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Chaney K. Morgan

University of Tennessee, Knoxville, cmorga39@vols.utk.edu

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**Evaluation of single vs. pair housing Holstein calves on specific
antibody concentrations to KLH**

Chaney K. Morgan

Chancellor's Honors Thesis

Gina M. Pighetti, Ph.D.

December 11, 2017

Evaluation of single vs. pair housing Holstein calves on specific antibody concentrations to KLH

C. K. Morgan, C. B. Kesterson, P. D. Krawczel, G. M. Pighetti. Dept. of Animal Science, Chancellor's Honors Program, The University of Tennessee, Knoxville, TN.

ABSTRACT

Dairy calf management for the past few decades has leaned towards individual calf housing to reduce spread of disease. Since then better farm practices have reduced such disease and offers the opportunity to reevaluate housing. Our objective was to determine the effect of pair vs. individual housing of calves on the humoral immune response. Calves with successful passive transfer of immunoglobulins from colostrum feeding (STP ≥ 5.5 g/dL) were enrolled into paired ($n = 28$) or individual ($n = 14$) housing by 5 d (± 1.4 d) of age. Treatment ended at weaning, 56 d (± 7 d) of age. Single calf housing was implemented by using individual pens. Calf pairing was implemented by combining two individual pens by removing the middle divider; one calf was used for actual data collection and the other merely to implement treatment. Humoral immunity was evaluated by inoculating calves with a 1 mL injection of keyhole limpet hemocyanin (KLH; 0.1 mg), Quil-A adjuvant (0.5 mg) and pyrogen-free saline at 7 d. Another injection at d 21 contained KLH (0.1 mg), Quil-A adjuvant (0.5 mg), and heat-killed *Candida albicans* (CA, 2×10^6 cells) in pyrogen-free saline. Blood samples were collected at d 3, 14, 28, & 35. Enzyme-linked immunosorbent assays (ELISAs) were performed to detect antibodies specific to KLH. A highly positive d 3 calf sample serially diluted from 500-32000 was used as a standard curve. The calf samples were plated with a dilution factor of 1000. The mean concentration of IgG antibodies was calculated relative to the standard curve. A mixed model ANOVA (SAS 9.4), was used to analyze relative expression of antibodies to KLH. At d 14, regardless of housing treatment, concentrations were lower than other collection days ($P = 0.0003$). Interaction between housing treatment*day was not evident ($P = 0.6500$). Housing treatment has no effect on relative expression ($P = 0.9332$). This suggests that under well-managed conditions single and paired housing were both capable of generating antibodies to an immune stimulus and suggests disease resistance would be similar. The potential for humoral immunity presenting similarly in single and paired housed calves, paired housing would be preferred based on other social benefits to the calf. Further evaluation of dairy calf immune competence needs to be conducted in order to better understand the immune development of pre-weaned dairy calves.

INTRODUCTION

Dairy calf management for the past few decades has leaned towards individual calf housing to reduce spread of disease and allow for individual nutrition/management plans while maintaining visual and auditory contact (USDA, 2016). Better farm practices such as

administration of good quality colostrum in a timely manner (Godden, 2008), monitoring serum total protein to assess antibody transfer (Tyler et al., 1998), and ventilation to minimize respiratory diseases (Lago et al., 2006)) have reduced such disease. Colostrum is the first secretion of milk from a cow after giving birth, this first milk gives the calf the maternal antibodies which they do not receive in utero (Quigley, 2004). Colostrum needs to be given to the calf within the first 24 hours of birth as the efficiency of absorption of antibodies starts to decrease soon after birth, decreases significantly at 12 hours and completely ceases around 24 hours (Bush and Staley, 1980). Colostrum containing higher concentrations of antibodies gives calves a better chance of survival, with one study showing calves receiving >1000 mg/dL of IgG had a better chance of survival than those receiving <1000 mg/dL (NAHMS, 1993). Individual calf housing is valuable to reduce disease transmission and allow for easier individual care of the calf (Svensson et al., 2003). Group housing on the other hand at a young age (from day 3-35 of age to weaning around 56 days of age) has been shown to increase instance of disease depending on the size of the group (Svensson et al., 2006). Other studies however have shown no disadvantage in health when the calves were housed in pairs with the only criteria to enter being adequate quantity consumption of colostrum (Chua et al., 2002; Bolt et al., 2017). Because of these better management practices that promote immunity, it may be time to reevaluate housing.

Multiple studies have focused on the behavioral aspects of calf housing. These studies indicate that paired calf housing reduces stress-indicating behaviors in calves, such as vocalization (De Paula Vieira et al., 2010; Bolt et al., 2017). Stress increases the level of cortisol in the body which in turn suppresses the immune response (Palacios and Sugawara, 1982; Borysenko and Borysenko, 1982). Therefore, there could be value in having a partner in housing to reduce stress and aid the immune system. Most dairy farms house their weaned and adult cows

in large groups. Starting calves in pairs, instead of in isolation, could help better prepare them for housing changes later in life. However, housing calves in groups of 10 or more from birth to 6-8 weeks of age has been shown to be unfavorable for health and growth reasons (Svensson and Liberg, 2006). Studies have shown that putting calves in pairs reduced weaning (ceasing milk substitute feeding in calves) stress and improved performance past weaning in terms of feed intake and weight (De Paula et al., 2012). The humoral immune response in calves also is affected by weaning, with calves having a higher antibody response to KLH when weaned early and experiencing transport stress (Mackenzie et al., 1997).

Similar studies have been done evaluating the effects of dairy calf housing type (individual pens vs. individual hutches) on IgG concentrations to KLH (Cummins et al., 1991). KLH is a large protein from the giant keyhole limpet and is a protein that calves will not come across in their lifetime. As such, this protein is a useful agent to illicit an immune response and study antibody production under certain conditions. In order to avoid non-specific binding to pre-immune IgM with KLH, IgG testing is preferred for primary immune response measures (Korver et al., 1984).

Immune evaluation of the effects of paired housed dairy calves has yet to be fully understood. The objective of this study was to determine the effect of pair vs. individual housing of Holstein dairy calves on the humoral immune response through evaluation of antibody concentrations. It was hypothesized that, if antibody concentration is related to calf housing, then paired calves will have a higher mean concentration than single calves.

MATERIALS AND METHODS

All animal procedures used in this study were approved by IACUC. Holstein calves



Photo 1. Individual calf housing (left) and paired calf housing (right). Paired calf housing made by removing middle divider between two individual pens.

(n=42) were enrolled in the study from December 2016 to May 2017 with 14 calves housed individually and 28 calves housed in pairs (only 14 calves were used to take samples while the other 14 were used to impose the paired housing treatment and did not receive any injection or have blood collected).

Individual housing was implemented by housing the calf in a standard calf pen (roughly (1.9m X 0.57m) and 1.1 meters in depth) used by East Tennessee Research and Education Center – Little River Animal

and Environmental Unit (Walland, TN) where the study was conducted. Paired calf housing was implemented by taking a divider out between two

pens allowing for the same space per calf as an individual pen with the ability to interact fully with its pair. Both individual and paired housing is shown in Photo 1. Pens are outdoors and have a metal roof covering all of the pens. Approximately day 3, (3 days after the calf's birth) blood was collected via jugular venipuncture with a vacutainer and an 18-21-gauge needle. Blood was then tested for serum total protein (STP) as an indicator of antibody absorption from colostrum. Calves with a STP of less than 5.5 mg/dL were deemed at risk for infection and were not entered in the study due to failure of passive antibody transfer. Calves that passed this were enrolled in

the study and treatment was implemented at 5 days of age (± 1.4). On day 7, the calves received a primary injection of KLH (1 mL injection of KLH (0.1 mg), Quil-A adjuvant (0.5 mg) and pyrogen-free saline). The calves received the secondary injection of KLH (KLH (0.1 mg), Quil-A (0.5 mg), and heat-killed *Candida albicans* (CA, 2×10^6 cells) in pyrogen-free saline) on day 21. (*Candida albicans* was used in the second injection to measure delayed type hypersensitivity and will not be covered in this paper.) Blood was collected on the calves 7 days after each injection and again on day 35. Calves weaned on day 56 (± 7) ending their time in the study.

All calves were given milk substitute in accordance to normal farm procedure at the dairy. Feed was kept at 5 lbs. every day, allowing for equal feed for each calf. Each calf whether paired or single was given its own water and feed bucket. Pens were regularly cleaned and bedding replenished as needed. Weekly health exams were performed on every calf including: observation of ear droop or head tilting, coughing, rapid or difficult breathing, rectal temperature, eye discharge, and nasal discharge. Health exams were performed based on visual observation and rectal temperature. Scoring for each observation was given based on the protocol as shown in Photo 2 in the Appendix.

Serum samples were generated from centrifuging blood collections and placing the sera in cryo-vials at -80°C . When it was time to run ELISA's on the serum samples to test for relative antibody concentration specific to KLH, the sera samples were thawed. Costar high binding 3669 plates were coated with $100\ \mu\text{L}/\text{well}$ of KLH ($1\ \mu\text{g}/\text{mL}$) in carbonate coating buffer and were incubated at 4°C overnight. Plates were then washed 4x with 10x phosphate buffer-Tween (PBT). Plates were blocked with PBT plus 2.5% goat serum at $200\ \mu\text{L}/\text{well}$ for 30 min at room temperature (RT). Washing was performed the same way as before. Calf sample 456 day 3 was

chosen as the standard curve, as it had the highest concentrations. The positive control (calf 456 d 3) was serially diluted with PBT + 2.5% goat serum on the plate (100 μ L/well) from 1/500 to 1/32000 in duplicate. The negative control [fetal bovine serum (FBS)] (Atlanta biologicals. cat. S11550, lot. A1061. Flowery Branch, GA) was diluted 1/1000 in PBT + 2.5% goat serum and plated in duplicate at 100 μ L/well. Each calf's four sera samples were diluted 1/1000 with PBT + 2.5% goat serum (Gibco. ThermoFisher. 16210-072, lot. 1671329. Waltham, MA) and plated in duplicate at 100 μ L/well. The plates then were incubated at RT for 1 hour. Plates were washed as before after incubation. Secondary goat anti-bovine antibody (Jackson ImmunoResearch. 101-035-165. West Grove, PA) diluted 1/2500 with PBT were plated at 100 μ L/well and incubated for 1 hour at RT. Plates were washed for the last time. Substrate (BD OptEIA TMB substrate reagent set cat. 555214, lot. 5327556. San Diego, CA) added at 100 μ L/well and incubated for 15 min at RT. Plates were then read at 2:630nm.

STATISTICAL ANALYSIS

KLH data was analyzed as a randomized block design with repeated measures in days, using the mixed procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Animals were blocked by birth date and sex with housing treatment as the fixed effect. The least square means and standard errors through the LSMEANS statement in the mixed procedure was conducted to compare relative expression between the housing treatment*day interaction. Relative expression was determined by the standard curve set from FBS (negative control) and our most positive sample. A mixed model ANOVA was used.

RESULTS AND DISCUSSION

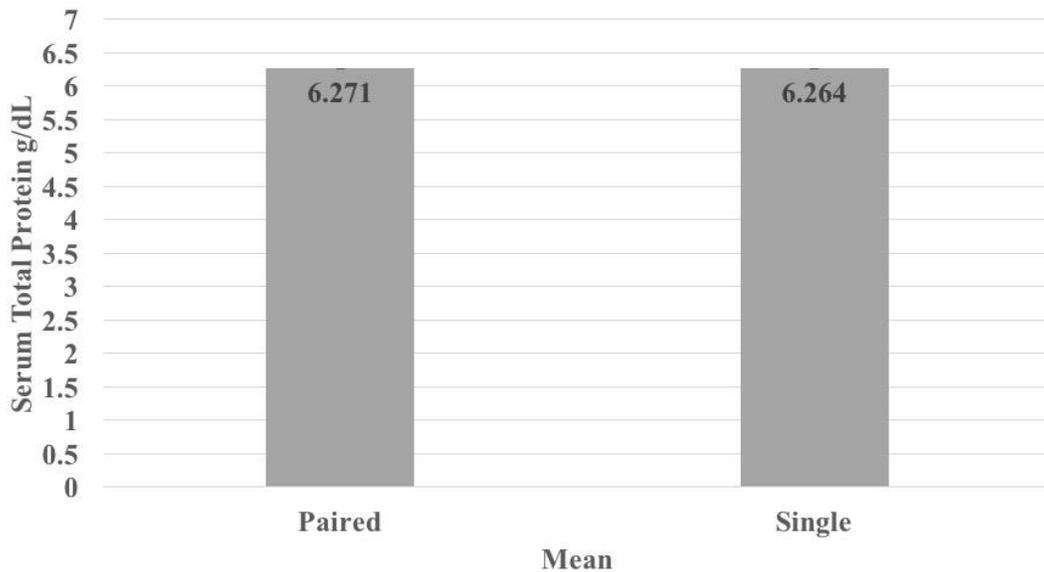


Figure 1. Graph of the mean serum total protein for passive transfer for paired vs. single housing. Data collected from calves before they were allowed enrollment in the study. Means between both treatments are not significantly different, $P>0.05$.

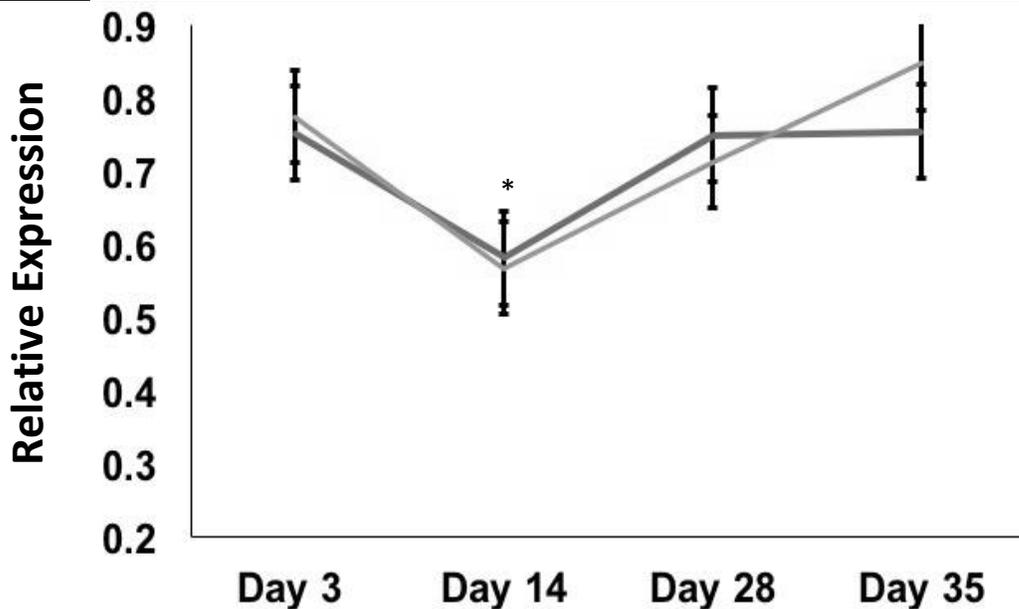


Figure 2. Mean relative expression of antibody concentration to KLH by blood collection day for each treatment. No significance between the two treatments or from 1st collection to last collection. *Represents significant difference for day 14 between time points, $P=0.003$,

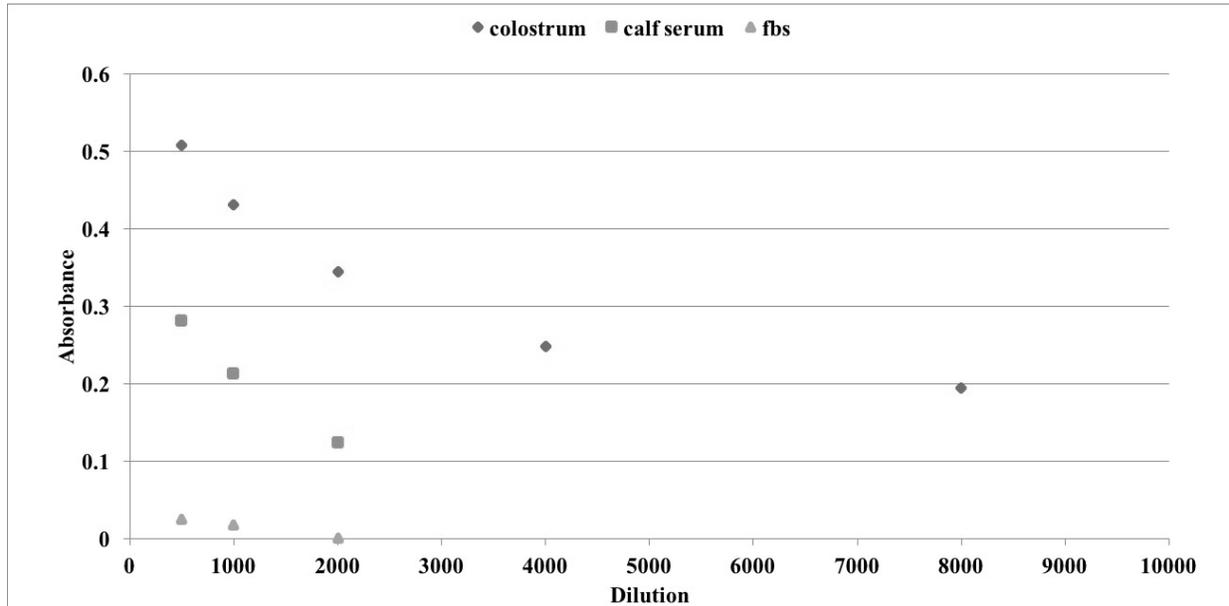


Figure 3. Titration of KLH antibody concentration of colostrum fed to calf 4854 and calf 4854 sera from d 3 collection.

Table 1. Average health scores \pm standard error and number of instances of high scoring for respiratory and fecal scoring on calves. Scale 0-6 for respiratory and 0-3 for fecal, with the highest number being the worst. $P = 0.307$ for respiratory averages between treatment and P -value = 0.177 for fecal averages between treatments.

| <i>Average Health Score</i> | | |
|-----------------------------|----------------|----------------|
| Treatment | Respiratory | Fecal |
| Single | 1.21 \pm 0.1 | 0.5 \pm 0.07 |
| Paired | 1.1 \pm 0.08 | 0.4 \pm 0.05 |

| <i>Number of Incidences of a High Score</i> | | | |
|---|----------|----------|----------|
| | Resp. =4 | Resp. =5 | Resp. =6 |
| Single | 7 | 3 | 4 |
| Paired | 2 | 2 | 0 |

| | Fecal =2 | Fecal=3 |
|--------|----------|---------|
| Single | 20 | 13 |
| Paired | 20 | 5 |

Calves are born without maternal antibodies and must obtain these from colostrum. Testing serum total protein estimates the number of antibodies in the blood. Blood collections were taken around day 3 of age for each calf and was used to determine enrollment in the study based off STP results. Figure 1 shows similar antibody levels were observed between both treatments and were >5.5 g/dL, indicating successful transfer. Normal industry management practice deems >5.2 g/dL in 90% of its herd and >5.5 g/dL in 80% as meaning that herd has had successful antibody transfer (USDA, 2016). The average STP from d 3 collection between treatments was not significantly different and indicates colostrum quality and quantity will not be a major contributor to differences between treatments.

In Figure 2, calves in paired vs. single treatment did not show significant differences between KLH antibody concentrations relative to the established standard curve. Sex and birth date did not show significant differences ($P < 0.05$) between the two treatments. The only significant difference in the data was d 3 to d 14 in comparison to the other time points within each treatment. There was a significant drop in antibody concentration from d 3 collection to d 14. High antibody concentration around d 3 were unexpected because the calves would not have been exposed to KLH before, nor had the dams. To determine whether colostrum may have been the source of high initial KLH antibody concentrations in calf sera, we assessed colostrum provided to the calf in addition to the calf sera (Figure 3). Such a high spike in antibody concentration around d 3 is influenced by the strong presence of the maternal antibodies just received from colostrum intake on the first day of life. The colostrum contained antibodies capable of binding to KLH and was greater than the calf sera. Even though cows or calves were not exposed to KLH before, this protein is very bulky and has been demonstrated to illicit a

humoral immune response (Korver et al., 1984). When the calf consumed the colostrum, they were able to absorb the dam's mature IgG through the GI tract. The calf's own immune system doesn't begin to completely take over and make its own antibodies until around 2-4 weeks of age while maternal antibodies only stick around for 2-3 months (Chase, 2008). This explains the raise in mean antibody expression with both treatments from d 14 to d 28. Future research with an increased sampling time would be of interest since it looks as though the paired data seems to increase while single decreases the further out in time it went. Following the calves past weaning and into production would be of interest to see if there are lasting effects on immunity from paired vs. single housing.

Overall, there was no significant difference in antibody expression between single vs. paired housed calves. Table 1, however is indicative of a higher instance of disease in individually housed calves than paired calves although the difference in disease is not significant. In this study, healthy calves with a STP >5.5 g/dL were enrolled. This suggests that under well-managed conditions pair housing calves does not increase disease risk and provides other social benefits.

SUMMARY AND CONCLUSION

Although this study hypothesized the results to bring more antibody expression with paired housing vs. single, having no significance between the two does not discourage the possible advantages in pairing pre-weaned calves. Housing dairy calves in pairs has both benefits and disadvantages. The reported behavioral and stress advantages of the paired housing shown in previous studies, work in the calves' favor, but producers lose the ease of individual planning and management. It is harder to measure feed intake and keep the calf on its own personalized

diet if it has a partner interfering. Past research has shown social housing pre-weaning to positively affect the calves' production later on, because they are quicker to begin eating feed and put on more weight (De Paula et al., 2012). The feed and weight aspect may have economic implications for producers since grain is typically cheaper than milk replacer. With no difference in immunity and advantages in performance, paired housing could have implications for positive effects on the industry.

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APPENDIX

| UC DAVIS VETERINARY MEDICINE <small>Teaching • Medicine • Research and Diagnostic Center</small> | | UC CE University of California Agriculture and Natural Resources Cooperative Extension | | UC DAVIS ANIMAL SCIENCE | |
|--|---|--|------------------------------|----------------------------|--|
| Bovine respiratory disease scoring system for pre-weaned dairy calves^{1,2} | | | | | |
| Clinical sign | Score if normal | Score if abnormal (any severity) ³ | | | |
| Eye discharge | 0  | 2  <small>or</small>  <small>or</small>  | | | |
| Nasal discharge | 0  | 4  <small>or</small>  <small>or</small>  | | | |
| Ear droop or Head tilt | 0  | 5  <small>or</small>  <small>or</small>  | | | |
| Cough | 0 No cough | 2 | Spontaneous cough | | |
| Breathing | 0 Normal | 2 | Rapid or difficult breathing | | |
| Temperature | 0 < 102.5° F | 2 | ≥ 102.5° F | | |
| Add scores for all clinical signs, if total score is ≥ 5, calf may be positive for bovine respiratory disease | | | | | |

Photo 2. Chart showing the designations for scoring based on visual observation of clinical signs for calves. (UC Davis, 2014)

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