

ANIMAL WELFARE

Title: Transport lairage effects on well-being of 18 kg pigs using a multi-disciplinary approach - **NPB# 04-134**

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Abstract: Long distance transports may significantly affect the health of pigs; thus, adding a rest stop (lairage) during long journeys may improve their well-being. The objective of this study was to determine whether a mid-journey lairage was beneficial to swine behavior, intestine microbial populations, and immune variables during a 16-h transport. Four replications were conducted, one in each of four seasons. Eighteen-kg pigs were housed in 16 pens (13 to 16 pigs/pen) with 8 pens/treatment. Lairage pigs were transported for 8 h, given a rest with food and water for 8 h, then transported 8 h. Continuous (Co) pigs were continuously transported for 16 h. Ambient temperatures reflected 3 seasons. The temperatures on the truck had less variability. Body weight loss was not different between the treatments. Behavior was evaluated by scan sampling of the pens prior to and after transport, pens on the truck, and latencies to eat and latencies to drink during lairage. Drinking and walking after transport was greater for the continuously transported pigs. Latencies to eat and latencies to drink during the lairage varied by season. No vomiting was observed during the transport, so the ingestion of food was not detrimental. There were some differences in whether the pigs were lying. The continuously transported pigs continued to lie after the second group was loaded. Lying increased in the lairage group over the transport until they were equivalent to the continuously transported group.

Intestine microbial population shifts were detected in the jejunum contents on d 1 and 7, in the cecum contents on d 3 and 14, in the cecum tissue on d 14, and the jejunum tissue on d 14. On d 14 the ileum tissue was altered. In all but the ileum tissue, the variability of bacterial populations decreased for the continuously transported treated pigs. Jugular blood samples were collected from 16 pigs (8/treatment) on d 1, 3, 7 and 14 post-transport. Hematocrit and white blood cell (WBC) counts were obtained and neutrophil cell functions (phagocytosis and oxidative burst) and phenotypic cell markers (CD14 and CD18) were analyzed using flow cytometry. There were no treatment by season interactions. In continuously transported pigs, total WBC count was higher on d 1 than lairage pigs. As expected, granulocyte count in continuously transported pigs was higher than in lairage pigs on d 1; further, granulocyte count was lowest on d 3 in continuously transported pigs. In both treatments, lymphocyte count was lower on d 14

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than on d 1. There were more cells expressing CD14 in continuously transported pigs than lairage pigs on d 1. In addition, continuously transported pigs on d 1 and 14 had the highest percentage of CD14 and CD18 positive cells and lairage pigs had the highest percentages of both on d 14. Percent phagocytosis was highest on d 7 in the continuously transported pigs; however, in both treatments oxidative burst was highest on d 7. In both treatments, CD18 percentage was lowest on d 0. The anti-microbial peptide mRNA expression by blood leukocytes was greater for continuously transported pigs on d 14, however at d 3 PR-39 expression from jejunum tissue was greater for the lairage pigs. Toll-like receptor 2, 4, and 5 expression, and the cytokine, IL-8, and the chemokine, CCL20 did not differ between treatments. This study indicates that extended transport without lairage alters few behaviors, but changes the microbial populations of the jejunum and cecum contents and the microbial populations of jejunum, ileum and cecum tissues, and alters innate immune functions which may cause greater susceptibility to pathogens.

Introduction: Transport is a stressful time for farm animals. Pigs are routinely transported for at least 16 h. The need for a mid-transport rest-stop allowing feed and water consumption as well as rest appears to have the potential for both benefits and liabilities. Through this research we determined if behavior problems are exacerbated by the increased handling and mixing required to provide a 8 h rest period. We also examined physiological measures to test the effect of this practice on health, both immediately and for several weeks post-transport. Therefore, as well-being standards are being determined by commercial entities, a scientific basis will be available to evaluate the recommendation of a mid-journey lairage for early grower pigs. Cumulatively, this and other research of swine transport will begin to reduce the variables that are associated with transportation research, so that more accurate comparisons between systems can be made.

Objectives:

- 1) To determine social interactions and maintenance behaviors during and up to 72 hours after transport associated with lairage or continuous transport that may affect pathogen susceptibility.
- 2) To determine changes in intestinal microbial populations with and without lairage during a 16 hour transport of grower pigs.
- 3) To determine alterations in peripheral and intestinal innate immunity associated with feed and water withholding of continuous transport and the social and physical stressors of lairage
- 4) To develop recommendations as to the benefit of an 8 hour rest-stop including access to feed and water for 18 kg pigs during long transports (16 hour).

MATERIALS AND METHODS

A. Experimental Design

An experiment was conducted with 4 replicates in various seasons; January, April, August, and October, (169, 247, 231, and 247 animals were used in each replication, respectively). This project was conducted at Purdue University's Animal Science Research and Education Swine unit. This is a 200-sow operation, farrowing on average, 36 sows per month in standard industry farrowing crates. The January farrowing was smaller than anticipated, so numbers were adjusted accordingly to maintain equivalent space per pig in all replications. Piglets were weaned at 3 weeks of age and the operation was managed under normal industry practices and is

representative of commercial conditions. After blocking by sex, litter, and weight, the pigs were randomly assigned to one of the two transport treatments.

The lairage group was loaded at a stocking density of about 0.125 m² per pig onto the bottom deck of the trailer. After 8 h of transport these pigs were off loaded and given access to food and water for 2 h with an additional 6 h of rest until their next leg of the journey. Meanwhile, the continuous transport group was loaded at the same stocking density onto the sections of the lower deck that had not been used in the previous transport. No pens were within the front 2.4 m nor the rear 1.8 m. They were transported for 8 h and returned to the unit where the second group was loaded onto the truck and all pigs transported the final 8 h together (but in separate pens). Pigs were off loaded and placed into 8 pens per treatment with approximately 16 pigs per pen. We expect an extreme difference between transported and non-transported in the variables that we are measuring. However, with limited funding we did not include a non-transported control since the question that we are addressing is the need for a lairage during transport.

B. Sampling and data collection

Treatment placement could not be varied among the transports because of the truck loading being from the rear of the deck. This allowed us to not disturb the pigs on the continuous transport treatment. HOBOS[®] were placed throughout the trailer to record temperature and humidity throughout the transports (Figure 1). Pigs were identified for sampling and individual weights taken prior to the first transport and after the final transport.

Eight pigs per treatment were humanely euthanized. Telazol, xylazine, and ketamine cocktail, 1 mL/23 kg (50#) was used to sedate pigs. Jugular blood samples were obtained and then pigs were euthanized with sodium pentobarb (5 mL/pig) and exsanguination. Sample pigs from each pen were taken at time 0 (n = 4 per treatment, baseline prior to transport), immediately post-transport (d 1, n = 8 per treatment), and at 3, 7, and 14 days post-transport (n = 8/treatment).

Behavioral Measures

Observation pre-transport. Behavior was observed over 24 h immediately prior to transport. Each pen of pigs was individually marked and 4 pigs were selected as focal pigs based on sex and weight. The focal pigs were comprised of the heaviest pig, the lightest pig and two “average” weight pigs. Behavior was recorded in each home pen using ceiling-mounted cameras attached to time-lapse video recorders, set to record in 24 h mode. The video data was analyzed to determine daily time budgets, using a scan sampling technique, with behavior, location and posture of each focal pig in a pen recorded every 10 min. The behaviors recorded were inactive, active (including walking, rooting, manipulating pen-mates and pen components and belly-nosing, tail-biting, playing, aggression, and positive social interactions), alert, drinking and feeding. Data from each pig then were combined to determine a behavioral time budget with each pen as an experimental unit. The video data were analyzed to determine the amount of agonistic interactions involving focal pigs using all-occurrences sampling.

Observation during transport. Behavior was recorded in each pen on the trailer using ceiling-mounted cameras attached to time-lapse video recorders, set to record in real-time mode allowing for the greatest detail. The video data were analyzed to determine time budgets during transport, using a scan sampling technique, with behavior, location and posture of each pig in a pen recorded every 10 min. Because of crowding and lying on top of one another, pen usage and posture were the only

behaviors recorded on the truck. Data from each pig then were combined to determine a behavioral time budget with each pen as an experimental unit. The video data also was analyzed to determine if incidences of travel sickness involving focal pigs using all-occurrences sampling (ascertained by posture). For pigs in the rest treatment, latency to eat and latency to drink were recorded after off-loading for the rest period.

Observation post-transport. Behavioral observations were carried out over the 3 d immediately post-transport and on d 6 and 13 post-transport. Behavior was recorded as described above, including time-budgets, all-occurrences agonistic interactions and latency to eat and drink during the lairage.

Intestinal Microbial Populations

Samples were collected aseptically after slaughter, placed in sterile containers and stored on ice until assayed in the lab. Tissue samples were homogenized and genomic DNA from both tissues and contents were extracted using the MO-Bio Fecal DNA extraction kit.

To detect shedding of *Salmonella*, intestinal contents were diluted 10-fold in buffered peptone broth (BPB) and incubated overnight at 37°C. Samples (0.3 mL) were added to 3.0 mL tetrathionate broth and incubated for 48 h at 37°C. Then, 5 ml Rappaport-Vassalides (RV) broth were inoculated with 0.1 mL of each tetrathionate enriched sample and incubated for 24 h at 37°C. Loops of the RV enrichment were streaked onto XLT4 plates and incubated for 24 h at 37°C. Black-colored colonies were presumptively identified as *Salmonella* positive using TSI/LIA slants and Rambach agar.

Extracted genomic DNA was amplified by PCR using a reverse primer (534r) and a forward primer with a GC clamp (341FGC) that amplifies the variable V3 region of bacterial 16s rDNA. The amplicons were separated using denaturing gradient gel electrophoresis. Changes in banding pattern were determined using Bionumerics software.

Peripheral and Intestinal Immune Measures

Peripheral blood measures. Blood was collected into one 10-ml acid citrate dextrose (ACD) tube and one 5-mL EDTA tube, then placed on ice for transport to the laboratory. The EDTA blood was used to determine hematocrit percentage, and granulocyte, peripheral blood mononuclear cell, and total white blood cell counts. One milliliter of the blood from the ACD tube was placed into each of four 5 mL tubes. The four 5mL tubes were incubated for 1 h at 37°C in a hot water bath. The first tube was used for the phagocytosis and chemiluminescence assay (Böhmer et al., 1999). The second tube was used to measure the Fc receptor independent phagocytosis of fluorescently labeled latex beads. The last two tubes were used for control (cells only) and PE labeled anti-CD14 (DAKO) and FITC labeled anti-CD18 (DAKO) phenotypic analysis.

Quantitative real time RT-PCR. The remaining blood from the ACD tubes was used for RNA extraction using the QIAamp RNA blood minikit by Qiagen. The abundance of anti-microbial peptide (PR-39) was determined with real-time RT-PC using Taqman primers and probes. The threshold was determined for each gene and relative total RNA quantified. Data are presented as a ratio of the gene of interest to the reference gene, 18S.

Real-time RT-PCR analysis of toll-like receptors 2, 4, and 5, IL-8, CCL20, and PR39. Intestinal samples were collected from the ileum and jejunum (5 cm in length). These were washed immediately with sterile (37°C) Hank's Balanced Salt Solution (HBSS) and placed into RNALater for transport to the laboratory. In the laboratory, the sections were washed, ground and RNA extracted using RNEasy mini kits (Qiagen).

After reverse transcription, the relative abundance of toll-like receptors 2, 4, and 5 and of the cytokine, interleukin-8, the chemokine CCL20, and the anti-microbial peptide, PR-39 were determined by real-time PCR using Taqman primers and probes. Dr. Ernie Minton, Kansas State University collaborated by performing the RT-PCR of RNA collected from these samples.

Statistical analysis

Data were analyzed using Mixed models procedures of SAS. Random variables included replicate. Sex and litter were balanced in selecting pigs to sample from each pen. Data were checked for normality and homogeneity of variance, then transformed when necessary. Covariance structures were tested and the Bayesian criterion used to select the best fit of the data.

Care and use of all pigs were in accordance with Purdue University's Animal Care and Use Committee following the guidelines established in the Guide for the Care and Use of Agricultural Animals in Agriculture Research and Teaching (1999). Animal handling was by experienced animal care technicians and truck driving by persons experienced in animal transport. Inserts for the trailer walls were used to reduce temperature extremes (Figure 1).

RESULTS

General:

Temperatures were not as varied as anticipated (Figure 1 and Table 1), however three distinct seasons were observed. The truck temperature and humidity were not recorded for the first replication in January. Weights prior to and after transport did not differ between treatments.

Objective 1: Behavior

Truck behavior. Behaviors on the truck included location within the pen and posture at 10 min intervals. Incidences of posture indicating vomiting were not seen during the observations. The percentage of observations in which the lairage group was sitting at the front of their pens was greater ($P < 0.01$) than for the continuously transported pigs. There were no differences in the percentage of observations that continuous and lairage treated pigs were lying at the front of their pens. Lairage pigs stood at the front during more ($P < 0.05$) observations than did the continuous transported pigs. The lairage pigs also stood more ($P < 0.05$) in the back of their pens for the first half of the final leg of the journey. Additionally the continuously transported pigs lay more ($P < 0.05$) at the back of their pens than did the lairage pigs.

Lairage barn latency to eat and drink. All pigs in the lairage group were observed until the feed was removed at 2 h post-transport after the first leg of their journey. The experimental unit was pen and therefore the data presented are means, SD, and ranges of the pens. Average latency to drink was 10 min and 49 s with a SD of 7 min 52 s. The range was 1 min 42 s to 34 min. The latency to eat was 10 min 13 s with a SD of 8 min 10 s. The range for latency to eat was 56 s to 38 min 38 s. The least latency to eat was in the January transport, 4.7 min which was less than the Aug and Oct latencies. In contrast the least ($P < 0.05$) latencies to drink were in October, 3.3 min and April, 8.4 min.

Time budgets prior to and after transport. Lying, standing, belly nosing (initiating and receiving), eating, inactive, rooting, and positive social interactions received all had day effects. Only initiating play was determined to have treatment differences ($P < 0.05$). The lairage pigs initiated more play at the observations than did the continuous pigs. A day by treatment interaction was observed for drinking and for walking. During

baseline, the pigs that were to be assigned to the lairage treatment drank more ($P < 0.05$) than the other pigs, however after the transport the continuously transported pigs drank most ($P < 0.05$). Similarly, the lairage pigs walked more ($P < 0.05$) prior to transport, but walked less ($P < 0.05$) for d 1 to 3 after transport.

Objective 2: Intestinal Microbial Populations

To determine changes in the intestinal microbial populations due to treatment effects, denaturing gradient gel electrophoresis (DGGE) was used. DGGE is a technique that identifies microbial species' diversity based on the genetic makeup of the organism; each band present on a polyacrylamide gel correlates to a separate bacterial species present in the jejunal, ileal, or cecal content or wall of the pig sampled. Relatedness of bacterial populations present were determined using Bionumerics Software (Applied Maths, Austin, TX), which determined similarity coefficients (SC) based on the number and location of the bands for each sample. The number of bands is indicative of the number of bacterial species present within a specific intestinal ecosystem. Similarity coefficients are based on the number and position of bands in pairwise comparisons. These similarity coefficients both within treatment and across treatment (cross products) were then statistically analyzed using Mixed Models procedures of SAS and the Tukey-Kramer means separation. The within treatment comparisons are used to determine how similar the gut bacterial population profiles are within the treatment sample population (Table 2). The cross products, however, are comparisons between each individual pig and all the other individuals of the opposite treatment, indicating differences resulting if there were no treatment effects.

The jejunum contents on d 1 of the lairage pigs had a lower similarity coefficient than that observed in the continuously transported pigs ($P < 0.05$). Additionally, on d 3 in the cecal contents the lairage pigs had a lower SC than both the continuously transported pigs and the cross products ($P < 0.05$) while in the cecal tissue, only the continuously transported treatment was higher than the lairage treatment ($P < 0.05$). Again on d 7, the continuously transported pigs had a higher SC than the lairage pigs in the jejunal contents ($P < 0.05$). Finally, on d 14, in the jejunal and ileal tissues and in cecal contents, there were differences observed in SC between lairage and continuously transported pigs. In the jejunal tissue and in the cecal contents, the continuously transported pigs had a higher coefficient, while the opposite was seen in the ileal tissues ($P < 0.05$). Occurrences of *Salmonella* in the intestinal contents or tissues were not different between the treatments. *Salmonella* was detected in 2 pigs following the January transport (1 per treatment), 4 after the August transport (3 from lairage and 1 from continuous), none in the April transport, and 9 in the October transport (5 in the lairage group and 4 from the continuous group).

Objective 3: Peripheral and Intestinal Immune Measures.

Peripheral blood measures. Hematocrit (Figure 2) was not different between the treatments throughout the study. White blood counts (Figure 2) were greater ($P < 0.05$) for the continuously transport treated pigs immediately following transport (d1). Similarly, granulocyte counts were greatest ($P < 0.05$) for the continuously transported pigs immediately following transport, but returned to the pre-transport counts by d 3 following transport. A significant time effect ($P < 0.05$) was found for both treatments following transport. Peripheral blood mononuclear cells, including both lymphocytes and monocytes, increased ($P < 0.05$) on d 1 post-transport compared to d 14 post-transport. Phagocytosis of propidium iodide labeled *Staphylococcus aureus* by neutrophils (Figure 3) was greatest ($P < 0.05$) on d 7 after transport. Oxidative burst corresponded with the increased ($P < 0.05$) phagocytosis. Phagocytosis of latex bead was greater ($P < 0.05$)

for pigs on the continuous transport treatment on day 7 post-transport. Both treatments responded to the transport with greater ($P < 0.05$) phagocytosis of latex beads by neutrophils on d 3, 7, and 14.

The continuously transported pigs had more ($P < 0.05$) cells with CD14 fluorescence (Figure 4) immediately after transport, but both treatments had greater ($P < 0.05$) CD14 expressing cells on d 14 post-transport. Cells positive for CD18 expression were not different between treatments, however more ($P < 0.05$) fluorescence was detected on d 1 and 14 for the continuous transported pigs than on d 0 and more ($P < 0.05$) fluorescence was detected on the lairage treatment pigs on d 14 than for previous days. *Quantitative real time RT-PCR.* The two chemoattractants, IL-8 for neutrophils, and the chemokine CCL20 for dendritic cells were not different between treatments (Figure 5). The continuously transported pigs had greater ($P < 0.05$) relative abundance of IL-8 on d 3 post-transport than on the other days. On d 7 and 14 post-transport in the ileal tissues of the continuous transported pigs, the CCL20 relative abundance was greater ($P < 0.05$) than on d 1. In contrast, the IL-8 expression in the jejunum peaked for the continuous transported pigs immediately after transport and on d 7 for the lairage treated pigs. No treatment differences were detected. The jejunum CCL20 mRNA expression was greatest on d 3 and 7 for the lairage treatment, but no time differences were detected for the continuously transported pigs. No treatment differences were detected for jejunum CCL20.

Toll-like receptor 2, TLR4 and TLR5 mRNA expression in the ileum tissue (Figure 6) did not differ over time nor between treatments. A trend ($P < 0.10$) for greater TLR2 expression in the ileal tissue for lairage and continuous treated pigs was detected on d 7 and again for the continuous treated pigs on d 14. The expression in the jejunum (Figure 7) also did not differ over time nor between treatments for TLR2, but a trend ($P < 0.10$) for the greatest expression for TLR4 for both treatments was detected on d 3. The mRNA expression of those toll-like receptors was greatest ($P < 0.05$) for both treatments on d 3 post-transport.

The anti-microbial peptide, PR39, mRNA expression (Figure 8) was not different between treatment in the ileum. But a time effect ($P < 0.05$) was detected for both treatments. The mRNA expression in the jejunum for PR39 was greater ($P < 0.05$) for the lairage treated pigs than for the continuously transported pigs on d 3 post-transport. This was because of an increase ($P < 0.05$) in PR39 expression on d 3 for the lairage pigs. In contrast, by d 14 the PR39 expression was greater ($P < 0.05$) for the continuously transported pigs.

Objective 4: Recommendations

Lairage in a known clean environment has benefits of diminished stress and immune stimulation, even 2 wk post-transport. This is evidenced by the differences seen between treatments in the intestinal tissue and content microbial populations, and anti-microbial peptide expression in blood leukocytes. The pens of pigs were not mixed in this experiment, so behavior problems were not significantly altered by transport or treatment. Only increased drinking and walking were associated with the continuous transport treatment. Therefore, the rehydration of the lairage was beneficial. Additionally, access to feed improved the intestinal microbial population stability. With a clean lairage environment and maintained social structure, a lairage of 8 h was beneficial. However, effects of mixing on that benefit needs to be determined and the minimum time of the rest stop necessary to see these benefits should be determined.

Discussion: Although we achieved some seasonal effects, the ambient temperatures were not as varied as anticipated for the months we selected for this study. There were

some differences, particularly in latencies to eat and drink during the lairage, which reflected seasonal effects.

The immunological data demonstrated traditional measures of stress; increased granulocyte counts and neutrophil and monocyte activation. These data also show that there are alterations immediately after transport that can lead to changes up to 2 wk post-transport. The increased granulocyte number is indicative of increased stress.

The microbial population results indicate that on d 1 and 3 in the jejunal contents and cecal tissues of lairage pigs, respectively, there was increasing diversity of the bacterial community. Also on d 3, the difference seen in the cecal contents may indicate that there was no treatment effect regarding the continuous transport; however, when pigs were offered a lairage, the bacterial population within the gut shifted to obtain a more diverse populace. One week and still at two weeks after transport, again a more diverse bacterial population within the jejunal contents, jejunal tissues, and cecal contents of lairage pigs was observed, respectively. In the ileum, however, the opposite was seen. This may indicate that the ileal tissues, while not as acidic of an environment as the jejunum but with higher flow rates than the cecum, a select bacterial population is able to exist which is less variable over time. In addition, when variability is small, more dominant bacteria may be able to localize and reside in favorable conditions in the gut due to stress, consuming the nutrients and space required by a less dominant species.

Lay Interpretation: Fifty pound pigs were transported across seasons, January, April, August, and October. Measures of behavior on the truck, during the lairage, and after the transport showed some effects of increased transport stress in the continuously transported pigs (increased time spent drinking). The immune measures reflected the transport stress as well for the continuous transported group (increased white blood cell population counts and activation markers). Additionally, the microbial population measures also indicated differences in intestinal microbial populations between the two treatments, indicating that the degree of stress may be affecting this variable. Alterations in microbial populations could alter the ability of the pig to use the nutrients that it eats. Overall, in this setting of a controlled lairage environment without mixing of the pens, lairage lessened changes after transport.

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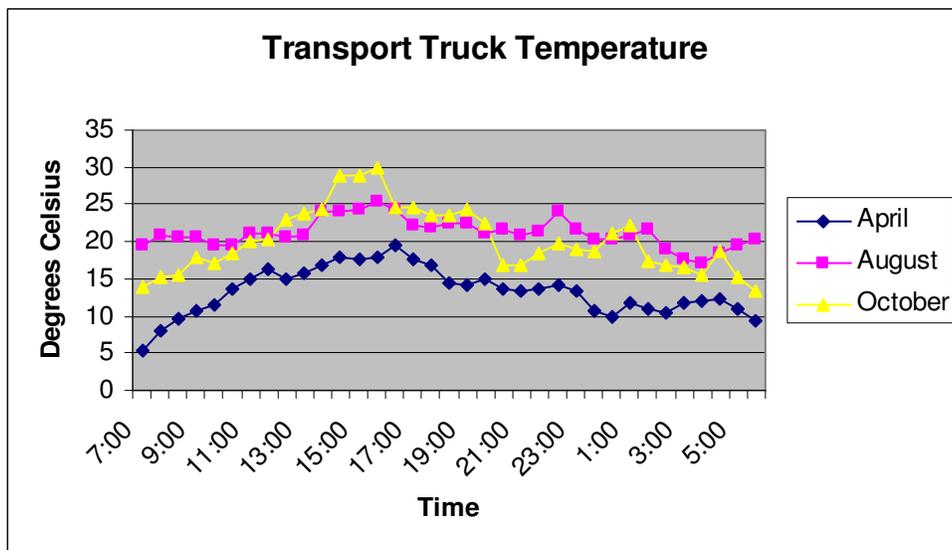
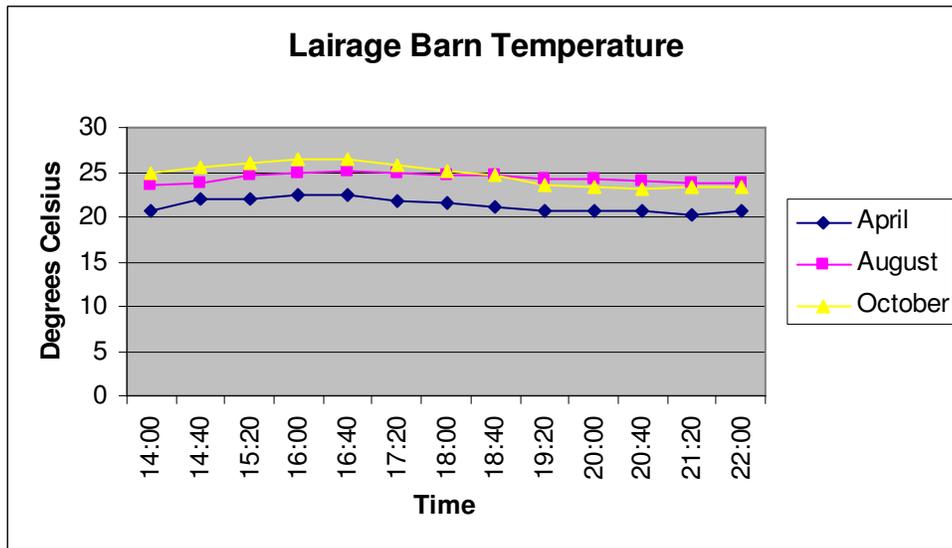


Figure 1. Temperature in the lairage barn and on the truck beginning at 0700 prior to loading until 0630 after arrival the following day.

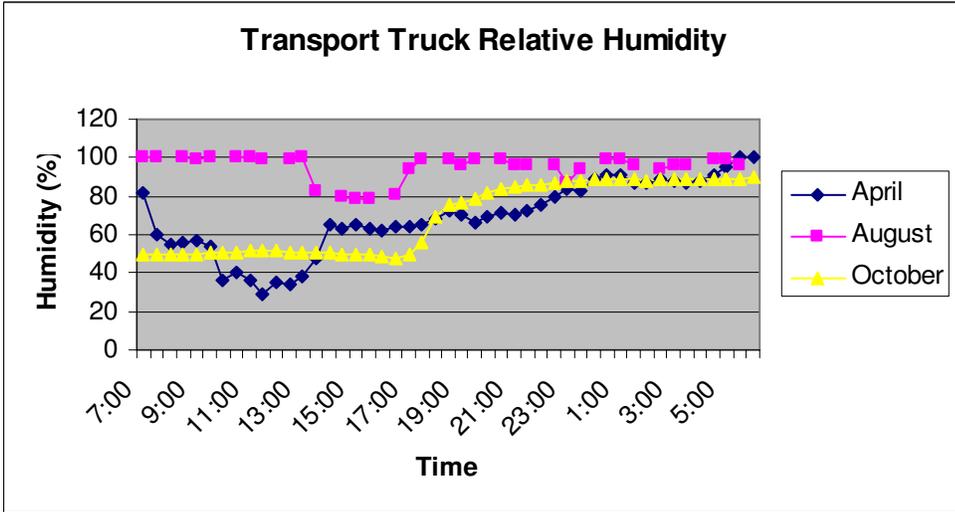
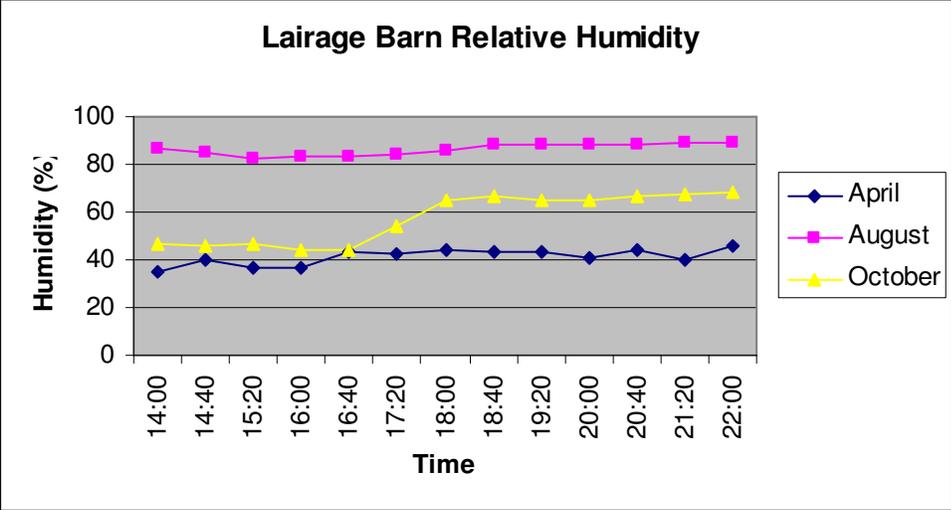


Figure 2. Humidity in the lairage barn and on the truck beginning at 0700 prior to loading until 0630 after arrival on the next day.

Table 1. Minimum and maximum ambient temperatures and precipitation over the 2 transport days.

Transport Replication	1 st d °C Maximum	2 nd d °C Minimum	Precipitation D1/d2
1	5.6	0	0.67/0.83
2	10.6	1.7	trace/0.23
3	22.8	16.7	0.18/0.03
4	20.0	8.9	0/0.05

Table 2. Similarity coefficients for microbial populations

Day 1

Treatment Comparison	Jejunum Content	Tissue	Ileum Content	Tissue	Cecum Content	Tissue
Lairage	17.07 ± 1.51 ^b	26.17 ± 1.17	35.91 ± 1.60	30.00 ± 3.37	46.49 ± 1.38	28.14 ± 1.50
Continuous	24.51 ± 1.60 ^a	26.06 ± 1.15	40.80 ± 1.56	35.87 ± 3.40	44.00 ± 1.38	30.81 ± 1.42
Cross Product	20.89 ± 1.09 ^{ab}	26.83 ± 0.82	39.05 ± 1.11	33.25 ± 2.36	45.24 ± 0.97	30.37 ± 1.03

Day 3

Treatment Comparison	Jejunum Content	Tissue	Ileum Content	Tissue	Cecum Content	Tissue
Lairage	19.58 ± 1.16	30.75 ± 1.39	35.00 ± 2.03	24.66 ± 1.73	43.64 ± 0.94 ^b	29.90 ± 0.96 ^b
Continuous	22.55 ± 1.36	32.12 ± 1.39	38.78 ± 2.03	30.11 ± 1.73	48.72 ± 0.95 ^a	34.53 ± 0.93 ^a
Cross Product	20.90 ± 0.87	32.27 ± 0.98	36.97 ± 1.43	28.44 ± 1.30	46.56 ± 0.67 ^a	32.60 ± 0.66 ^{ab}

Day 7

Treatment Comparison	Jejunum Content	Tissue	Ileum Content	Tissue	Cecum Content	Tissue
Lairage	18.60 ± 1.21 ^b	37.90 ± 1.37	35.47 ± 1.44	28.79 ± 1.30	43.21 ± 2.39	41.35 ± 1.27
Continuous	23.55 ± 1.36 ^a	33.38 ± 1.36	35.68 ± 1.39	28.79 ± 1.17	46.24 ± 2.39	44.05 ± 1.26
Cross Product	19.74 ± 0.89 ^{ab}	34.23 ± 0.96	37.37 ± 0.99	29.82 ± 0.85	45.93 ± 1.65	42.64 ± 0.89

Day 14

Treatment Comparison	Jejunum Content	Tissue	Ileum Content	Tissue	Cecum Content	Tissue
Lairage	21.68 ± 1.18	34.81 ± 1.32 ^b	35.14 ± 1.85	30.09 ± 1.12 ^a	49.36 ± 1.81 ^b	38.02 ± 1.47
Continuous	21.25 ± 1.15	40.99 ± 1.33 ^a	34.89 ± 1.83	24.19 ± 1.10 ^b	57.52 ± 1.85 ^a	39.82 ± 1.45
Cross Product	21.77 ± 0.82	38.22 ± 0.93 ^{ab}	35.29 ± 1.30	26.89 ± 0.77	52.43 ± 1.29 ^{ab}	38.02 ± 1.03

^{ab} Means with differing superscripts differ ($P < 0.05$).

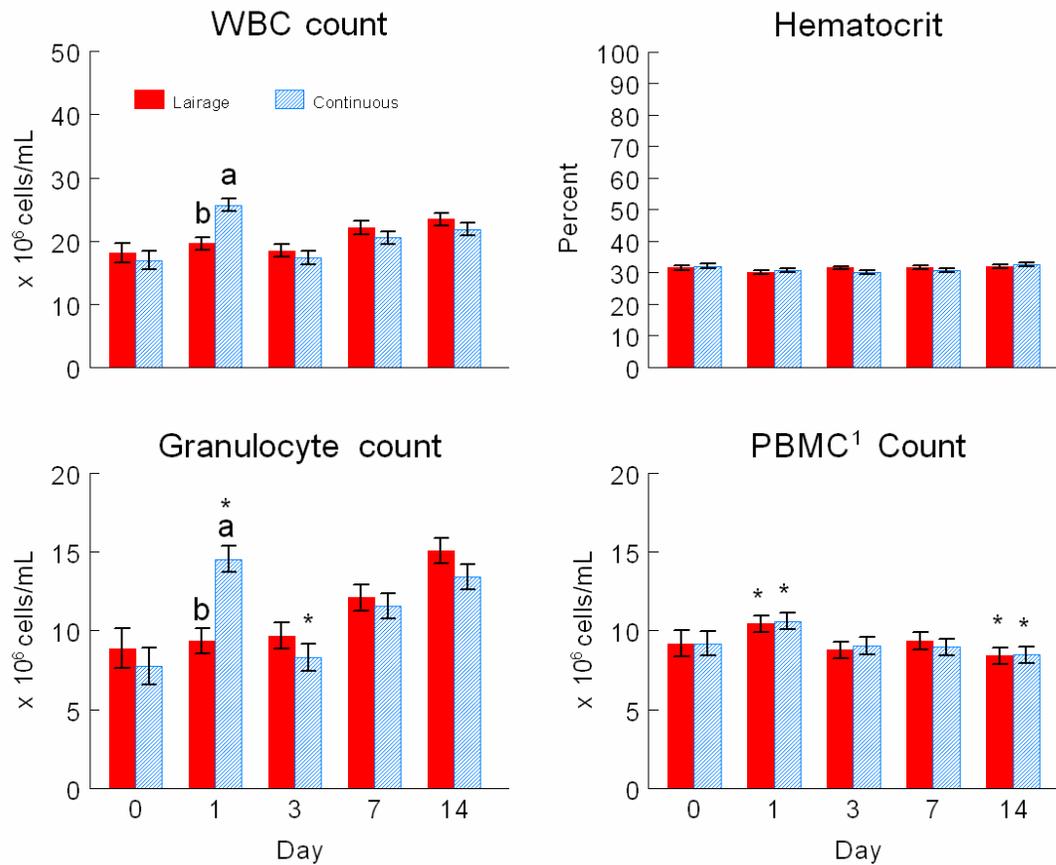


Figure 2. Total white blood cell counts (upper left panel), hematocrit percentage (upper right panel), granulocyte counts (lower left panel), and peripheral blood mononuclear cell (PBMC) counts (lower right panel) of control and lairage treatments. ^{ab}Treatment means within a day with differing superscripts differ ($P < 0.05$). * Time effect within a treatment ($P < 0.05$).

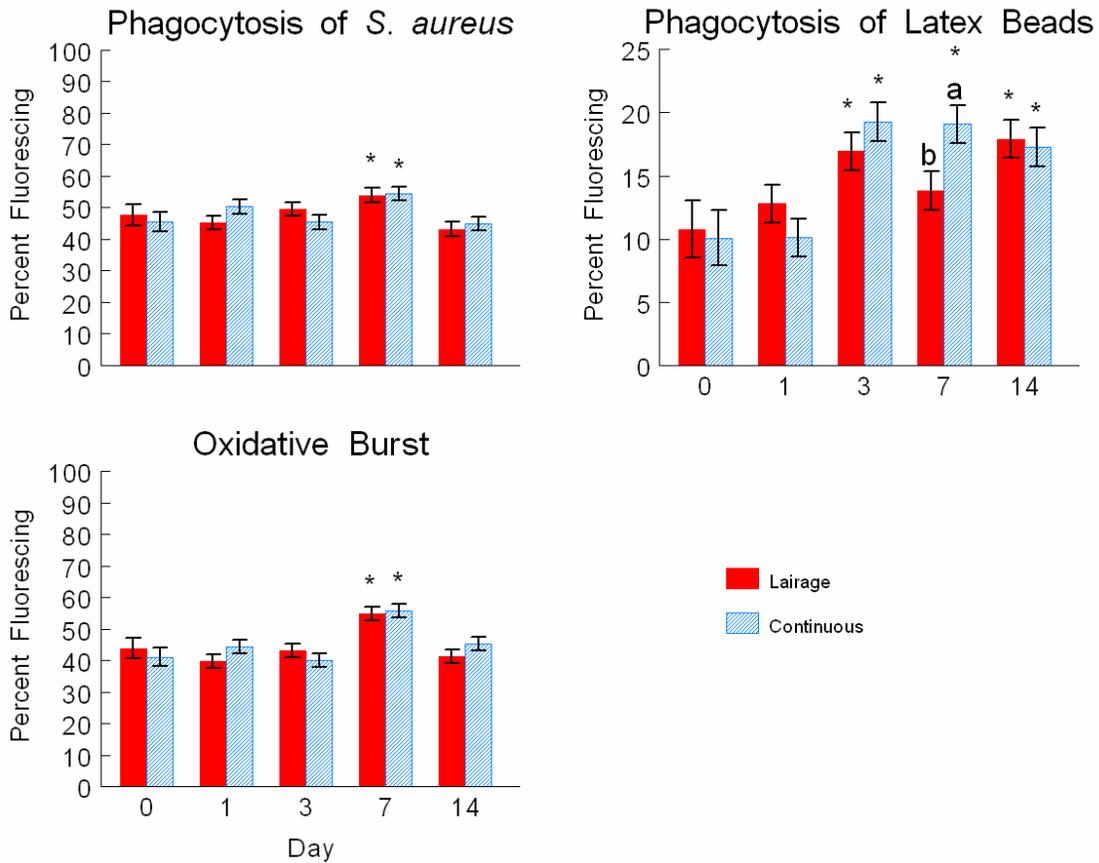


Figure 3. Phagocytosis of propidium iodide labeled *S. aureus* and oxidative burst (upper and lower left panels) and phagocytosis of fluorescently labeled latex beads (right panel). ^{ab}Treatment means within a day with differing superscripts differ ($P < 0.05$). * Time effect within a treatment ($P < 0.05$).

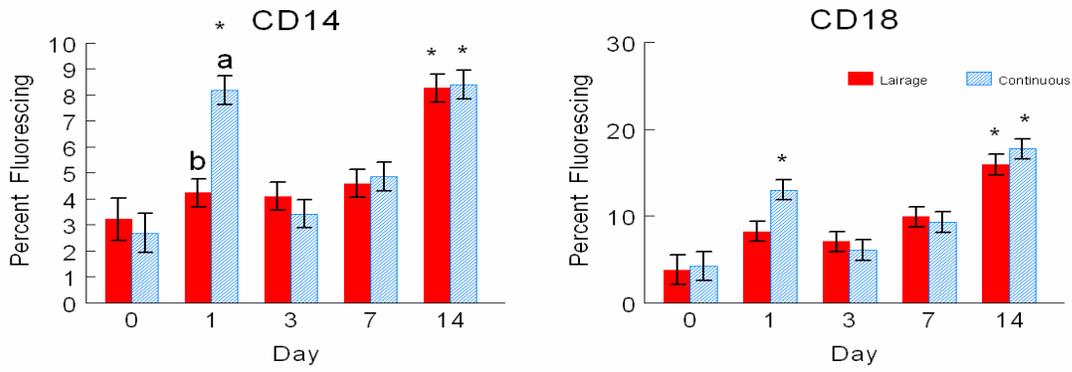


Figure 4. Cluster of differentiation (CD) 14 and CD18 expression on blood leukocytes from lairage or continuous treatments. ^{ab}Treatment means within a day with differing superscripts differ ($P < 0.05$). * Time effect within a treatment ($P < 0.05$).

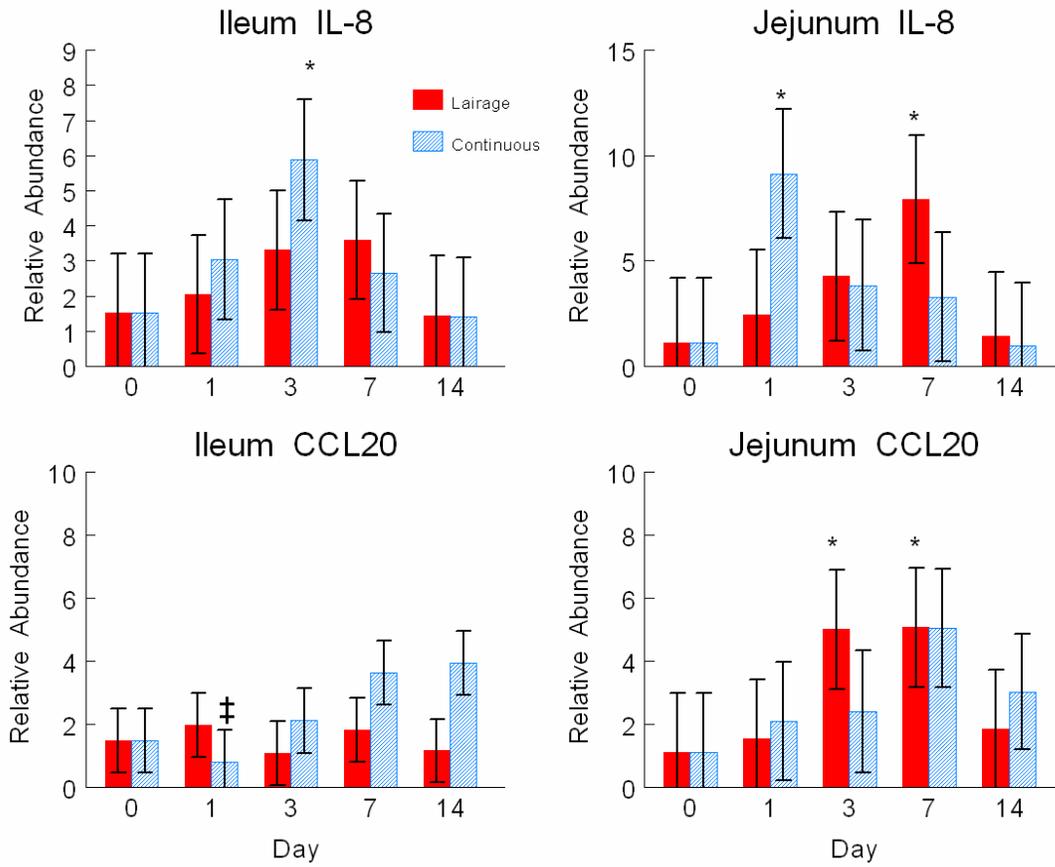


Figure 5. Relative abundance of mRNA expression of IL-8 from ileum (upper left panel) and jejunum (upper right panel) and CCL20 from ileum (lower left panel) and jejunum (lower right panel). * Time effect within a treatment ($P < 0.05$). ‡ Time effect within a treatment ($P < 0.10$).

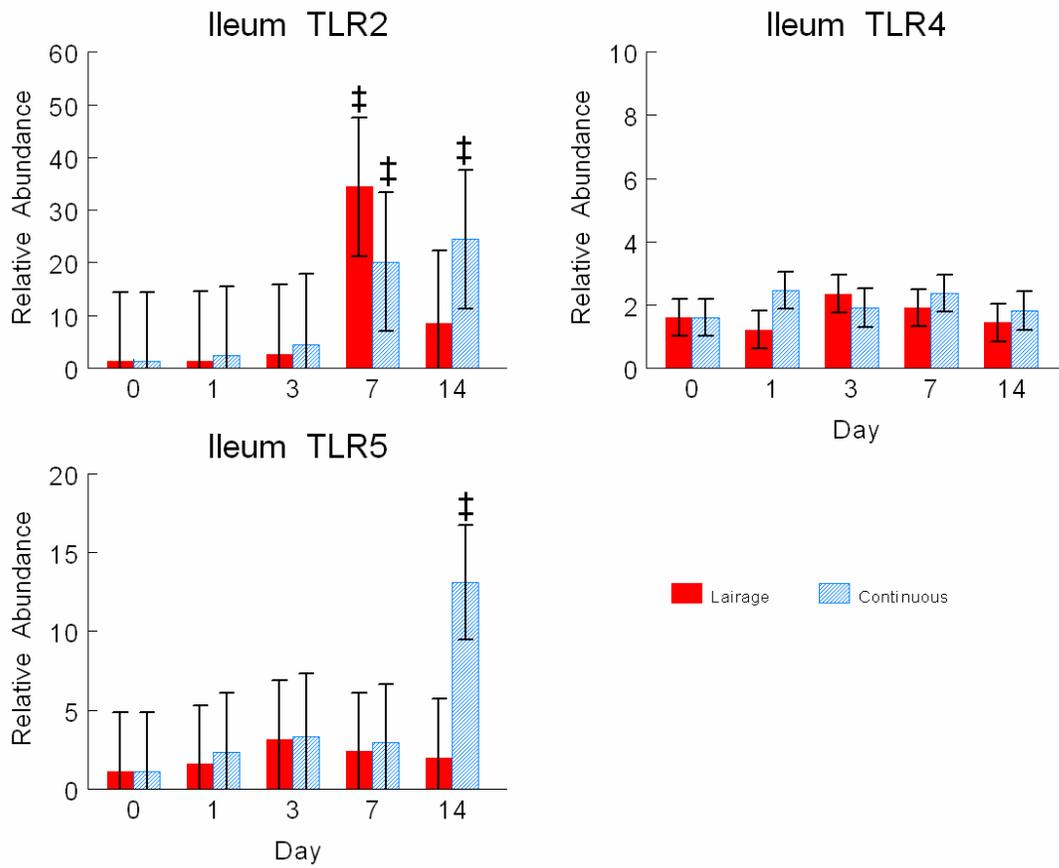


Figure 6. Relative abundance of mRNA expression of toll-like receptor (TLR) 2, TLR4, and TLR5 from ileum tissue. ‡Time effect within a treatment ($P < 0.10$).

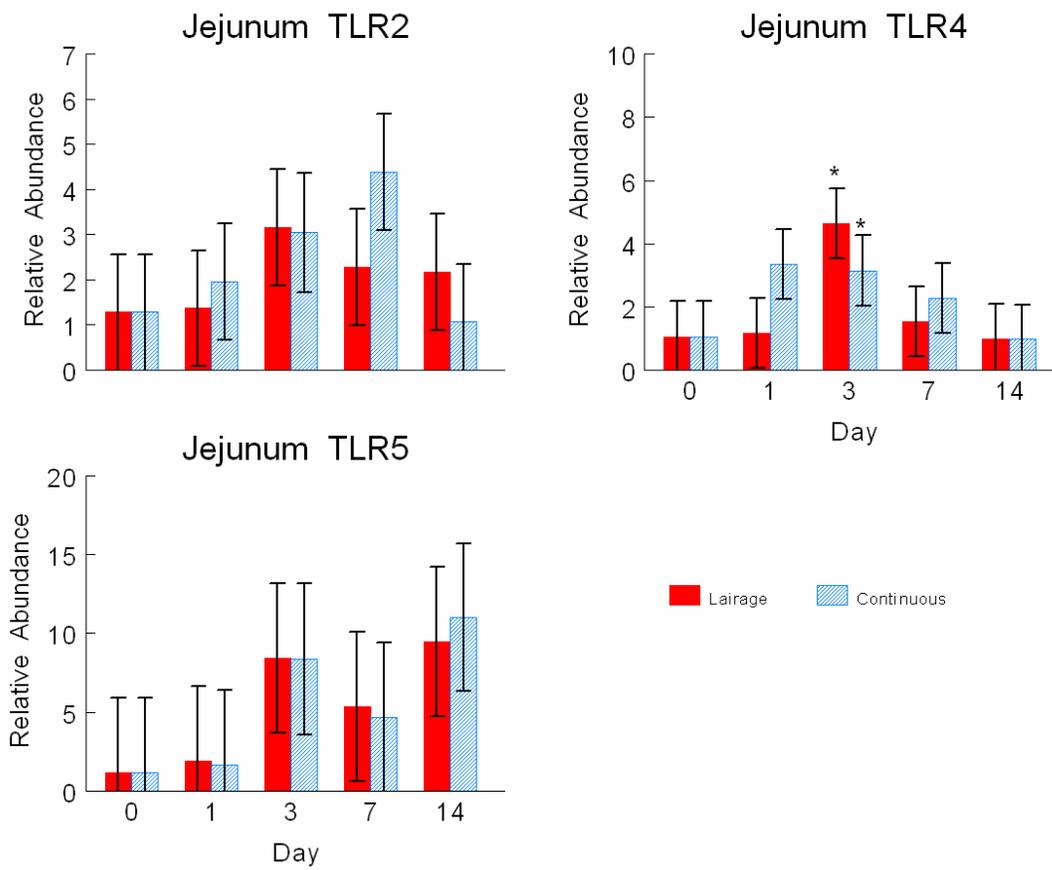


Figure 7. Relative abundance of mRNA expression of toll-like receptor (TLR) 2, TLR4, and TLR5 from jejunum tissue. No treatment differences were detected. * Time effect within a treatment ($P < 0.05$).

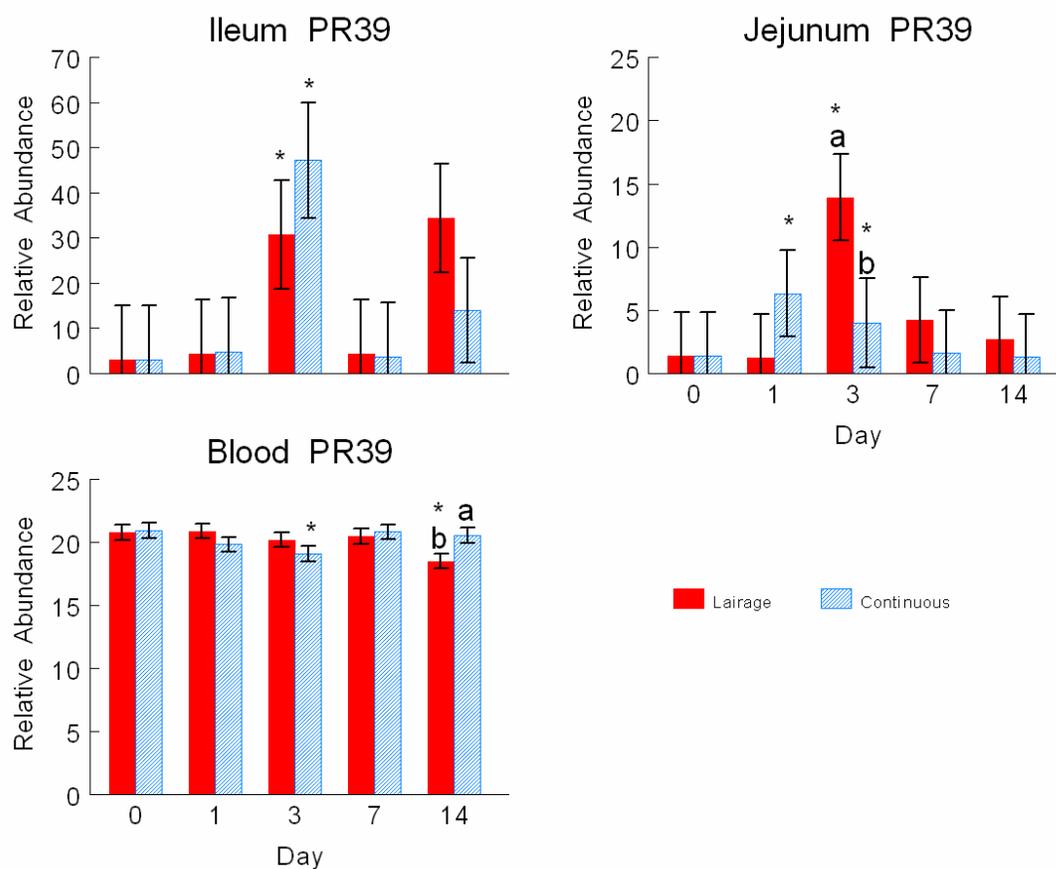


Figure 8. Relative abundance of mRNA expression of Proline Rich (PR)39 anti-microbial peptide from ileum (upper left panel) and jejunum (upper right panel) tissue and from peripheral blood leukocytes (lower left panel). ^{ab}Treatment means within a day with differing superscripts differ ($P < 0.05$). * Time effect within a treatment ($P < 0.05$).