MEASURING THE EFFECTS OF HOUSING STYLES ON HOLSTEIN-CROSS DAIRY CALVES

by

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A THESIS

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Philip Washbourne, Ph.D.

The aim of this study was to investigate the effects of paired versus individual housing of approximately three-month old dairy calves on stress levels and overall calf health and performance. To do this, the study analyzed serum cortisol levels, fecal parasite loads (via a McMaster fecal egg count technique), and body condition scores for each of our two groups before and after our 35-day study. Each study group contained ten Holstein-cross dairy calves each. The individually housed group was housed in individual hutches, fed twice daily with ad libitum access to water. The pair-housed group was housed in a paired hutch setup, where two hutches and loafing areas were connected to create a larger, shared pen for two calves. Pair-housed animals had two feeder buckets and water buckets and were fed according to the same twice-daily protocol. Data collections were made on day zero when calves were randomly selected into their assigned group and again on day 35 when the study was finished. Paired t-test analyses were performed using before and after-study values for the three measurements conducted. Cortisol concentration results showed a significant increase in pair-housed calf serum cortisol concentrations before and after the study ($p=0.012$); individually housed calves saw no significant change in serum cortisol concentration (p=0.443). McMaster fecal egg count results were insignificant for both groups, although a general declining trend in eggs per gram of feces (ePG) was observed. Average body condition scores (BCS) for both groups were identical, minimally varying from the healthy BCS score of 3 (out of 5). The results suggest paired housing of dairy calves could cause increased stress, due presumably to social stressors, because this study found no correlation with paired-housing of dairy calves and decreased productivity or calf health. This study was the first of its kind to employ McMaster fecal egg counting techniques to quantify enteric parasite loads in calves for the purpose of evaluating calf health.

Acknowledgements

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Introduction

Dairy calf housing in the United States has become a topic of great discussion in the last 20 years because of an increase in consumer awareness of animal welfare conditions. This newfound interest in animal housing types has been brought about by waves of animal rights groups advocating for the humane treatment of livestock. Farmers and agriculture policy makers are being challenged to increase space allocations and socialization time for animals in commercial agricultural settings. The suggestion of investing in larger, more expensive calf structures or group housing systems has been met with fairly low compliance and measurable resistance by many longtime members of the agricultural community. For example, Reservation Ranch, the family dairy farm in rural Northern California that served as the location for this study, has been using essentially the same individual calf housing system for over 75 years with what has been seen as great success. With the long-term success of the current methods of housing, why invest tens of thousands of dollars in new equipment?

In order to better inform practices in agriculture, as in any other progressive field, dairymen rely on the work of scientists and researchers to support the idea that an investment in better infrastructure and a shift toward contemporary practices can indeed improve animal welfare while also sustaining or improving the production of livestock. In the case of calf housing systems, researchers play a key role in fostering a shift away from the widespread view that group housing promotes the spread of disease and parasites through a herd of calves more quickly than individual housing. It is the goal of this thesis to investigate the effects of individual housing systems and paired housing systems of Holstein-cross dairy calves.

This study will take a holistic approach to this investigation by evaluating individual animal stress level by measuring serum cortisol concentration, along with subject fecal parasite load via a modified McMaster Fecal Egg Count technique, subject performance via growth (weight gain in the form of a calculated average daily gain) and body condition scoring.

Background

Dairy Calf Housing Structures

The majority of American dairy farms that raise calves use individual housing systems. In a 2012 survey performed by the United States Department of Agriculture, 78.9% of dairy farmers were using some form of individual housing system (USDA, 2012). For decades conventional views in the dairy industry maintained that individual housing systems worked to minimize the passage of pathogens and various diseases between calves, however that convention has been challenged in recent years. In the past decade, studies have been performed investigating the effects of various forms of paired or group housing on behavior and health of dairy calves (Pempek et al., 2016; Chua et al., 2002; Jensen et al., 1997). The vast majority of these studies have demonstrated that, group housing can have positive effects on calves without debilitating herd health.

These studies examined a multitude of indicators of animal production, health, and wellness including: weight gain or average daily gain (ADG), fecal scoring, total serum protein concentration, feed intake and conversion efficiency, serum immunoglobulin concentrations, and behavioral measures (Pempek et al., 2016; Kung et al., 1997; Quigley et al., 1995). This study integrated portions of these previous studies into a single experiment, looking at three very different indicators of animal health, wellness, and production. By measuring fecal egg counts of parasites, while also quantifying stress levels via serum cortisol concentrations, and average daily gain and body condition scores, this study will serve to comprehensively compare individual and paired (group) housing systems in three different manners.

Stress, Blood Cortisol Levels, & Effect on Animal Growth & Production

The hormone cortisol has been well documented as an indicator of stress levels in dairy cattle for years. Studies have shown that dairy cows of all ages and stages react to stressful scenarios physiologically; several studies of different interests have shown a direct correlation between stressful events for dairy cattle and an increase in blood cortisol concentration (Wohlt et al., 1994; Van Reenen et al., 2005; Hickey et al., 2003; Stilwell et al., 2010). Although these studies are generally centered on the use of blood cortisol concentration testing to measure stress level after an acutely stressful event, they first must establish a baseline cortisol concentration (before stressor is introduced – in multiple studies that stressor was dehorning or disbudding) to which they can compare the post-stressor measurement (Wohlt et al., 1994; Stilwell et al., 2010). Because this experiment is not centered around the effect of a single stressful event or procedure performed on calves, this study compared the resting serum cortisol concentrations of each grouping. As a frame of reference to ensure appropriate concentrations have been obtained, a comparison of these measurements to the baseline values for cortisol concentration that were identified in previous studies.

Bovine Fecal Parasites & Host Interactions

According to a study done by the University of Missouri (Corwin & Randle, 1993) some of the most common and equally harmful parasites found in the intestinal tracts of dairy cattle in the United States are: Brown stomach worms (*Ostertagia ostertagia*), Lungworms (*Dictyocaulus viviparous*), Hookworms (*Bunostomum phlebotomum*), and Coccidia (*Eimeria bovis*). Young dairy calves are especially susceptible to these parasites and when severely infected, calf parasitism can be fatal.

These parasites, with the exception of lungworms, disrupt or harm a portion of the gastrointestinal tract, causing a spectra of symptoms: from indigestion that can cause animals to go off feed, to scouring (diarrhea), dehydration, bloody stools, anemia, weight loss, and ultimately if untreated, death (Foreyt, 2001).

A commonality shared by this entire suite of harmful parasites is that their eggs, larvae, or oocysts are released from their host organism via feces. Parasite life cycles and parasitic success rely on the release of eggs by a host animal via excrement and their reproduction from a larval state, thus infecting an environment inhabited by hosts carrying adult worms. Calves that come in contact with the feces of animals that are hosts to these various adult parasites are at an increased risk of infection (Corwin & Randle, 1993; Foreyt, 2001). These parasite eggs or oocysts are all easily viewed under a microscope at 10X magnification.

McMaster Fecal Egg Count Technique

The McMaster fecal egg count technique is popular for two main reasons: it can be performed quickly and is a fairly accurate manner to quantitatively measure parasite load. The speed and quantitative nature of the McMaster fecal egg count technique make it perfect for fecal analysis of large groups of animals. Various labs and scientists have designed modifications to the baseline fecal egg quantification technique, making it suitable for livestock species of all ages, sizes, and levels of infection (Vadlejch et al., 2011). By suspending the eggs in solution, and utilizing a special chambered glass slide with overlaid grids, scientists are able to quickly count the numbers and species of eggs present in a given chamber, and then use a basic mathematic equation to calculate the total number of eggs each gram of animal feces contains, expressed in eggs per gram (ePG) (URI & VT, 2014). This study measured ePG totals for oocysts and eggs rather than total numbers of specific parasitic eggs. In order to determine results from these tests, ePG values found in samples from animals housed individually were compared to those found in samples from animals housed in pairs.

Body Condition Scoring & Average Daily Gain In Dairy Cattle

One of the most implicit forms of gauging animal performance, especially in young animals, is weight gain and body condition scoring. We as humans naturally deduce that the healthiest animals are the biggest animals of a group. This assumption in many cases proves to be true, as was found in a recent study of Jersey heifer calves comparing the two different housing styles to be explored in my study (Pempek et al., 2016). In the study, calves raised in paired housing finished out the study at a heavier weight across the board, although their frames were essentially the same size. This indicates more efficient food conversions, because the amount of food consumed by calves was found to be nearly identical in both groups.

The idea that weight and average weight gain are strong indicators of health status and productivity is widely accepted. This study went beyond this convention, however, by assigning a body condition score to each study subject at the same time they were weighed and had samples collected. The subject’s body condition will be scored on a defined five-point scale, with a score of one being significantly underweight and a score of five being significantly overweight (Kellogg, University of Arkansas Division of Agriculture). In general, a score of three is an ideal body condition score for dairy calves.

By using both body weight –deriving an average daily gain from this measurement – and body condition score, the goal of the study is to get a stronger representation of truly how *healthy* calves are, rather than just how *quickly* they are growing.

Purpose and Hypothesis

The purpose of this study and thesis is to determine the effects, either positive or negative, of paired and individual housing systems on dairy calves. The measurements this study conducted to test these effects were: parasite load (an indirect indicator of health via parasite concentration), serum cortisol concentration (an indicator of animal stress level which could influence overall health), and body condition score (an indicator of animal performance, and health). *It was hypothesized that calves housed in a paired housing system would have higher and more diverse fecal parasite loads, however, their serum cortisol concentrations would be lower, and their average body condition scores (BCS) would be higher when compared to the individually housed group of calves.*

Methods

Subjects

A total of 20 Holstein-cross calves (made up of 60% Holstein/40% Jersey genetics based on herd pedigree), approximately 75 days of age (+/- 14 days) at the start of the study, were used for this experiment. The 20-calf subject roster was made up of 8 heifer calves, 9 steer calves (castrated males), and 3 bull calves (uncastrated males). This study was performed on Reservation Ranch in Smith River, California. All veterinary sampling and handling of study subjects was carried out under the supervision of the veterinarians of Town & Country Animal Clinic in Brookings, Oregon.

Study Groups

Two study groups were established at random, with 10 unrelated animals being put into each group. Group 1 contained 10 animals that were individually housed in single animal hutches. Group 2 contained 10 animals that were housed in a paired hutch arrangement. The paired-hutch housing was double the size of the individual calf hutch to accommodate the second calf. Both groups received feedings of a total mixed ration with chopped alfalfa added twice daily. They also had *ad libitum* access to water in water buckets at all times. Both groups were housed outdoors at the Reservation Ranch calf barn facility in standard indoor/outdoor fiberglass hutches with associated outdoor loafing areas. These indoor/outdoor setups are comprised of fully enclosed 7'x4.5'x4' hutches with an adjacent outdoor loafing area enclosed by rubbed wire fencing, as seen in Appendix Figure 1. In the case of the paired housing, two of these hutches next to each other had their loafing areas joined to create a single, larger, shared area in front of two hutches. The paired animals had equal access to both enclosed hutch structures as well as the shared loafing area; the paired pens had two sets of feed and water buckets.

Fecal/Blood Sample Collection

Samples were collected from each subject on the first day of the study (12/11/16) and the thirty-fifth day of the study (1/15/17) three hours after morning feedings (approximately 11:00 AM). All necessary collections were performed by the primary investigator, with the help of a licensed veterinarian from Town & Country Animal Clinic, and the assistant herdsman from Reservation Ranch. 5 ml of blood was collected into 6ml Vacutainer red top blood collection tubes via jugular venipuncture using sterile 22 gauge needles. Blood samples in 5ml red top tubes were labeled and placed on ice immediately following collection and transported to the laboratory at Town & Country for centrifugation. Blood samples were centrifuged for 15 minutes at 3,000 times gravity (x g) (Hernandez et al., 2014); once centrifuged, serum was pipetted into 3 ml red top tubes, tubes were labeled with calf identification numbers, and samples were frozen at -18 degrees Celsius. Upon completion of the sample collection phase of the study, all serum samples were delivered to the Veterinary Diagnostic Laboratory at the Oregon State University College of Veterinary Medicine, where cortisol concentration screens were measured on all serum samples collected.

Fecal samples were obtained digitally by insertion into the rectum of subjects; approximately 5 grams of feces were collected per subject. These feces were tied off inside two inverted examination gloves, and labeled with the animal subject's identification number. Samples were then placed in a cooler and transferred to a refrigerator upon completion of sample collection. Samples were stored in refrigerator at 0 degrees Celsius until McMaster counts were performed (approximately 24 hours after collection).

Body Condition Scoring & Weight Analysis

During sample collection from each study subject at the first and thirty-fifth days, animal weights were recorded using a portable livestock weigh scale. Each individual's body condition score was also scored using the accepted bovine standardized BCS scale of 1-5 (described in Table 1), where 1 is grossly underweight or emaciated and 5 is grossly overweight or obese (Kellogg, 2010).

BCS Score	**Explanation of Score**
1	Depression around tailhead, deep depression in loin; rib, shoulder, pin and hook bones exposed with no fat deposits. Animal has minimal fat deposits anywhere on body.
2	Minor depression around tailhead, depression in loin; Thin layer of fatty tissue over ribs, hook and pin bones easily viewed with large, deep depression between them.
3	No depression around tailhead, slight depression in loin; Fatty tissue layer covering ribs (although still palpable), hook and pin bones visible with visible shallow curved depression between them.
4	Rounded tailhead with evidence of fatty tissue deposite, no depression in loin; rib bones cannot be felt, hook bones palpable but covered with considerable fatty tissue; no depression between hook and pin bones.
5	Tailhead covered in layer of fatty tissue; rib, shoulder, hook and pin bones not visible or accessible by palpation.

Table 1: Body Condition Scoring Index

Body Condition Scoring Index with scores explained (Kellogg, 2010).

McMaster Fecal Egg Count (FEC) Technique

Fecal samples collected were processed at Town & Country Animal Clinic. McMaster fecal egg counts were performed following the guidelines set forth by the

University of Rhode Island's College of Environmental and Life Sciences (URI & VT, 2014). To start, 2 grams of feces were weighed into a plastic cup, and then 28 ml of fecal flotation solution (Ovasol – zinc sulfate) was added. The feces and flotation solution were mixed appropriately and let sit for 5 minutes – allowing the eggs to disassociate from the feces and float to the top of the flotation solution. After 5 minutes, the solutions were strained using a small strainer into a second cup; the fecal suspensions were forced through the strainer using a wooden tongue depressor. The majority of fecal contents left behind on the strainer – grass and other digested materials – were discarded and the strainer washed.

Upon completion of straining, McMaster slide chambers were filled individually with the strained solutions and allowed to sit for 5 minutes – again to allow for eggs to float to the top of the solution. Slides were observed under light microscopy at 10x magnification. Total numbers of nematode eggs (a blanket term used in this study to denote the larvae of hookworms, brown stomach worms and lungworms, which all present in a similar shape and size under 10x magnification) and coccidia oocysts in a chamber were counted for each sample. To calculate eggs per gram (ePG), the number of eggs observed in the chamber of the slide (volume = 0.15ml) was multiplied by a factor of 100. This factor was calculated using the equation given in Table 2, by taking into account the amount of feces used, the volume of the fecal solution used, and indicates that for the above protocol used, a minimum of 100 eggs per gram of feces can be detected.

ePG Multiplication Factor Calculation	
Sample Equation:	$ePG\ mult\ factor$ $= \frac{volume\ of\ flotation\ solution + volume\ of\ feces}{volume\ of\ feces * volume\ of\ slide\ chamber}$
Multiplication Factor Calculation:	$ePG\ mult\ factor$ $= \frac{(28\ ml\ Ovasol) + (2\ grams\ feces)}{(2\ grams\ feces) * (0.15\ ml\ chamber)} = 100$

Table 2: ePG Multiplication Factor Calculation

McMaster ePG multiplication factor equation and study calculation (Whitlock, 2010).

Serum Cortisol Level Testing

Serum cortisol concentration tests were performed using the frozen serum samples obtained during sample collection. Cortisol tests were run at the Veterinary Diagnostic Laboratory at the Oregon State University College of Veterinary Medicine. As a point of reference, cortisol concentration levels in this study were compared to measurements from previous studies looking at stress and associated cortisol levels in dairy calves to ensure the reliability of our concentration measurements (Wohlt et al., 1994; Van Reenen et al., 2005; Hickey et al., 2003; Stilwell et al., 2010).

Data Analyses

Statistics for each test were run through paired, one or two tailed t-tests. T-tests were chosen to analyze results because they allow for the expedient analysis of differences in mean value for a given result. An analysis of differences in mean value aligned well with the study format of a comparison of two relatively small, randomly selected study groups, and enabled the assessment of before and after-treatment values to quantify the effects of housing style on dairy calf health. Statistical significance was determined based on comparison to an accepted p-value of 0.05, anything less than which was considered significant.

Results

Body Condition Scoring

Body Condition Scoring (BCS) results were judged and recorded utilizing the conventional 1-5 scale (described in detail in Table 1 and depicted in Appendix Figure 2) and BCS were calculated for individual and pair-housed calves. There were no significant changes in either group's calf BCS between the onset and completion of the 35-day study period as can be seen in Figure 1; both groups' averages varied by just tenths of a point. The vast majority of total subjects – 18/20 at the onset of the study, and 13/20 after the completion of the study – scored a 3/5 (healthy, normal body condition score) on the BCS scale. In addition to BCS scores, calf weight results were obtained with the intention of calculating average daily gain, however, results were thrown out due to weigh scale inaccuracy and lack of precision. Average daily gain calculations were not made.

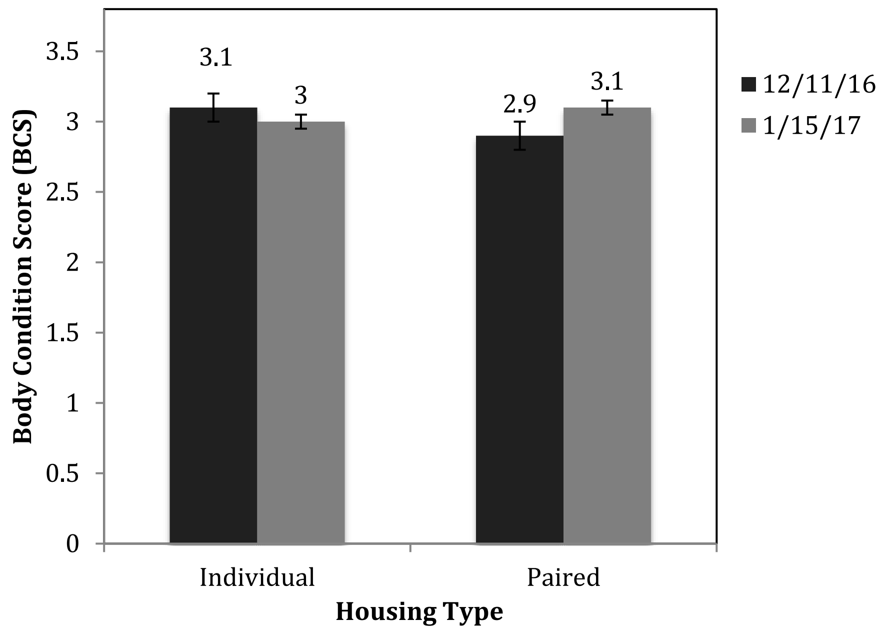


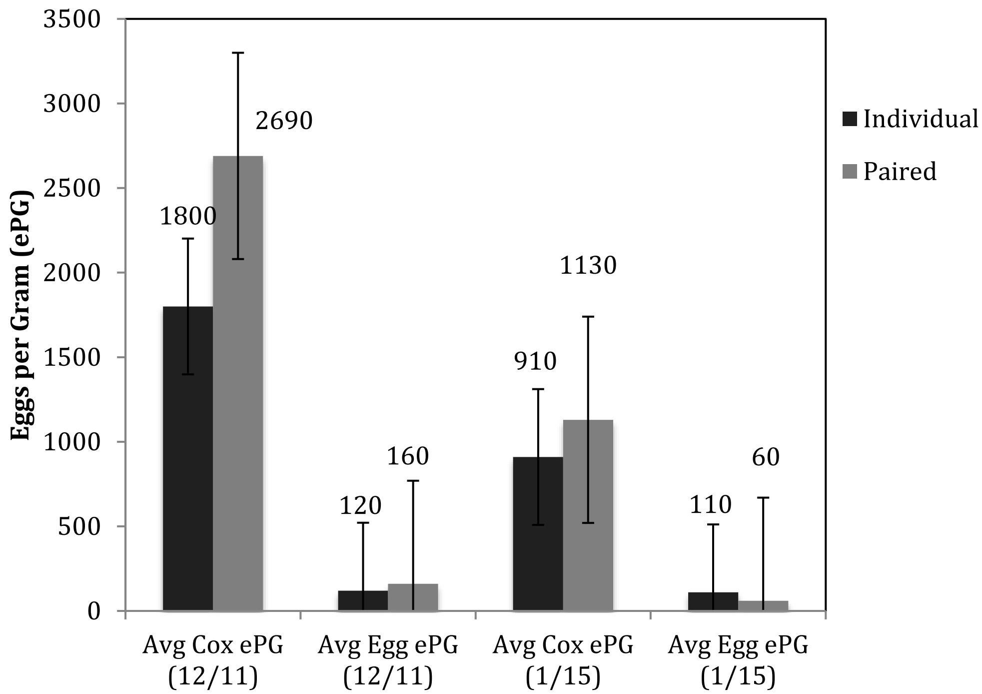
Figure 1: Average Body Condition Score Results

Average BCS results given for both collection dates. No significant changes in average body score were observed for either group (Individual p-value = 0.59; Paired p-value = 0.34). A majority of both groups scored a 3 for body condition score – 18/20 subjects beginning and 13/20 end of study.

McMaster Fecal Egg Count (FEC) Analysis

McMaster Fecal Egg count analysis of fecal samples showed a decreasing trend in eggs per gram (ePG) of feces in both groups over the duration of the study. Quantitative analyses of fecal suspensions measured numbers of Coccidia oocysts and "strongyle" eggs – a blanket term referring to various parasitic nematode worms (hookworms, brown stomach worms, and lungworms) that look very similar at 10x magnification in their larval stage – present within the grid of the McMaster slide. Eggs per gram (ePG) measures were calculated from these data using a multiplication factor calculated (as shown in Table 2) and can be found in Figure 2. Although a decreasing trend in average ePG was observed for both Coccidia oocysts and strongyle eggs in the individually housed and pair-housed calves, neither decrease was statistically significant when compared to an accepted p-value of 0.05.

Figure 2: McMaster Fecal Egg Count Results



McMaster Fecal Egg count results given in ePG. Average Coccidia oocyst ePG was decreased by 50% and 58% respectively. Average Strongyle egg ePG measurements were decreased by 8% and 62% respectively. None of the decreases were considered significant ($p<0.05$) decreases, as is indicated by p-values.

Serum Cortisol Level Testing

Serum cortisol results revealed that calves in paired housing had significantly elevated cortisol levels after the 35-day study duration. Figure 3 shows the before and after cortisol concentration values for both study groups. The study observed nearly identical average start cortisol concentrations for both the individually housed (1.044 ug/dl +/- 0.132) and pair-housed (1.066 ug/dl +/- 0.198) groups (both n=10). However, after 35 days, pair-housed calf cortisol concentrations significantly increased (1.405 ug/dl +/- 0.366). Individually housed calves did not display the same significant increase in serum cortisol concentration (1.036 ug/dl +/- 0.077) – in fact, the average cortisol concentration for these animals decreased after the month-long study, although not significantly. These changes in cortisol concentration are indicative of increased stress levels in calves and showed that calves housed in pairs were experiencing higher levels of stress than their individually housed counterparts.

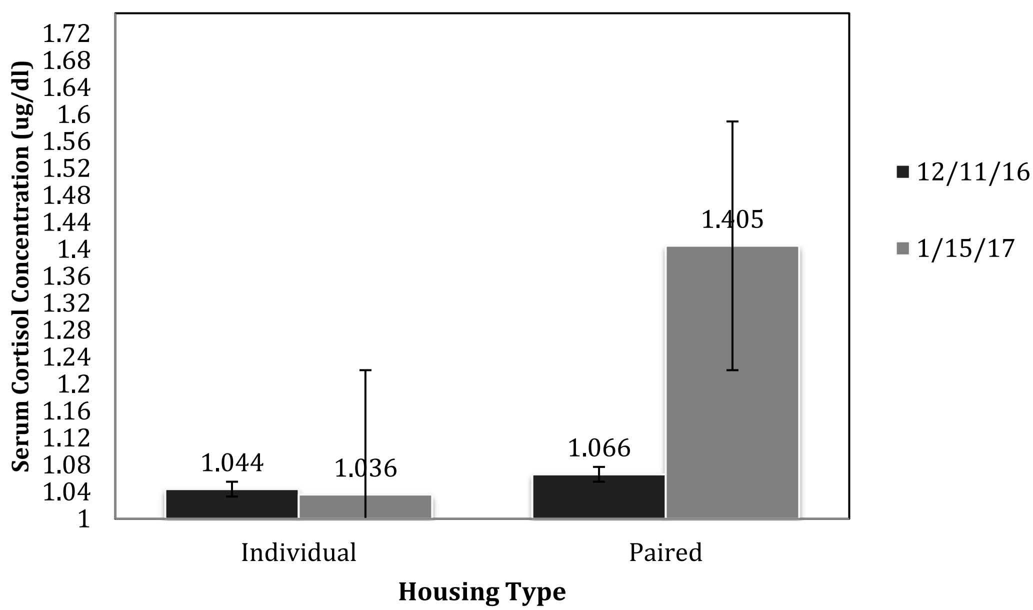


Figure 3: Serum Cortisol Concentration Results
Cortisol concentrations for individually housed and pair-housed calves, indicating a significant increase ($p = 0.012$) in basal cortisol concentrations for calves housed in pairs during our 35-day study.

Discussion

Paired Housing Increases Dairy Calf Stress in the Short Term

As has been observed in previous studies, increased blood cortisol concentration in test subjects is a manifestation of animal stress, and can be detrimental to calf health, growth, and performance in the long term (Van Reenen et al., 2005; Chua et al., 2002). Some studies have attributed this increase in animal stress to stressful social factors such as close-quartered housing with unknown conspecifics, food competition, or novel object presentation (Pempek et al., 2016). Other studies have theorized this stress is caused by bodily factors – such as high parasite load, low body condition, and weight loss (an indicator of a myriad of possible problems including lack of sufficient nutrition, disease, parasite infestation, dehydration, etc.). Inversely, additional studies found increased stress to have a negative influence on calf immune response and less directly, calf health and growth success – which exposes calves to the various aforementioned health issues (Hickey et al., 2003). The results of this study serve to address the chicken and egg debate about stress and its negative impacts on dairy calf health.

It was the finding of this study that at Reservation Ranch, pair-housed Holstein-cross calves had increased serum cortisol levels of expression when compared to individually housed conspecifics of similar age and identical rearing conditions – besides paired or individual housing. These pair-housed calves were otherwise healthy, as is evidenced by their very healthy post-study average body condition score of 3.1, and their average ePG, which decreased more than 50% throughout the duration of the study (a nearly identical decrease to their individually housed counterparts). With these other gauges of health and growth indicating no health-related stressors were present, this study contends that the increased stress levels observed in the pair-housed calf group was caused by social stressors such as food competition or close proximity housing with an unfamiliar conspecific.

Previous studies that investigated the negative impacts of paired housing on individual calf health and performance evaluated a variety of additional factors along with animal stress – with many of these studies analyzing calf behavior and how housing system influenced the development of social skills (Costa et al., 2016; Wagner et al., 2012). In the studies that were geared more directly to calf health and performance, fecal analyses were a consistently under-investigated portion of calf health. Previous studies that did analyze fecal matter in any way only went as far as basic fecal scoring, which assigns a score to feces based on consistency. No previous studies available quantified parasite load as an indicator of calf health.

For this study, the use of the McMaster fecal egg count technique was an expedient way to easily and reasonably accurately quantify subject parasite load based on number of parasites in fecal samples – giving an easily attained "picture of health" in terms of parasite load for each subject being tested. Fecal analysis results were not significantly different for either of the two study groups, and thus could not support an alternate hypothesis that pair-housed calves would have increased parasite load because of increased calf-calf interactions. This lack of a significant difference coupled with a very similar decrease in before and after-study ePG measures for both groups led us to believe that the trend was not the effect of the housing system, but instead could have been the result of an environmental change, a living quarters size increase, a food change, or a combination of these factors. A trend of this nature should be of major interest to Reservation Ranch.

Calf Barn-Linked Parasite Increases in Study Subjects

As can be found in Figure 2, at the onset of the study, calves had relatively high eggs per gram concentrations of both coccidia oocysts and strongyle eggs in their feces. All calves studied had been housed in Reservation Ranch's 75+ year-old calf barn from the time they were moved to the barn (at less than 24 hours old) until the time the study was started on the first collection date (approximately 2.5 months spent in the barn). It is well documented that once present in an environment, enteric parasites such as coccidia are very challenging to completely remove from a given housing area, especially during the cold, wet winter months coastal Northern California endures annually. Because of the immense challenge of emptying, sanitizing, and efficiently drying a flat barn in the wintertime, Reservation Ranch cannot perform complete monthly calf barn cleanouts and sanitization spray-downs to try and limit parasite colonization of calf stalls. Instead, they employ a rotating housing model, in which calves are brought in and moved out of the long, two-rowed barn in "blocks" – effectively using pens for one to two months to house animals and then leaving them empty to sanitize and leave resting for a month before a new group of calves is born and brought into the barn to spend their first two months of life.

Study results suggest that this rotation scheme is not working effectively. Once removed from the calf barn – regardless of stress or growth, egg per gram values went down by upwards of 50% in all calves. This could be the result of maturing immune systems or it could be the result of removing the animals from a constant colonized environment. A few of the possible explanations for this persistent colonization of the barn are: steady "aerolization" of the microorganisms causing infection (as explained by Chua et al., 2002), fecal-oral transmission of the microorganisms from neighboring calf contact at the front openings of pens (Chua et al., 2002), possible water contamination due to contamination of large, open water tanks at the opening of the barn, and of course, subpar cleaning and sanitation techniques. The aerolization explanation is one that if true, presents a particular problem during the winter months, because for those months the barn curtains remain closed almost all the time to keep wind and rain off the animals. This practice minimizes fresh airflow and allows the stagnation of air inside the barn; this lack of fresh airflow could lead to higher concentrations of microorganisms in the barn and thus higher frequency of airborne parasite transmission.

Additionally, the explanation of fecal-oral transmission should be limited to within the individual "blocks" of calves that are moved into the barn in sections. However, the use of an automated feeder wagon with a single nipple for a four-minute milk feeding and shared troughs for free-fed grain for three, four-minute settings could facilitate the passage of microorganisms from one "block" of animals to another. Space restrictions in times of heightened calving also bring calf blocks into contact with one another and limit "rest" times for pens between usage. Ultimately, the question for Reservation Ranch management that must be addressed with this intriguing parasite load data in mind is "what risk is greater, housing calves in a barn that is colonized by parasites or moving calves out into the harsher weather conditions of hutches at a younger age?" This decision cannot be made based solely on the trends discovered in this study, and instead would ideally be made after additional testing of larger groups of calves of various exposure timeframes to the calf barn. The possibilities for future research are nearly endless in the realm of dairy calf management, as farmers each and every day are encouraged to update management practices by veterinarians and animal rights activists alike.

Industrial Relevance

The industrial implications of studies such as this are quite high, not only from an animal welfare standpoint, but also from an economic standpoint. As is the case in other slim-margin industries, changes in agricultural practices are dictated by economic success; additional studies of various calf housing systems could lead to breakthrough discoveries explicitly showing that group or individual housing methods increase animal welfare while also bolstering a dairy's production or economic success. Studies like this one could be the key to spanning the divide between animal welfare motivated protocols and economically motivated protocols. Therefore, it is in the best interest of activists and farmers alike to find a common ground in supporting studies that investigate the pros and cons of changing calf-housing protocols along with other facets of the daily lives of production dairy animals. These studies, in addition to longer-term studies looking at the effects of social interaction of calves on their success and production later in life as producing cows, could give a more holistic, lifelong picture of the effects of housing styles and other common calf protocols on the production of animals.

A plethora of future research questions exist that will directly influence the dairy industry. Studies like this one and many others should help to inform future research, and develop new questions to investigate. Some of those future questions could include investigations into the effects of milk-weaning time on calf stress, success, and social skills, or how automated feeding and more human-centric forms of feeding in calves affect cow stress levels later in life when they are placed in dairy settings where they are forced to interact with people at various junctures of each and every day. Other research could be done to study the effects of weather patterns and how they might be related to the success or failure of various housing styles in reducing stress and fostering better growth environments for calves. This seemingly unrelated study would be of interest because of the extreme variation in the findings of previous studies investigating paired and individual calf housing, and how systems positively and negatively affect calves. Many of these studies that have investigated housing effects on calf health and wellness have been performed in areas of varied climate and at varied times of the year. A probe of these studies and how their findings were correlated with weather could provide intriguing results.

A final study that would be of great interest would be one that looked at the effects of housing styles on different breeds of dairy cows. Previous studies used a litany of different breeds of calves, with the vast majority being Jersey with Holsteins a close second. Cross-bred calves (as were used in our study) and still other breeds of less common calves could possibly react differently to housing styles because of any of a number of genetic factors associated with their various breed pedigrees. A study of this nature could be the key to unlocking a hidden genetic gem, the next crossbreeding fad brought on because of a certain trait or advantage that could be “bred into” a string of cows by making a single cross with the breed that possessed the advantageous trait. The avenues for future research involving the dairy industry and calf husbandry are neverending, with the possible implications for the findings of said research being just as broad and intriguing.

Limitations

This study has some important limitations to note. Firstly, the sample size of animals studied was relatively small due to a limited number of age-appropriate calves available during the winter months. This study was ended a month prematurely at the request of Reservation Ranch due to a lack of calf housing space during the months of December and January – two of the coldest and harshest weather months in the calendar year in Del Norte County. It was the original plan of the study to calculate average daily gain for each subject involved in the study, however, due to weigh-scale issues and a lack of reputable readings, the weight data for this study was thrown out and average daily gain results were not calculated. It is of important note that the machine at the Veterinary Diagnostic Lab at Oregon State University used to calculate serum cortisol concentration had a minimum sensitivity of 1 ug/dl, which is higher than the accepted bovine serum cortisol biochemical reference range of 0.45-0.75 ug/dl (Jackson & Cockroft, 2002).

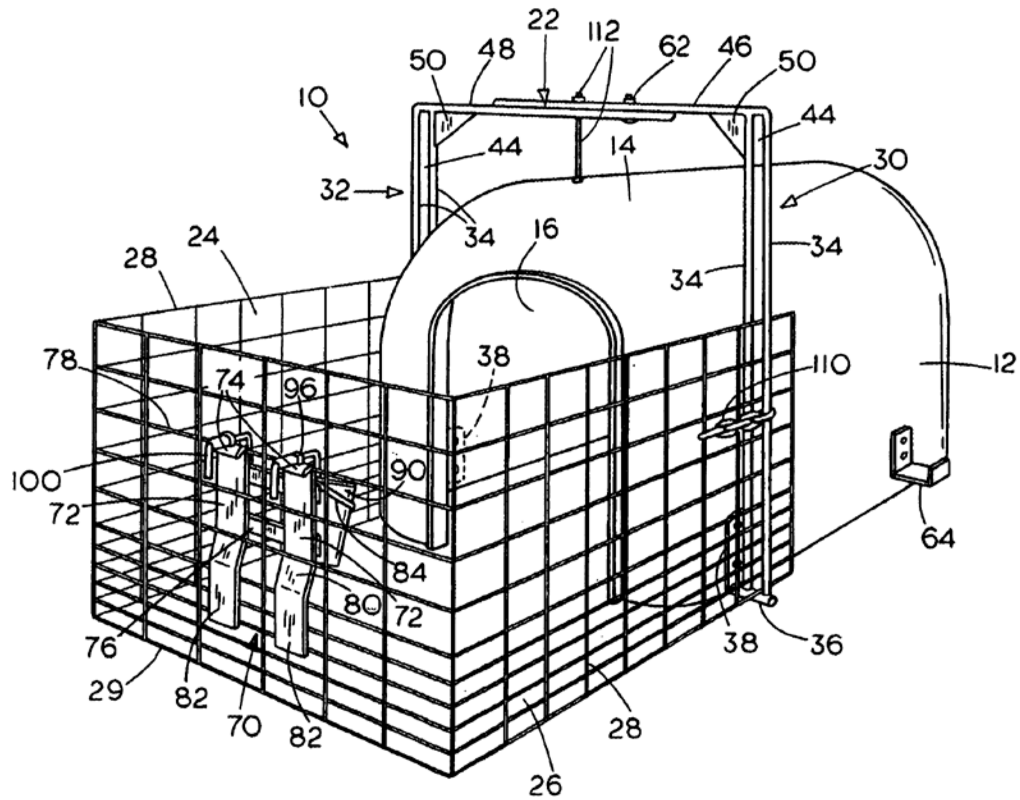
The subjects involved in this study were not fully related, and because of this, genetic variance may play a role in any differences in readings or measurements for subjects in the study. However, to perform a study such as this at a dairy the size of Reservation Ranch (milking on average 750 cows), it is not possible to get 20 or more subjects of the same age with an identical genetic pedigree. With that in mind, this study investigated trends observed in the two study groups while also being aware of the inherent genetic variation associated with having different dams and sires of each subject in the study, just as others have done in past studies (Pempek et al., 2016; Jensen et al., 2014).

If this study were to be expanded upon, the authors suggest a larger test subject pool be analyzed, for a longer period of time – a minimum two-month study instead of a one-month study. The authors would also recommend the use of a reliable weigh scale to give a proper representation of calf growth success via average daily weight gain calculations. Additionally, serum cortisol levels should be evaluated using a machine with a higher sensitivity than was utilized in this study to give the most accurate results possible.

Conclusions

Grouped housing was shown to increase serum cortisol levels in approximately two and a half month-old Holstein-cross dairy calves – an indication of correlation between novel social interaction with an unknown conspecific and increased stress levels in test subjects. It is the belief of this study that this correlation was the byproduct of group feedings and the competition for food associated with paired access to food for both calves in a paired housing setup. Additionally, this study found no correlation between shared physical space and increased subject fecal parasite load counts or increased body condition scores. This study combined various measures utilized in past investigations of calf housing systems (Jensen et al., 2014; Van Reenen et al., 2005; Chua et al., 2002) to be the first of its kind to holistically assess calf success by quantifying subject stress levels, parasite loads, and body condition scores. It is the conclusion of this study that there are no negative influences on calf health and wellness associated with single-subject calf housing, however our results suggest social housing can lead to higher stress levels in calves – presumptively due to feeding competition. These results offer insight into the pros and cons of housing systems and should be considered by farmers and future researchers alike interested in the field of dairy calf husbandry and housing protocols.

Appendix



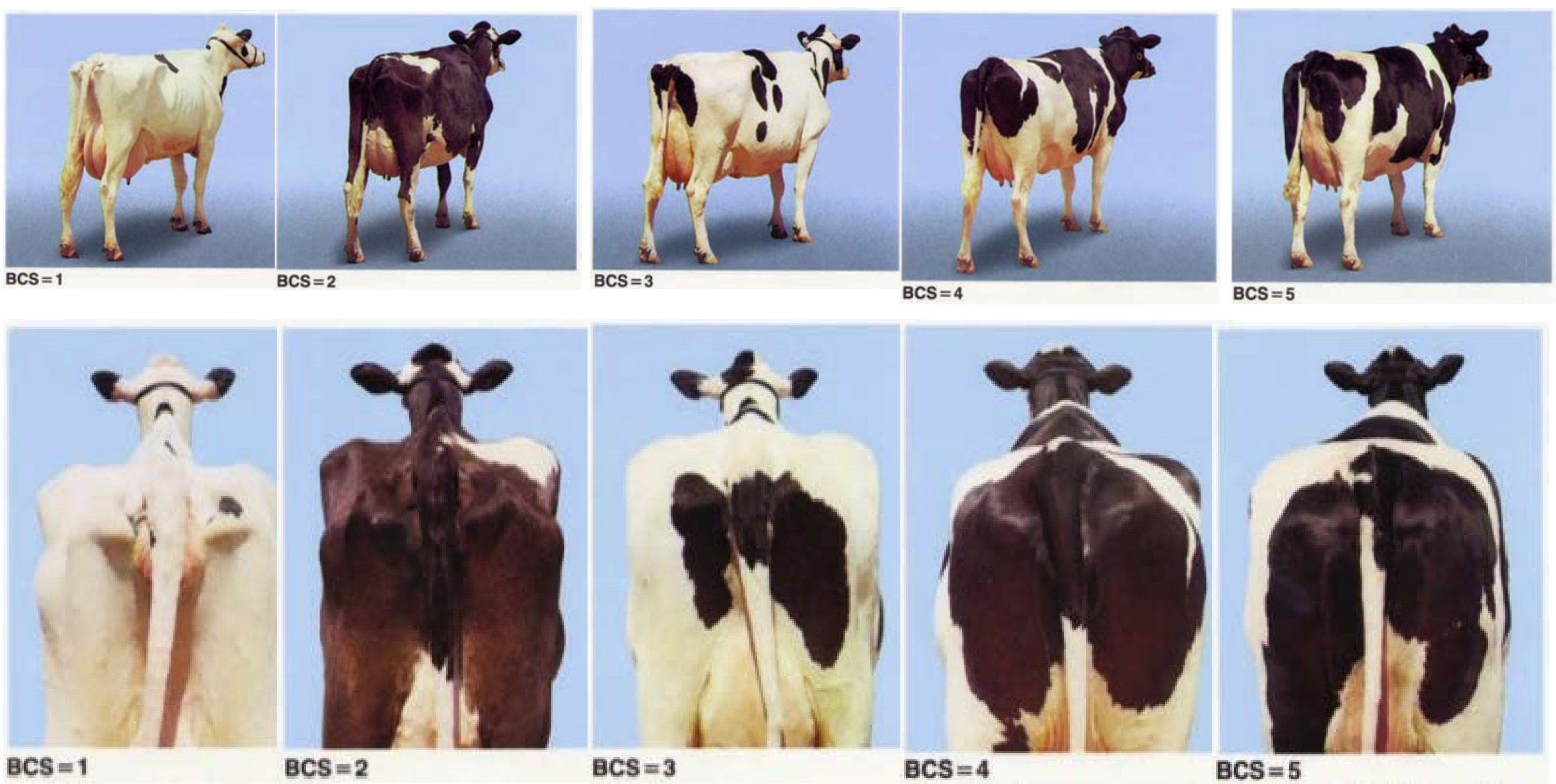
Appendix Figure 1: Calf Hutch Schematic Drawing

Calf hutch schematic drawing indicating hutch enclosure and associated loafing area enclosed by rubbed-wire fencing with feeder attached to front. (Anderson, 2004)

Appendix Figure 2: Body Condition Score Examples

Body Condition Score Examples given for each score 1-5

(Kellogg, 2010)



Glossary of Terms

Calf hutch – a plastic/fiberglass house or shelter with approximate dimensions of 7'x4'x4'; calves are housed in these structures from weaning age (~2months) until about 5 months of age – See Figure 1 for schematic drawing

Rubbed wire – rounded, non-abrasive wiring used in agricultural enclosures and fencing

Loafing area – an outdoor area for an animal to walk around and relax in adjacent to enclosed hutch

Cortisol – an adrenal hormone secreted into the blood when organism is under psychological or physiological stress; documented stress indicator in dairy cattle

Venipuncture – the puncture of a vein, in this case for a blood draw.

McMaster slide – specific type of chambered slide overlaid with etched or printed grids to facilitate the counting of fecal eggs

ePG – eggs per gram

Fecal Suspension – solution used to suspend fecal samples, thus separating the manure from the parasite eggs

Fecal loop – small, smooth plastic loop used to obtain fecal samples from small animals

Body condition score (BCS) – a system used to assign a numerical score to an animal's body composition, the fatter the animal, the larger the BCS score

Disbudding – Dehorning; the act of removing the horn bulbs of calves, usually performed using a hot iron under mild sedation

Heifer – a female dairy animal that has not calved or is not currently in milk; some consider a heifer a female dairy animal that has not yet calved (< 2 years old)

Steer – a castrated male dairy animal

Bull – a non-castrated (intact) male dairy animal

Dam – the female parent of a calf

Sire – the male parent of a calf

Conspecific – members of the same species

“Go off feed” – a general loss of appetite observed in livestock

Scouring/scours – diarrhea generally associated with parasitic pathogenesis or other enteric diseases and disorders

Total mixed ration – a feed mix assembled at the dairy made up of various commodities and grains along with chopped alfalfa hay

Enteric – relating to or occurring in the intestines

Strongyle – a type of nematode egg, usually long and elliptically shaped; ~20 nm in length

Oocyst – a cyst containing a zygote formed by a parasitic protozoan such as *Eimeria bovis*

Aerolization – the spread of microorganisms via small airborne particles

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