

THE USE OF BEHAVIORAL MEASURES TO QUANTIFY WELFARE IN DAIRY COWS
AND LAMBS

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Title

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ABSTRACT

The primary objective of the experiments described in this thesis was to use quantitative behavioral measures of livestock to evaluate animal welfare. In one study, the effects of a therapeutic drug in lame dairy cows were evaluated for alleviating pain associated with lameness and hoof trimming. Lameness pain was measured using lying and standing times as well as locomotion scoring. No effect on lameness indicators or milk production was found for hoof trimming or drug administration. In the second study, behaviors associated with maternal nutritional plane during gestation were measured in lambs. Lambs born to nutritionally restricted dams behaved differently from those born to adequately-fed dams after a pen change, implying a difference in adaptation to a stressful event. Continuing to find quantitative behavioral measures for pain and adaptation to stressors will aid in future work to improve livestock welfare.

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You all have made the past brighter and the future easier.

DEDICATION

To my family-

*“Our world may not be good in an abstract sense,
But it is the very best we could have.
Each trait plays its part and must be as it is.”
Gould and Lewontin (1979)*

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LIST OF ABBREVIATIONS

BW	Body Weight
Ca	Calcium
cm	centimeter
CP	Crude Protein
d	day
DM	Dry Matter
DIM	Days in Milk
DVR	Digital Video Recorder
FDA	Food and Drug Administration
g	gram
h	hour
IU	International Units
kg	kilogram
lb	pound
m	meter
min	minute
mg	milligram
mL	milliliter
mo	month
NaCl	Sodium Chloride
NRC	National Research Council
NRS	Numerical Rating System

NSAID Non-steroidal anti-inflammatory drug
P Phosphorus
ppm parts per million
SAS Statistical Analytical Software
Se Selenium
TMR Total Mixed Ration
wk week

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

Presented here is a collection of work performed with the aim to improve animal welfare. Finding valid, quantifiable behavioral measures of animal welfare directed our work throughout each study. This thesis will move through an introductory literature review, with the two following chapters each describing a research project. Each is a unique study with a similar theme of quantifying behavioral measures of the animal to evaluate welfare. The first study evaluates the behavior of lambs born to undernourished and adequately fed ewes when lambs undergo a pen change. As pen changes are a stressful event, the lamb behaviors were used to evaluate each lamb's ability to adjust to change following a stressor. The second study examines the use of pharmaceutical therapeutics to mitigate pain associated with lameness and hoof trimming in dairy cows. A final chapter includes concluding remarks and possible future research directions.

What is Welfare?

The welfare of an animal influences the animal's health, survival, and well-being. Animal welfare has been broken down into five necessary domains (Mellor and Reid, 1994; Mellor, 2004). The first three domains are nutrition, environment, and health. Nutrition includes food and water quality and availability. Environment focuses on any environmental challenge including heat or cold stress, and the health domain includes disease, bodily injury, or any form of impairment. These domains have commonly been the focus of research. The last two domains, behavior and mental state, are more difficult to quantify. The behavior domain includes anything that will inhibit a natural behavior of an animal, for example restraining a dairy cow in a tie stall, which inhibits cow's natural behavior to walk around and act as a herd.

The final domain, mental state/experience, is influenced by the previous four domains, and includes states such as fear, pain, anxiety, thirst, and hunger. Work throughout our studies will be focused on quantifying animal's health and behavior.

Welfare domains may be described using grades, with a grade O being optimal conditions, followed by grades A, B, C, and E being the poorest welfare state, resulting in death. Whichever domain has the highest grade assigned will dictate the overall welfare of the animal. By creating a scale, we can judge welfare of animals across all aspects of the animal's life. For example, any grade of O is considered no insult to that domain for an animal. Grade A is considered a minor insult to the animal which is readily reversible. This includes a variety of insults, such as a pen change. Grade B is a short-to-moderate term insult from which the animal will completely recover. For example, a grade B insult can include common surgeries. Grade C is an insult from which a serious compromise has been made and may result in euthanasia to end the insult. This includes an animal contracting a disease from which it is unable to recover. Finally, grade E has the animal severely debilitated and will result in the animal having an unpleasant death. Grade E insults include severe undernourishment. These grades allow us to evaluate all aspects of an animal's life during research (Mellor, 2004; Mellor, Patterson-Kane, and Stafford, 2009).

Evaluating Welfare

As animal welfare is influential on our management of livestock, it is important to use clear measures to evaluate the animal. Both studies presented in this thesis share the objective of quantifying behavioral measures indicative of good (or bad) welfare statuses. By objectively quantifying behavior, we can use behavior to evaluate animal welfare and improve an animal's overall well-being.

Welfare Measurements in Lambs

Animals are born with their welfare state influenced by maternal fitness, which has a life-long influence on the animal's welfare domain related to health. For example, lambs born as triplets have the lowest birth weight, when compared to those born as singletons or twins. Though triplets are desirable in production due to a higher yield per ewe, they also are more likely to have lower vitality and are more likely to die from dystocia (Everett-Hincks and Dobbs, 2008). Triplets will also suckle less frequently and for shorter duration of time compared to twins or singleton lambs, contributing to the negative welfare of the animal (Hess et al., 1974). Maternal age also influences lamb health. Lambs born to two-year old ewes are less likely to survive to weaning than those lambs born to ewes which are three or older (Everett-Hincks and Dodds, 2008). Maternal nutrition also influences lamb health; this is discussed further in Chapter 2.

Lamb-dam interactions have a measurable impact on lamb welfare and behavior. Lambs separated from dams at 24 h after birth have shorter standing times, more social interactions with other lambs, and will eat less solid food than those raised with a dam present (Napolitano et al., 2003). Lambs housed with only other lambs will play with an unknown lamb sooner than those raised with a dam present, and those raised alone will bleat more than those housed with a group (Zito et al., 1977).

Frequently, animals in a decreased welfare state are physiologically compromised, which can be measured. When a lamb is raised in the presence of a dam but unable to suckle it will have higher cortisol levels than those removed from the dam (Napolitano et al., 2003). There is varying data in weight gain results for lambs raised in a group without dam, with Napolitano et al. (2003) reporting no differences in weight gain after the first 7 days and Zito et al. (1977)

reporting a measurably lower average daily gain in group housed lambs. Though weight change can be a measurement of welfare, it is not always reflective of the current welfare state of the animal.

Video recordings are commonly used to observe behavior of multiple species, including sheep and lamb observations. For example, video recordings have been used to observe suckling behavior in triplets, twins, and singleton lambs (Hess et al., 1974). Video recordings also are used for training new observers, to prevent bias and ensure consistency. Video recordings allow for a single animal to be observed at multiple time points for an extended period of time. This allows the observer to maintain consistency and accuracy, as quick judgments are not necessary with video. Videos can also be used when live observation is not an option, such as when sheep are placed in a maze situation (Erhard et al., 2004), 24 h monitoring of cattle is needed (von Keyserlingk et al., 2008), or the presence of a human will modify the result as seen in pigs (DeBoer et al., 2013).

It is important that behaviors are verified for accurately measuring what they are intended to measure. Romeyer and Bouissou (1992) evaluated fear responses in lambs with a focus on verifying each behavior. All lambs were measured for latency to enter the test room. Following this result, all subsequent behaviors measured were compared to the initial test. All behaviors were compared to the initial latency and were found to be connected. For example, if a lamb was slow to enter a testing area, then that lamb was also slower to approach the feed available and ate the feed quickly. By understanding the connections between behaviors exhibited in animals, one may be able to draw similar conclusions to the animal's welfare. If a lamb is seen eating quicker than the other lambs and is slow to approach the feed, the animal's mental welfare status is being compromised as that lamb is exhibiting a fear response (Viérin and Bouissou, 2003).

Welfare Measurements in Cattle

Pain occurs in livestock as both acute pain, which involves the animal experiencing a rapid increase in pain, and chronic pain in which the animal sustains pain over an extended period of time (usually days to weeks). Each of these is evaluated differently and causes different insults to an animal's welfare. Pain in livestock can be caused from an insult in at least 2 domains and across all grades. Pain can be a result from environment (producers hot-iron dehorn calves, causing pain from handlers) or health (animal develops an illness which results in pain over time). Pain can cause an insult in the animal's health (the animal is impaired from performing a normal function when in pain), behavior (the animal cannot express normal behaviors due to subsequent pain), mental state/experience (the animal will suffer from distress from pain), and sometimes nutrition (if the animal in in pain, it may not intake proper levels of nutrients).

Within each domain, chronic or acute pain can have effects across all grades.. In the case of hot-iron dehorning, this would occur as acute pain, with a grade B level insult. The animal is undergoing a common procedure and will recover to normal health with time. In the case of compromised health, this can be presented as either chronic or acute pain. If an animal becomes ill acutely, this may result in death from illness, which will result in a grade E insult within the health domain. If an animal is suffering from chronic pain, such as some mastitis cases in cattle, this may result in a grade C insult, where the animal is eventually culled or euthanized due to levels of constant pain.

In order to make accurate judgment calls regarding an animal's welfare, there must be a method to quantify pain levels. Quantifying pain can be difficult. For example, in a study by Lay et al. (1992) evaluating pain associated with hot-branding cattle, it was found all the animals

exhibited an increase in cortisol due to handler effect and being placed in confinement. It is important to verify measurements to ensure the behaviors and biology observed accurately reflects what is occurring.

Efforts have been made to quantify pain across conditions cattle undergo during their lifetime. Pain has been successfully evaluated in livestock using behavioral measures associated with hot-iron dehorning (Faulkner and Weary, 2000), mastitis (Fitzpatrick et al., 2013), and lameness (Chapinal et al., 2010A) and biochemical measures, such as plasma cortisol concentration in scoop dehorning (McMeekan et al., 1997) and band castration (Molony et al., 1995) and salivary cortisol during band castration (González et al., 2010).

One way that pain may be quantified is using behavioral markers. Behaviors used to evaluate pain during disbudding include head shaking, ear flicking, and head rubbing. Calves will exhibit these behaviors less frequently immediately following disbudding when lidocaine is used than when calves are disbudded without any therapeutics (Stilwell et al., 2009). During hot-iron branding cattle will show similar behaviors of discomfort, such as tail-flicking, kicking, vocalizing, and falling down in the chute more often than when cattle are freeze branded. Cattle also will exert higher amounts of force on a head gate when hot-iron branded than when cattle are freeze branded as an attempt to avoid discomfort (Schwartzkopf-Genswein et al., 1998).

Technology can be used to measure behavior through data recorders such as accelerometers and weight scales. Numeric scales can be created for defined behaviors, which allow for a systematic recording of behavior using video recordings and live observers. Technology also allows for precise measurements to be taken, and can decrease the level of human error in the work or can gather measurements a human is unable to collect. For example, accelerometers can collect continual data about lying and standing times for multiple days.

Accelerometers have been used to show that dairy cows have longer lying bouts due to lameness and this can be observed for weeks (Chapinal et al., 2010C). Also, rumination collars can collect data related to feed intake in cows. Rumination collars have measured that lame cows spend less time feeding and ruminating than those which are not lame (Miguel-Pacheco et al., 2014).

Scales have been used to automatically collect measurements related to weight, such as weight distribution and weight shifting. These measures have been used to diagnose lameness in dairy cows, as larger variation of weight between back legs, i.e. weight shifting, is indicative of soreness in the hind feet (Chapinal et al., 2010AB). Weight shifting is best used to detect pain associated with lameness in dairy cows, as pain from mastitis is not detectable by weight shifting (Madrano-Galarza et al., 2012).

Visual appraisal of cows can be systematically used to make animal behaviors into quantitative measures, similarly to the use of observing behaviors in lambs to draw conclusions regarding welfare. The locomotion of dairy cows can be visually scored to evaluate lameness. Flower and Weary (2006) have created a 5-point scale to evaluate lameness in dairy cows when walking past the observer. This scale contains explicit variables of cow movement such as head bobbing, length of stride, and arching of back, and has been proven for intraobserver consistency. This scale has been accepted as a valid measure of dairy cow lameness despite its subjectivity, when cows are viewed by trained observers.

Visual appraisal of animal welfare can be performed as both live observer and using video recordings of animals. Live observations involve explicit classifications of behaviors and frequently involve one observer across a set time period. Live observations have been used to evaluate young animals, such as feeding behavior in dairy calves (Jensen and Budde, 2006). Live

observations may require a quick scan of behavior of multiple animals across a pen, or they may entail a set period of time focused on an individual animal.

Besides behavioral measures, physiological and biochemical measures can be used to measure welfare in cattle. Cortisol is a commonly used indicator of stress. Blood cortisol will increase when using caustic paste disbudding when compared to sham disbudding (Stilwell et al., 2009) or scoop dehorning (McMeekan et al., 1998). An increase in salivary cortisol may be associated also with band castration (González et al., 2010), however Fisher et al. (2001) observed no change in plasma cortisol during band castration. This variation may be due to the difference in sensitivity of cortisol taken through salivary samples compared to blood sampling. Infrared thermography has shown an increase in heat to a branding site, indicative of an inflammatory response and likely pain associated with branding (Schwartzkopf-Genswein and Stookey, 1997).

Welfare Measurements and Conclusions across Species

Research has been using behavior to quantify welfare. Animals will adapt to stressful events and will attempt to minimize negative welfare interactions. Observing behaviors and quantifying them using research may help generate information that can be used to improve welfare states for livestock.

As animal scientists we should have a desire to improve and maintain a positive welfare status in livestock. If this is not motivation enough, frequently an economic loss will exist when negative welfare statuses occur. Many examples exist in production, such as lamb mortality due to environment and nutritional stress (Everett-Hincks and Dodds, 2008). Also, dairy cows will decrease in milk production due to clinical lameness (Enting et al., 1997). Finding methods to

measure behaviors in animals is the first step in using management to control pain and decrease stress in livestock.

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CHAPTER 2. EVALUATION OF THE EFFECTS OF MATERNAL NUTRITION AND REGROUPING ON LAMB BEHAVIOR

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Abstract

The objective of this study was to investigate the effects of undernutrition during pregnancy on offspring behavior during a period of regrouping. A total of 28 lambs were enrolled in the study. Lambs were born to ewes fed one of three rations beginning on day 54 of pregnancy. Dams received a control diet (CON; $n = 9$) meeting 100% of NRC requirements, or a restricted diet at 60% of NRC requirements, with or without supplemental arginine (RES; $n = 19$). Lambs were weighed and removed from dams at parturition and placed in group housing pens. At 38 ± 7 d of age, the lambs were blocked by treatment and randomly regrouped into new pens. Following this regrouping, lamb behavior was recorded using a digital camera for 30 h across 3 d. Each pen contained a raised platform (61 cm x 122 cm x 12 cm). Using digital recordings, individual lamb behaviors were evaluated every 3 minutes. Lambs increased the proportion of time spent lying over the 3 d observational period, with CON lambs having a larger increase in time spent lying alone during the study ($P = 0.006$). Control lambs spent proportionally more time ingesting milk than RES lambs ($P = 0.034$). Also, those lambs born in the bottom quarter of birth weights spent more time ingesting a feed source than those lambs born to the top quarter of birth weights ($P < 0.001$). Although all lambs decreased the proportion of time spent interacting with other lambs during the duration of our study ($P < 0.001$), the CON lambs had a greater decrease than RES lambs ($P = 0.002$). For all lambs, an increase was seen in

the proportion of time spent alone. Control lambs had a greater increase in time alone and a larger decrease in proportion of time spent interacting with other lambs than RES lambs. Control lambs may be experiencing a quicker adaptation to the stress of regrouping as evidenced by quicker increase in time spent lying and time spent away from the flock. Maternal nutrition may affect how quickly lambs adapt to stressors such as novel environments and regrouping.

Key words: Lamb behavior, maternal nutrition, pen change, social interactions

Introduction

In livestock species, undernourishment of the dam during pregnancy is known to have adverse effects on fetal and neonatal growth. Low body weight and condition during pregnancy in ewes result in lower birth weight lambs (Vonnahme et al., 2003, Ford et al., 2007, Everett-Hincks and Dodds, 2008).

Our knowledge regarding the physiological effects of maternal health during pregnancy on offspring is expanding, but little is known about the long-term effects of undernourishment during pregnancy on offspring behavior. Undernourishment during pregnancy and lactation has been reported to affect rat behavior. After weaning, rats that suckled from undernourished dams completed a maze with a food reward faster and with fewer errors compared with rats that suckled from adequately fed dams (Smart et al., 1973).

Erhard et al. (2004) investigated the effects of maternal undernutrition on cognitive ability in offspring sheep at 18 months of age. Sheep born to undernourished dams showed greater activity when placed in confinement conditions, regardless of their birth weight. However, maternal nutrition did not affect offspring locomotion, jumping, or vocalizing during periods of isolation. Control-born lambs were quicker to approach a novel stimulus and were more likely to correctly complete a T-maze when compared to undernourishment-born lambs.

Lamb rearing can also affect lamb behavior and growth. Lambs separated from dams 24 h after parturition have shorter daily standing times when compared to lambs raised with dams (Napolitano et al., 2003). Lambs separated from dams early also have a greater number of social interactions with other lambs than those raised with dams (Napolitano et al., 2003). Despite strong social bonds formed between lambs, group-raised lambs with no dam present have a lower mean body weight than lambs reared alone, with dams in a group, or lambs raised with only their dam. This may be explained by increased competition for feed in a group setting without dams present. Also, lambs raised without dams who are either group raised or individually raised would attempt to avoid an unfamiliar ewe when placed with one in a pen. Lambs placed with an unfamiliar ewe once a week for eight weeks would attempt to butt the ewe during the final three weeks (Zito et al. 1977).

Artificial rearing, defined by Napolitano et al. (2003) as when “lambs are separated from the dam at an earlier age (0-2 days)”, is a common management tool that can affect lamb behavior and growth. Artificially raising lambs without interactions with their dam can have negative consequences on lamb outcomes, including lower weight gain during the first 7 days of life (Zito et al., 1977; Napolitano et al., 2003). Artificially raised lambs are known to have stronger social connections and exhibit more play behavior with other lambs, when compared to lambs raised individually or with only a dam (Zito et al., 1977).

Mixing unfamiliar animals together is stressful and results in behavioral changes. Plasma cortisol and leukocyte concentrations increase in calves at times of pen randomization, showing pen changes to be a physiologically stressful event for animals (Hickey et al., 2003). When pen changes occur associated with weaning, pre-exposing animals to new pen conditions will result in fewer antagonistic behaviors than when animals are placed in the new situation immediately

(Walker et al. 2007, Hessel et al. 2006). In dairy production, mature cows will be displaced more frequently from the feed bunk up to two days following a pen move, and cows exhibit shorter lying bouts the day of the change (von Keyserlingk et al., 2008). Knowing pen changes are a stressful event in an animal's life with negative effects on animal behavior, a pen change can be used to evaluate behaviors associated with adapting to stress across livestock species.

As work in undernutrition, coupled with behavioral effects of pen changes and artificial raising may suggest, we hypothesized that those lambs born to undernourished dams would decrease social behaviors and would perform more food focused actions compared to lambs born to adequately fed dams during pregnancy. Our objectives included validation of quantitative, objective behavioral methods to measure vitality in lambs born to adequately fed and undernourished ewes, and associating these quantitative behavioral measurements with lamb growth, illness, and survival.

Materials and Methods

Animals and Housing

Twenty-eight lambs from a larger group born to a nutritional study were enrolled, based on observed good health at the start of the study. Enrollment included 3 sets of twins (2 CON; 1 RES) and 22 singleton lambs (6 CON; 8 RES; 8 RES-ARG), with 11 males (4 CON; 5 RES; 2 RES-ARG) and 17 females (6 CON; 6 RES; 5 RES-ARG). Lambs were between 31 and 45 days old during the time of observation. All lambs were housed at the North Dakota State University Animal Nutrition and Physiology Center in Fargo, ND. Lambs were housed in five rectangular pens (2.79 m²), blocked by treatment and randomly assigned in groups of 4-7 lambs. Lambs were fed milk replacer (Super Lamb Milk Replacer, Merrick's Inc., Middleton, WI; DM basis: 24% CP, 30% fat, 0.10% crude fiber, 0.5 to 1.0% Ca, 0.65% P, 0.3 ppm Se, 66,000 IU/kg vitamin A,

22,000 IU/kg vitamin D, and 330 IU/kg vitamin E) in buckets ad libitum and had a mixture of long stem mid-bloom alfalfa hay and creep feed (DM basis: 20% CP, 6% fat, 8% crude fiber, 1.4 to 1.9% Ca, 0.4% P, 0.5% to 1.5% NaCl, 0.3 ppm Se, 11,000 IU/kg vitamin A, 6,000 IU/kg vitamin D, and 100 IU/kg vitamin E) and water available ad libitum as well. Both milk replacer and creep feed were commercially available feeds and met NRC guidelines. Fresh milk replacer was delivered daily at 8:00 h and 18:00 h, with creep delivered as needed during feedings. Procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee.

Experimental Procedure and Treatments

Lambs in this study were born of ewes which were assigned to one of three treatment groups on d 54 of pregnancy and continued on treatment diets through the duration of pregnancy as part of another study (Peine et al., 2013). The diets were comprised of alfalfa meal (34.0%), beet pulp (27.0%), wheat middlings (25.0%), ground corn (8.4%), soybean meal (5.0%) and a trace mineral premix (0.6%). The control group (CON; n = 9) consisted of ewes who received a diet meeting NRC requirements. The restricted group (RES; n = 11) was comprised of dams who received the same diet as control ewes, at 60% of NRC recommendations. In the arginine-supplemented group (RES-ARG; n = 8) dams were fed the restricted diet, supplemented with rumen-protected L-Arginine at 180 mg arginine/kg body weight per day. Arginine was mixed with 50 g of fine ground corn and fed once daily at 8:00 h before offering the pelleted diet. Both CON and RES ewes were also provided 50 g of fine ground corn daily, without the added rumen protected arginine. After parturition, lambs were immediately removed from dams. Lambs were bottle fed colostrum (Lifeline Rescue Colostrum, APC, Ankeny, IA) at 0, 2, and 4 h, then every 4 h until 24 h of age, and then fed milk replacer in buckets ad libitum. All lambs were group

housed for the duration of the study. Lamb housing contained a 12 h on, 12 h off automatic light cycle, with lights turning on at 7:00 h and off at 19:00 h. Each pen contained a raised platform (61 x 122 x 12.7 cm) to allow lambs further interaction with the environment.

Data Collection

Data were collected through video recordings, using four AgCam cameras (DMAC-RC, Dakota Micro, Inc. Cayuga, ND, USA) recorded to an EverFocus DVR (ECOR Series, EverFocus Electronics Corp., Taipei, Taiwan). Lambs were filmed and observed for 3 days following the pen change. Day 1 observation began after the completion of the pen switch and after each pen had been fitted with the raised platform, which was completed at 13:00 h that day. The following 2 days of observed footage began at 7:00 h and ended at 19:00 h each day, with a total of 30 h of footage. Each lamb's activity was recorded every 3 minutes throughout the observation period. Birth weights were also recorded and used to investigate correlations with behaviors.

Lamb behaviors, as represented in Table 2.1, were categorized as follows: lying anywhere within the pen, lying alone (defined as the lamb not in physical contact with another lamb), lying in contact with at least one other lamb, ingesting some form of feed (either milk or creep), interacting with either milk or creep (including sniffing of feeders), interacting with water (including ingesting), standing upon the elevated area (defined as at least one leg placed on the platform), and interacting with the elevated area either alone or with other lambs. Other behavior categories included interacting with other lambs through sniffing or grooming behaviors and interacting with the environment, specifically sniffing walls or pen fencing.

Table 2.1. Description of criteria for behaviors used to evaluate lambs

Behavior	Definition
Lying	Lamb is lying in pen, either alone or with other lambs
Lying with others	Lamb is lying in physical contact with another lamb
Lying alone	Lamb is lying without physical contact with another lamb
Ingesting	Lamb is ingesting feed, either creep or milk
Ingesting milk	Lamb is ingesting milk defined as the lamb's head upward and appearing latched onto the milk bucket, with frequently the tail 'wagging' while ingesting
Ingesting Creep	Lamb is ingesting creep feed defined as the lamb's nose in the creep feeder
Interacting with milk	Lamb is interacting in some way, except consuming, with milk bucket
Interacting with creep	Lamb is interacting in some way, except eating, with creep feeder
Standing on mound	Lamb is standing with at least one foot on heightening area
Alone on mound	Lamb is standing alone on heightened area (no other lamb has one foot or more placed on heightened area)
Interacting with mound	Lamb is interacting but not standing in some way with the heightened area
Interacting with lambs	Lamb sniffing, chewing, touching, or otherwise interacting with another lamb
Interacting with water	Lamb is ingesting or otherwise interacting with automatic waterer
Interacting with environment	Lamb in sniffing floor, walls, pen railing, chewing on pen railing, or otherwise interacting with the environment within the pen in some way

Statistical Analyses

Two sets of analyses were performed. In the first set of analyses, a binary logistic regression model was fit to the data for each behavior using the GLIMMIX procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). The GLIMMIX procedure was implemented due to the binary

nature of the behavior data as well as the need for both fixed and random effects in the model. Treatment, day, and treatment by day interaction were included as fixed effects. Lamb, pen, and twin status were included as random effects. Sex was removed from the model due to no observed effect on results. Additionally, an AR(1) correlation structure was estimated for observations that correspond to the same lamb and the same day (lamb 1 & day 1, lamb 1 & day 2, lamb 1 & day 3, lamb 2 & day 1, lamb 2 & day 2, etc.). This correlation structure accounts for higher correlations among observations that were recorded close to each other in time for each lamb.

Due to the variation of the amount of time observed across days, results are presented as proportions of days. Graphs were developed using a generalized linear mixed model, modeled using the log odds for each behavior, where:

$$\text{Log Odd } (p) = \text{Log} \left(\frac{p}{p-1} \right)$$

where p is the probability that a lamb is performing a certain behavior. Specifically, an estimation was made for the expected value of the log odds (\widehat{LO}) with the following formula:

$$\widehat{LO} = \hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_2 + \hat{\beta}_3 x_3 + \hat{\beta}_4 x_1 x_2 + \hat{\beta}_5 x_1 x_3$$

where $x_1 = 1$ for CON lamb, or 0 for RES and RES-ARG lamb, $x_2 = 1$ for Day 1, and 0 for any day other than Day 1, and $x_3 = 1$ for Day 2, and 0 for any day other than Day 2.

Birth weight data were sorted from heaviest to lightest lambs and the top and bottom 25% taken for analysis, resulting in 7 lambs per group (high 5.76 ± 0.5 kg; low 3.98 ± 0.4 kg), as listed in Table 2.2. These data were used in the second set of analyses, which were focused on evaluating correlations between behavior and birth weights. Similarly to the first set of analyses, a binary logistic regression model was fit to this subset of data for each behavior. Birth weight (low or high), day, and birth weight by day interaction were included as fixed effects. As with

the first set of analyses, lamb, pen, and twin status were included as random effects, and an AR(1) correlation structure was estimated for observations that correspond to the same lamb and the same day.

Table 2.2. A list of all lambs' birth weight and treatment, sorted from lowest birth weight to highest birth weight, split into quartiles. Lamb assignment to the low birth weight group (3.68 ± 0.4 kg) or high birth weight group (5.76 ± 0.5 kg) was not correlated with dam treatment.

Treatment	Birth weight
RES	3068
RES	3163
RES	3786
CON	3833
RES-ARG	3891
RES	3931
CON	4089
RES	4353
RES-ARG	4385
CON	4600
RES	4730
RES-ARG	4820
RES	4828
RES	4835
CON	4849
RES-ARG	4880
RES-ARG	4900
CON	4946
RES	5000
CON	5090
RES-ARG	5174
RES	5325
RES-ARG	5364
CON	5442
RES-ARG	5489
CON	5854
RES	5925
CON	6947

Results

When differences between RES-ARG born lambs and RES lambs are present, these will be represented in each behavior. When no differences between RES-ARG treatment and RES treatment were found, the group was combined and presented as RES.

Lying Behavior

Differences in time spent lying were found between days ($P < 0.001$). As seen in Figure 2.1, lambs spent proportionally less time lying day 1 than day 2 ($P < 0.001$) and proportionally less time day 1 than day 3 ($P < 0.001$). Differences in lying time between day 2 and day 3 were not found to be different. Also, a difference was found between RES and RES-ARG lambs, with RES born lambs spent more time lying overall compared to RES-ARG throughout the study ($P = 0.014$).

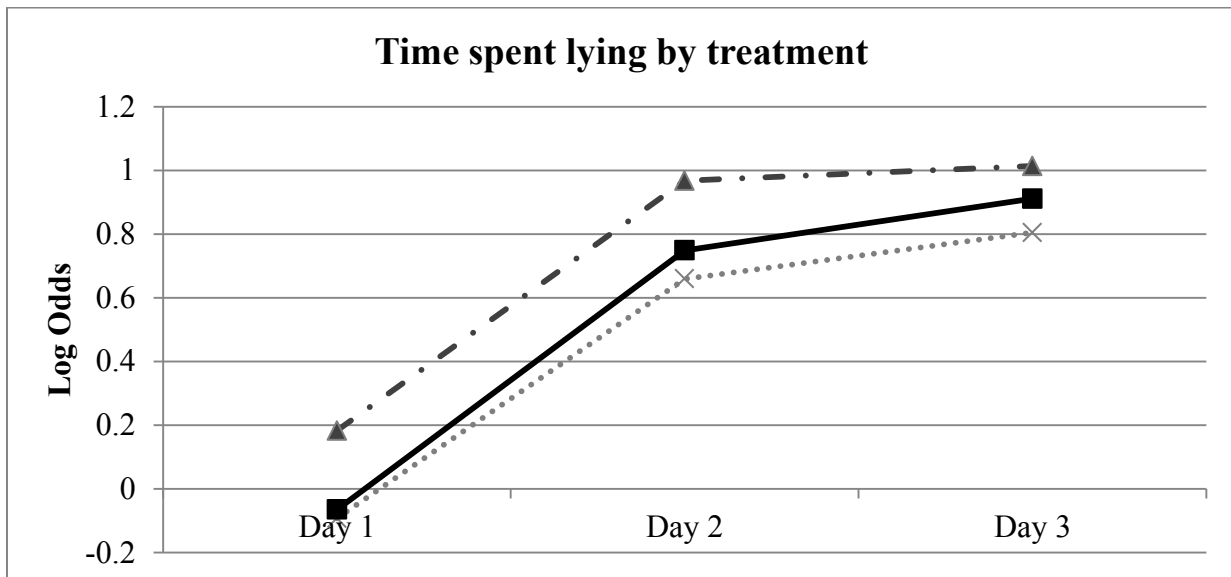


Figure 2.1. Time spent lying either alone or with others expressed as group means of log odds of the proportion of time lying. Control (CON ■; $n = 9$), restricted (RES ▲; $n = 11$), and restricted plus arginine (RES-ARG ×; $n = 8$) are represented across 30 h observed within 3 d on the x-axis. Time spent lying by treatment was not different ($P = 0.46$). Time spent lying was significant across day ($P < 0.001$), with an increase in time spent lying throughout the duration of the study. A significant day affect was present from day 1 to day 2 ($P < 0.0001$) and day 1 to day 3 ($P < 0.0001$).

Differences in time spent lying alone were significant for treatments across each day ($P < 0.001$) and significant for a treatment \times day interaction ($P = 0.006$), as represented in Figure 2.2. Lambs spent a significantly larger proportion of time lying alone on day 2 than day 1 ($P = 0.004$), more time on day 3 than day 2 ($P = 0.002$) and a larger proportion of time on day 3 than day 1 ($P < 0.001$).

When RES and RES-ARG was combined due to no differences between the groups, a treatment \times day interaction was present for RES versus CON for day 1 versus day 3 and day 2 versus day 3. The increase in proportion of time spent lying alone from day 1 to day 3 was greater for lambs born from dams receiving the CON diet than the RES diet ($P = 0.016$). The increase in proportion of time spent lying from day 2 to day 3 was also greater for CON lambs than those RES ($P = 0.004$). Birth weights did not correlate with time spent lying alone or across days.

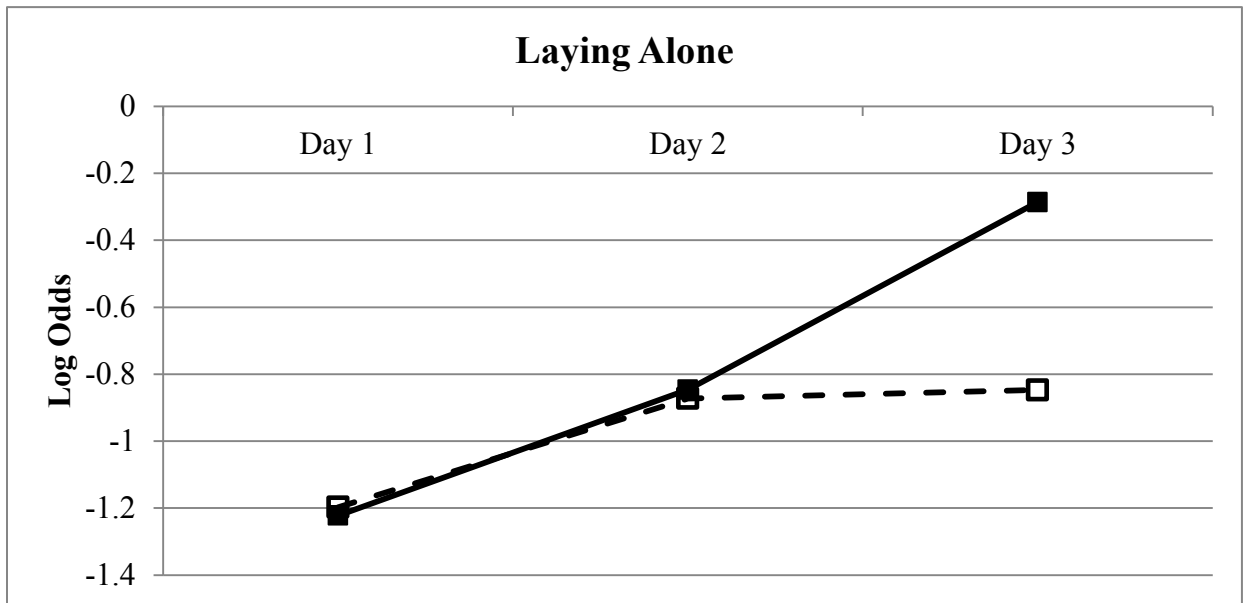


Figure 2.2. Time spent lying alone expressed as group means of log odds of the proportion of time lying. Group mean values for control lambs (CON ■; $n = 9$) and restricted plus restricted arginine lambs (RES □; $n = 19$) are represented across 3 d on the x-axis. Time lying alone was significant across day ($P < 0.001$), with a larger increase in time spent lying alone in CON lambs ($P = 0.006$), but not significant across treatment ($P = 0.49$).

Differences in time spent lying in contact with other lambs were significant across each day ($P = 0.003$) and significant for treatment \times day ($P = 0.032$). Lambs spent proportionally less time lying in contact with other lambs on day 1 when compared to day 2 ($P = 0.001$) and on day 1 compared to day 3 ($P = 0.049$). No difference was found from day 2 to day 3. Treatment \times day effects were present for RES versus CON for day 1 versus day 3 and day 2 versus day 3 when RES and RES-ARG were combined, represented in Figure 2.3. From day 1 to day 3 lambs born to RES dams had a greater increase in proportion of time spent lying in contact with other lambs than those lambs born to CON dams ($P = 0.023$). Lambs in the RES group also increased in time spent lying with other lambs from day 2 to day 3 when compared to lambs born to CON dams, which actually decreased time spent lying with other lambs from day 2 to day 3 ($P = 0.032$).

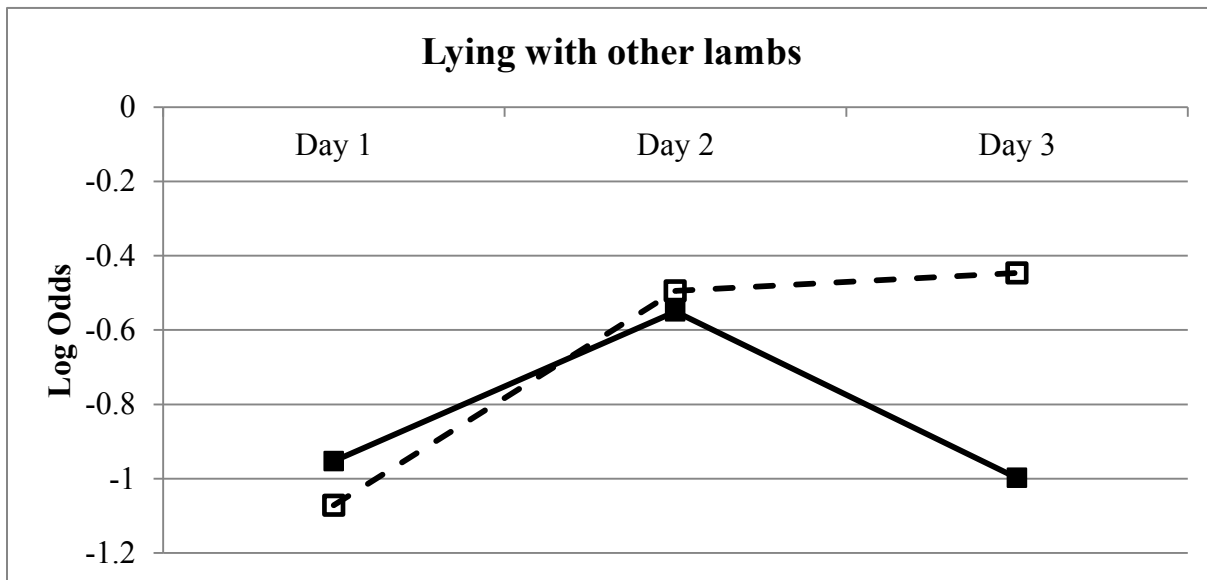


Figure 2.3. Time spent lying with other lambs expressed as group means of log odds of the proportion of time lying. Group mean values for control lambs (CON ■; $n = 9$) and restricted plus restricted arginine lambs (RES □; $n = 19$) are represented across 3 d on the x-axis. Time lying with others was significant across day ($P < 0.002$), with a larger decrease in time spent lying with others in CON lambs ($P = 0.032$). No treatment effect was present ($P = 0.47$).

Ingesting Behavior

When birth weight is broken into quartiles, those lambs in the lowest quarter organized by birth weight spent more time ingesting either milk or creep than those lambs born with a higher birth weight ($P < 0.001$), represented by Figure 2.4.

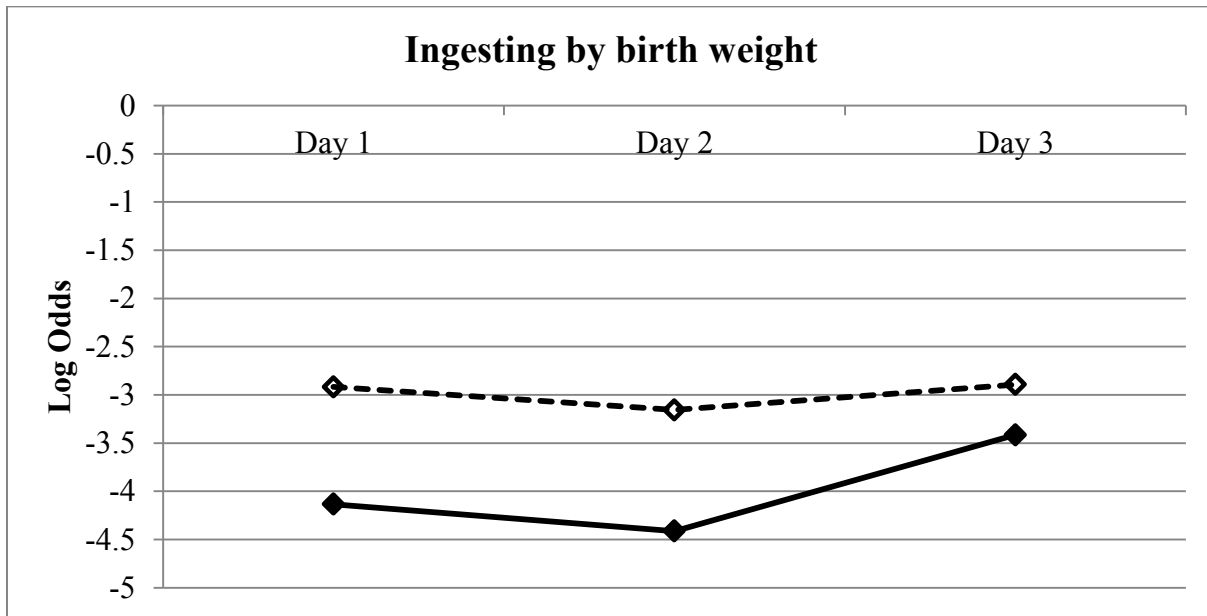


Figure 2.4. Mean log odds of the proportion of time spent ingesting either milk or creep are compared across birth weight groups. Highest birth weight lambs (High \blacklozenge ; $n = 7$) and lowest birth weight lambs (Low \blacklozenge ; $n = 7$) are represented across 3 observational days. Significant birth weight ($P = 0.03$) and day effects from day 2 to day 3 ($P = 0.04$) were present.

Differences in time spent ingesting milk were present with a treatment effect ($P = 0.034$) and day effect ($P = 0.019$). Control lambs spent proportionally more time ingesting milk than RES and RES-ARG combined lambs ($P = 0.034$), as seen in Figure 2.5. Also, lambs spent proportionally more time ingesting milk on day 1 than day 2 ($P = 0.015$) and more time on day 3 than day 2 ($P = 0.015$).

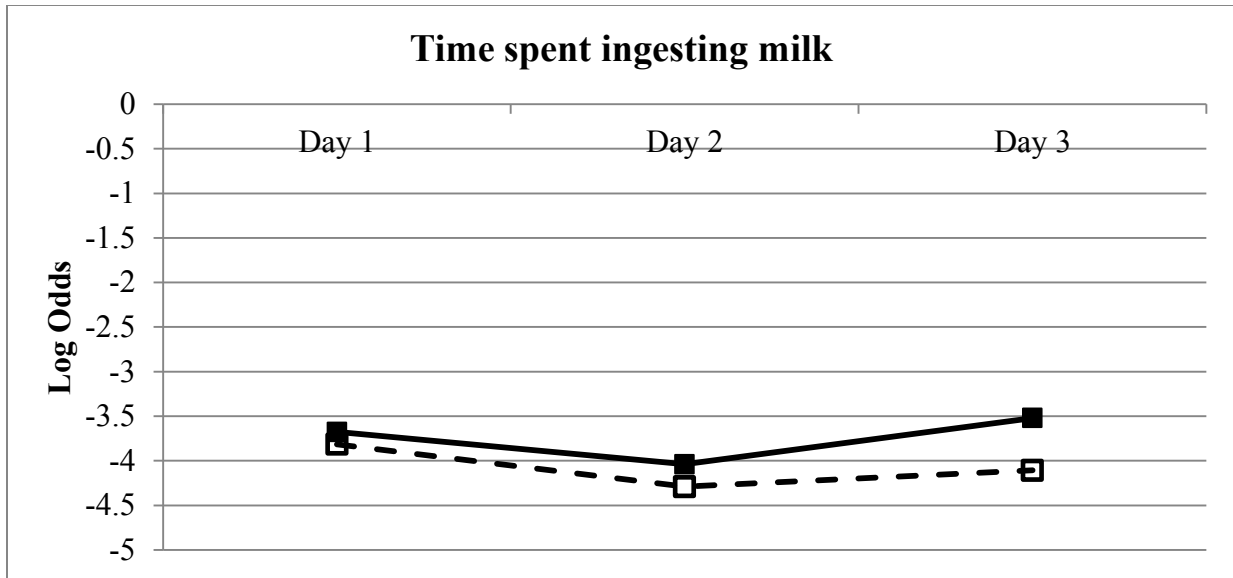


Figure 2.5. Time spent ingesting milk expressed as group means of log odds of the proportion of time spent ingesting. Group mean values for control lambs (CON ■; n = 9) and restricted plus restricted arginine lambs (RES □; n = 19) are represented across 3 d on the x-axis. Time spent ingesting milk was significant across treatments, with CON lambs spending more time ingesting milk than RES ($P = 0.03$), with a day effect ($P = 0.012$), with an overall decrease of activity on day 2.

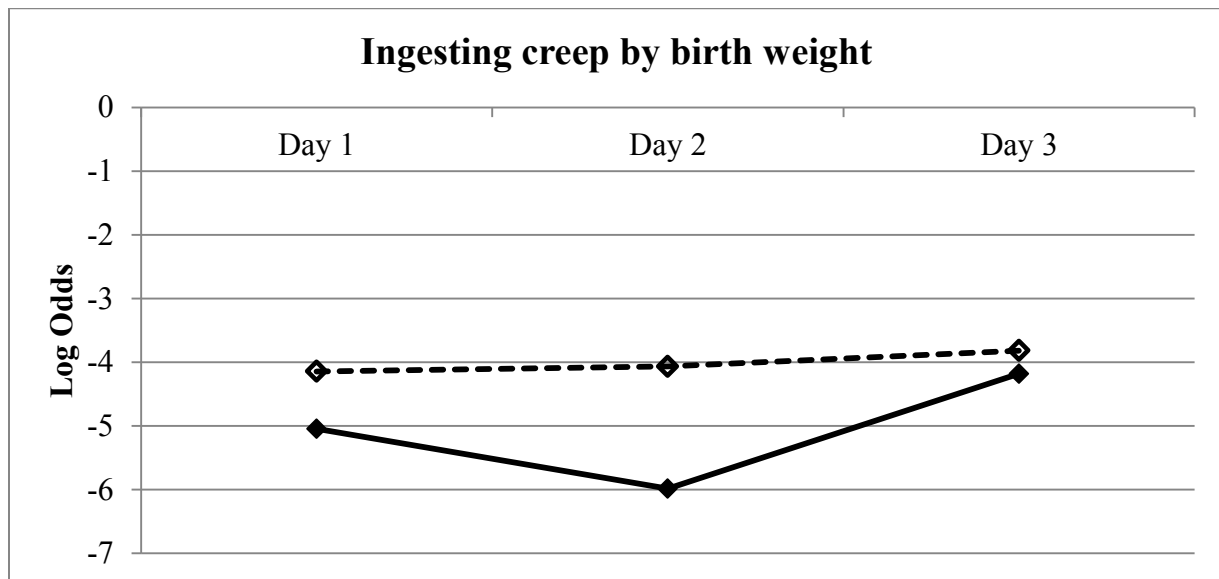


Figure 2.6. Groups means for log odds of the proportion of time spent ingesting creep are compared across lamb birth weight groups. High birth weight lambs (High ◆; n = 7) and the lowest birth weight lambs (Low ◇; n = 7) are represented across 3 observational days. A birth weight by day interaction was observed with High, having a larger increase in time spent ingesting creep from day 2 to day 3 than Low ($P = 0.039$).

Differences in time spent ingesting creep were significant for each day ($P = 0.022$). Lambs spent a larger proportion of time ingesting creep on day 3 than day 1 ($P = 0.032$) and more time on day 3 than day 2 ($P = 0.020$). A birth weight by day interaction was present with high birth weight lambs, having a larger increase in time spent ingesting creep from day 2 to day 3 than low birth weight lambs ($P = 0.039$), as presented in Figure 2.6.

Interaction with Milk

Differences in time spent interacting with milk buckets were significant for both treatment ($P = 0.005$) and day ($P < 0.001$), as seen in Figure 2.7. Lambs in the CON group spent a higher proportion of time interacting with the milk buckets than lambs in the RES and RES-ARG combined group ($P = 0.005$). Also, RES-ARG spent more time interacting with the milk bucket than those born to RES ($P = 0.018$). Finally, lambs spent proportionally more time interacting with the milk buckets on day 1 than day 2 ($P < 0.001$) and more time on day 3 than day 2 ($P < 0.001$).

Interaction with Creep

No differences between treatment or birth weight groups were present in time spent interacting with creep.

Interaction with Mound

No effects were seen regarding maternal plane of nutrition or birth weight on time spent standing on the platform.

Represented in Figure 2.8, both day and treatment \times day effects were observed for time spent alone on the mound. Lambs spent proportionally more time alone on the mound on day 2 than day 1 ($P = 0.026$), proportionally more on day 3 than day 1 ($P < 0.001$) and more time on day 3 than day 2 ($P = 0.007$). A treatment \times day effect was present for RES and RES-ARG born

lambs combined versus CON lambs. Control lambs had a proportionally larger increase in time spent alone on the mound from day 1 to day 3 when compared to RES lambs, and a greater increase in time spent alone on the mound from day 2 to day 3. Also, lambs presented a birth weight \times day interaction for time spent alone on the mound as well. There was a larger increase in time spent alone on the mound from day 2 to day 3 for low birth weight lambs compared to high birth weight lambs ($P = 0.026$).

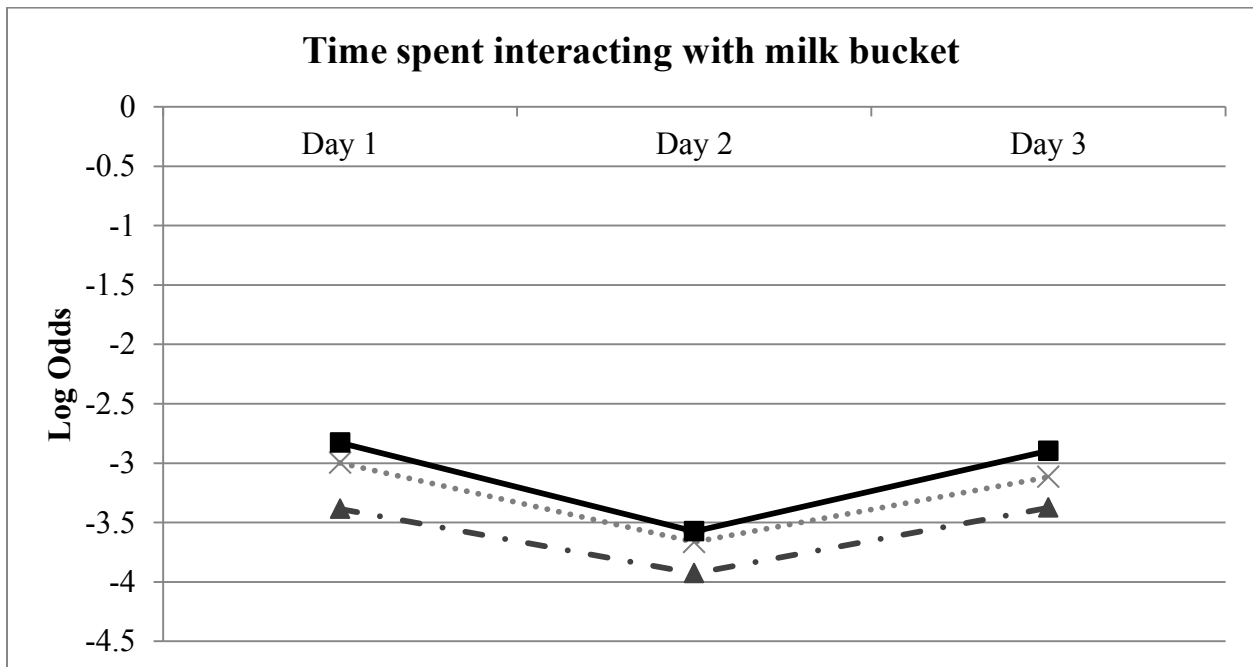


Figure 2.7. Time spent interacting with the milk bucket expressed as log odds of the proportion of time. Group mean values for control (CON ■; $n = 9$), restricted (RES ▲; $n = 11$), and restricted plus arginine (RES-ARG ×; $n = 8$) are represented across 30 h observed within 3 d on the x-axis. Time spent interacting with the milk bucket varied across treatments with a significant treatment affect ($P = 0.001$), with an overall decrease of activity on day 2 ($P < 0.001$).

Differences in time spent interacting with the mound were significant across days and a treatment \times day interaction was present. Lambs spent a larger portion of time interacting with the mound on day 1 than day 2 ($P < 0.001$) and larger proportion of time on day 1 than day 3 ($P < 0.001$). Lambs also spent more time interacting with the mound on day 2 than day 3 ($P = 0.018$). When RES and RES-ARG were combined, a treatment \times day effect was present for

interacting with the mound for lambs born to the RES versus CON dams from day 1 to day 3 and day 2 to day 3, represented in Figure 2.9. Lambs in the CON group decreased more in

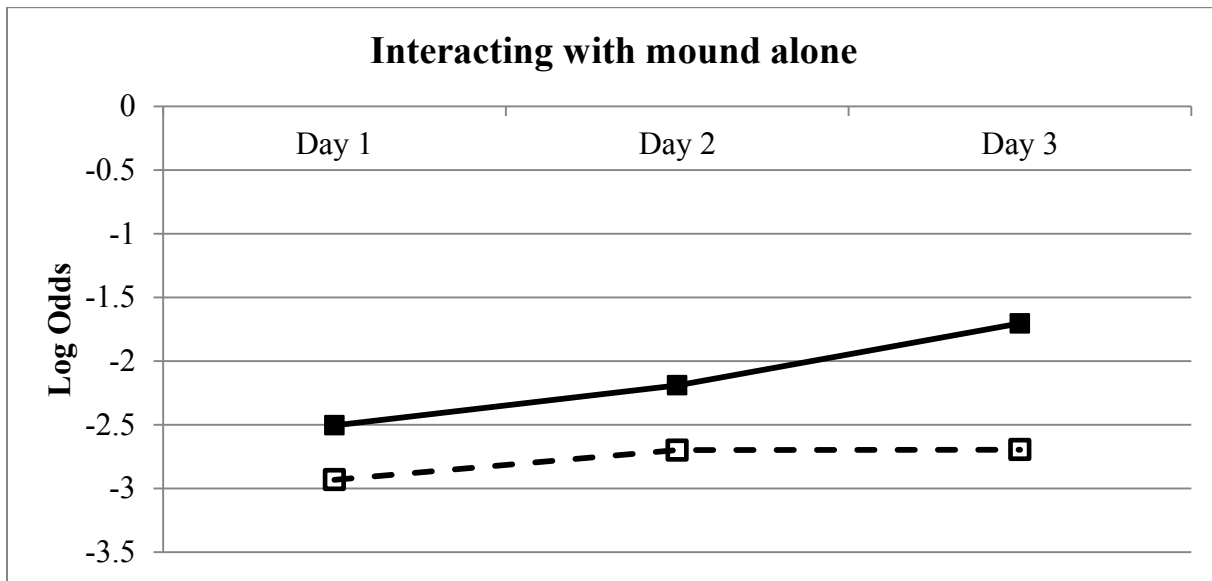


Figure 2.8. Time spent interacting with the mound alone expressed as group means of log odds of the proportion of time spent interacting with the mound. Group mean values are represented for control lambs (CON ■; n = 9) and restricted plus restricted arginine lambs (RES □; n = 19). Time spent interacting was different between days ($P < 0.0001$), with a larger increase in those lambs from CON ewes ($P = 0.01$), but no difference was seen between treatments ($P = 0.27$).

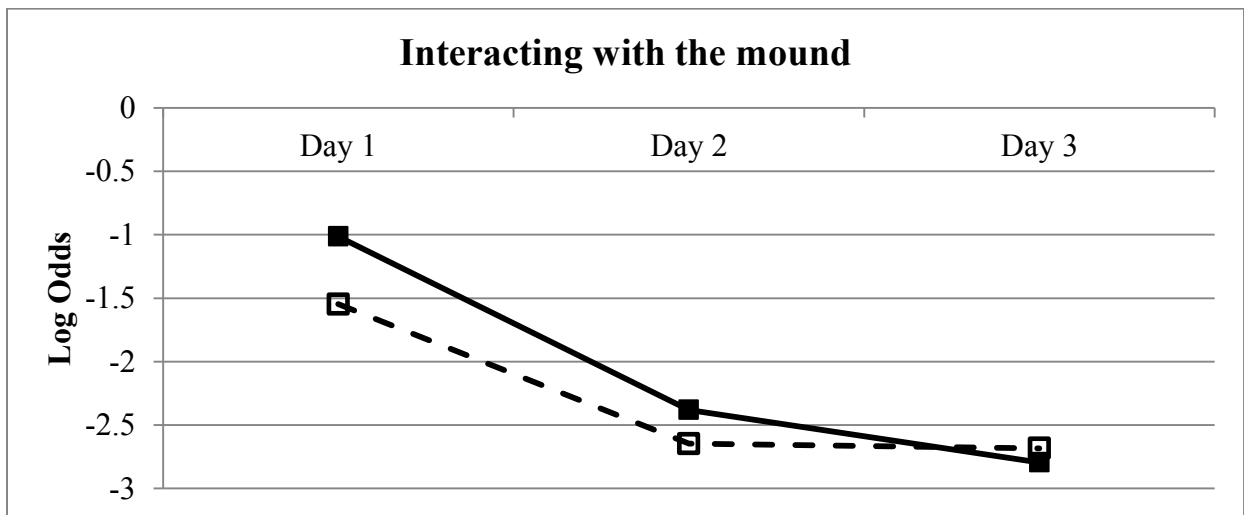


Figure 2.9. Group means for log odds of the proportion of time spent interacting with the mound across treatment. Group mean values are for control lambs (CON ■; n = 9) and restricted plus restricted arginine lambs (RES □; n = 19) across 3 d. Time spent interacting was different between days ($P < 0.0001$), with a larger decrease in those lambs from CON ewes ($P = 0.002$), but no difference was seen between treatments ($P = 0.10$).

proportion of time spent interacting with the mound from day 1 to day 3 when compared to those in the RES group ($P = 0.005$) and had a greater decrease in proportion of time spend interacting with the mound from day 2 to day 3 when compared to those lambs in the CON group ($P = 0.046$).

Lambs

Differences in time spent interacting with other lambs were significant for day ($P < 0.001$) and treatment \times day ($P = 0.002$) effects. Lambs spent more time proportionally interacting with other lambs on day 1 than day 2 ($P < 0.001$) and day 3 ($P < 0.001$) as seen in Figure 2.10. Lambs born to RES and RES-ARG dams decreased in proportion of time spent interacting with other lambs less than those lambs born to CON dams from day 1 to day 2 ($P = 0.002$) and from day 1 to day 3 ($P = 0.001$).

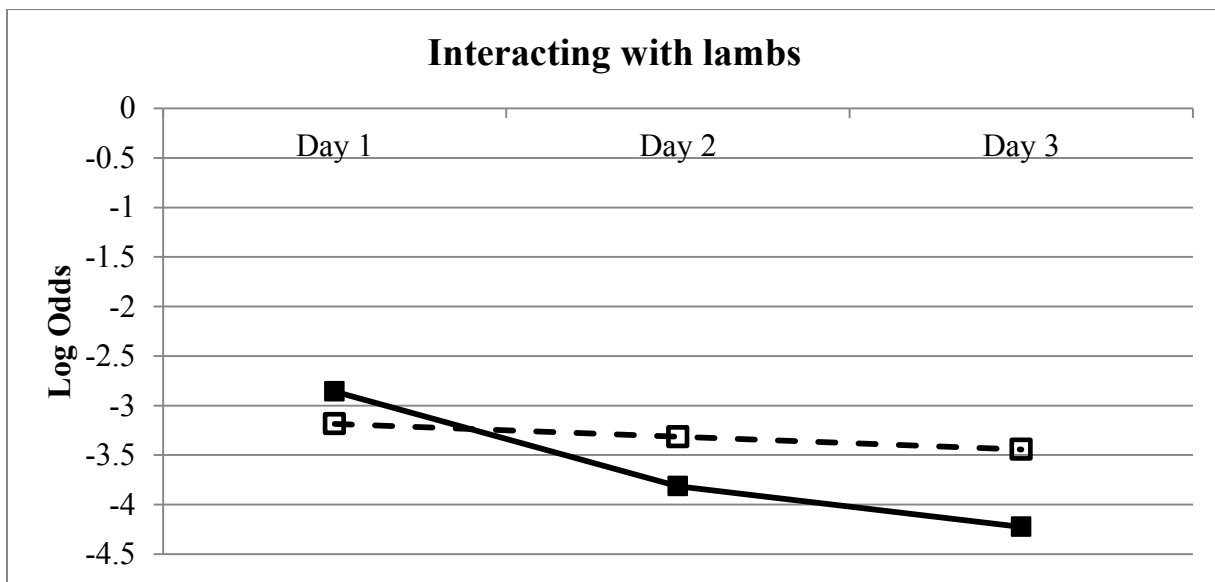


Figure 2.10. Time spent interacting with other lambs expressed as group means of log odds of the proportion of time spent with other lambs. Group mean values are for control lambs (CON ■; $n = 9$) and restricted plus restricted arginine lambs (RES □; $n = 19$) across 3 d on the x-axis. Time spent interacting was different between days ($P < 0.001$), with a larger decrease in those lambs from CON ewes ($P = 0.002$), but no difference was seen between treatments ($P = 0.13$).

Lambs presented a birth weight \times day effect for time spent interacting with other lambs. There was a larger decrease in proportion of time spent interacting with other lambs from day 1 to day 3 for high birth weight lambs than low birth weight lambs ($P = 0.006$) and a greater decrease from day 2 to day 3 for high birth weight lambs when compared to low birth weight lambs ($P < 0.001$).

Water

Differences in time spent interacting with water were only significant for day ($P = 0.014$) and lambs spent more time interacting with water on day 2 than day 3 ($P = 0.004$). No differences were present in time spent interacting with water when lambs were grouped by weight.

Environment

Differences in time spent interacting with the environment were significant across days ($P < 0.001$), as represented in Figure 2.11. Lambs spent proportionally more time interacting with the environment on day 1 than day 2 ($P = 0.006$) and day 3 ($P < 0.001$). Lambs also spent more time interacting with the environment on day 2 than day 3 ($P < 0.007$). Also, RES-ARG born lambs spent more time interacting with their environment than RES born lambs ($P = 0.0044$). Interacting with the environment was not significantly different between top and bottom birth weight lambs.

Discussion

In this study, the behaviors evaluated were chosen based on our objectives and commonly observed lamb behaviors. Behaviors previously observed in calves (Kondo et al., 1983), as well as those seen in lambs which have undergone early separation from a dam (Napolitano et al., 2003; Zito et al., 1977) were chosen for evaluation. All evaluations of feeding behaviors

including ingesting milk and creep and interacting with milk, creep and water were chosen because calves have modified feeding behaviors when housed in a larger group (Jensen and Budde, 2006). In a range setting, adult sheep have been observed spending 11.6 h lying on average (Arnold, 1984) and standing and lying times are known to be affected by pen change, therefore each of these behaviors were included in our analysis. In a typical production setting, lambs will use an elevated area and play ‘king of the hill’ or other games involving climbing on the area. With this in mind, we included an elevated area in each pen, the platform, to observe if a similar behavior would be exhibited and if so, by which lambs. Finally, alone and social interactions were included across parameters as distance from one calf to other calves while lying has been used (Kondo et al., 1983) in previous pen change work.

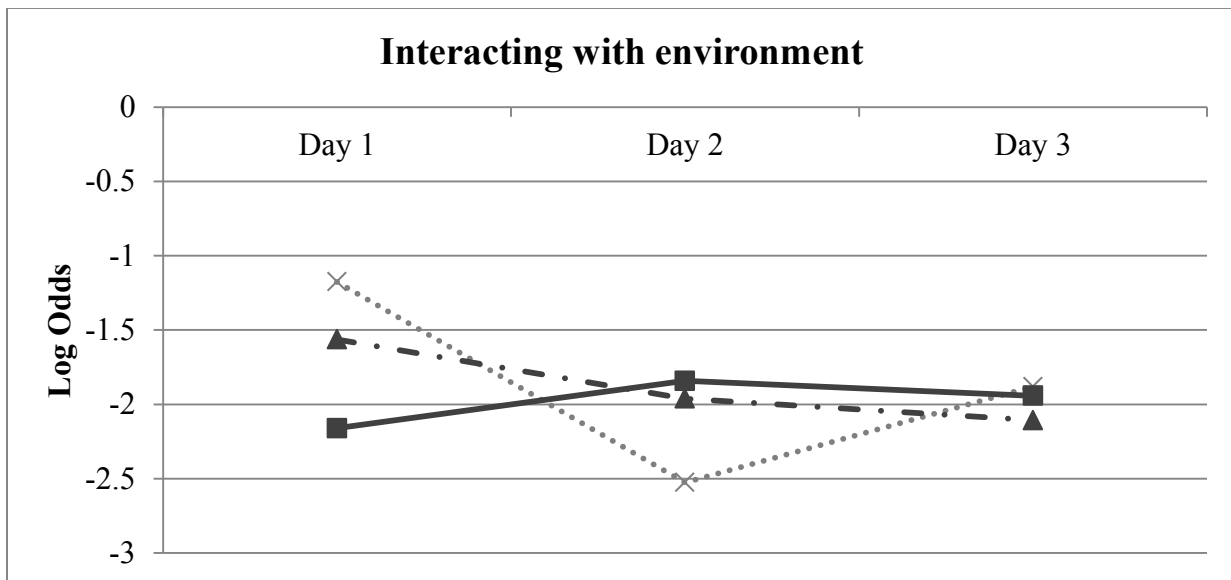


Figure 2.11. Time spent interacting with the environment expressed as log odds of the proportion of time. Mean values for control (CON ■; n = 9), restricted (RES -▲-; n = 11), and restricted plus arginine (RES-ARG ·x·; n = 8) treatment groups are represented across 30 h observed within 3 d. A significant treatment ($P = 0.016$) and day ($P < 0.001$) effect was observed, with a decrease of time spent interacting with the environment observed over time.

Few differences in behaviors were seen between RES lambs and RES-ARG lambs.

Restricted-Arginine lambs spent a higher proportion of time lying, interacting with the

environment, and interacting with the milk bucket than RES lambs. No other differences were observed in behaviors between RES and RES-ARG lambs.

Overall, lambs spent more time alone with the progression of the observation period. Lambs are typically gregarious and might be expected to spend more time together in groups as group interactions were established. However, lambs were 72.3% more likely to be seen interacting with each other on day 1 than day 2, and 125.6% more likely to be seen interacting with each other more on day 1 than day 3. There was a significant decrease from day 1 of the study to the completion of observations. This could be a response of lambs exploring their environment and adjusting to the stress of the pen change.

Despite few treatment effects, a clear interaction between day and treatment was seen for many outcomes. Across the study, lambs born to CON ewes spent more time alone on the heightened area in the pen than those lambs born to RES ewes. Likewise, CON lambs overall increased in time spent lying alone greater than RES lambs. When evaluating overall lamb interest in interacting with others, CON lambs decreased in the number of daily interactions more than RES lambs. There appears to be a pattern that lambs from ewes on a nutritionally adequate diet prefer to be alone more than in a group with other lambs. These results present a pattern counter-intuitive to a natural flock behavior. However, CON lambs' comfort to be alone may represent the lambs adapting to the new pen more quickly than RES lambs. As lambs and sheep will flock together when scared or stressed, control lambs' time alone may represent a decreased level of stress, or a quicker recovery from stress, than RES lambs.

Lambs appeared to be more interested in interacting with novel items on the first day of the study, and decreased interest as the study progressed. Lambs spent more time the first day on investigative behaviors, and less time as the study progressed. Lambs were 25.4% more likely

to investigate the new pen on day 1 than day 2, and 60.4% more likely on day 1 than day 3. Also, lambs were 27.9% more likely to investigate the pen on day 2 than they were on day 3. Likewise, lambs spent more time sniffing and investigating the elevated area within the pen at the beginning of the study than at the end. On day 1 of the study, lambs were 4.30-fold more likely to interact with the heightened area than on day 3, and likewise 3.43-fold more likely to interact with it on day 1 than day 2. Lambs are interested in a novel item and will investigate it. A similar pattern can be found in time investigating the rest of the environment, with a larger proportion of time spent investigating the new pen on day 1, decreasing over time. Also, animals investigated each other proportionally more on the first day than the third. As lambs acquainted themselves with other lambs and novel items in a pen, it appears that lambs became less interested in each other and the environment.

When animals are spending their time investigating their environment, they have less time available for consuming nutrients. Lambs spent more time ingesting either milk or creep feed on day 3 than day 2 (with lambs being 40.4% more likely to be seen ingesting a food source on day 3 than they were on day 2) while spending an equal amount of time lying on day 2 and day 3. Animals were observed spending 52.2% of their time lying on day 1, while spending 70.0% and 72.9% on day 2 and day 3, respectively. These findings agree with Walker et al. (2007) who found cattle will lay more and eat less on the second day following a pen move. This is likely due to abnormally high levels of activity the first day, coupled with probable high levels of stress following the event of the move.

Low birth weight lambs spent more time ingesting either milk or creep than high birth weight lambs. Lambs born to a lower weight may be trying to compensate compared to those lambs born to a higher birth weight. However, high birth weight lambs had a larger increase in

time spent ingesting creep from day 2 to day 3. Greenwood et al. (1998) found that those lambs born with a low birth weight (below 2.9 kg) overall had a higher feed intake when compared to high birth weight lambs (4.3 kg). Possible differences may exist with digestive maturity, with low birth weight lambs ingesting more of all possible feed sources and high birth weight lambs moving toward ingesting a larger amount of creep at the end of the study.

Overall CON lambs spent more time ingesting milk than RES, with the odds of a CON lamb to be ingesting milk at any given time being 38% higher than the odds that a RES lamb would be ingesting milk. Although we do not know the volume of milk ingested by each lamb, lambs will spend less time eating when exhibiting a fear response (Viérin and Bouissou, 2003) and may be a response by RES born lambs to the stress of pen change.

Although lambs decreased their interactions with the elevated area in the pen over time, a treatment by time interaction was present. Control lambs decreased interaction more over time than those in the RES group. If CON lambs engage in less investigative activities than the RES lambs, these lambs may be adjusting quicker into the environment and are more comfortable with their new surroundings. Restricted born lambs put more energy over time into exploration within the pen, which may affect future growth. Despite this pattern across treatments, no significance was seen comparing birth weights with behaviors.

In summary, our study found CON lambs seem to adapt more quickly to a novel environment following a pen change than those lambs born to RES dams. Lambs will decrease investigation of a novel environment over time. Also, CON lambs exhibited increased motivation for food, suggesting that those lambs born to undernourished ewes are less able to adapt to an environmental change. In addition, lambs with low birth weights spent more time ingesting feed

than those lambs with a high birth weights, suggesting some compensatory mechanism. Results suggest that maternal nutrition influences ability of lambs to adapt to a stressor.

Acknowledgements

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CHAPTER 3. THE EFFECTS OF FLUNIXIN MEGLUMINE AND HOOF TRIMMING ON GAIT SCORES AND LYING TIMES OF LACTATING DAIRY COWS

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Abstract

Lameness in dairy cows is a common problem in U.S. herds. Lameness is associated with decreased milk production and increased lying times. Hoof trimming can be used as a preventive measure in cows; however hoof trimming itself may lead to increased lying times. A total of 68 lactating Holstein cows were enrolled in a study designed to determine the effects of flunixin meglumine and hoof trimming on time spent standing and lying and locomotion. All cows were fitted with an accelerometer one week prior to hoof trimming to collect baseline standing and lying data. The day before hoof trimming, cows were filmed to be evaluated for locomotion scoring. Thirty minutes prior to hoof trimming, animals were given intravenous injections of one of two treatments, flunixin meglumine (n = 34) administered at the highest dosage of 2.2 mg/kg, or isotonic sterile saline solution (n = 34) at the same volume. The same drug treatment was repeated 24 h later. All cows were filmed for locomotion scoring the day following hoof trimming, and 7 and 28 d later. Accelerometers were removed 28 d following hoof trimming. Total daily milk production was recorded for each cow 1 wk prior to and 2 mo following trimming. Overall, 10 cows in each treatment group had a locomotion score ≥ 3 . No differences in locomotion scores, lying times, or milk production were found between flunixin and saline treated cows. Most of the cows in our study were not severely lame, which may have limited treatment effects on lying times and locomotion scores. Other methods of detecting

lameness may be more sensitive for identifying the effects of hoof trimming in moderately lame cows.

Key words: hoof trimming, flunixin meglumine, lameness, locomotion scoring

Introduction

Lameness is a common problem across dairy herds as seen in one study in Minnesota (Espejo et al, 2006), which found on average 24.6% of dairy cows are clinically lame, with producers able to identify approximately half of the lame cows in their herd. Milk production and milk protein levels are inversely related to lameness scores in dairy cows (Juarez et al. 2003), leading to 1.5 to 2.8 kg/d decrease in milk yield for 2 wk following diagnosis (Rajala-Schultz et al., 1998). In the Netherlands, it was found that on average, lameness accounted for approximately \$50 loss per cow per year, with a \$230 loss per lame cow per year (Enting et al. 1997). As lameness is a preventable disease, this is an unnecessary loss in production.

Gait or locomotion scores are common tools used to assess lameness in dairy cows. The Numerical Rating System (NRS) from Flower and Weary (2006) is an accurate tool for predicting lameness due to sole ulcers in dairy cows, which may take about 8 to 10 wk to become visible (Lucey et al., 1986), as well as sorting healthy cows from those with ulcers. Cows with lameness due to sole ulcers may be identified by jerky head movement, shorter strides, decreased joint flexion, more pronounced back arch, and uneven hoof placement. The NRS is a 1-to-5 scale, with 1 being sound and 5 representing a severely lame cow, which evaluates several factors of a cow's gait such as head movement while walking, curving of the back, uniformity of foot placement, and willingness to bear weight evenly on all 4 feet. This system allows for subjective but quantitative measuring of lameness, with high intraobserver reliability (Flower and Weary, 2006).

Lying behavior in cows is directly related to lameness scores (Juarez et al. 2003). Automated measuring devices can measure increases in lying behavior, which is indicative of lameness (Chapinal et al. 2010A, 2010B). Chapinal et al. (2010B) observed nonlame cows lying approximately 12.2 h/day compared to lame cows lying 13.4 h/day. Dairy cows in deep bedded stalls with NRS ≥ 4 will lay down 1.6 h longer per day and have longer lying bouts than those which were scored 3 or lower (Ito et al. 2010). Lying time can be used to clearly distinguish those cows which are severely lame from those cows which are less lame or sound. An increase in lying times and locomotion scores are likely indicative of pain in cattle, causing it to be a welfare concern, and motivating the search for methods to prevent or alleviate lameness-related pain.

When dairy farms trim cows' hooves on a yearly or biannual schedule, those herds have a lower prevalence of lameness than herds trimmed only on an 'as needed' basis (Espejo et al., 2007). Consistent hoof trimming throughout a herd leads to a decrease in disease occurrence (Manske et al., 2002). Despite the long-term preventive effects of hoof trimming, both lame and non-lame dairy cows have been found to lie down longer the day of and up to 5 wk following hoof trimming (Chapinal et al., 2010B, 2010C), suggesting a possible temporary increase in lameness pain due to the procedure.

Based on previous work it has been proposed that therapeutic drug use may offset the increase in lying time associated with hoof trimming. Chapinal et al found that cows treated with flunixin prior to hoof trimming had increased lying times for 2 days following hoof trimming, while saline-treated cows had increased lying times for the duration of the 5 day observation period after hoof trimming (Chapinal et al., 2010C).

We hypothesized that cows treated with flunixin meglumine before and after hoof trimming would spend more time standing and less time lying in the period following hoof trimming, when compared to control cows which had their hooves trimmed but did not receive analgesic drug treatment. Cows treated with flunixin meglumine around the time of hoof trimming were expected to have improved locomotion scores and milk production when compared to untreated cows. The study objectives were to determine the effect of flunixin meglumine treatment on time spent standing and lying in dairy cows after hoof trimming and on locomotion score and milk production following hoof trimming.

Materials and Methods

Animals and Housing

We enrolled 73 lactating Holstein cows from the North Dakota State University Teaching and Research Unit in Fargo, North Dakota. Cows were housed in 2 pens with mattress-bedded free-stalls with approximately 30 to 60 cows in each pen. Cows were fed a TMR diet ad libitum formulated to meet NRC requirements once daily at 07:00 h and pushed up twice daily. Water was freely available from automatic waterers. Cows were milked twice daily at 04:00 h and 15:00 h. All procedures involving animals were approved by the North Dakota State University Institutional Animal Care and Use Committee.

Experimental Procedure and Treatments

Cows were enrolled in the study based on a need for hoof trimming as determined by the farm management and investigators, working together. Nine cohorts were enrolled, with each cohort containing 7 to 10 cows.

Drug Treatment

The study schedule is shown in in Figure 3.1. Cows were blocked by cohort and assigned randomly to one of two treatments; either 2 mL/100 lb BW sterile isotonic saline solution (n = 35; SAL; DIM = 192 ± 134 ; BW = 682 ± 99) or flunixin meglumine (n = 38; FLUN; Banamine, Merck Animal Health, Summitt, NJ; DMI = 194 ± 139 ; BW = 666 ± 76) at the highest labeled dose (2.2 mg/kg of 50 mg/mL solution, 2 mL/100 lb BW). Cows were weighed the day before treatment to accurately calculate drug dosage. The hoof trimmer and cow handlers were masked to treatment. Both treatments were given by injection into the jugular vein. Cows were trimmed less than 30 min following treatment by an experienced hoof trimmer, using the Dutch method (Toussaint-Raven et al., 1985). All observed hoof lesions were recorded. Treatments were repeated the day following hoof trimming, within $24 \text{ h} \pm 2 \text{ h}$ from the previous day's injection.

Data Collection

Locomotion Scoring

Cows were locomotion scored through video recordings taken the day before hoof trimming, the day after hoof trimming, and 7 d and 28 d after hoof trimming, as seen in the schedule in Figure 3.1. Recordings were performed using a digital camera (Canon PowerShot 110 HS, Canon Americas, Melville, NY). Cows were video recorded walking a minimum of 5 strides at a consistent pace per video with at least 3 videos per day for each cow. Cows were walked down the central feed aisle, which consists of non-grooved poured concrete. Cows were encouraged forward in a consistent manner and were able to walk freely without being touched by handlers during filming. Cows were recorded from a profile and were recorded moving both directions. Locomotion scores were performed by a single masked observer using a 1-to-5 numerical rating system (NRS; where 1 = perfect gait and 5 = severely lame) adapted from

Flower and Weary (2006) and Chapinal et al. (2009). Both lame and non-lame cows were enrolled to allow for a variety of locomotion scores for each treatment.

Activity

Seven to 5 days prior to treatment, cows were fitted with an accelerometer (HOBO Pendant G Data Logger, Onset, Cap Cod, MA) to record time spent lying prior to hoof trimming as baseline data. The accelerometer was wrapped in terry cloth to prevent rubbing and secured to the cow's leg using veterinary cohesive wrap and tape. Accelerometers were placed on either the right or left rear leg in the milking parlor prior to milking. Leg choice was based on leg cleanliness and any lesions or abrasions present. In the case of moderately to severely lame cows, preference was given to the sound or less lame rear leg to prevent any further discomfort to the cow. Units were attached on the lateral aspect of the cow's leg, between the hock and fetlock, to record orientation of the leg in 3 directional planes once a minute. Accelerometers allow for analysis of duration of standing/lying bouts, number of bouts and the total time spent standing and lying each day. Accelerometers were checked regularly to ensure that they were secure and any shifting was recorded.

Accelerometers were removed and replaced every 7 to 10 days to download recorded measurements and check cows' legs for any abrasions, lesions or swelling present. Accelerometers were removed approximately 2 h prior to milking while cows were in the free stalls by cutting bandaging off the leg with scissors. Data were downloaded to a single computer (Dell Latitude d630, Dell Inc., Round Rock, TX) and accelerometers were programmed to begin collecting data after the cow had finished milking or approximately 2.5 h after removal of accelerometers. Accelerometers were reapplied using the previously mentioned method and were removed for a final time 28 d after hoof trimming.

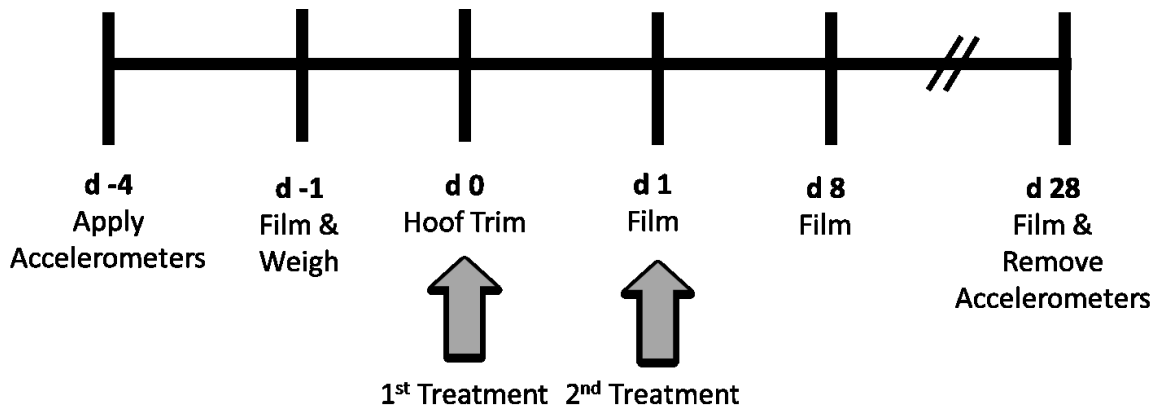


Figure 3.1. Schedule of study procedures.

Statistical Analyses

Treatment effect was tested using a mixed model with repeated measures. The cow was considered the experimental unit, time (relative to first injection and hoof trimming) a repeated measure over cow and enrollment cohort as a block. A baseline value was calculated for each variable and used as a covariate in the model. Initial DIM and parity at enrollment were also included as covariates in each model. Initial locomotion score (as an ordinal variable with 9 possible values – from 1 to 5 with half integers - or as a binary variable that classified cows into lame or not using the cutpoint Locomotion Score ≥ 3) was included as a covariate for lying behavior and milk production models. Two-way interactions were also tested. Residuals were examined after each model to verify normality and homogeneity of variance.

Results

Five cows were removed from the study. Data from three of those cows were not used at all in the analysis; one of the cows had an allergic response to the drug and the other two developed joint soreness. Accelerometer data from one cow was used up to and including d 30 and locomotion scoring including d 8, with a leg injury developing after d 30. Data from the final cow were used until d 15, at which time she contracted mastitis. This resulted in 34 cows

with complete data for each treatment group, with 2 additional cows in the flunixin meglamine group with partial data. There were 19 primiparous and 15 multiparous cows in each treatment group, with cows in mid-lactation during the study.

Milk Production

There were no differences post treatment in milk production between treatment groups ($P = 0.88$) after controlling for baseline milk production, initial locomotion score, and enrollment cohort. There were no significant interactions ($P > 0.05$).

Locomotion

In each treatment group, 10 cows had locomotion scores ≥ 3 at the time of enrollment in the study, and 24 cows had scores below 3 when enrolled. There was no treatment effect on locomotion score ($P = 0.82$), after controlling for initial locomotion score and parity, shown in Figure 3.2. There were no significant interactions ($P > 0.05$). Locomotion scores following hoof trimming did not differ between prior to hoof trimming to the day after ($P = 0.06$), d 8 ($P = 0.28$), or d 28 ($P = 0.17$).

Lying Behavior

There was no difference between treatments in post treatment daily lying time ($P = 0.61$) after controlling for baseline lying time, as seen in Figure 3.3. There were no significant interactions ($P > 0.05$). Hoof trimming with or without drug treatment had no effect on lying time ($P = 0.99$).

No differences were found in post treatment daily frequency of lying bouts between treatments ($P = 0.41$) after controlling for baseline frequency of bouts. There were no significant interactions ($P > 0.05$).

Post treatment average lying bout duration did not differ between treatments ($P = 0.24$) after controlling for baseline average lying bout duration and enrollment group. There were no significant interactions ($P > 0.05$).

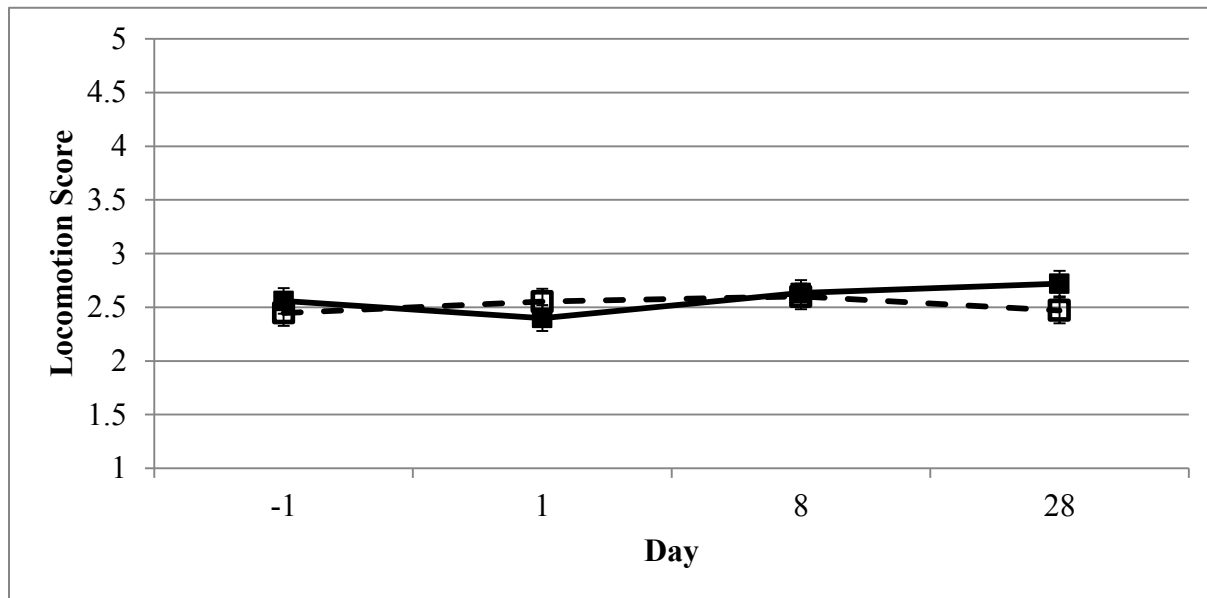


Figure 3.2. Mean locomotion scores over time. Cows were scored one day prior to treatment, as well as 1 d, 1 wk, and 4 wk after treatment. No differences were seen between those which received flunixin meglumine (FLUN; \square) treatment and those which received saline (SAL; \blacksquare ; $P = 0.82$), nor did hoof trimming affect locomotion score on d 1 ($P = 0.062$), d 8 ($P = 0.28$) or d 28 ($P = 0.17$).

Discussion

This study was performed as a follow-up to work performed by Chapinal et al. (2010C). In that study, cows were treated prior to hoof trimming and on the following day with flunixin meglumine or sterile isotonic saline solution. Daily standing and lying times were recorded for 5 days following hoof trimming. An increase in time spent lying down was seen following hoof trimming, with cows receiving flunixin returning to normal lying times 2 d following treatment. Control cows treated with saline solution did not return to pre-treatment lying times during the 5 d post-trimming observation period. These observations lead to the current study hypothesis, that treatment of dairy cows with flunixin meglumine around the time of hoof trimming would reduce

the number of days of increased lying time observed in dairy cows after hoof trimming. Our study was intended to continue this work and measure the effects of hoof trimming and flunixin on parameters for a month following procedures.

In this study, neither drug treatment nor hoof trimming were found to affect milk production, locomotion scores, or lying behavior in dairy cows. Cows in this study were not severely lame; only 10 cows total had an NRS ≥ 3.5 , compared to work by Chapinal et al., (2010C) in which 41 of 66 cows had an NRS > 3 . The effects of intervention intended to mitigate lameness may have been more difficult to detect in the current study because as a low level of lameness was seen across our experimental herd. As lying behavior is most correlated with severely lame cows (NRS ≥ 4 ; Ito et al., 2010), it is possible that our measures were not sensitive enough to detect the analgesic effects of our therapeutic drug or the effects of hoof trimming.

High variability in milk production may make it difficult to detect differences between treatment groups. Milk production can be very sensitive to diet, weather change (Ravagnolo et al., 2000), and even handler interactions (Waiblinger et al., 2002), contributing to the lack of differences seen between treatments as different handlers milked the cows day to day and weather varied across seasons. Chapinal et al. (2010A) showed no effect on lying time when lame cows were treated with ketoprofen, which is a non-steroidal anti-inflammatory drug (NSAID) similar to flunixin meglumine. Their study did find a significant effect of ketoprofen on weight shifting behavior in dairy cows. Both flunixin meglumine and ketoprofen are shorter-acting drugs with short half-lives, and more potent, longer-acting drugs may be more likely to affect lying time. As currently no pharmaceuticals are FDA-approved for pain relief in cattle in

the United States, new drugs should be tested and entered into the market, to alleviate pain in lameness and other painful conditions.

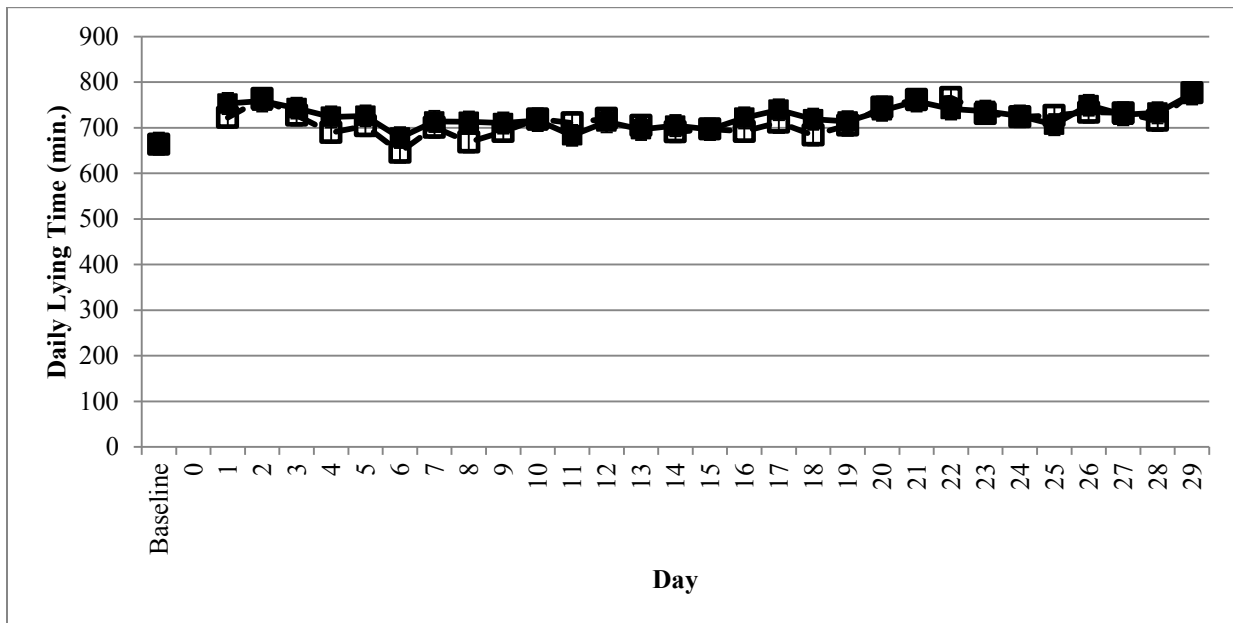


Figure 3.3. Mean time spent lying in min/d. Baseline value is a mean of data collected for 5 to 7 days prior to treatment of cows. No differences were seen for total daily lying time ($P = 0.61$) for cows treated with flunixin meglumine (FLUN; \square) and those treated with saline (SAL; \blacksquare). No differences in lying time were observed following hoof trimming ($P = 0.9989$).

Hoof trimming had no effect on the cow behaviors we measured. Chapinal et al. (2010C) reported no effect of hoof trimming on locomotion scores; however, as described above, an increase was seen in daily lying time following hoof trimming. Chapinal et al. (2009) reported an increase in lying time in both nonlame and lame cows for 5 wk following hoof trimming. The effects of hoof trimming are likely to depend on the training and skill level of the hoof trimmer as well as the method used to perform trimming.

In conclusion, flunixin meglumine did not affect lying behavior or locomotion scoring for lame or non-lame dairy cows following hoof trimming. Also, hoof trimming did not affect locomotion scores or lying behavior. Indicators of lameness other than lying time and locomotion scores, such as weight shifting between legs (Chapinal et al., 2010A, 2010C) may be

more sensitive to the effects of drug treatment or hoof trimming, or other hoof trimming methods or drugs may produce greater effects on outcomes used to evaluate lameness in dairy cows.

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CHAPTER 4. OVERALL CONCLUSIONS AND FUTURE DIRECTIONS

Currently, researchers are working toward using behavioral measures to understand and improve animal welfare. The main objective of the two studies described in this thesis was to find quantifiable behavioral means to evaluate animal welfare. In the first study, behaviors were quantified and measured in lambs. Following a pen change, lambs born to undernourished dams were less likely to leave their pen mates for up to 3 days and spent less time ingesting milk when compared to lambs born to adequately fed ewes. The behaviors of these lambs suggest a possible negative effect on adaptation following a stressful event, such as a pen change, for offspring born to underfed dams.

In the second study, lameness in dairy cows was quantified using both subjective and objective measures. Some cows were measurably lame when evaluated with locomotion scores, however no differences in locomotion scores were observed following hoof trimming with or without anti-inflammatory drug treatment. Although differences between lame and sound cows in the amount of time spent lying down each day have been observed by other researchers, we did not detect differences between lame and sound cows using daily lying times. Lying time as a measure of lameness identifies severely lame cows more reliably, which may account for the lack of differences observed, as the dairy cows in our study were most often moderately lame. Also, no changes in lameness measures were observed following hoof trimming, implying that the methods used to measure lameness were not sensitive enough to detect an effect, or that hoof trimming did not cause lameness in sound cows or change the degree of lameness in cows diagnosed as lame.

Future research directions will include developing and validating more objective methods for evaluating behaviors of animals. These behaviors can then serve as a way to measure welfare

in livestock, by providing a means to understand which practices produce a positive welfare state, which practices are harmful to an animal's welfare, and the degree to which the animal's well-being is affected.