively managed breeding protocols, including several factors such as the use of vasectomized teaser rams which effectively synchronized ewes

prior to fertile ram introduction. Socio-sexual interactions in sheep have proven to be a powerful way to manipulate reproductive activity in sheep (Rosa et al., 2000; Ungerfeld, 2012; Ungerfeld & Bielli, 2012). The ram effect refers to introducing rams to a previously isolated group of ewes so as to advance the breeding season and induce ovulation in response to olfactory and auditory cues among ewes and rams (Martin & Scaramuzzi, 1983). The ram effect has been proven extensively in the literature to hasten the onset of estrous in seasonally anestrous ewes, and synchronize reproductive cyclicity in ewes during the both breeding seasons (Delgadillo et al., 2009; Fabre-Nys et al., 2015; Martin & Scaramuzzi, 1983; Ungerfeld et al., 2005). It is suggested that the success of the ram effect is dependent on season, with ewes further away from the breeding season being less responsive than those on the cusp of the breeding season (Martin et al., 1981). In the present study, rams were isolated from ewes until the commencement of the pre-breeding treatment period, during both long and short-day seasons. In the long day period, day length is steadily increasing, and ewes are several months away from the transition to the traditional breeding season found in late summer. Regardless, synchronization of ewes by the introduction of teaser rams was successful in both long and short-day seasons, given that 92% of ewes conceived during the first cycle post-fertile ram introduction.

Studies into breed (Rosa et al., 2000, Scott and Johnstone 1994) have revealed that polled Dorset rams are more effective in stimulating ewes than sires of other breeds, regardless of season, and that ewes with a shorter anestrous period are more receptive to stimulation by the ram effect (Rosa et al., 2000). In the present study, polled Dorset rams and Dorset x Polypay ewes were used, and based on the literature, should be more apt to respond to the ram effect, and advancement of the breeding period. The majority of ewes (92%) in the present study conceived within the first cycle after fertile ram introduction across season, and there was no

significant seasonal effect on timing of conception, further pointing to successful synchronization by vasectomized rams prior to the introduction of fertile rams.

Not only does the sudden introduction of rams to anestrus ewes prove to advance the breeding season and synchronize ovulation (ram effect), but exposure of anovulatory females to ovulatory females may also induce ovulation in noncyclic ewes (Nugent and Notter 1990; Sunderland et al., 1990). Because of this female-female interaction, a portion of ewes in the present study who were noncyclic may have resumed cyclicity due to exposure to cyclic pen-mates, thus further improving the number of cyclic ewes during the mating period. There is evidence in sheep that suggest that competition among breeding rams leads to suppression of libido (Price et al., 1991). This competition was eliminated in our study by housing rams separately. However, frequent rotation of rams (12-h intervals) exposed rams to new pens of ewes as performed in this study could potentially contribute to increased ram libido (Gonzales et al., 1988), leading to higher conception rates across seasons. In addition, rams were managed such that ram to ewe ratios were not greater than 1 ram per 25 ewes. Bryant and Tompkins (1975) reported that, when rams were assigned between 6-30 ewes per ram, the timing of first service and the efficacy of service declined as number of ewes increased. Despite our ram:ewe ratio being on the higher end of this study, a high degree of breeding activity and conception was achieved. It is not known whether the resumption of reproductive cyclicity was due to the ram effect, female-female interaction, or frequent exposure of rams to new ewes in the present study. Plasma progesterone concentrations throughout the pre-breeding period are necessary to elucidate this.

Litter Size

Season of conception proved to influence litter size, regardless of dietary treatment with a reduction evident in the long day season. This could be explained by a reduction in ovulation rate

and/or increased fetal loss. Our findings were consistent with those of Lewis (1996), Notter and Copenhaver (1980), and others. Notter (2000) found that Polypay ewes mated in spring and summer exhibited reduced litter sizes by 0.31 lambs when compared to fall mating seasons. These findings are in line with the 0.28 lamb reduction in spring versus fall lambing found in the current study.

One contributing factor to reduced litter sizes in the long day breeding season could be increased incidence of embryonic and fetal loss. Shi et al (2015) reported higher ovulation rates in ewes super-ovulated in May versus September, but higher viability of embryos in September versus May. In this experiment, ewes bred in the spring displayed a strong tendency for greater fetal loss, regardless of dietary treatment. Higher numerical differences in fetal loss for all measured time intervals were observed, however these were not significant. Despite this, a tendency for higher incidence of fetal loss was found overall, when accounting for the total number of lambs lost between conception and term, including stillborn lambs. In the present study, we detected a strong trend ($P=0.06$) of a seasonal influence on fetal loss with 9% of ewes in the short-day season and 15% of ewes in the long day season exhibiting fetal loss. Dixon and colleagues used serial ultrasound measurements in early pregnancy to determine patterns of embryonic and fetal loss in ewes that conceived in either the optimal or suboptimal mating season, and found that in 19% of ewes experienced embryonic or fetal loss prior to term in the optimal season (Dixon et al., 2007). They also revealed no effect of season of conception on patterns of embryonic or fetal loss. Another study conducted by Pope et al (1989) investigated the incidence of early embryonic loss and its interaction with season in Polypay, Dorset, St. Croix, and Targhee ewes. Notably, there was a significant breed by season interaction in this study, Targhee showing a much higher incidence of embryonic loss in the autumn season when compared to the other

breeds. Additionally, there was a marked seasonal impact on the incidence of embryonic loss, with ewes displaying higher incidence of loss in spring matings than fall matings, in all breeds except Dorset. Potential clues into the seasonal effect on embryonic loss may be gleaned from the work of Jenkinson et al. (1995) who found evidence for less extensive placentation in ewes conceiving during long day periods. Jenkinson and colleagues studied pregnant ewes at day 140 of gestation and measured placental weight, number of placentomes, and number of occupied vs. unoccupied caruncles, and found that ewes bred in the natural mating season had heavier placentae, as well as a higher ratio of occupied to unoccupied caruncles, as well as more placentomes overall.

Gestational Length

Typical gestational length in sheep varies from breed to breed, with terminal breeds raised for meat production having shorter gestational lengths than fine wool breeds (Tilton 1964), and larger mature size breeds having shorter gestations than smaller framed breeds (Terrill and Hazel 1947). Other factors that influence gestation length are parity, breed of sire, and litter size (Anderson et al 1981). Breed of sire, parity, and litter size were all controlled for in the present analysis, which revealed seasonal differences in gestational length (145 days in Long vs, 147 days in Short). Terrill and Hazel (1947) concluded that season affected gestation lengths, and that gestation length decreased at a steady rate with the advancement of the breeding season. Wheaton and colleges found that gestation length was reduced by 2 days in ewes bred in March versus ewes bred in December (Wheaton et al., 1992). Jenkinson et al., (1995) found that ewes lambing in fall or winter had significantly shorter gestational lengths than those lambing in spring or summer. This study suggested that gestational length differences could be attributed in differences in placental development and ultimate size (Jenkinson et al., 1995), as previously

described, which also lead to smaller lamb birthweights in autumn and winter birth periods. Our findings align with the findings of Jenkinson (1995), in that both lamb birth weights and gestational lengths were reduced in the long day (autumn) breeding season. These findings suggest that differential placentation between seasons may play a role in determining the length of pregnancy in sheep.

IMPLICATIONS AND FURTHER DIRECTIONS

The present study indicates that moderate increase and decrease of plane of energy intake relative to those recommended in current feeding during both lactation and the ensuing flushing period do indeed invoke significant changes in energy status and adiposity. These changes in energy status and adiposity were present in both long and short day breeding periods in our multiparous ewes on an accelerated production system. These changes in energy status however were insufficient to alter reproductive outcomes regardless of season. The absence of the effect of plane of nutrition during the flushing period on litter size in particular, was surprising given the large body of literature indicating that energy status during this period can have a large impact on ovulation rate and subsequently litter size (Downing & Scaramuzzi, 1991; Scaramuzzi & Martin, 2008). Current models seeking to understand this flushing effect suggest that dynamic changes in energy status are more important than static differences in invoking this effect (Blache et al., 2006; McCann et al., 1992; Scaramuzzi et al., 2006a) In the present study, a group of ewes (LH treatment) were fed a low plane of nutrition during late pregnancy and then a high plane of nutrition during the flushing period to meet current feeding standard recommendations (1.6 times maintenance; NRC 1985). The LH treatment ewes clearly exhibited dynamic changes in energy status in both seasons, yet their litter sizes were not

significantly greater than other treatment groups. This suggests that either the magnitude or timing of energy changes was not sufficient to invoke a flushing response in prolific multiparous ewes on an accelerating lambing system. Furthermore, this indicates current and past NRC standards (either 1985 or 2007) for energy intake during the 3-week pre-breeding period are not adequate to invoke a flushing response in prolific, multiparous ewes in an 8-month accelerated lambing system.

The reproductive outcomes in general and particularly, the conception rates of ewes during the less optimal, long-day breeding period were high, and remarkably not different between seasons in this natural mating system. While there was a significant impact of season on litter size, the high conception in the long day season regardless of dietary treatment were notably higher than reported in the literature on accelerated lambing systems (Iniguez et al., 1986a; Lewis et al., 1996b; Notter, 1981a; Wheaton et al., 1992). This was particularly notable given that a natural mating system was utilized in this system and this flock been on this management system for a mere 3 years with little time for intensive selection for long day breeding success. We speculate that the intensive breeding management utilizing the ram effect, low ram to ewe ratios and frequent movement of rams between pens (every 12 hours) may have contributed to this high rate of conception during the long-day breeding season by increasing both ram libido, ewe receptivity, and socio-sexual interactions between rams and ewes. Given this high rate of conception, further studies are warranted looking at the effect of the presence of teaser rams on the induction of ovulation in the suboptimal season, as well as the effect of frequent exposure of rams to new ewes on libido and ewe receptivity to optimize this response. These results indicate that it is possible to achieve a high rate of conception without the use of exogenous hormones or photoperiod treatment in an 8-month accelerated lambing system.

Clear seasonal impacts were evidenced in this study on various aspects of reproductive outcomes including litter size, gestational length, lamb birth weight and fetal loss. These outcomes were all less optimal in long day as compared to short day mating periods. We did not measure ovulation rate or aspects of implantation/placentation in the present study however, our findings are consistent with literature in sheep suggesting that these are reproductive processes that may be compromised in the long-day breeding seasons, even in ewes capable of breeding throughout the year, such as those in the present study. A closer look at the endocrine status of these ewes would reveal greater insight as the underlying basis for the seasonal impacts on reproduction observed. Analysis of plasma progesterone concentration prior to mating is necessary to determine the exact timing of ovulation relative to the onset of the flushing dietary treatment and the introduction of teaser rams. Additionally, the analysis of plasma concentrations of various hormones and metabolites known to affect ovulation rate in sheep, such as glucose, insulin, leptin, and estradiol may aid in the understanding of seasonal differences in the response to nutritional flushing. It is important to understand the interaction between season and nutrition with respect to reproductive performance in an intensively managed system. These analyses would also provide an important understanding of the role of the ram effect in potentially inducing ewes into estrus following lactation and how this might be impacted by season. We currently do not know if modestly seasonal ewes such as those found in the present study exhibit an anestrus period during periods of increasing day length and how this relates to lactational status. In summary, a greater understanding of the management of the ram effect and breeding management in general along with a more refined understanding of the magnitude and timing of pre-mating energy nutrition in the ewe will be important in optimizing the reproductive management of accelerated production systems.

APPENDIX

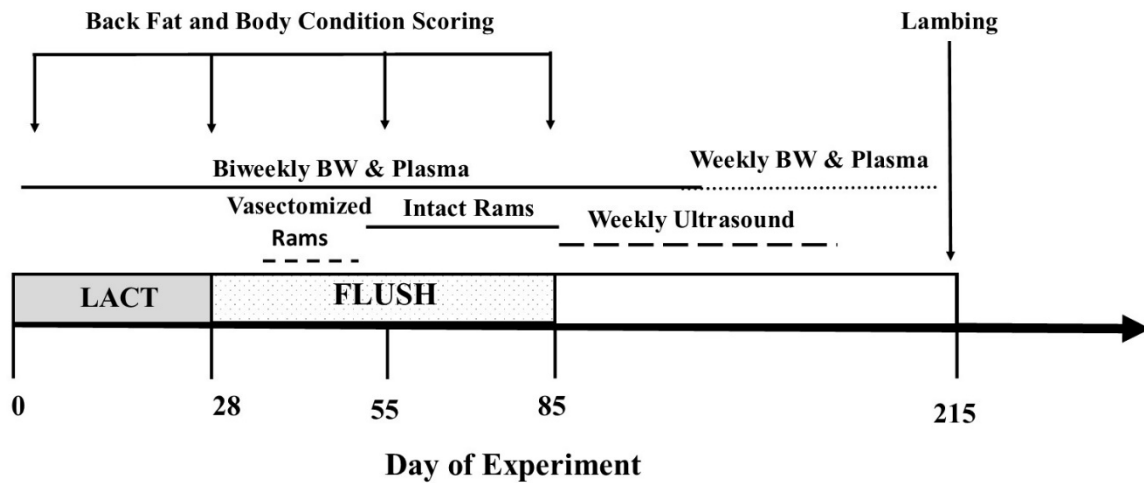


Figure 1. Experimental design

Ewes were fed either H (160%), L (70%), or C (100%) of energy requirements for late lactation according to rearing status during the last 28 days of lactation. At weaning, L and H ewes were then fed either H (160%) or L (70%) of energy requirements for maintenance. C ewes remained at 100% energy requirements for maintenance during a 21-d pre-breeding and 34-d breeding period (Flushing). At the end of the Lactation period, lambs were weaned, and ewes were allowed five days on a low energy dry off diet. Vasectomized rams were introduced at day 7 of Flushing. Fertile rams were introduced at day 21 of Flushing. Ewes were weighed on a biweekly basis for the duration of the experiment. This experiment was repeated during the Long and Short-day season.

Table 1. Ingredient and Nutrient Composition of treatment diets for lactation and flushing.

		Treatment ¹							
		Lactation						Flushing	
		L		C		H			
Item	Season ²	Short	Long	Short	Long	Short	Long	Short	Long
Ingredient,% DM ³									
Corn Silage				47.3	47.9	33.4	37.4	49.0	49.0
Alfalfa Silage				41.9	41.8	28.4	19.5	24.5	24.5
Grass Silage		81.5	84.6					24.5	24.5
Soybean Meal		14.0	10.9	6.3	5.7	14.4	18.7		
Corn Grain						19.3	19.9		
Min & Vit									
Premix ⁴		2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Decoquinat premix ⁵		2.5	2.5	2.5	2.5	2.5	2.5		
Nutrient_Composition ⁶									
DM %		43.0	47.0	38.5	40.0	44.0	47.0	35.0	40.0
CP%		15.4	14.5	16.6	16.0	17.8	17.4	13.6	12.8
ME ⁷ , Mcal/kg DM		2.35	2.14	2.53	2.45	2.58	2.56	2.37	2.43
NDF		55.0	55.0	38.0	35.0	31.0	32.0	46.0	48.0

¹Treatment: Ewes were fed either H (150%), L (70%), or C (100%) of energy requirements for late lactation according to rearing status during the last 28 days of lactation. At weaning, Low and High ewes were then fed either H (160%) or L (70%) of energy requirements for maintenance. C ewes remained at 100% energy requirements for maintenance during a 21-d pre-breeding and 34-d breeding period (flushing)

² Season: Season in which dietary treatments were administered, Okemos, MI, USA: Short (November-January) and Long (March-May)

³DM: All feed ingredients are expressed on a dry matter basis

⁴Mineral and vitamin premix contained 49.25% ground corn grain, 25% limestone, 12.5% trace mineralized salt (Sheep Trace Mineral Salt, Marvo Mineral Co, Inc. Hillsdale, MI containing 95-98.5% sodium chloride, 0.35% zinc as zinc oxide, 0.34% iron as iron carbonate, 0.2% manganese as manganous oxide, 0.03% iodine as calcium iodate, 0.012% selenium as sodium selenite and 0.005% cobalt as cobalt carbonate), 12.5% Vitamin E (44 I.U. per gram), and 0.75% Vitamin A (30,000 IU RE per gram) on a DM basis.

⁵Decoquinat premix contained 88% ground corn grain and 12% Deccox[®] (0.5% decoquinat) on a DM basis.

⁶Nutrient composition was calculated by averaging weekly samples for each treatment period. The samples analyzed as a composite of daily samples taken over a 3-day period each week.

⁷ME was calculated according to NRC 2001.

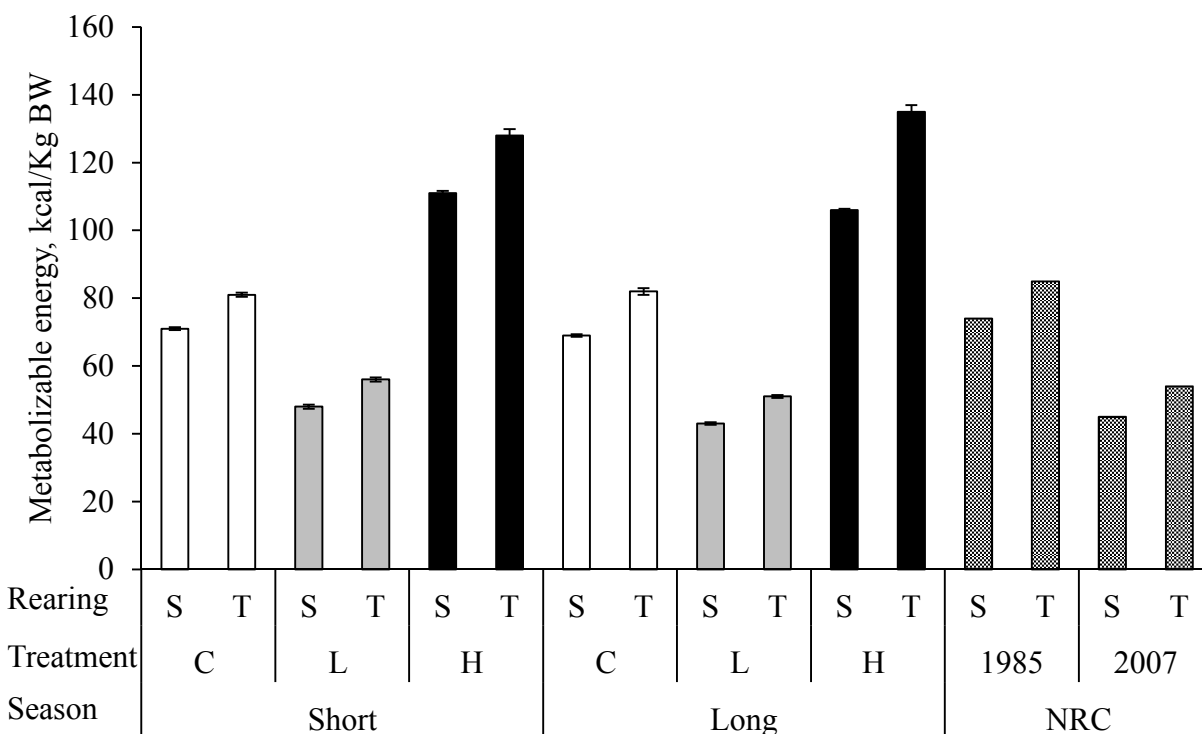


Figure 2. Metabolizable energy intake during lactation compared to NRC 1985 and 2007.

Daily metabolizable energy (ME) intake for ewes fed L (70%), C (100%), or H (150%) of energy requirements for late lactation, as defined by NRC 1985, for either Single (S) or Twin (T) rearing status during the last 28 days of lactation during the Short (November 1-28, 2018), and Long (March 1-28, 2018) seasons. Ewes were fed each diet according to litter size (S or T) with all ewes of each litter size and diet housed within a single pen. Pen ME intake was calculated daily after correcting for feed residual and using the analysis of a composite feed sample taken over 3 days each week. ME intake per bodyweight was calculated on pen bodyweight calculated weekly over the treatment period. Each bar represents mean daily pen intake calculated over the treatment period.

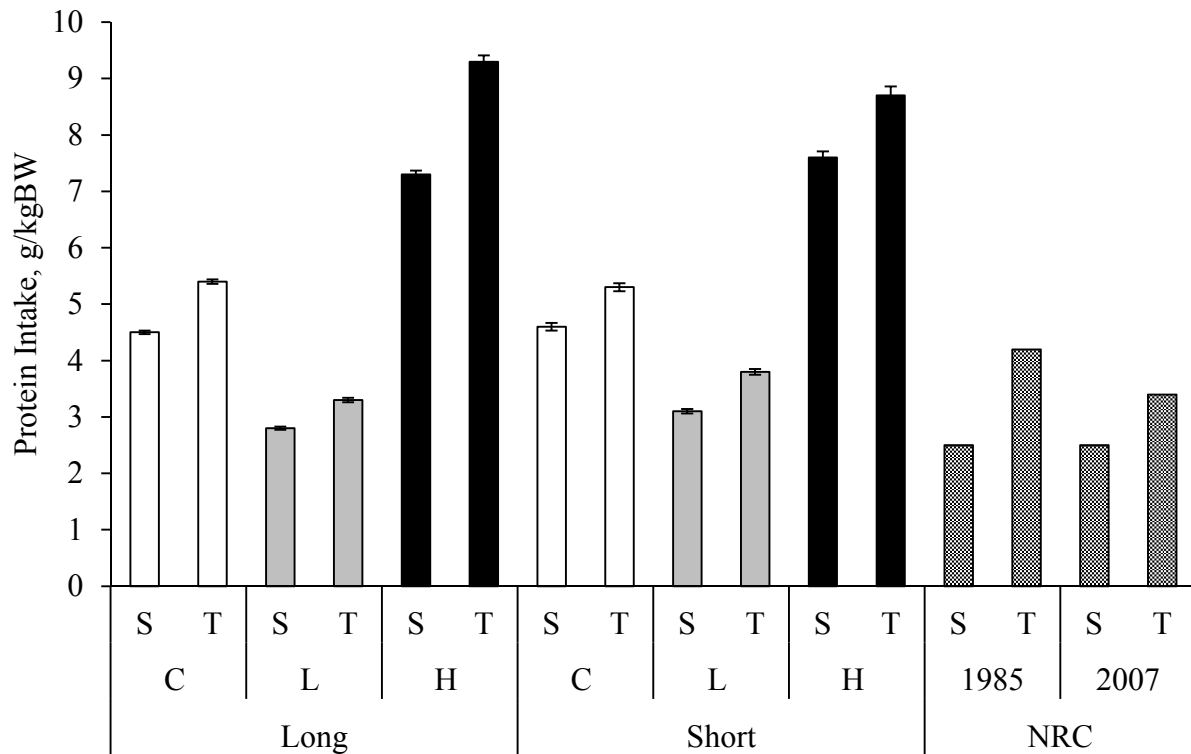


Figure 3. Calculated protein intake during lactation compared to NRC 1985 and 2007.

Crude protein (CP intake for ewes (n = 107 Short, n = 117 Long) fed L (70%), C (100%), or H (150%) of energy requirements for late lactation, as defined by NRC 1985, for either Single (S) or Twin (T) rearing status during the last 28 days of lactation during the Short (November 1-28, 2018), and Long (March 1-28, 2018) seasons. Ewes were fed each diet according to litter size (S or T) with all ewes of each litter size and diet housed within a single pen. Pen CP intake was calculated daily after correcting for feed residual and using the analysis of a composite feed sample taken over 3 days each week. CP intake per bodyweight was calculated on pen bodyweight calculated weekly over the treatment period. Each bar represents mean pen intake calculated over the treatment period.

Table 2. Least Square Means of body weight, body condition score, and back fat depth for ewes fed C, L or H diets during the last 28 days of lactation.

Variable	Treatment ¹				P-Value ²					
	C	L	H	SEM	Diet ³	RS ⁴	Season ⁵	Diet x	RS x	Diet x
								RS	Season	RS x Season
BW, kg	77.1 ^b	73.8 ^b	89.7 ^a	2.6	<0.01	0.31	0.75	0.72	0.87	0.91
BW Change, kg	-2.5 ^b	-7.8 ^c	7.5 ^a	0.5	<0.01	0.81	0.64	0.27	0.96	<0.01
BCS	3.03 ^b	2.78 ^c	3.46 ^a	0.05	<0.01	<0.01	0.62	<0.01	<0.01	<0.01
BCS Change	-0.06 ^b	-0.33 ^c	0.37 ^a	0.04	<0.01	0.03	<0.01	<0.01	<0.01	0.07
BF, cm	0.27 ^b	0.22 ^c	0.43 ^a	0.02	<0.01	<0.01	0.06	0.19	0.02	<0.01
BF Change, cm	-0.05 ^b	-0.11 ^c	0.11 ^a	0.01	<0.01	0.05	<0.01	0.09	<0.01	<0.01

¹ Treatment: Ewes were fed either H (150%), L (70%), or C (100%) of energy requirements for late lactation according to rearing status (NRC 1985), for the last 28 days of lactation.

² P-Value: Refers to the ANOVA model accounting for the effects of treatment, rearing status and the interactions among them. Tukey's test was used for multiple comparisons. Significance was determined at $P < 0.05$

³ Diet: Dietary treatments fed to ewes during the last 28 days of lactation.

⁴ RS: Rearing status, either single or twin rearing ewes.

⁵ Season: Season in which dietary treatments were administered, Okemos, MI, USA: Short (November-January) and Long (March-May)

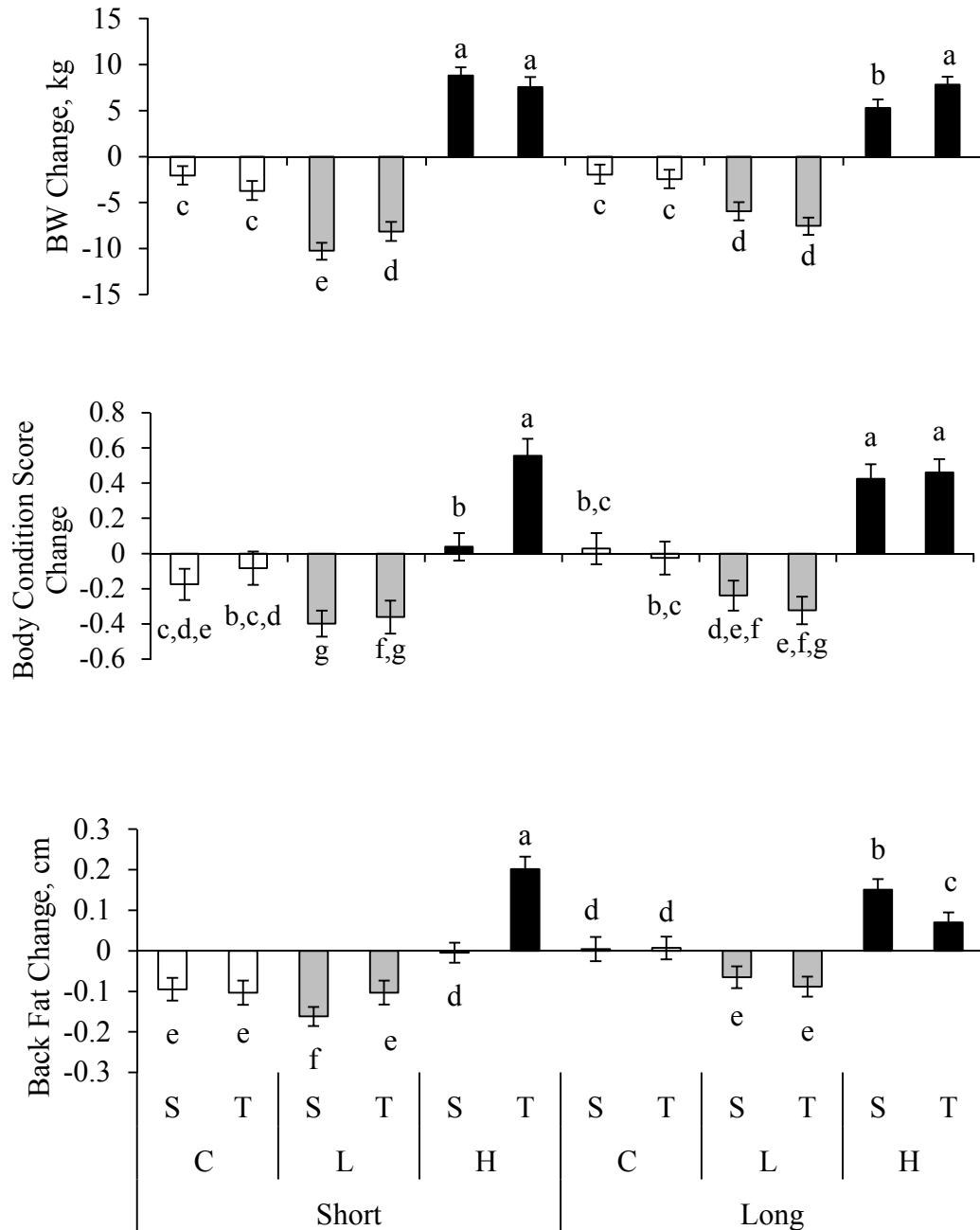


Figure 4. Effect of dietary treatment, rearing status, and season on BW, BCS, and BF during lactation.

LS-means of body weight change (kg), body condition score, and subcutaneous backfat depth (cm) of ewes fed C (100%), L (70%), or H (150%) of NRC requirements for late lactation by rearing status (S, single; T, twin) during the last 28 days of lactation. Tukey's test was used to test all pairwise comparisons. Error bars represent SEM. Bars assigned different letters are significantly different overall ($P < 0.05$).

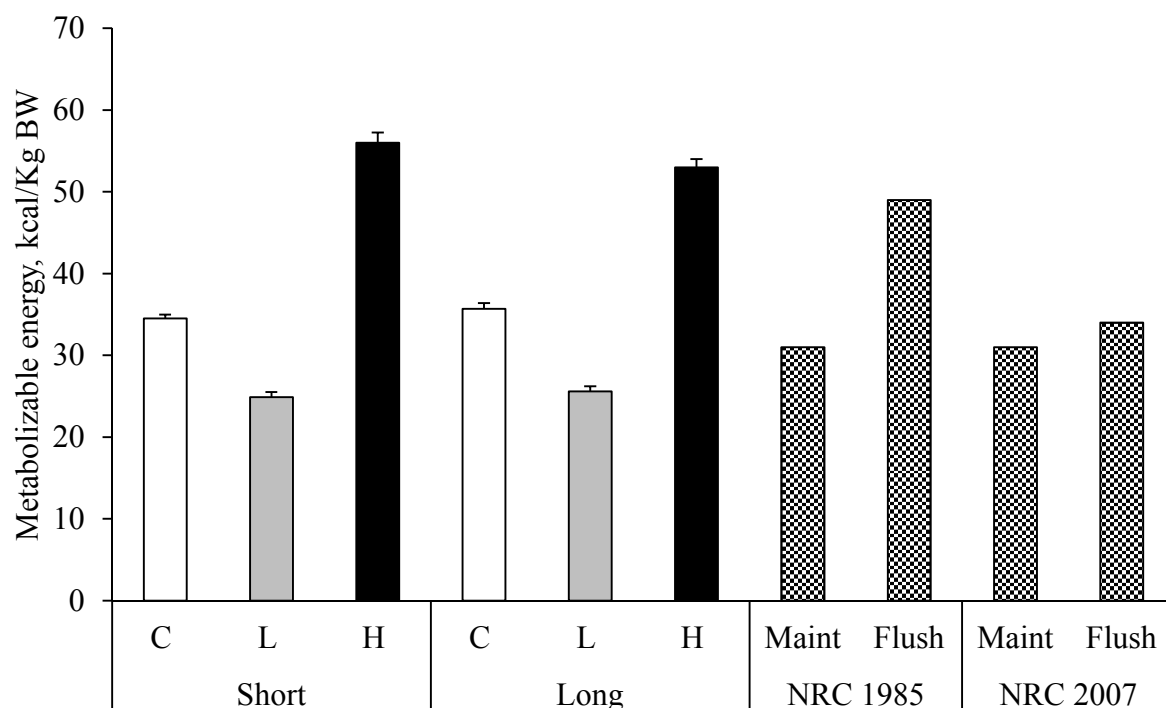


Figure 5. Metabolizable energy intake of C, L, and H diets during the flushing period

Metabolizable energy (ME) intake for ewes fed L (70%), C (100%), or H (160%) of energy requirements for maintenance, as defined by NRC 1985 during a 21 day pre-breeding period and 34 day breeding period during Long, (April 1 – May 28) and Short (December 4 – January 26). Ewes fed the C diet were housed in a single pen whereas those on the L and H diets were randomly assigned to one of 2 pens. Mean ME intake was calculated daily after correcting for feed residual and using the analysis of a composite feed sample taken over 3 days each week. ME intake per bodyweight was calculated on pen bodyweight calculated weekly over the treatment period. Each bar represents mean pen intake calculated over the treatment period.

Table 3. Least Square Means of BW, BCS, and BF during flushing and cumulatively during lactation and flushing

Variable ³	Treatment ¹					SEM ⁴	P-Value ²		
	C	LL	LH	HL	HH		Diet ⁵	Season ⁶	Diet x Season
<u>Flushing⁷</u>									
BW, kg	75.5 ^{b,c}	66.2 ^d	80.7 ^b	74.9 ^c	88.4 ^a	2.6	<0.01	0.59	0.99
BW Change, kg	-1.8 ^c	-9.2 ^d	7.4 ^a	-15.8 ^e	-0.1 ^b	0.5	<0.01	0.02	0.03
BCS	3.09 ^c	2.64 ^d	3.35 ^b	3.04 ^c	3.70 ^a	0.05	<0.01	0.96	<0.01
BCS Change	0.06 ^c	-0.13 ^d	0.55 ^b	-0.43 ^c	0.25 ^a	0.03	<0.01	0.29	<0.01
Back Fat, cm	0.28 ^c	0.15 ^d	0.42 ^b	0.26 ^c	0.55 ^a	0.02	<0.01	<0.01	<0.01
BF Change, cm	0.01 ^c	-0.06 ^d	0.20 ^a	-0.19 ^e	0.14 ^b	0.02	<0.01	<0.01	<0.01
<u>Overall⁸</u>									
BW Change, kg	-4.3 ^c	-16.6 ^e	-0.76 ^b	-7.73 ^d	7.0 ^a	0.7	<0.01	0.03	<0.01
BCS Change	-0.01 ^c	-0.45 ^d	0.21 ^b	-0.09 ^c	0.65 ^a	0.03	<0.01	0.29	<0.01
BF Change, cm	-0.04 ^c	-0.16 ^e	0.09 ^b	-0.08 ^d	0.24 ^a	0.02	<0.01	<0.01	<0.01

¹ Treatment: Ewes were fed either C (100%), LL (70%, 70%), LH (70%, 160%), HL (150%, 70%), or HH (150%, 160%) of NRC requirements for late lactation and subsequently for maintenance.

²P-Value: Significant differences were determined for main effects of diet and season, and their interaction. Tukey's test was used for all pairwise comparisons. Significance was declared at $P < 0.05$.

³BW, BCS, and BF measurements taken at the end of the flushing treatment period. Change values were calculated by subtracting the flushing values from the values determined at the end of the lactation period

⁴SEM: Standard Error of the mean.

⁵Diet: Effect of nutritional treatment on BW, BCS, BF changes during the flushing period

⁶Season: Season of breeding, as defined by either Short (late fall, early winter) or Long (late spring).

⁷Flushing: The flushing treatment period is defined as 21 days prior to ram introduction and a subsequent 34-d breeding period.

⁸Overall: The overall BW, BCS, and BF change during both the last 28 days of lactation and flushing

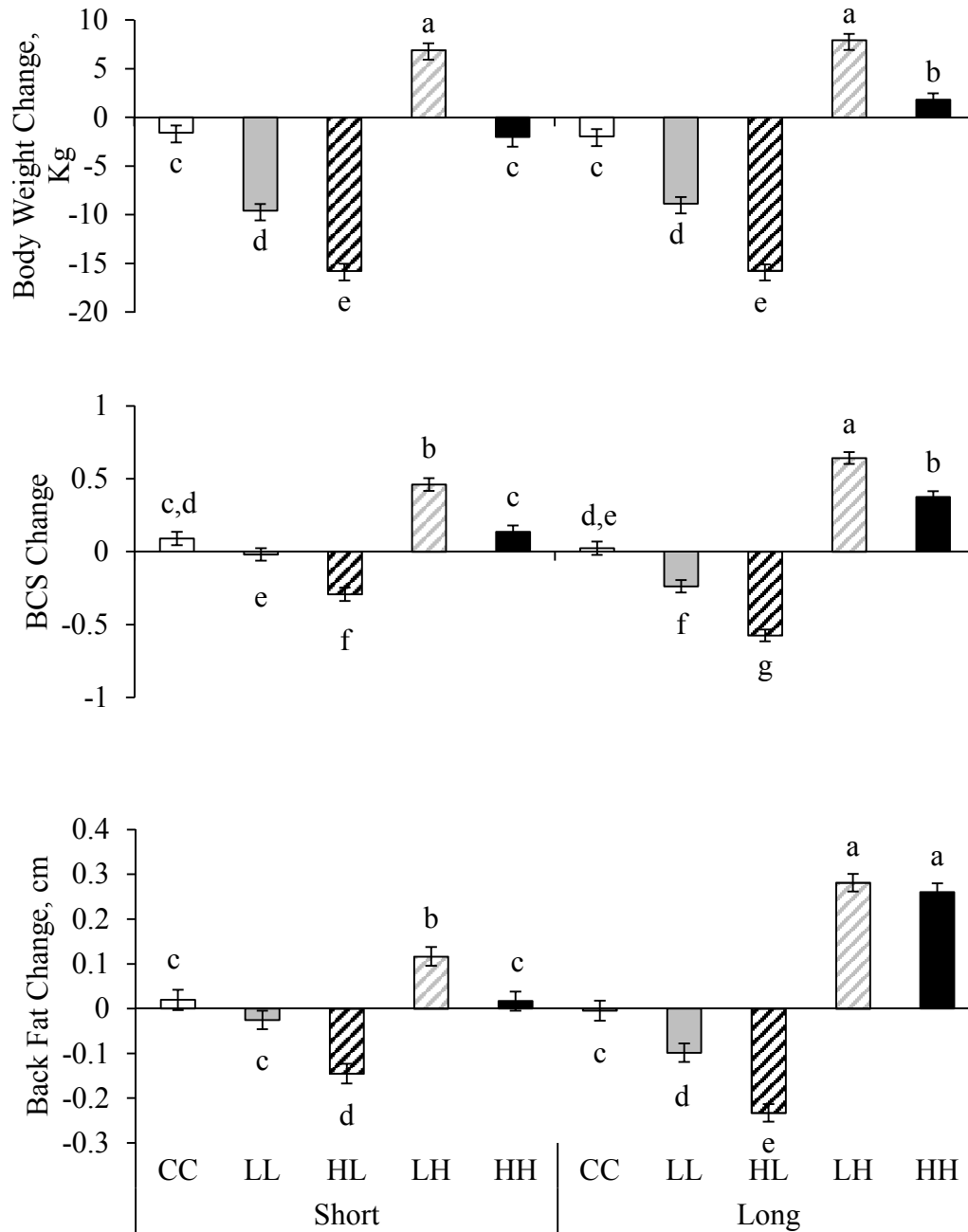


Figure 6. Effect of dietary treatment, rearing status, and season on BW, BCS, and BF change during flushing.

LS-means of body weight change (kg), body condition score, and subcutaneous backfat depth (cm) of ewes fed either C (100%), LL (70%, 70%), LH (70%, 150%), HL (150%, 70%), or HH (150%, 160%) of NRC requirements for late lactation and subsequently for maintenance. Solid bars represent ewes who were on the same treatment for both late lactation and pre-breeding, whereas striped bars represent ewes on different treatments for late lactation and pre-breeding. Error bars represent SEM. Tukey's test was used for all pairwise comparisons. Bars with different letters are significantly different ($P < 0.05$).

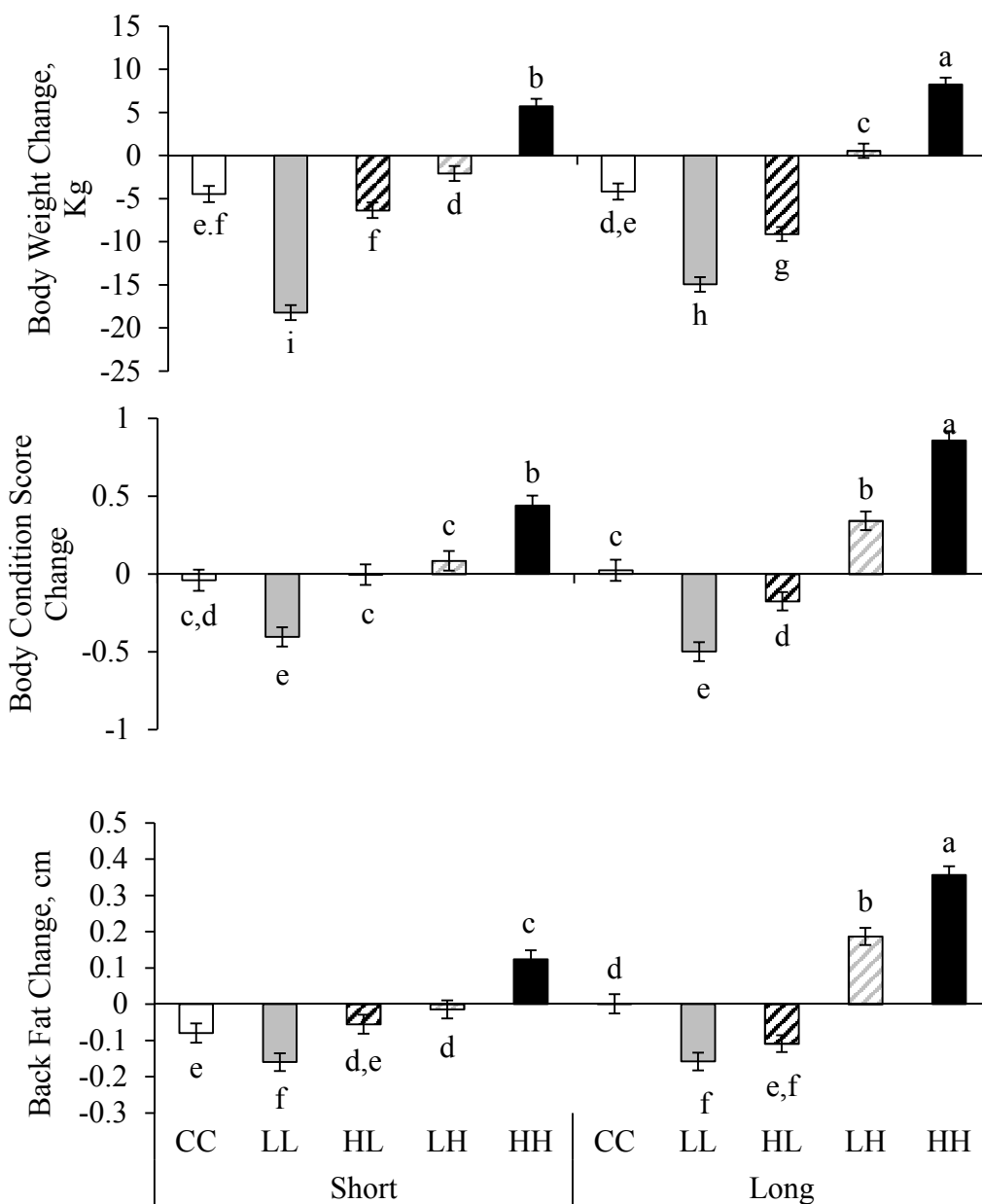


Figure 7. Effect of dietary treatment, rearing status, and season on BW, BCS, and BF change during lactation and flushing.

LS-means of body weight (kg), body condition score, and subcutaneous backfat depth (cm) change of ewes fed either C (100%), LL (70%, 70%), LH (70%, 160%), HL (160%, 70%), or HH (160%, 160%) of NRC requirements for late lactation and subsequently for maintenance. Solid bars represent ewes who were on the same treatment for both late lactation and pre-breeding, whereas striped bars represent ewes on different treatments for late lactation and pre-breeding. Error bars represent SEM. Tukey's test was used for all pairwise comparisons. Bars with different letters are significantly different ($P < 0.05$).

Table 4. Effect of dietary treatment and season on reproductive outcomes in an accelerated lamb production system.

Variable	Treatment ¹					SEM	P-value ²		
	C	LL	LH	HL	HH		Diet ³	Season ⁴	Diet x Season
Conception, %	97.4 ^a	93.5 ^a	98.0 ^a	95.6 ^a	96.0 ^a	0.03	0.98	0.33	0.59
Fetal Number ⁵	1.64 ^a	1.63 ^a	1.57 ^a	1.49 ^a	1.62 ^a	0.09	0.71	<0.01	0.40
Lambs Born ⁶	1.57 ^a	1.61 ^a	1.55 ^a	1.45 ^a	1.57 ^a	0.09	0.70	<0.01	0.14
Gestation Length ⁷	145.3 ^b	146.3 ^a	146.2 ^{a,b}	146.1 ^{a,b}	145.6 ^{a,b}	0.38	0.24	<0.01	0.59

¹¹ Treatment: Ewes were fed either C (100%), LL (70%, 70%), LH (70%, 160%), HL (160%, 70%), or HH (160%, 160%) of NRC requirements for late lactation and subsequently for maintenance.

²²P-Value: Significant differences were determined for main effects of diet and season, and their interaction. Significance was declared at $P < 0.05$.

³The fixed effect of dietary treatment during the last 28 days of lactation and 21 d pre-breeding and 34 d breeding period (flushing) on reproductive outcomes.

⁴The fixed effect of breeding season on reproductive outcomes.

⁵Fetal number was determined via transabdominal ultrasonography at day 69 *post-coitus*.

⁶Lambs born alive.

⁷Gestation length was calculated using the difference in days between the last standing heat observation and lambing

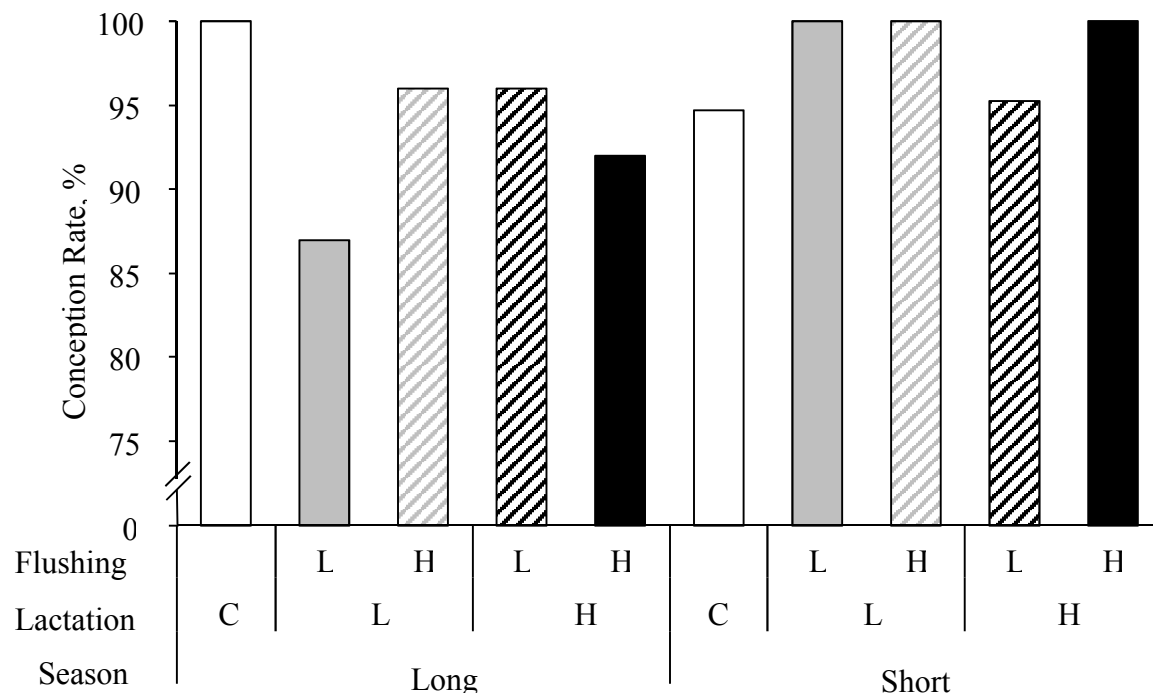


Figure 8. Effect of dietary treatment and season on conception rate.

H (160%), C (100%), and L (70%) of energy requirements for late lactation (NRC 1985) were fed during last 28-d of lactation. During flushing (21-d pre-breeding and 34-d breeding periods), L or H lactation treatment ewes were then fed either 70% (L) or the NRC (1985) flushing recommendation of 160% (H) of energy requirements for maintenance (C ewes remained at 100% maintenance during flushing).

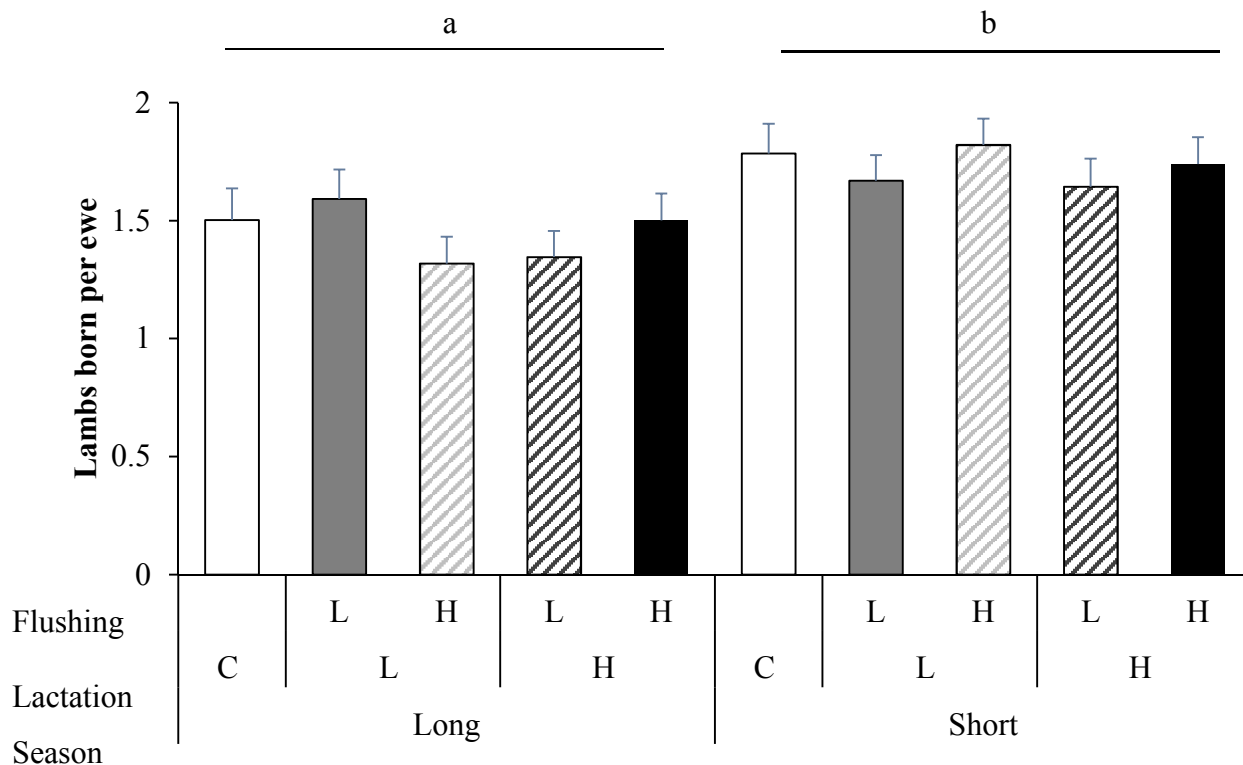


Figure 9. Effect of dietary treatment and season on litter size.

H (150%), C (100%), and L (70%) of energy requirements for late lactation (NRC 1985) were fed during LACT the last 28 days of lactation. During Flushing (21-d pre-breeding and 34-d breeding periods), L or H Lactation ewes were then fed either 70% (L) or 160% (H) of the NRC (1985) energy requirement for maintenance (C ewes remained at 100% maintenance during Flushing). Litter size is represented in lambs born per ewe, measured at day 69 ± 0.04 PC. Error bars represent SEM, and Tukey's test was used for all pairwise comparisons. Bars with differing letters are significantly different.

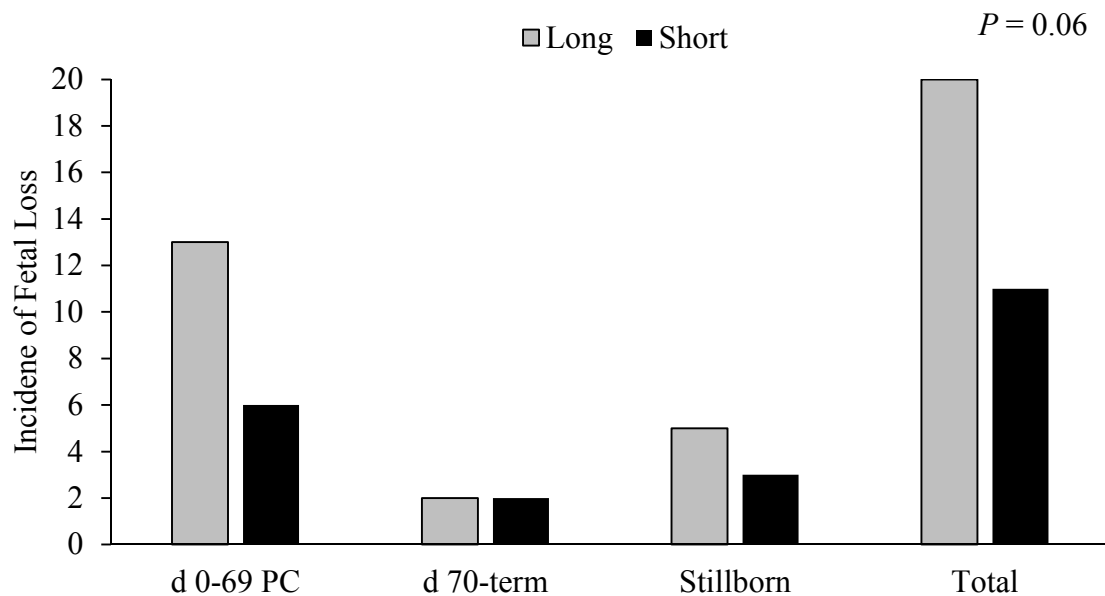


Figure 10. Effect of season of conception on incidence of fetal loss.

H (150%), C (100%), and L (70%) of energy requirements for late lactation (NRC 1985) were fed during last 28-d of lactation. During Flushing (21-d pre-breeding and 34-d breeding periods), L or H Lactation ewes were fed either 70% (L) or 160% (H) the NRC (1985) energy requirements for maintenance (C ewes remained at 100% maintenance during Flushing). Ewes were bred in either Long (April/May) or Short (December/January).

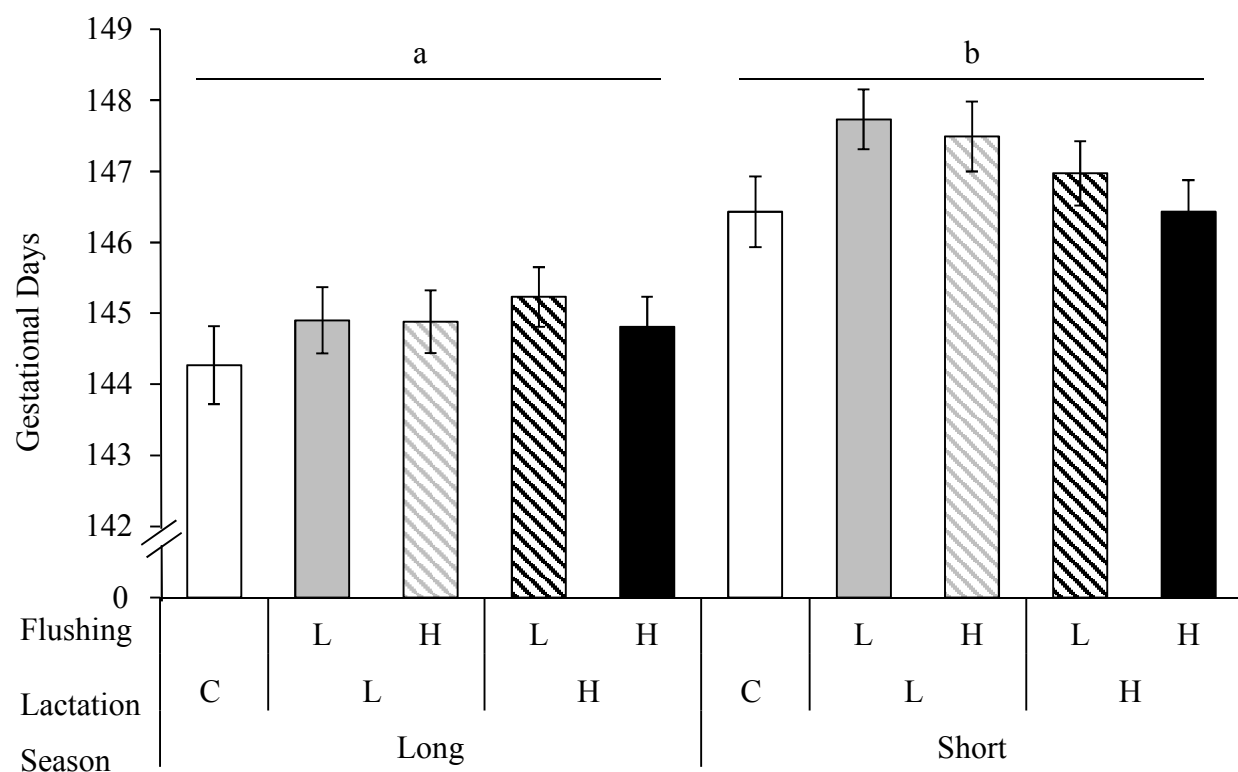


Figure 11. Effect of dietary treatment and season of conception on gestational length. H (150%), C (100%), and L (70%) of energy requirements for late lactation (NRC 1985) were fed during the last 28 days of lactation. During Flushing (21-d pre-breeding and 34-d breeding periods), L or H Lactation ewes were fed either 70% (L) or 160% (H) of the NRC (1985) of energy requirements for maintenance (C ewes remained at 100% maintenance during Flushing). Ewes were exposed to rams in either Long (April/May) or Short (December/January). Error bars represent SEM. Bars with differing letters are significantly different.

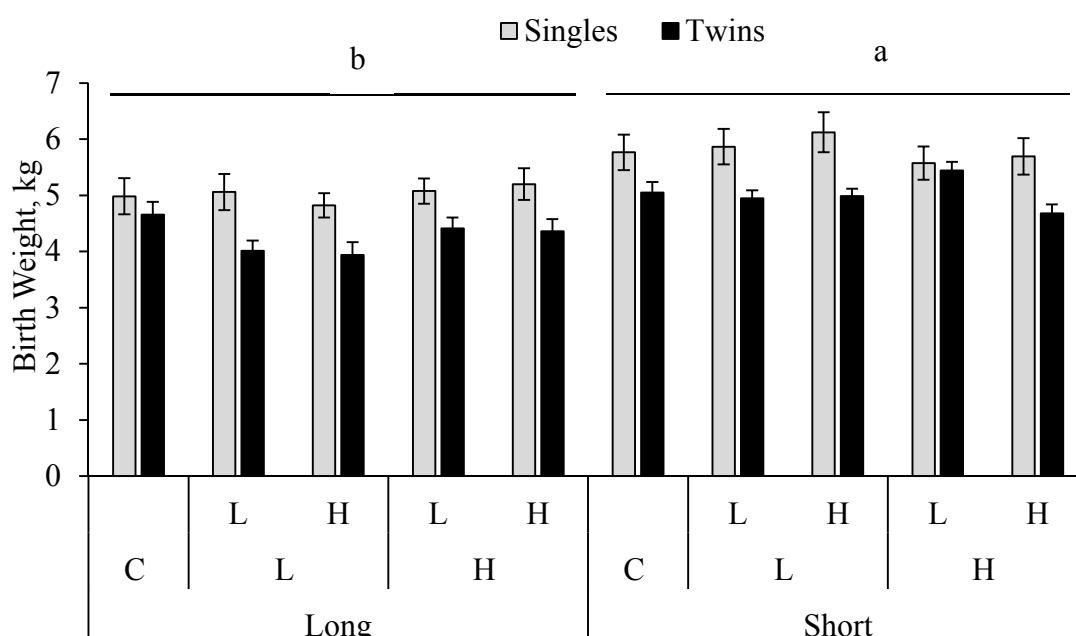


Figure 12. Effect of dietary treatment, season, and litter size on birth weight.

H (150%), C (100%), and L (70%) of energy requirements for late lactation (NRC 1985) were fed during late lactation. During Flusing (21-d pre-breeding and 34-d breeding periods), L or H Lactation ewes were then fed either 70% (L) or 160% (H) of the NRC (1985) energy requirements for maintenance (C ewes remained at 100% maintenance during Flushing). Ewes were bred in either Long (April/May) or Short (December/January). Bars with differing letters are significantly different.

REFERENCES

REFERENCES

- Abecia, J. A., Forcada, F., Palacín, I., Sánchez-Prieto, L., Sosa, C., Fernández-Foren, A., & Meikle, A. (2015). Undernutrition affects embryo quality of superovulated ewes. *Zygote*, 23(1), 116.
- Adams, V. L., Goodman, R. L., Salm, A. K., Coolen, L. M., Karsch, F. J., & Lehman, M. N. (2006). Morphological plasticity in the neural circuitry responsible for seasonal breeding in the ewe. *Endocrinology*, 147(10), 4843–4851. <https://doi.org/10.1210/en.2006-0408>
- Anderson, G. B., Bradford, G. E., & Cupps, P. T. (1981). Length of gestation in ewes carrying lambs of two different breeds. *Theriogenology*, 16(1), 119–129. [https://doi.org/10.1016/0093-691X\(81\)90120-5](https://doi.org/10.1016/0093-691X(81)90120-5)
- Annett, R. W., & Carson, A. F. (2006). Effects of plane of nutrition during the first month of pregnancy on conception rate, foetal development and lamb output of mature and adolescent ewes. *Animal Science*, 82(6), 947–954. <https://doi.org/10.1017/ASC2006111>
- Bartlewski, P. M., Beard, A. P., Cook, S. J., & Rawlings, N. C. (1998). Ovarian follicular dynamics during anoestrus in ewes. *Reproduction*, 113(2), 275–285. <https://doi.org/10.1530/jrf.0.1130275>
- Bartlewski, Pawel M., Baby, T. E., & Giffin, J. L. (2011). Reproductive cycles in sheep. *Animal Reproduction Science*. <https://doi.org/10.1016/j.anireprosci.2011.02.024>
- Bauman, D. E., & Bruce Currie, W. (1980). Partitioning of Nutrients During Pregnancy and Lactation: A Review of Mechanisms Involving Homeostasis and Homeorhesis. *Journal of Dairy Science*, 63(9). [https://doi.org/10.3168/jds.S0022-0302\(80\)83111-0](https://doi.org/10.3168/jds.S0022-0302(80)83111-0)
- Blache, D., Zhang, S., & Martin, G. B. (2006). Dynamic and integrative aspects of the regulation of reproduction by metabolic status in male sheep. *Reproduction Nutrition Development*, 46(4), 379–390. <https://doi.org/10.1051/rnd:2006019>
- Cannas, A., & Atzori, A. S. (2005). Development and evaluation of a model to predict sheep nutrient requirements and feed utilisation. *Italian Journal of Animal Science*, 4(SUPPL. 1). <https://doi.org/10.4081/ijas.2005.1s.15>
- Chemineau, P., Malpaux, B., Delgadillo, J. A., Guérin, Y., Ravault, J. P., Thimonier, J., & Pelletier, J. (1992). Control of sheep and goat reproduction: Use of light and melatonin. *Animal Reproduction Science*, 30(1–3), 157–184. [https://doi.org/10.1016/0378-4320\(92\)90010-B](https://doi.org/10.1016/0378-4320(92)90010-B)
- Chilliard, Y., Bocquier, F., & Doreau, M. (1998). Digestive and metabolic adaptations of ruminants to undernutrition, and consequences on reproduction. *Reproduction Nutrition Development*, 38(2), 131–152. <https://doi.org/10.1051/rnd:19980201>

- Clarke, I. J., & Arbabi, L. (2016). New concepts of the central control of reproduction, integrating influence of stress, metabolic state, and season. *Domestic Animal Endocrinology*, Vol. 56, pp. S165–S179. <https://doi.org/10.1016/j.domaniend.2016.03.001>
- Cochran, K. P., Notter, D. R., & McClaugherty, F. S. (1984). A comparison of Dorset and Finnish Landrace crossbred ewes. *Journal of Animal Science*, 59(2), 329–337. <https://doi.org/10.2527/jas1984.592329x>
- Delgadillo, J. A., Gelez, H., Ungerfeld, R., Hawken, P. A. R., & Martin, G. B. (2009). The “male effect” in sheep and goats-Revisiting the dogmas. *Behavioural Brain Research*, 200(2), 304–314. <https://doi.org/10.1016/j.bbr.2009.02.004>
- Dixon, A. B., Knights, M., Winkler, J. L., Marsh, D. J., Pate, J. L., Wilson, M. E., ... Inskeep, E. K. (2007). Patterns of late embryonic and fetal mortality and association with several factors in sheep. *Journal of Animal Science*, 85(5), 1274–1284. <https://doi.org/10.2527/jas.2006-129>
- Downing, J., & Scaramuzzi, R. (1991). Nutrient effects on ovulation rate, ovarian function and the secretion of gonadotrophic and metabolic hormones in sheep. *Reproduction, Fertility and Development*. <https://doi.org/10.1530/biosciprocs.2.017>
- Edwards, L. J., & McMillen, I. C. (2002). Impact of Maternal Undernutrition During the Periconceptional Period, Fetal Number, and Fetal Sex on the Development of the Hypothalamo-Pituitary Adrenal Axis in Sheep During Late Gestation1. *Biology of Reproduction*, 66(5), 1562–1569. <https://doi.org/10.1095/biolreprod66.5.1562>
- El-Saied, U. M., de la Fuente, L. F., & San Primitivo, F. (2006). Lifetime traits comparison between annual and accelerated lambing systems for dairy ewes. *Livestock Science*, 101(1–3). <https://doi.org/10.1016/j.livsci.2005.11.003>
- Fabre-Nys, C., Chanvallon, A., Dupont, J., Lardic, L., Lomet, D., Martinet, S., & Scaramuzzi, R. J. (2016). The “ram effect”: A “non-classical” mechanism for inducing LH surges in sheep. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0158530>
- Fabre-Nys, C., Kendrick, K. M., & Scaramuzzi, R. J. (2015). The “ram effect”: New insights into neural modulation of the gonadotropic axis by male odors and socio-sexual interactions. *Frontiers in Neuroscience*. <https://doi.org/10.3389/fnins.2015.00111>
- Forbes, J. M. (1967). Factors affecting the gestation length in sheep. *The Journal of Agricultural Science*, 68(2), 191–194. <https://doi.org/10.1017/S0021859600016221>
- Forcada, F., Zarazaga, L., Abecia, J. (1995). Effect of exogenous melatonin and plane of nutrition after weaning on estrous activity, endocrine status and ovulation rate in salt ewes lambing in the seasonal anestrus. *Theriogenology*, 43.
- Forcada, F., Abecia, J. A., Cebrián-Pérez, J. A., Muiño-Blanco, T., Valares, J. A., Palacín, I., &

- Casao, A. (2006). The effect of melatonin implants during the seasonal anestrus on embryo production after superovulation in aged high-prolificacy Rasa Aragonesa ewes. *Theriogenology*, 65(2), 356–365. <https://doi.org/10.1016/j.theriogenology.2005.05.038>
- Gardner, D. S., Buttery, P. J., Daniel, Z., & Symonds, M. E. (2007a). Factors affecting birth weight in sheep: Maternal environment. *Reproduction*. <https://doi.org/10.1530/REP-06-0042>
- Gootwine, E., & Rozov, A. (2006). Seasonal effects on birth weight of lambs born to prolific ewes maintained under intensive management. *Livestock Science*, 105(1–3), 277–283. <https://doi.org/10.1016/j.livsci.2006.06.018>
- Gutierrez, C. G., Ferraro, S., Martinez, V., Saharrea, A., Cortez, C., Lassala, A., ... Hernandez, J. (2011). Increasing ovulation quota: More than a matter of energy. *Acta Scientiae Veterinariae*, 39(SUPPL. 1).
- Hogue, D. E. (1987). Frequent lambing systems. In *New Techniques in Sheep Production* (pp. 57–63). <https://doi.org/10.1016/b978-0-408-10134-9.50011-x>
- Hulet, C. V., Ercanbrack, S. K., & Knight, A. D. (1984). Development of the Polypay breed of sheep. *Journal of Animal Science*. <https://doi.org/10.2527/jas1984.58115x>
- Iniguez, L. C., Quaas, R. L., & Van Vleck, L. D. (1986a). Lambing performance of Morlam and Dorset ewes under accelerated lambing systems. *Journal of Animal Science*, 63(6), 1769–1778. <https://doi.org/10.2527/jas1986.6361769x>
- Jenkinson, C. M. C., Peterson, S. W., Mackenzie, D. D. S., McDonald, M. F., & McCutcheon, S. N. (1995). Seasonal effects on birth weight in sheep are associated with changes in placental development. *New Zealand Journal of Agricultural Research*, 38(3), 337–345. <https://doi.org/10.1080/00288233.1995.9513135>
- Johnson, L. E. (1943). Differences in Lengths of Gestation Periods of Breeds of Beef Cattle, Swine, and Sheep. *Academy of Science*.
- Jordan, R. M., & Hanke, H. E. (1977). Effect of Level of Grain Fed Ewes During Late Lactation on Lamb Production. *Journal of Animal Science*, 45(5), 945–947. <https://doi.org/10.2527/jas1977.455945x>
- Karsch, F. J., Legan, S. J., Ryan, K. D., & Foster, D. L. (1980). Importance of Estradiol and Progesterone in Regulating LH Secretion and Estrous Behavior During the Sheep Estrous Cycle1. *Biology of Reproduction*. <https://doi.org/10.1095/biolreprod23.2.404>
- Kelley, R. (1943). The Length of Gestation Periods Preceding Single and Multiple Births in Sheep. *Australian Veterinary Journal*, 19, 43–45.
- Kendall, N. R., Gutierrez, C. G., Scaramuzzi, R. J., Baird, D. T., Webb, R., & Campbell, B. K.

- (2004). Direct in vivo effects of leptin on ovarian steroidogenesis in sheep. *Reproduction*, 128(6), 757–765. <https://doi.org/10.1530/rep.1.00256>
- Kenyon, P. R., Maloney, S. K., & Blache, D. (2014). Review of sheep body condition score in relation to production characteristics. *New Zealand Journal of Agricultural Research*, 57(1), 38–64. <https://doi.org/10.1080/00288233.2013.857698>
- Knickerbocker, J. J., Wiltbank, M. C., & Niswender, G. D. (1988). Mechanisms of luteolysis in domestic livestock. *Domestic Animal Endocrinology*, 5(2), 91–107. [https://doi.org/10.1016/0739-7240\(88\)90011-2](https://doi.org/10.1016/0739-7240(88)90011-2)
- Knights, M., Baptiste, Q. S., Dixon, A. B., Pate, J. L., Marsh, D. J., Inskeep, E. K., & Lewis, P. E. (2003). Effects of dosage of FSH, vehicle and time of treatment on ovulation rate and prolificacy in ewes during the anestrus season. *Small Ruminant Research*, 50(1–2), 1–9. [https://doi.org/10.1016/S0921-4488\(03\)00111-1](https://doi.org/10.1016/S0921-4488(03)00111-1)
- Lassoued, N., Rekik, M., Mahouachi, M., & Ben Hamouda, M. (2004). The effect of nutrition prior to and during mating on ovulation rate, reproductive wastage, and lambing rate in three sheep breeds. *Small Ruminant Research*, 52(1–2), 117–125. [https://doi.org/10.1016/S0921-4488\(03\)00250-5](https://doi.org/10.1016/S0921-4488(03)00250-5)
- Legan, S. J., Karsch, F. J., & Foster, D. L. (1977). The endocrine control of seasonal reproductive function in the ewe: A marked change in response to the negative feedback action of estradiol on luteinizing hormone secretion. *Endocrinology*, 101(3), 818–824. <https://doi.org/10.1210/endo-101-3-818>
- Lenz Souza, M., Leandro Gressler, M., & Uribe Velásquez, L. (2014). Interrelationships of nutrition, metabolic hormones and reproduction of female sheep. *Revista CES Medicina Veterinaria y Zootecnia*, 9(2). <https://doi.org/10.21615/3148>
- Lewis, R. M., Notter, D. R., Hogue, D. E., & Magee, B. H. (1996a). Ewe Fertility in the STAR Accelerated Lambing System. *Journal of Animal Science*, 74(7). <https://doi.org/10.2527/1996.7471511x>
- Ley, W. B., Sprecher, D. J., Thatcher, C. D., Pelzer, K. D., & Umberger, S. H. (1990). Use of the point-score system for breeding soundness examination in yearling Dorset, Hampshire and Suffolk rams. *Theriogenology*, 34(4), 721–733. [https://doi.org/10.1016/0093-691X\(90\)90027-Q](https://doi.org/10.1016/0093-691X(90)90027-Q)
- Llewellyn, S., Fitzpatrick, R., Kenny, D. A., Murphy, J. J., Scaramuzzi, R. J., & Wathes, D. C. (2007). Effect of negative energy balance on the insulin-like growth factor system in pre-recruitment ovarian follicles of post partum dairy cows. *Reproduction*, 133(3), 627–639. <https://doi.org/10.1530/REP-06-0122>
- Macías-Cruz, U., Vicente-Pérez, R., Correa-Calderón, A., Mellado, M., Meza-Herrera, C. A., & Avendaño-Reyes, L. (2017). Undernutrition pre- and post-mating affects serum levels of

- glucose, cholesterol and progesterone, but not the reproductive efficiency of crossbred hair ewes synchronized for estrus. *Livestock Science*, 205, 64–69.
<https://doi.org/10.1016/j.livsci.2017.09.016>
- Malpaux, B., Migaud, M., Tricoire, H., & Chemineau, P. (2001). Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. *Journal of Biological Rhythms*, 16(4), 336–347. <https://doi.org/10.1177/074873001129002051>
- Martin, G. B., Cognie, Y., Schirar, A., Nunes-Ribeiro, A., Fabre-Nys, C., & Thiéry, J. C. (1985). Diurnal variation in the response of anoestrous ewes to the ram effect. *Journal of Reproduction and Fertility*, 75(1), 275–284. <https://doi.org/10.1530/jrf.0.0750275>
- Martin, Graeme B., & Scaramuzzi, R. J. (1983). The induction of oestrus and ovulation in seasonally anovular ewes by exposure to rams. *Journal of Steroid Biochemistry*, 19(1 PART 3), 869–875. [https://doi.org/10.1016/0022-4731\(83\)90026-2](https://doi.org/10.1016/0022-4731(83)90026-2)
- Martin, Graeme B., Scaramuzzi, R. J., & Lindsay, D. R. (1981). Induction of ovulation in seasonally anovular ewes by the introduction of rams: Effects of progesterone and active immunization against androstenedione. *Australian Journal of Biological Sciences*, 34(6), 569–576. <https://doi.org/10.1071/BI9810569>
- McCann, J. P., Bergman, E. N., & Beermann, D. H. (1992). Dynamic and static phases of severe dietary obesity in sheep: Food intakes, endocrinology and carcass and organ chemical composition. *Journal of Nutrition*, 122(3), 496–505. <https://doi.org/10.1093/jn/122.3.496>
- Miller, L. R., Shiflett, J. S., Marsh, D. J., & Rodgers, P. (2016). *U . S . Sheep industry reseach, development , and education priorities*.
- Nestor, C. C., Bedenbaugh, M. N., Hileman, S. M., Coolen, L. M., Lehman, M. N., & Goodman, R. L. (2018). Regulation of GnRH pulsatility in ewes. *Reproduction*, 156(3), R83–R99. <https://doi.org/10.1530/REP-18-0127>
- Notter, D. R. (1981). Repeatability of Conception Rate and Litter Size for Ewes in an Accelerated Lambing System. *Journal of Animal Science*, 53(3). <https://doi.org/10.2527/jas1981.533643x>
- Notter, D. R. (2012). Genetic improvement of reproductive efficiency of sheep and goats. *Animal Reproduction Science*, 130(3–4), 147–151. <https://doi.org/10.1016/j.anireprosci.2012.01.008>
- Nottle, M. B., Kleemann, D. O., Grosser, T. I., & Seamark, R. F. (1997). Evaluation of a nutritional strategy to increase ovulation rate in Merino ewes mated in late spring-early summer. *Animal Reproduction Science*, 47(4). [https://doi.org/10.1016/S0378-4320\(97\)00025-0](https://doi.org/10.1016/S0378-4320(97)00025-0)
- Nugent, R. A., Notter, D. R., & Beal, W. E. (1988). Effects of ewe breed and ram exposure on

- estrous behavior in May and June. *Journal of Animal Science*, 66(6), 1363–1370.
<https://doi.org/10.2527/jas1988.6661363x>
- Nugent, R. A., Notter, D. R., & McClure, W. H. (1988). Effects of ram preexposure and ram breed on fertility of ewes in summer breeding. *Journal of Animal Science*, 66(7), 1622–1626. <https://doi.org/10.2527/jas1988.6671622x>
- Oldham, C., Martin, G. B., & Knight, T. (1979). Stimulation of seasonally anovular merino ewes by rams. *Animal Reproduction Science*, 1, 283–290.
- Ortavant, R., Bocquier, F., Pelletier, J., Ravault, J. P., Thimonier, J., & Volland-Nail, P. (1988). Seasonality of Reproduction in Sheep and its Control by Photoperiod. *Australian Journal of Biological Sciences*, 41(1), 69–86. <https://doi.org/10.1071/BI9880069>
- Parr, R. A. (1992). Nutrition-progesterone interactions during early pregnancy in sheep. *Reproduction, Fertility and Development*, 4(3), 297–300.
<https://doi.org/10.1071/RD9920297>
- Parr, R. A., Davis, I. F., Fairclough, R. J., & Miles, M. A. (1987b). Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. *Journal of Reproduction and Fertility*, 80(1). <https://doi.org/10.1530/jrf.0.0800317>
- W. F. Pope, K. E. McClure, D. E. Hogue, M. L. Day, Effect of Season and Lactation on Postpartum Fertility of Polypay, Dorset, St. Croix and Targhee Ewes, *Journal of Animal Science*, Volume 67, Issue 5, May 1989, Pages 1167–1174,
<https://doi.org/10.2527/jas1989.6751167x>
- Quinlivan, T. D., Martin, C. A., Taylor, W. B., & Cairney, I. M. (1966). Estimates of pre- and perinatal mortality in the New Zealand Romney Marsh ewe. I. Pre- and perinatal mortality in those ewes that conceived to one service. *Journal of Reproduction and Fertility*, 11(3), 379–390. <https://doi.org/10.1530/jrf.0.0110379>
- Redden, R., Sanko, E., Ehrhardt, R. A., & Heimke, C. (2018). *Seasonality of the US Lamb Industry*.
- Rosa, H. J. D., & Bryant, M. J. (2003b). Seasonality of reproduction in sheep. *Small Ruminant Research*. [https://doi.org/10.1016/S0921-4488\(03\)00038-5](https://doi.org/10.1016/S0921-4488(03)00038-5)
- Rosa, H. J. D., Juniper, D. T., & Bryant, M. J. (2000). The effect of exposure to oestrous ewes on rams' sexual behaviour, plasma testosterone concentration and ability to stimulate ovulation in seasonally anoestrous ewes. *Applied Animal Behaviour Science*, 67(4).
[https://doi.org/10.1016/S0168-1591\(00\)00086-1](https://doi.org/10.1016/S0168-1591(00)00086-1)
- Scaramuzzi, R. J., Adams, N. R., Baird, D. T., Campbell, B. K., Downing, J. A., Findlay, J. K., ... Tsonis, C. G. (1993). A model for follicle selection and the determination of ovulation rate in the ewe. *Reproduction, Fertility and Development*, 5(5), 457–478.

<https://doi.org/10.1071/RD9930459>

- Scaramuzzi, R. J., & Martin, G. B. (2008). The Importance of Interactions Among Nutrition, Seasonality and Socio-sexual Factors in the Development of Hormone-free Methods for Controlling Fertility. *Reproduction in Domestic Animals*, 43(SUPPL.2), 129–136. <https://doi.org/10.1111/j.1439-0531.2008.01152.x>
- Scaramuzzi, Rex J., Campbell, B. K., Downing, J. A., Kendall, N. R., Khalid, M., Muñoz-Gutiérrez, M., & Somchit, A. (2006b). A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. *Reproduction Nutrition Development*, 46(4), 339–354. <https://doi.org/10.1051/rnd:2006016>
- Schinckel, P. G. (1954). the Effect of the Ram on the Incidence and Occurrence of Oestrus in Ewes. *Australian Veterinary Journal*, 30(7), 189–195. <https://doi.org/10.1111/j.1751-0813.1954.tb08198.x>
- Stewart, R., & Oldham, C. M. (1986). Feeding lupins to ewes for four days during the luteal phase can increase ovulation rate. *Proceedings of the Australian Society of Animal Production*, 16, 367–370.
- The role of nutrition on reproduction of sheep. (2014). *Acta Veterinaria Brasilica*, 8(supl.2). <https://doi.org/10.21708/avb.2014.8.0.3955>
- Tilton, J. E. (1964). *Factors influencing the gestation length in sheep*. [Master's Thesis, Illinois State Normal University].
- Ungerfeld, R., Carbajal, B., Rubianes, E., & Forsberg, M. (2005). Endocrine and ovarian changes in response to the ram effect in medroxyprogesterone acetate-primed corriedale ewes during the breeding and nonbreeding season. *Acta Veterinaria Scandinavica*, 46(1–2), 33–44. <https://doi.org/10.1186/1751-0147-46-33>
- Ungerfeld, R., Pinczak, A., Forsberg, M., & Rubianes, E. (2002). Ovarian responses of anestrous ewes to the “ram effect.” *Canadian Journal of Animal Science*, 82(4). <https://doi.org/10.4141/A02-005>
- Ungerfeld, Rodolfo. (2012). Sexual behavior of medium-ranked rams toward non-estruual ewes is stimulated by the presence of low-ranked rams. *Journal of Veterinary Behavior: Clinical Applications and Research*, 7(2). <https://doi.org/10.1016/j.jveb.2011.05.023>
- Ungerfeld, Rodolfo, & Bielli, A. (2012). Seasonal and social factors affecting reproduction. *Livestock Reproduction: Bovine, Swine, and Ruminants” Encyclopaedia of Life Support Systems (EOLSS)*.
- USDA NASS. (2020). Sheep and Goats. *Critical Quarterly*, 2(1), 64–71. <https://doi.org/10.1111/j.1467-8705.1960.tb00840.x>

- Viñoles, C., Paganoni, B., Glover, K. M. M., Milton, J. T. B., Blache, D., Blackberry, M. A., & Martin, G. B. (2010). The use of a “first-wave” model to study the effect of nutrition on ovarian follicular dynamics and ovulation rate in the sheep. *Reproduction*, 140(6). <https://doi.org/10.1530/REP-10-0196>
- Webb, R., Baxter, G., McBride, D., Ritchie, M., & Springbett, A. J. (1992). Mechanism controlling ovulation rate in ewes in relation to seasonal anoestrus. *Journal of Reproduction and Fertility*, 94(1), 143–151. <https://doi.org/10.1530/jrf.0.0940143>
- Wheaton, J. E., Windels, H. F., & Johnston, L. J. (1992). Accelerated lambing using exogenous progesterone and the ram effect. *Journal of Animal Science*, 70(9), 2628–2635. <https://doi.org/10.2527/1992.7092628x>
- Xu, S. S., Gao, L., Xie, X. L., Ren, Y. L., Shen, Z. Q., Wang, F., ... Li, M. H. (2018). Genome-wide association analyses highlight the potential for different genetic mechanisms for litter size among sheep breeds. *Frontiers in Genetics*, 9(APR), 1–14. <https://doi.org/10.3389/fgene.2018.00118>