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Research paper

Acute phase cytokines, TAC1, and toll-like receptor4 mRNA expression and health associated with group size in veal calves

E.M. Abdelfattah[a], M.M. Karousa[a], M.M. Schutz[b], D.C. Lay Jr.[c], J.N. Marchant-Forde[c], S.D. Eicher[c,*]

[a] Department of Animal Hygiene, Behavior and Management, Faculty of Veterinary Medicine, Benha University, Qalyubia, Moshtohor 13736, Egypt
[b] Purdue University, Department of Animal Sciences, West Lafayette, IN 47907, USA
[c] USDA-ARS, Livestock Behavior Research Unit, West Lafayette, IN 47907, USA

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ABSTRACT

Chronic stressors are a major health and well-being issue in animals. Immune status of animals under chronic stress is compromised, thus reducing disease resistance and compromising well-being of the animal. The objective of this study was to determine the influence of group size of veal calves on immune status and leukocyte mRNA expression of acute phase cytokines, toll-like receptor 4 (TLR4) and tachykinin 1 (TAC1) over a five-month finishing period. Holstein bull calves ($n = 168$), 44 ± 3 days of age were assigned to one of three treatments; 2, 4, or 8 calves/pen (pen space allowance of 1.82 m^2/calf). Jugular blood samples were collected at the day of grouping and then monthly for 4 months. The differential leukocyte counts were determined and mRNA was extracted from the leukocytes. Reverse transcription-qPCR was used to measure the gene expression of interleukin-1 (IL-1β), IL-1 receptor antagonist (IL-1Ra), tumor necrosis factor (TNF-α), TLR4, and TAC1 in leukocytes. Health was evaluated before grouping and monthly for 4 months. On the 1st month after grouping, veal calves that were housed in groups of 8 have greater expression of IL-1β mRNA than calves housed in groups of 4 or 2 (treatment × month, $P = 0.04$). Also at 1 month, groups of 8 had greater TAC1 expression ($P < 0.05$) than calves housed in groups of 4 or 2. However, the expression of IL-1Ra, TNF-α, and TLR4 were not influenced by group size. In the first month of the trial, calves in groups of 8 coughed more ($P < 0.05$) than calves in groups of 2 and coughed more than calves in groups of 4 and 2 during the 2nd month (treatment × month, $P = 0.03$). Calves housed in groups of 8 tended to have greater neutrophil percentage ($P = 0.09$), neutrophil to lymphocyte ratio ($P = 0.06$), and had lower lymphocyte percentage ($P = 0.06$) than those housed in groups of 4 or 2. In conclusion, the number of veal calves in a group, given the same space during the finishing period did not alter IL-1Ra, TNF-α, and TLR4 mRNA expression. However, housing of calves in groups of 8 was associated with greater expression of IL-1β and TAC1 mRNA in peripheral blood leukocytes, and coughing during the first 2 months after grouping. Therefore, housing of veal calves in larger groups may lead to greater susceptibility to respiratory disease and stress.

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Abbreviations: mRNA, messenger RNA; N/L ratio, neutrophil to lymphocyte ratio; RT-qPCR, quantitative reverse transcription PCR; TAC1, tachykinin 1; TLR4, toll-like receptor 4.

* Corresponding author. Present address: USDA-ARS, Purdue University, 125 S. Russell Street, 216 Poultry Building, West Lafayette, IN 47907, USA. Tel.: +1 7654963665; fax: +1 7654961993.
E-mail address: Susan.Eicher@ars.usda.gov (S.D. Eicher).

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1. Introduction

Grouping calves is an issue common to the dairy heifer; dairy beef; and veal industries and may represent a source of chronic stress for the calf (Hulbert and Ballou, 2012). Chronic stress often results in less discernible changes to animals than acute stress. Acute stress typically causes increased cortisol concentrations and neutrophil populations. Chronic stress on the other hand; is hallmarked by greater leukocyte numbers and cytokine changes (Eicher et al., 2013). Immune status of animals under chronic stress can be compromised thereby reducing disease resistance and well-being; and may even lead to death (Muir and Woolf, 2001). This was underscored by research (Brscic et al., 2012) showing that increasing group size lead to more hampered respiration and others (Bähler et al., 2012) who showed that respiratory disease was identified as a major cause of death in group-housed veal calves in Switzerland.

Stress is known to influence both innate and adaptive immune responses in both young and adult animals (Nonnecke et al., 2009). Traditionally, assessment of physiological stress has been estimated by measuring levels of adrenal hormones, mainly cortisol (Veissier et al., 1998). However, there are associated drawbacks, such as the potential for rapid changes in hormone levels in response to short-termed acute stressors, which potentially leave changes undetectable as seen with our assessment of cortisol in the companion paper (Abdelfattah et al., 2013). Acute stress causes a concurrent rise in neutrophil (N) number, and drop in lymphocyte (L) number, and thus a composite measure that is often used to assess the stress response is the N:L ratio in cattle (Friend et al., 1987).

An alternative method of assessing physical stress that has the potential to reflect a more chronic state of the animal is an investigation of relative white blood cell counts and acute phase responses. Peripheral blood leukocytes provide a broad picture of inflammatory response during exposure to stressors. Tachykinin 1 (TAC1) is produced by leukocytes which also have a receptor for TAC1and is involved in the increase of acute phase cytokines such as IL-1β, its receptor antagonist, and TNFα. Additionally, TAC1 is related to respiratory infections. Currently, 10 TLR have been identified in cattle (Jungi et al., 2011). Toll-like receptors, particularly TLR4 are proving to be critical in the initiation of many immune (Akira and Takeda, 2004) and pain responses (Gárate et al., 2013).

Real-time PCR has been developed in recent years for the quantitation of genes and provides a simple and the rapid analysis for quantitation of cytokine expression and cytokine profiles associated with stress in cattle (Satoru et al., 2003). Recent work (O'Loughlin et al., 2011, 2012) has shown that cytokines are upregulated in response to weaning and social regrouping stress for up to 7 days in beef calves. A previous report (a companion paper to the current study), described the behavioral and physiological responses of those calves in groups of differing sizes (Abdelfattah et al., 2013). Presently, no study has assessed the leukocyte markers of stress that we propose to measure on an experimental study of group housing for veal calves in North America. In the current study, the relative gene expression of a number of pro-(IL-1β, TNF-α) and anti- (IL-1Ra)inflammatory cytokines was measured to assess the inflammatory stress response following various sizes of group housing. Therefore the objectives of this study were (1) to determine the effect of group size on health and innate immunity of veal calves, and (2) to evaluate leukocyte gene expression of major pro- and anti-inflammatory cytokines mRNA of veal calves housed in different group sizes during the finishing period. We hypothesized that calves housed in larger groups will have impaired immune function, altered expression of cytokine mRNA (IL-1β, TNF-α), toll-like receptor (TLR4), and neurotransmitter associated with pain and respiratory disease (TAC1) in leukocytes than those in smaller group.

2. Materials and methods

2.1. Experimental design

The experimental protocol was approved by Purdue University Animal Care and Use Committee (Protocol number, 1112000434). A total of 168 Holstein-Friesian bull calves, 44 ± 3 (mean ± SE) day (d) of age, originated from Strauss Feeds calf starter barns. Two replications were conducted 1 week apart, each in one of two identical rooms in one barn with common feeding equipment and personnel. Upon arrival, calves within a given weight range were placed into 84 pens with 2 calves per pen. The calves were given visual assessment for health and blood collected for iron and leukocyte profiles. After grouping a percentage of the calves in the barn were retested throughout the growing period and twice daily management assessed and recorded injuries and potential illnesses (diarrhea, not readily approaching the trough during feeding, or coughing). Calves requiring more thorough assessment were removed to another pen, however none on this experiment required removal. The experimental pens were sequential in outside rows of the barns and consisted of stainless steel partitions that enabled visual and tactile contact between calves. After 1 week of acclimation, the group pens that had been assigned randomly (prior to the beginning of the experiment) to treatments of 2, 4, or 8 calves per pen were created by removal of stainless steel panels between pens. Those assigned to 2 calves per pen remained as they were. Those assigned to 4 or 8 calves per pen had the metal partitions between neighboring pens removed to form the larger groups. Calves in the larger groups had access to the same area that they had been in plus additional space and penmates. Calves were not moved, only the partitions. Twelve replications of each treatment were formed, 6 replicates per room. The total pen area per calf was kept constant on 1.82 m^2 per calf for all group sizes. Initial mean BW (±SE) of calves was 65.3 (3.7), 66.5 (3.7), and 67.6 (3.7) kg for calves in groups of 2, 4, and 8, respectively. Calves were fed milk replacer (Agri-Best Balancer 51/12 and LiquiKalf veal feed; Strauss Veal Feeds Inc., North Manchester, IN) and solid feed twice daily at 12-h intervals (0500 and 1700 h). Once the milk was fed, calves were allowed 15 min to eat the dry-grain mixture (12% CP and 4% fiber) in the same trough before water was added to the trough.