Title: Space requirements of weaned pigs during transportation **(#06-011) REVISED**

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Industry Summary

Currently there are no trucking quality assurance recommendations for space allowance of weaned pigs during transport. The objective of this research was to establish a first estimate of the space requirements of weaned pigs during transport in winter, summer, and spring/fall based on measures of animal well-being. A commercial semi-trailer was fitted with compartments that provided 0.5, 0.6, and 0.7 square feet per pig (on the upper and lower deck) with a constant 100 pigs per compartment. Cameras were placed in each experimental compartment to record behaviors and postures of the pigs during transport. The frequencies of standing, lying, sitting, standing on another pig, and lying/huddling on top of another pig were recorded every minute during the entire duration of transport to determine if space allowance affected the behavior of pigs during transport. Blood samples were taken and weights and lesion scores recorded from 32 pigs per space allowance for physiological and immune measures before and after transport (n = 32 pigs/treatment) per season. This study was replicated over all 4 seasons. Blood samples were analyzed for cortisol concentrations, blood chemistry values, and immune measures. These variables were chosen as they can all change in response to stress in pigs. Body weight was measured as changes in body weight can be used as an indication of dehydration. Finally, lesion scores were used as an indicator of fighting or extreme crowding during transport. Pigs were transported between 60 and 142 min to the wean-to-finishing site using the same route each season. Temperature and relatively humidity for each season were; 10.5±6.15ºC and 44.8±9.71% (winter), 28.4±1.23 ºC and 59.8±4.42 % (summer), 20.4±4.00ºC and 52.7±10.82% (fall), and 22.0±2.49ºC and 58.5±10.48 % (spring). The neutrophil to lymphocyte ratio and cortisol concentrations were elevated in pigs after transport regardless of space allowance or season, suggesting that these pigs were experiencing stress. The blood chemistry measures, blood urea nitrogen, total protein, albumin, aspartate aminotransferase, and creatine kinase were elevated and body weight was reduced in pigs after transport, suggesting that these pigs were experiencing mild dehydration and mild muscle break down due to transport, however space allowance did not further impact these blood chemistry values regardless of season. All blood chemistry values were within the upper limit of the normal range for pigs after transport, but were significantly greater than values prior to transport, suggesting that transport affected the physiology of the pig but did not compromise the welfare of these animals. Different space allowances during transport did not influence any performance or physiological measures measured in this study regardless of season, except in summer the higher neutrophil to lymphocyte ratio in pigs transported at 0.5 ft^2/pig, suggest that space allowances of 0.6 and 0.7 ft^2/pig are preferable when transporting pigs in summer. Decreased standing behavior performed by pigs transported at 0.6 ft^2/pig in winter, decreased lying behavior performed by pigs transported at 0.5 ft^2/pig in summer, and increased sitting behavior performed by pigs transported at 0.5 ft^2/pig in fall and spring, suggests that space allowances of 0.6 or 0.7 ft^2/pig is preferable to 0.5 ft^2/pig when transporting weaned pigs in winter, summer, fall or spring.

Scientific abstract

Currently there are no trucking quality assurance recommendations for space allowance of weaned pigs during transport. The objective of this research was to establish a first estimate of the space requirements of weaned pigs during transport in winter, summer, and spring/fall based on measures of animal well-being. A commercial semi-trailer was fitted with compartments that provided 0.05, 0.06, and 0.07 m^2/pig (on the upper and lower deck) with a constant 100 pigs per compartment. Cameras were placed in each experimental compartment to record behaviors and postures of the pigs during transport. The frequencies of standing, lying, sitting, standing on another pig, and lying/huddling on top of another pig were recorded using 1 min scan samples during the entire duration of transport. Blood samples were taken and weights and lesion scores recorded from 4 pigs (5.1±0.10 kg) per compartment for performance and physiology measures before and after transport (n=32 pigs/treatment, total of 96 pigs/season). The trial was replicated 4 times per season. Pigs were transported between 60 and 142 min to the wean-to-finishing site using the same route each

season. Temperature and relative humidity were; 10.5±6.15ºC and 44.8±9.71% (winter), 28.4±1.23 ºC and 59.8±4.42 % (summer), 20.4±4.00ºC and 52.7±10.82% (fall), and 22.0±2.49ºC and 58.5±10.48% (spring). Data were analyzed using the MIXED procedure of SAS. The trailer was the experimental unit. Wind speed in the trailer averaged 1.76 m/s over all four seasons. The neutrophil to lymphocyte ratio, Cortisol, blood urea nitrogen, total protein, albumin, aspartate aminotransferase, and creatine kinase increased ($P<0.05$) during transport regardless of space allowance or season. Body weight was reduced ($P<0.05$) during transport regardless of space allowance in summer and fall/spring. Lesion scores were increased ($P < 0.001$) during transport regardless of space allowance or season. Pigs transported at 0.06 m^2/pig spent less ($P < 0.001$) time standing than pigs transported at 0.05 and 0.07 m^2/pig during the last 15 min of transport during winter. Pigs transported at 0.05 m^2/pig spent more ($P < 0.05$) time standing than pigs transported at 0.06 and 0.07 m^2/pig during the last 15 minutes of transport during summer. Time spent performing total lying behavior differed ($P < 0.05$) between pigs transported at 0.06 and 0.07 m^2/pig between 76-120 and 135+ min after transport during spring/fall. In conclusion, space allowances of 0.06 or 0.07 m^2/pig are preferable when transporting weaned pigs during all seasons.

Introduction

Weaned pigs are transported on many USA farms. The behavioral and physiological response of weaned pigs to transport is not well documented, furthermore little is known about the optimal space requirement of weanling pigs during transport. Transport is a complex stressor involving temperature fluctuations, stocking density, withdrawal from food and water, mixing with conspecifics, and motion (Lambooij and van Putten, 1993). Stocking density is one important aspect of transport, which could affect animal health and welfare, especially in pigs already experiencing weaning stress which has been shown to affect the immune response, performance, and behavior in pigs (Hay et al., 2001; Kanitz et al., 2002).

Most studies evaluating the effects of space requirements of pigs during transport has been carried out on market weight hogs. Higher stocking densities have been associated with higher mortality rates (Warriss, 1998). Furthermore, Ritter et al. (2004) found that losses (dead and non-ambulatory pigs) during transport were more than two times greater at low (0.4 m^2/pig) compared with high (0.5m^2/pig) floor space allowances on trucks, 0.88 vs.0.36% total losses respectively (Ritter et al., 2004). Conversely, unacceptable skin damage was generally lowest at stocking density of 0.35 m^2/100 kg and highest at 0.42 m^2/100 kg (Barton-Gade and Christensen, 1998). Stocking density has also been shown to influence physiological measures in pigs during transport, including lactate dehydrogenase and creatine phosphokinase; indicators of transport stress and fatigue (Barton-Gade and Christensen, 1998; Warriss et al., 1998; Kim et al., 2004). Lactate dehydrogenase was lower in pigs transported at low (0.31 m^2/100 kg) compared with medium (0.35 m^2) or high (0.39 m^2) stocking densities (Kim et al., 2004). Creatine phosphokinase levels were greater in pigs kept at stocking densities lower than 0.5 m^2/100 kg during transport (Barton-Gade and Christensen, 1998; Warriss et al., 1998). The behavior of an animal during transit is also important as it influences how much room the animal may require. Adequate space allowance during transit is important as pigs generally prefer to lie down if the conditions are suitable, particularly if bedding is provided. However, pigs will stand on short journeys, but excessive vibration or uncomfortable flooring may influence this behaviour (Warriss, 1998). Kim et al. (2004) found that standing behavior during transport was lower in low (0.39 m^2/100 kg) compared with medium (0.35 m^2) and high (0.31 m^2) stocking density rates. However, Barton-Gade and Christensen (1998) did not find that giving pigs more space resulted in more lying behavior, on the contrary they observed continuous disturbances from other pigs and at stocking densities of 0.42 m^2/100 kg and at 0.5 m^2/100 kg pigs had more difficulty maintaining balance.

Environmental temperature is an important factor to take into account when implementing space requirements for animals during transport. Animals need the appropriate space allowance with which to use behavioral adaptations to regulate their body temperatures. During hot weather larger space requirement per pig may be necessary to allow for increased evaporative cooling and reduce the heat generated by pigs, however in winter pigs may use huddling behavior to help thermoregulate. Berry and Lewis (2001) found that weaned pigs transported at 30-35°C had the greatest live weight deficits. Mortality has also being shown to be greater at higher temperatures during transport (Warriss, 1998).

Transport stress has been shown to influence several physiological and behavioral measures in pigs. Furthermore, sub-optimal space allowance during transport can reduce the welfare and health of pigs. Therefore, space requirement during transport is not only an animal health issue but a welfare concern for the swine industry. To date there is very little information available on the optimum space requirements for weaned pigs during transport. To improve our knowledge and understanding of this subject and potentially the welfare of the pig more information is needed on the space requirements of weaned pigs during transport throughout the range of seasons by using a multi-disciplinary approach including animal performance, physiology, and behavior.

Objectives

To determine the effects of floor space over seasons on pig welfare (physiology, behavior, injury, and performance) during transport.

Materials and Methods

Animals, housing, and experimental design

Pigs used in this study were PIC USA genetics. All animals were fed a diet to meet or exceed NRC nutrient requirements. Water was provided ad libitum. All animal procedures were approved by the Texas Tech University Animal Care and Use Committee.

Ninety six weight matched (5.1 ± 0.10 kg) pigs (18 ± 1 d of age) from 16 sows/litters were allocated to 1 of 3 space allowances; 0.05 m^2/pig, 0.06 m^2/pig and 0.07 m^2/pig. Barrows and gilt were represented equally over all space allowances. In total, 32 pigs (16 gilts and 16 barrows) per treatment/compartment size were used (n = 96 total). The trial was then repeated each season (with 4 replications within each season). Transport time, temperature, humidity, wind speed and pig weight for each season are displayed in Table 1.

The initial component of this experiment was conducted at a gestation to weaning facility. Prior to weaning, a blood sample was taken from all experimental pigs and pigs were ear tagged, weighed, lesion scored and then returned to their home pen until weaning. Lesions were scored as 0 (no lesions), 1 (minor) and 2 (severe). Blood samples were taken by placing pigs in a supine position and collecting 5 mL of blood over heparin by anterior vena cava puncture (procedure lasts approximately 1 min) for analysis of hematological, blood chemistry and immune measures.

Approximately 30 min after sampling all pigs (experimental and non-experimental pigs) were removed from the gestation crates and moved into holding pens. Experimental piglets were placed with ‘filler’ pigs, so that group size was held constant at 100 pigs per experimental compartment. Pigs were loaded onto the semi-trailer. Male and female pigs were segregated on different decks of the trailer as is common practice on commercial swine facilities. Pig gender on each deck was alternated so as not to confound trailer deck level with gender effects. Once all piglets were loaded on the trailer, the trailer was transported to the wean-to-finish site. The truck followed the same route for each replication, but the route for a given season varied slightly.

At the wean-to-finish site, experimental pigs were located, weighed, lesion scored and a blood sample collected. Pigs were again held in a supine position and a 10 mL blood sample collected over heparin by anterior vena cava puncture (procedure lasts approximately 1 min) for analysis of hematological, blood chemistry, cortisol and immune measures.

Blood analysis

Whole blood (before and after transport) was analyzed to determine white and red blood cell counts, differential leukocyte counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentrations, red blood cell distribution width, and platelet counts using a cell counter (Cell-Dyn® 1800, Abbott laboratories, Abbott Park, IL) and the neutrophil to lymphocyte (N:L) ratio was calculated by dividing the percent of neutrophils by the percent of lymphocytes. Blood samples were centrifuged and plasma collected for analysis of cortisol concentrations and blood chemistry measures. Cortisol concentrations were analyzed using an enzyme immunoassay kit (Assay designs, Ann Arbor, MI). Blood chemistry were analyzed using the Roche/Hitachi 912 (Roche Diagnostics, Basel, Switzerland) for blood urea nitrogen (BUN), creatinine, glucose, total protein, albumin, aspartate aminotransferase (AST), creatine kinase (CK), alkaline phosphatase (Alk Phos), gamma-glutamyl transpeptidase (GGT) and total bilirubin.

Porcine neutrophils were isolated from 10 mL of whole blood by density gradient centrifugation using Histopaque-1077 (density = 1.077 g/mL; Sigma, St. Louis, MO) and Histopaque-1119 (density = 1.119 g/mL; Sigma). Whole blood was diluted with Roswell Park Memorial Institute (RPMI) media and layered over Histopaque-1077 and -1119 (Sigma), then centrifuged at 700 × g for 30 min at room temperature. Neutrophils and RBC were removed from the 1119 layer and washed once in RPMI. Red blood cells were lysed using cold endotoxin-free water and isotonicity was restored using 10× PBS. Neutrophils were centrifuged for 10 min at 475 × g, supernatant was decanted, pellet washed twice and resuspended in RPMI.

Chemotaxis

The neutrophil chemotaxis and chemokinesis assays were performed according to published methods (Salak et al., 1993; Hulbert and McGlone, 2006). Briefly, a modified Boyden chamber (Neuro Probe, Cabin John, MD) was used to measure the migration of neutrophils across a polyvinylpyrrolidone-free filter (pore size 5 μm; Neuro Probe) towards media (chemokinesis) or towards recombinant human complement-C5a (C5a; chemotaxis). The media or C5a (10^{-8} M) were added in duplicate to the bottom wells of the chamber and the neutrophils, adjusted to 1×10^{6} cells/mL, were added to the top wells of the chamber. The filter was fixed and stained using Lekostat I and II solution (Fisher Scientific, Houston, TX). Five fields per well of the cells were counted in a blind fashion at 1,000x magnification.

Phagocytosis

The phagocytosis assay was performed to determine the percent of latex beads engulfed by neutrophils, including the average number of beads phagocytized per cell, as previously described (Hulbert and McGlone, 2006). Briefly, opsonized latex beads (0.807 μm diameter, Sigma) at a concentration of 1×10^7 beads/mL were added to neutrophils adjusted to 1×10^6 cells/mL. The neutrophil/bead mixture was then incubated for 10 minutes in a humidified CO_2 chamber at 37°C. Tubes were spun at 40 x g for 7 minutes at 8°C and the supernatant was removed. The neutrophil/bead mixture was washed twice and then 200 μL of solution was obtained from the neutrophil/bead mixture and spun using a cytofuge microcentrifuge (Cytofuge 2, model M801-22, StatSpin, Inc., Norwood, MA) for 2 minutes at 9,693 x *g*. The slides were fixed and stained using Lekostat I and II solution (Fisher Scientific). A total of 100 neutrophils were counted and for each slide. The total percentage of cells that phagocyotyzed at least one bead was determined.

Behavior

Digital still cameras were placed in each experimental compartment to record the behavior of the pigs during transport. Cameras were programmed to take one picture a minute (1 min scan samples). Behaviors measured included lying, standing, sitting, standing on top of another pig (ST) and lying/huddling on top of another pig (LT). Behaviors were described in Table 2. All behaviors were mutually exclusive (Table 2). The frequency of each behavior was calculated over the entire transport period and divided into 15 min periods.

Trailer design

A Wilson brand straight deck stock trailer (Wilson Trailer Company, Sioux City, Iowa, USA) was used during all replications of this trailer. The trailer was fit with an upper and lower deck. The trailer contained compartments that were adjusted to provide 0.05, 0.06 and 0.07 m^2/pig based on pigs weighing approximately 5 kg and with each compartment holding 100 animals. Each of the three space allowances were represented on each of the upper and lower decks of the trailer. The compartments were established using standard metal compartment gates that were a feature of animal transport trailers (that came fitted in the truck at time of purchase). Compartments were constructed of steel beams (which are the usual material used in the industry). The trailer was instrumented with HOBO data logger to record temperature, humidity and wind speed. Cameras were placed in each experimental compartment to record behavior of the pigs during transport.

Statistical analysis

All data were tested for constant variance and departures from normal distribution. Data lacking normality were transformed logarithmically using $\log_{10}$ function. Data were subjected to analysis of variance using the mixed model procedure of SAS version 9.1 (SAS Inst., Inc., Cary, NC). All analyses were performed as two-tail tests. The trailer was the experimental unit. Data were analyzed as a random complete block with the trailer as the block. The experimental unit was the pen. The main fixed effects were block (4 levels), gender (2 levels), deck (2 levels), treatment (3 levels), and time (2 levels). Random effects in the model were litter (16 levels) and piglet (96 levels). The interaction between treatment and time (df = 4) and treatment and deck (df = 4) were included in the model. A total of 16 litters and 96 pigs (treatment, n = 32; gilts, n = 16 and barrows, n = 16) were used in this study. Gilts and barrows were balanced within each sampling period. Behavioral data were also analyzed using ANOVA using the mixed model procedure of SAS. The behavior observation period was divided into four 15-min periods. For behavioral measures, the main fixed effects were block (4 levels), gender (2 levels), deck (2 levels), treatment (3 levels), and period (4 levels). The interaction between treatment and period (df = 5) and treatment and deck (df = 4) were included in the model. Each season was analyzed separately.

Results

Winter

The percentage of neutrophils were elevated ($P < 0.001$) and conversely, the percentage of lymphocytes were decreased ($P < 0.001$) among pigs after transport regardless of space allowance (Table 3). The neutrophil: lymphocyte ratio (N:L) was elevated ($P < 0.001$) in pigs after transport regardless of space allowance (Table 3). No gender, deck or treatment*deck effects were observed for white blood cells measures ($P > 0.05$). Hematological values did not differ ($P > 0.05$) among pigs before or after transport regardless of space allowance (Table 3). No gender, deck or treatment*deck effects were observed for hematological values ($P > 0.05$). Cortisol concentrations were elevated ($P < 0.001$) among pigs after transport regardless of space allowance (Table 4). No gender, deck or treatment*deck effects were observed for cortisol concentrations ($P > 0.05$).

Blood urea nitrogen, total bilirubin, total protein, albumin, AST and CK were elevated ($P < 0.05$) in pigs after transport regardless of space allowance (Table 4). No gender, deck or treatment*deck effects were observed for blood chemistry values ($P > 0.05$).

The percentage of latex beads engulfed by neutrophils did not differ ($P > 0.05$) among pigs transported at different space allowances (0.05 m^2/pig: 61.2 ± 5.25 %, 0.06 m^2/pig: 53.4 ± 5.21 %, 0.07 m^2/pig: 45.9 ± 4.73 %).

Weight had a tendency to decrease ($P = 0.07$) in pigs after transport, regardless of space allowance (Before transport: 5.3 ± 0.09 kg and after transport: 5.1 ± 0.09 kg). Lesion scores were increased ($P < 0.001$) in pigs after transport, regardless of space allowance (Before transport: 0.03 ± 0.014 and after transport: 0.50 ± 0.070).

Pigs transported at 0.06 m^2/pig spent less ($P < 0.001$) time standing than pigs transported at 0.05 and 0.07 m^2/pig during the last 15 min of transport (Fig. 1). Pigs transported at 0.05 m^2/pig spent less ($P < 0.001$) time LT than pigs transported at 0.06 and 0.07 m^2/pig during the last 15 minutes of transport (Fig. 2).

Pigs spent more ($P < 0.05$) time lying during transport at 0.06 m^2/pig compared with pigs transported at 0.05 and 0.07 m^2/pig allowances (0.05 m^2/pig: 0.0 ± 0.57 %, 0.06 m^2/pig: 0.9 ± 0.20 %, 0.07 m^2/pig: 0.3 ± 0.18 %). Pigs spent less ($P < 0.01$) time ST during transport at 0.07 m^2/pig compared with pigs transported at 0.05 and 0.06 m^2/pig allowances (0.05 m^2/pig: 3.0 ± 0.88 %, 0.06 m^2/pig: 2.8 ± 0.30 %, 0.07 m^2/pig: 1.3 ± 0.28 %).

Summer

Total white blood cell counts and the percentage of neutrophils were elevated ($P < 0.001$) after transport in pigs regardless of space allowance, conversely the percentage of lymphocytes were decreased ($P < 0.001$) in pigs after transport regardless of space allowance (Table 5). No gender, deck or treatment*deck effects were observed for white blood cell measures ($P > 0.05$).

The neutrophil to lymphocyte ratio was elevated ($P < 0.001$) in pigs after transport regardless of space allowance (Table 5). The neutrophil to lymphocyte ratio was greater ($P < 0.005$) among pigs transported at 0.05 m^2/pig compared with pigs transported at 0.06 and 0.07 m^2/pig (Fig. 3).

Hematocrit was elevated ($P < 0.006$) among pigs after transport regardless of space allowance (Table 5). No other differences ($P > 0.05$) in hematological values were observed. No gender, deck or treatment*deck effects were observed for hematological values ($P > 0.05$).

Cortisol concentrations were elevated ($P < 0.001$) in pigs after transport regardless of space allowance (Table 6). No gender, deck or treatment*deck effects were observed for cortisol concentrations ($P > 0.05$).

Glucose concentrations were decreased ($P < 0.001$) in pigs after transport regardless of space allowance (Table 6). Blood urea nitrogen, albumin, AST, CK and GGT were elevated ($P < 0.01$) in pigs after transport regardless of space allowance (Table 6). No gender, deck or treatment*deck effects were observed for blood chemistry values ($P > 0.05$).

Chemotaxis in response to the mitogen C5a did not differ ($P > 0.05$) among pigs transported at different space allowances (0.05 m^2/pig: 9.2 ± 2.05 cells/5 fields, 0.06 m^2/pig: 6.2 ± 2.20 cells/ 5 fields, 0.07 m^2/pig: 5.0 ± 2.13 cells/5 fields). The percentage of latex beads engulfed by neutrophils did not differ ($P > 0.05$) among pigs transported at different space allowances (0.05 m^2/pig: 91.7 ± 2.54 %, 0.06 m^2/pig: 89.8 ± 2.76 %, 0.07 m^2/pig: 95.9 ± 2.65 %). No gender, deck or treatment*deck effects were observed for immune measures ($P > 0.05$).

Weight was decreased ($P < 0.05$) in pigs after transport, regardless of space allowance (Before transport: 5.2 ± 0.08 kg and after transport: 5.0 ± 0.07 kg). Lesion scores were increased ($P < 0.001$) in pigs after transport, regardless of space allowance (Before transport: 0.08 ± 0.030 and after transport: 0.67 ± 0.072).

Pigs transported at 0.05 m^2/pig spent less ($P < 0.05$) time lying than pigs transported at 0.06 and 0.07 m^2/pig from 16 to 60 minutes after transport (Fig. 4). Pigs transported at 0.05 m^2/pig spent more ($P < 0.05$) time standing than pigs transported at 0.06 and 0.07 m^2/pig during the last 30 minutes of transport (Fig. 5).

Over the entire transport period, pigs spent less ($P < 0.001$) time sitting during transport at 0.07 m^2/pig compared with pigs transported at 0.05 and 0.06 m^2/pig (0.05 m^2/pig: 12.7 ± 0.67 %, 0.06 m^2/pig: 12.2 ± 0.61 %, 0.07 m^2/pig: 7.4 ± 0.61 %). Pigs spent more ($P < 0.001$) time ST during transport at 0.05 m^2/pig compared with pigs transported at 0.06 and 0.07 m^2/pig (0.05 m^2/pig: 6.7 ± 0.30 %, 0.06 m^2/pig: 2.8 ± 0.27 %, 0.07 m^2/pig: 2.3 ± 0.27 %). Pigs spent more ($P < 0.001$) time LT during transport at 0.07 m^2/pig compared with pigs transported at 0.05 and 0.06 m^2/pig (0.05 m^2/pig: 1.8 ± 0.29 %, 0.06 m^2/pig: 2.2 ± 0.27 %, 0.07 m^2/pig: 3.0 ± 0.27 %).

Fall and Spring

Total white blood cell counts were elevated ($P < 0.001$) among pigs after transport regardless of space allowance (Table 7). The percentage of lymphocytes were decreased ($P < 0.001$) and conversely, the neutrophil and lymphocyte ratio was elevated ($P < 0.001$) among pigs after transport regardless of space allowance (Table 7). No gender, deck or treatment*deck effects were observed for white blood cells measures ($P > 0.05$).

Hematological values did not differ ($P > 0.05$) among pigs before or after transport regardless of space allowance (Table 7). No gender, deck or treatment*deck effects were observed for hematological values ($P > 0.05$).

Cortisol concentrations were elevated ($P < 0.001$) in pigs after transport regardless of space allowance (Table 8). No gender, deck or treatment*deck effects were observed for cortisol concentrations ($P > 0.05$).

Blood urea nitrogen, total bilirubin, total protein, albumin, AST, CK and GGT were elevated ($P < 0.05$) in pigs after transport regardless of space allowance (Table 8). No gender, deck or treatment*deck effects were observed for blood chemistry values ($P > 0.05$).

The percentage of latex beads engulfed by neutrophils did not differ ($P > 0.05$) among pigs transported at different space allowances (0.05 m^2/pig: 97.1 ± 5.15 %, 0.06 m^2/pig: 87.8 ± 5.80 %, 0.07 m^2/pig: 90.7 ± 5.36 %). Chemotaxis in response to the mitogen C5a did not differ ($P > 0.05$) among pigs transported at different space allowances (0.05 m^2/pig: 4.8 ± 2.56 cells/5 fields, 0.06 m^2/pig: 4.7 ± 2.77 cells/ 5 fields, 0.07 m^2/pig: 5.3 ± 2.66 cells/5 fields).

Weight was reduced ($P < 0.05$) in pigs after transport, regardless of space allowance (Before transport: 4 ± 0.12 kg; after transport: 5.1 ± 0.12 kg). Lesion scores were increased ($P < 0.001$) in pigs after transport, regardless of space allowance (Before transport: 0.35 ± 0.073; after transport: 0.71 ± 0.094).

Pigs transported at 0.07 m^2/pig spent less ($P < 0.05$) time sitting than pigs transported at 0.05 and 0.06 m^2/pig and pigs transported at 0.06 m^2/pig spent less ($P < 0.05$) time sitting than pigs transported at 0.05 m^2/pig (Fig. 6).

Pigs transported at 0.05 m^2/pig spent less ($P < 0.001$) time total standing than pigs transported at 0.07 m^2/pig between 16-30, 61-75 min after transport (Fig. 7). Pigs transported at 0.06 m^2/pig spent less ($P < 0.001$) time total standing than pigs transported at 0.05 and 0.07 m^2/pig between 91-105 and 135+ min after transport and conversely, pigs transported at 0.06 m^2/pig spent more ($P < 0.001$) time total standing than pigs transported at 0.05 and 0.07 m^2/pig between 106-120 min after transport (Fig. 7). Time spent performing total lying behavior differed ($P < 0.05$) between pigs transported at 0.06 and 0.07 m^2/pig between 91-120 and 135+ min after transport (Fig. 8).

Discussion

Increased cortisol concentrations, neutrophil to lymphocyte ratio, and reduced weight in weaned pigs after transport suggests that these pigs experienced stress regardless of space allowance and season (Table 9 and 10). The neutrophil to lymphocyte ratio was higher in pigs transported at 0.05 m^2/pig compared with pigs transported at 0.06 or 0.07 m^2/pig, suggesting that pigs transported at 0.05 m^2/pig may have experienced more distress than pigs transported at 0.06 or 0.07 m^2/pig in summer.

Blood urea nitrogen, total protein, albumin, AST, CK were increased in weaned pigs after transport regardless of space allowance and season (Table 10). Creatine kinase is released from muscle fibers into the circulation in response to exercise or tissue damage and is a good indicator of muscular activity or tissue damage. Values of CK measured in weaned pigs after transport were in the upper limit of the normal range for CK (Carr, 1998). Creatine kinase concentrations were reported to increase in market weight pigs during transport to the packing plant (Elbers et al., 1991). Creatine kinase concentrations were shown to increase in market weight pigs kept at stocking densities lower than 0.5 m2/100 kg during transport (Barton-Gade and Christensen, 1998; Warriss, 1998), however, space allowance did not affect CK concentrations in weaned pigs in this study. Therefore, elevated CK concentrations measured in transported weaned pigs were probably due to tissue damage caused by muscle exertion due to exercise as a result of transport, but concentrations were still within the normal range for these enzymes suggesting that the welfare of these pigs was not compromised.

Blood urea nitrogen is a waste product in the blood caused from the breakdown of protein. Blood urea nitrogen concentrations were in the upper limit or slightly above the normal range (Carr, 1998; Mersmann and Pond, 2001) in weaned pigs after transport. The high BUN concentrations among transported pigs may indicate muscle and protein break down which may result from the exertion/exercise of being loaded and unloaded onto the trailer as well as the effect of standing on the trailer. However, these concentrations did not exceed the normal range for weaned pigs so it is unlikely that this increase in BUN concentrations was an indicator of poor welfare or compromised health.

Aspartate aminotransferase is a hepatic enzyme released in the blood when certain organs or tissues, particularly the heart or liver, are injured. Aspartate aminotransferase was increased in transported pigs, but still within the normal range for weaned pigs (Carr, 1998). Therefore, increased concentrations of CK, BUN, and AST in pigs after transport suggest that pigs may have been in a catabolic state or starting to go into a catabolic state due to the exertion of transport but concentrations were still within the normal range for these measures suggesting that the welfare of these pigs was not compromised. It would be interesting to determine if these values would increase with longer transport durations or plateau as the animals adapted to the transport.

Total protein and albumin concentrations are markers for protein homeostasis, which increase with dehydration. Albumin concentrations usually parallel the total protein concentrations. Total protein and albumin were high, but still within the normal range for weanling age pigs (Carr, 1998;

Mersmann and Pond, 2001) suggesting that pigs may have been experiencing slight dehydration as a results of transport.

Space allowance influenced the behavior of weaned pigs during transport in winter, summer, fall and spring. In winter, pigs transported at 0.06 m^2/pig spent less time standing than pigs transported at 0.05 and 0.07 m^2/pig during the last 15 min of transport, suggesting that pigs transported at a space allowance of 0.06 m^2/pig may have experienced a more restful state than pigs transported at 0.05 or 0.07 m^2/pig. In summer, Pigs transported at 0.05 m^2/pig laid down less than pigs transported at 0.06 and 0.07 m^2/pig between 30 and 60 min after transport, suggesting that pigs transported at 0.05 m^2/pig were more restless than pigs transported at 0.06 or 0.07 m^2/pig. In fall and spring, pigs transported at 0.05 m^2/pig sat more than pigs transported at 0.06 or 0.07 m^2/pig. The behavior of pigs during transport suggested that a space allowance of 0.06 or 0.07 m^2/pig was preferable to 0.05 m^2/pig when transporting weaned pigs in winter, summer, fall or spring.

Summary

The increased blood chemistry values and reduced body weight in transported weaned pigs suggests that these pigs were experiencing dehydration and muscle break down due to transport, however space allowance did not further impact blood chemistry of pigs regardless of season. All blood chemistry measures were still within the normal range for these enzymes suggesting that the welfare of these pigs was not compromised.

Different space allowances during transport did not influence body weight or physiological measures measured in this study regardless of season, except in summer the higher neutrophil to lymphocyte ratio in pigs transported at 0.05 m^2/pig, suggest that space allowances of 0.06 and 0.07 m^2/pig are preferable when transporting pigs in summer.

Decreased standing behavior performed by pigs transported at 0.06 m^2/pig in winter, decreased lying behavior performed by pigs transported at 0.05 m2/pig in summer, and increased sitting behavior performed by pigs transported at .05 m^2/pig in fall and spring, suggests that space allowances of 0.06 or 0.07 m^2/pig is preferable to 0.05 m^2/pig when transporting weaned pigs in winter, summer, fall or spring.

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Table 1. Pig weights, transport duration, temperature, humidity and wind speed during transport in winter, summer, fall and spring

Measure	Winter	STDEV	Summer	STDEV	Fall	STDEV	Spring	STDEV
Weight, kg	5.2	0.10	5.1	0.10	5.2	0.95	5.3	1.15
Temperature, ºC	10.5	6.15	28.4	1.23	20.4	4.00	22.0	2.49
Humidity, %	44.8	9.71	59.8	4.42	52.7	10.82	58.5	10.48
Wind speed, m/s	1.35	0.111	1.77	0.574	1.93	0.799	1.98	0.970
Transport duration, min	112.5	6.45	60.0	0.5	150.5	0.90	142.0	0.30

Table 2. Description of behaviors

Behavior	Description
Sitting[1]	Resting on the caudal part of the body
Standing[1]	Assuming or maintaining an upright position on extended legs
Standing on another pig	Assuming or maintaining an upright position on extended legs while standing on another pig/s
Lying[1]	Maintaining a recumbent position and not in contact with other pigs
Lying/huddling on top of another pig	Maintaining a recumbent position while contacting another pig/s

[1]Hurnik, 1995

Table 3. Blood hematological values of weaned pigs before and after transport in winter

Measure	Time				P-value
	Before	SE	After	SE	
N	96		96		
Total white blood cell count, 10^3/µL	10.0	0.33	9.4	0.40	0.237
Neutrophils, %	19.7	1.08	48.5	1.58	0.001
Lymphocytes, %	74.1	1.20	46.9	1.68	0.001
Neutrophil to Lymphocyte ratio	0.30	0.023	1.29	0.10	0.001
Red blood cell count, 10^6/ µL	4.9	0.15	5.2	0.13	0.276
Hemoglobin, g/dL	11.2	0.35	11.8	0.28	0.235
Hematocrit, %	31.2	0.80	32.3	0.57	0.262
Mean copulsular volume, fL	59.8	0.68	59.7	0.63	0.892
Mean corpuscular hemoglobin, g/dL	22.7	0.31	22.5	0.30	0.620
Mean corpuscular hemoglobin conc, pg	38.1	0.15	37.8	0.13	0.183
Red cell distribution width, %	25.5	0.74	25.8	0.66	0.765

Table 4. Blood chemistry and cortisol concentrations of weaned pigs before and after transport in winter

	Time				
Measure	Before	SE	After	SE	P-value
N	48		48		
Cortisol, ng/mL	39.3	4.85	62.1	5.66	0.005
Glucose, mg/dL	129.5	2.18	126.5	2.69	0.329
Blood urea nitrogen, mg/dL	8.0	0.33	9.4	0.38	0.005
Creatinine, mg/dL	1.0	0.06	0.9	0.05	0.400
Total bilirubin, mg/dL	0.3	0.04	0.5	0.03	0.001
Total protein, g/dL	5.5	0.05	5.6	0.05	0.048
Albumin, g/dL	3.5	0.05	3.8	0.05	0.001
Aspartate aminotransferase, IU/L	50.4	2.15	65.6	5.41	0.001
Creatine kinase, IU/L	965.0	95.60	1626.0	179.25	0.001
Alkaline phosphatase, UL/L	52.8	3.19	49.1	2.63	0.992
Gamma-Glutamyl Transpeptidase, UL/L	39.3	4.85	62.1	5.66	0.428

Table 5. Blood hematological values of weaned pigs before and after transport in summer

Measure	Before	SE	After	SE	P-value
	Time				
N	96		96		
Total white blood cell count, 10^3/µL	11.3	0.43	14.3	0.55	0.001
Neutrophils, %	18.3	0.65	28.2	0.97	0.001
Lymphocytes, %	61.0	0.80	43.4	1.04	0.001
Neutrophil to Lymphocyte ratio	0.3	0.02	0.7	0.04	0.001
Red blood cell count, 10^6/ µL	5.5	0.08	5.8	0.08	0.064
Hemoglobin, g/dL	113.4	2.00	117.9	2.12	0.127
Hematocrit, %	0.31	0.005	0.33	0.006	0.006
Mean copulsular volume, fL	56.9	0.43	57.2	0.45	0.661
Mean corpuscular hemoglobin, g/dL	19.5	0.19	19.2	0.20	0.425
Mean corpuscular hemoglobin conc, pg	343.8	1.50	337.2	1.51	0.005
Red cell distribution width, %	25.1	0.34	25.4	0.35	0.530

Table 6. Blood chemistry and cortisol concentrations of weaned pigs before and after transport in summer

	Time				
Measure	Before	SE	After	SE	P-value
N	48		48		
Cortisol, ng/mL	92.7	17.68	144.6	17.37	0.001
Glucose, mg/dL	124.9	2.35	105.2	2.29	0.001
Blood urea nitrogen, mg/dL	6.8	0.27	8.1	0.25	0.005
Creatinine, mg/dL	0.9	0.04	0.8	0.03	0.111
Total bilirubin, mg/dL	0.4	0.02	0.4	0.03	0.900
Total protein, g/dL	5.0	0.06	5.1	0.06	0.053
Albumin, g/dL	3.2	0.04	3.5	0.05	0.001
Aspartate aminotransferase, IU/L	53.6	3.66	64.0	2.41	0.001
Creatine kinase, IU/L	903.4	121.43	1100.2	97.64	0.01
Alkaline phosphatase, UL/L	665.9	21.96	659.3	22.32	0.876
Gamma-Glutamyl Transpeptidase, UL/L	56.7	3.04	97.4	7.66	0.001

Table 7. Blood hematological values of weaned pigs before and after transport in the fall and spring

Measure	Time				P-value
	Before	SE	After	SE	
N	48		48		
Total white blood cell count, 10^3/µL	10.0	0.40	13.3	0.57	0.001
Neutrophils, %	13.4	0.66	14.7	0.81	0.001
Lymphocytes, %	65.7	1.03	45.7	1.17	0.245
Neutrophil to Lymphocyte ratio	0.21	0.014	0.35	0.030	0.001
Red blood cell count, 10^6/ µL	5.7	0.12	5.9	0.09	0.358
Hemoglobin, g/dL	116.2	2.32	117.3	2.40	0.751
Hematocrit, %	0.34	0.006	0.34	0.007	0.772
Mean copulsular volume, fL	59.0	0.76	57.9	0.79	0.338
Mean corpuscular hemoglobin, g/dL	19.8	0.23	19.5	0.23	0.345
Mean corpuscular hemoglobin conc, pg	337.4	1.35	334.8	1.26	0.151
Red cell distribution width, %	23.9	0.42	23.3	0.42	0.343

Table 8. Blood chemistry and cortisol concentrations of weaned pigs before and after transport in the fall and spring

	Time				
Measure	Before	SE	After	SE	P-value
N	48		48		
Cortisol, ng/mL	41.3	3.87	80.9	5.58	0.001
Glucose, mg/dL	124.3	1.74	126.3	1.73	0.420
Blood urea nitrogen, mg/dL	5.9	0.26	6.9	0.26	0.01
Creatinine, mg/dL	1.06	.034	0.93	.0381	0.005
Total bilirubin, mg/dL	0.38	0.025	0.46	0.030	0.05
Total protein, g/dL	5.01	0.04	5.3	0.05	0.005
Albumin, g/dL	3.3	0.04	3.5	0.04	0.001
Aspartate aminotransferase, IU/L	59.2	3.46	75.7	3.46	0.001
Creatine kinase, IU/L	903.3	118.96	1369.5	96.97	0.005
Alkaline phosphatase, UL/L	545.3	19.27	539.2	20.07	0.831
Gamma-Glutamyl Transpeptidase, UL/L	52.3	3.97	148.4	11.50	0.001

Table 9. The effect of transport on hematological values of weaned pigs in winter, summer, fall and spring. Table values are P-values.

Measure	Winter	Summer	Fall and Spring
Total white blood cell count, 10^3/μL	0.237	**0.001**	**0.001**
Neutrophils, %	**0.001**	**0.001**	**0.001**
Lymphocytes, %	**0.001**	**0.001**	0.245
Neutrophil to Lymphocyte ratio	**0.001**	**0.001**	**0.001**
Red blood cell count, 10^6/ μL	0.276	0.064	0.358
Hemoglobin, g/dL	0.235	0.127	0.751
Hematocrit, %	0.262	**0.006**	0.772
Mean copulsular volume, fL	0.892	0.661	0.338
Mean corpuscular hemoglobin, g/dL	0.620	0.425	0.345
Mean corpuscular hemoglobin conc, pg	0.183	**0.005**	0.151
Red cell distribution width, %	0.765	0.530	0.343

Table 10. The effect of transport on cortisol concentrations and blood chemistry of weaned pigs in winter, summer, fall and spring. Table values are P-values.

Measure	Winter	Summer	Fall and Spring
Cortisol, ng/mL	**0.005**	**0.001**	**0.001**
Glucose, mg/dL	0.329	**0.001**	0.420
Blood urea nitrogen, mg/dL	**0.005**	**0.005**	**0.01**
Creatinine, mg/dL	0.400	0.111	**0.005**
Total bilirubin, mg/dL	**0.001**	0.900	**0.05**
Total protein, g/dL	**0.048**	**0.053**	**0.005**
Albumin, g/dL	**0.001**	**0.001**	**0.001**
Aspartate aminotransferase, IU/L	**0.001**	**0.001**	**0.001**
Creatine kinase, IU/L	**0.001**	**0.01**	**0.005**
Alkaline phosphatase, UL/L	0.992	0.876	0.831
Gamma-Glutamyl Transpeptidase, UL/L	0.428	**0.001**	**0.001**

Figure Legends

Figure 1. The percentage of time pigs spent standing when shipped at 0.05 m^2/pig (◆), 0.06 m^2/pig (■) and 0.07 m^2/ pig (▲) during a 112 min transport period in winter. At each time period, least square means accompanied by a * signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.06 m^2/pig; # signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig; and + signifies that 0.06 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig.

Figure 2. The percentage of time pigs lying when shipped at 0.05 m^2/pig (◆), 0.06 m^2/pig (■) and 0.07 m^2/ pig (▲) during a 112 min transport period in winter. At each time period, least square means accompanied by a * signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.06 m^2/pig; # signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig; and + signifies that 0.06 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig.

Figure 3. The neutrophil to lymphocyte ratio of pigs shipped at 0.05 m^2/pig (n = 96), 0.06 m^2/pig (n = 96) and 0.07 m^2/ pig (n = 96) before and after transport in summer. Least square means accompanied by different subscripts are different at $P < 0.05$.

Figure 4. The percentage of time pigs spent lying when shipped at 0.05 m^2/pig (◆), 0.06 m^2/pig (■) and 0.07 m^2/ pig (▲) during a 60 min transport period in summer. At each time period, least square means accompanied by a * signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.06 m^2/pig; # signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig; and + signifies that 0.06 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig.

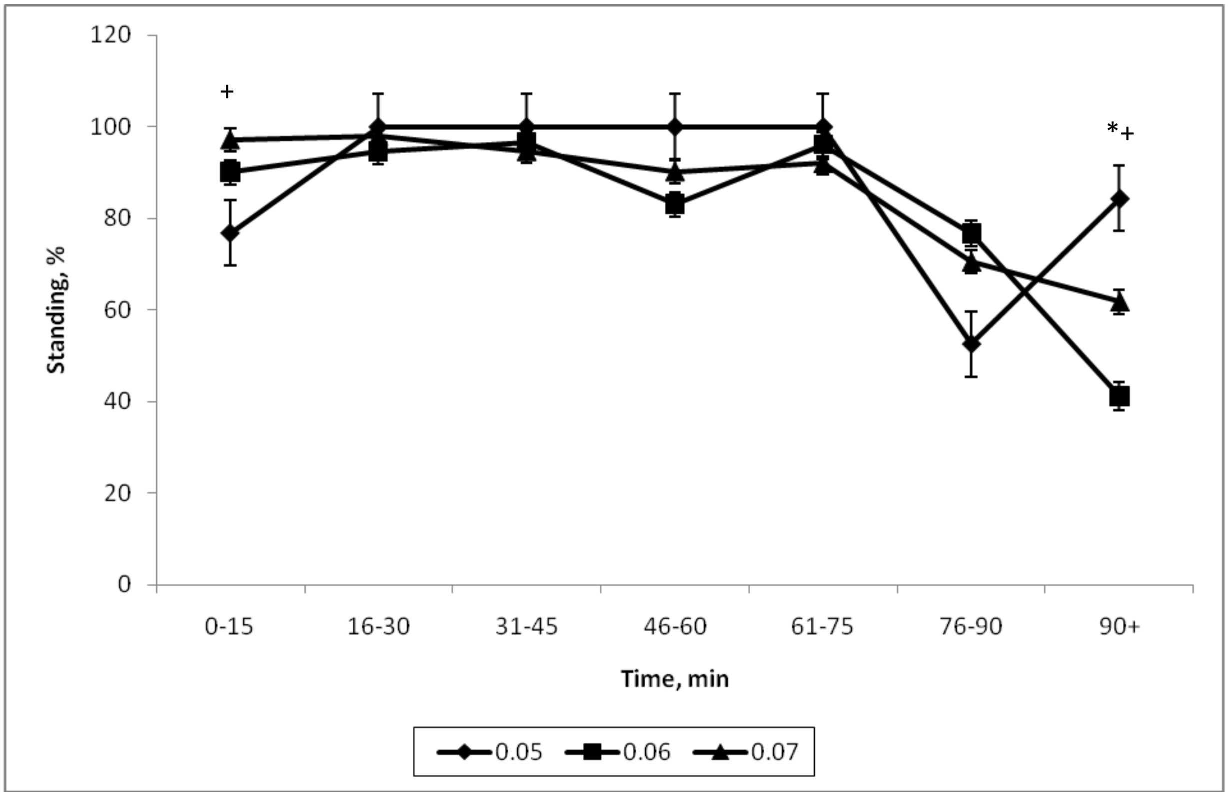
Figure 5. The percentage of time pigs standing when shipped at 0.05 m^2/pig (◆), 0.06 m^2/pig (■) and 0.07 m^2/ pig (▲) during a 60 min transport period in summer. At each time period, least square means accompanied by a * signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.06 m^2/pig; # signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig; and + signifies that 0.06 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig.

Figure 6. The percentage of time spent sitting when shipped at 0.05 m^2/pig, 0.06 m^2/pig and 0.07 m^2/ pig during a 150 min transport period in spring and fall. At each time period, least square means accompanied by different subscripts differ ($P < 0.05$).

Figure 7. The percentage of time pigs spent total standing when shipped at 0.05 m^2/pig (◆), 0.06 m^2/pig (■) and 0.07 m^2/ pig (▲) during a 150 min transport period in spring and fall. At each time period, least square means accompanied by a * signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.06 m^2/pig; # signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig; and + signifies that 0.06 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig.

Figure 8. The percentage of time pigs spent total lying when shipped at 0.05 m^2/pig (◆), 0.06 m^2/pig (■) and 0.07 m^2/ pig (▲) during a 150 min transport period in spring and fall. At each time period, least square means accompanied by a * signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.06 m^2/pig; # signifies that 0.05 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig; and + signifies that 0.06 m^2/pig differs ($P < 0.05$) from 0.07 m^2/pig.

Figure 1.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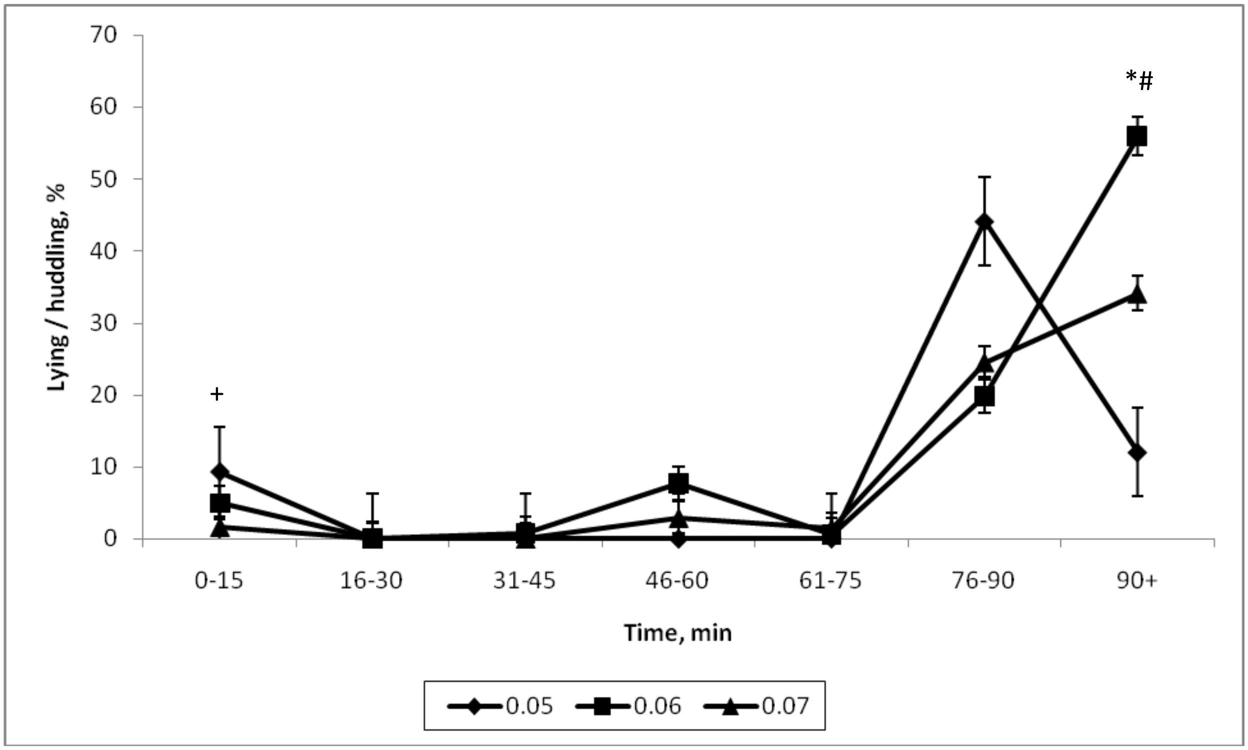
100
80
60
40
20
0
Standing, %
+
*+
0-15
16-30
31-45
46-60
61-75
76-90
90+
Time, min
0.05
0.06
0.07

Figure 2.



70
60
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40
30
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0
Lying / huddling, %
+
*#
0-15
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31-45
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76-90
90+
Time, min
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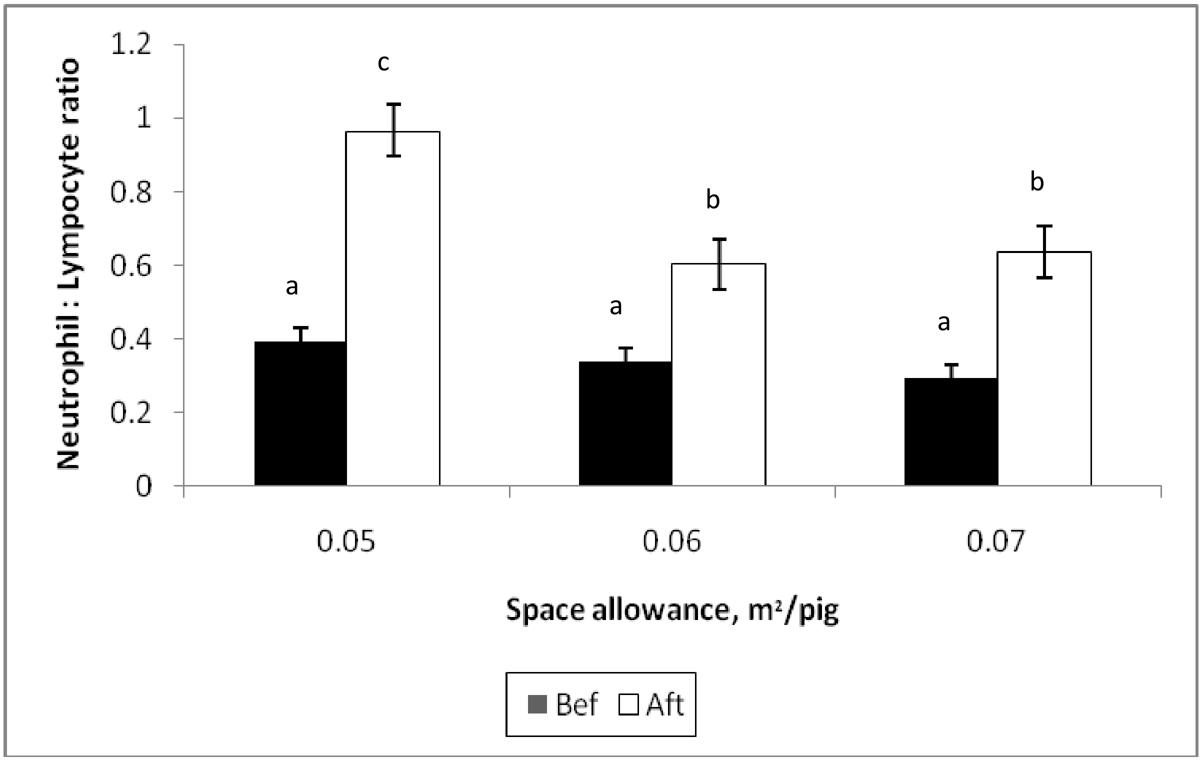
Figure 3.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1
0.8
0.6
0.4
0.2
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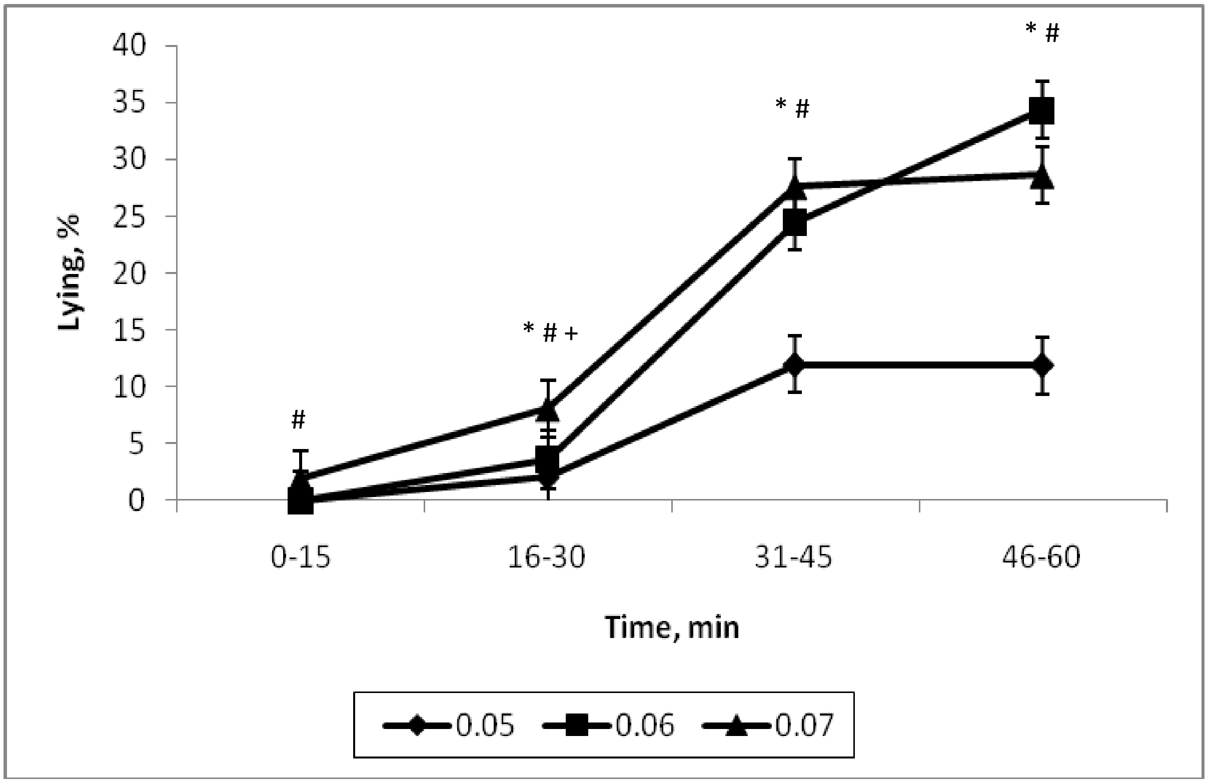
Neutrophil : Lympocyte ratio
c
a
b
a
b
a
0.05
0.06
0.07
Space allowance, m²/pig
Bef
Aft

Figure 4.



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Lying, %
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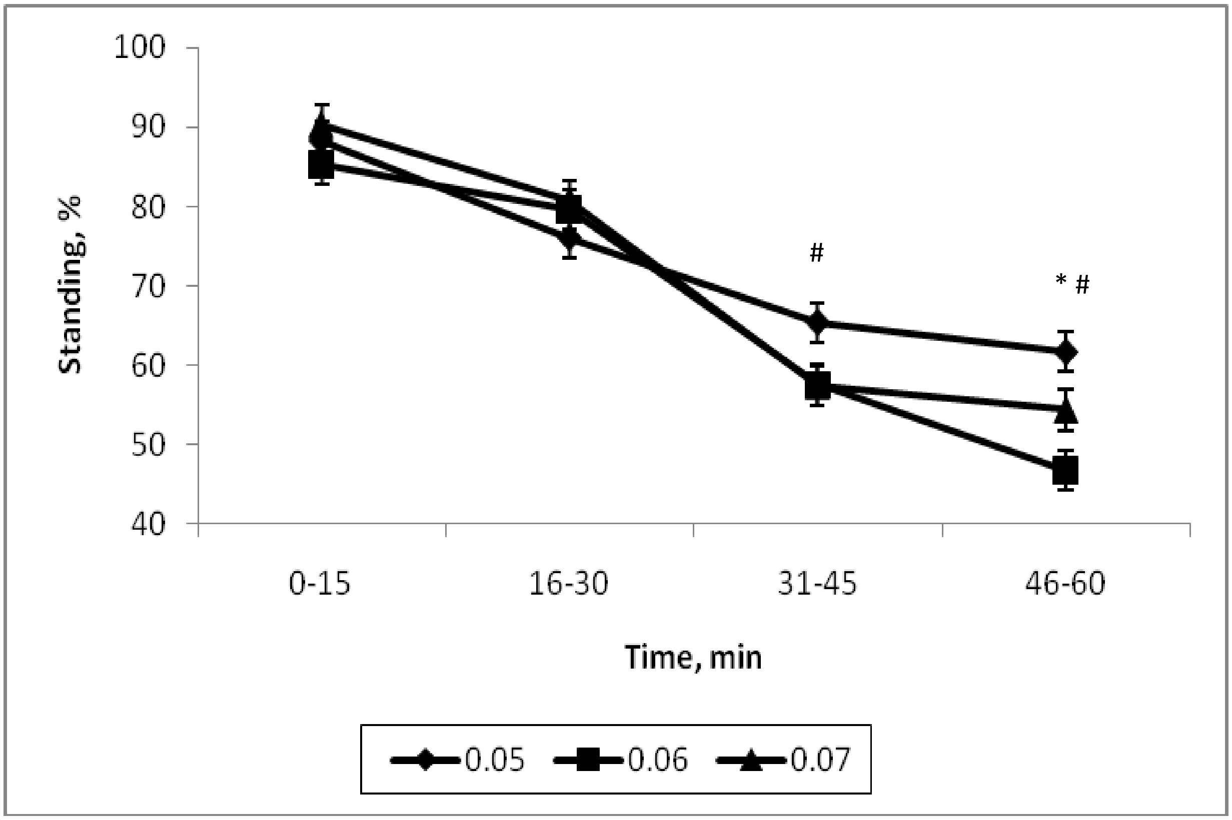
Figure 5.



100
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40
Standing, %
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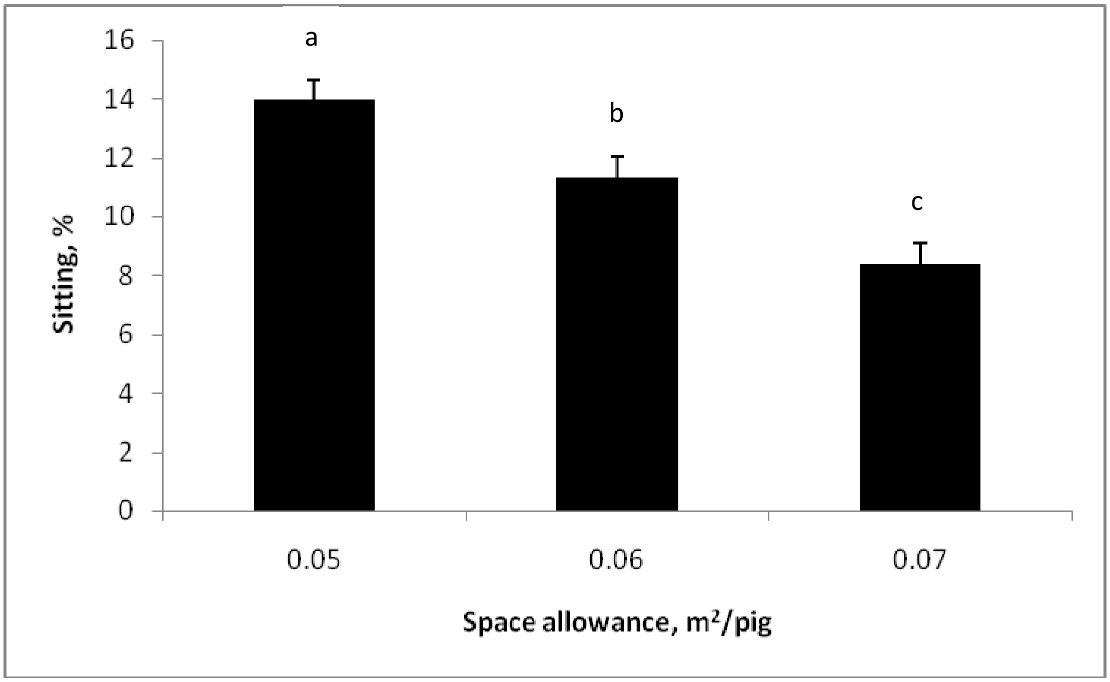
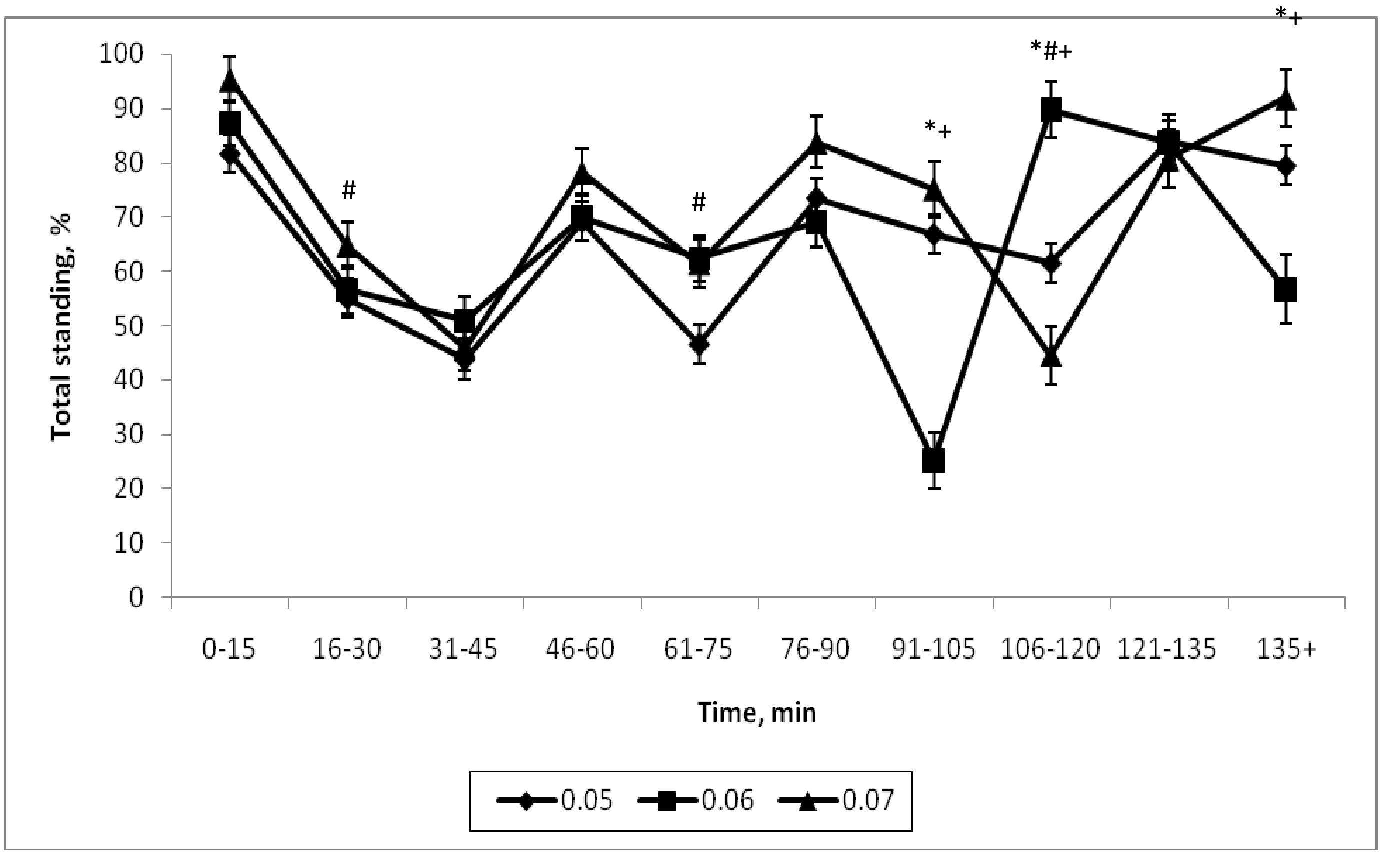
0-15
16-30
31-45
46-60
Time, min
0.05
0.06
0.07

Figure 6.



16
14
12
10
8
6
4
2
0
Sitting, %
a
b
c
0.05
0.06
0.07
Space allowance, m²/pig

Figure 7.



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100
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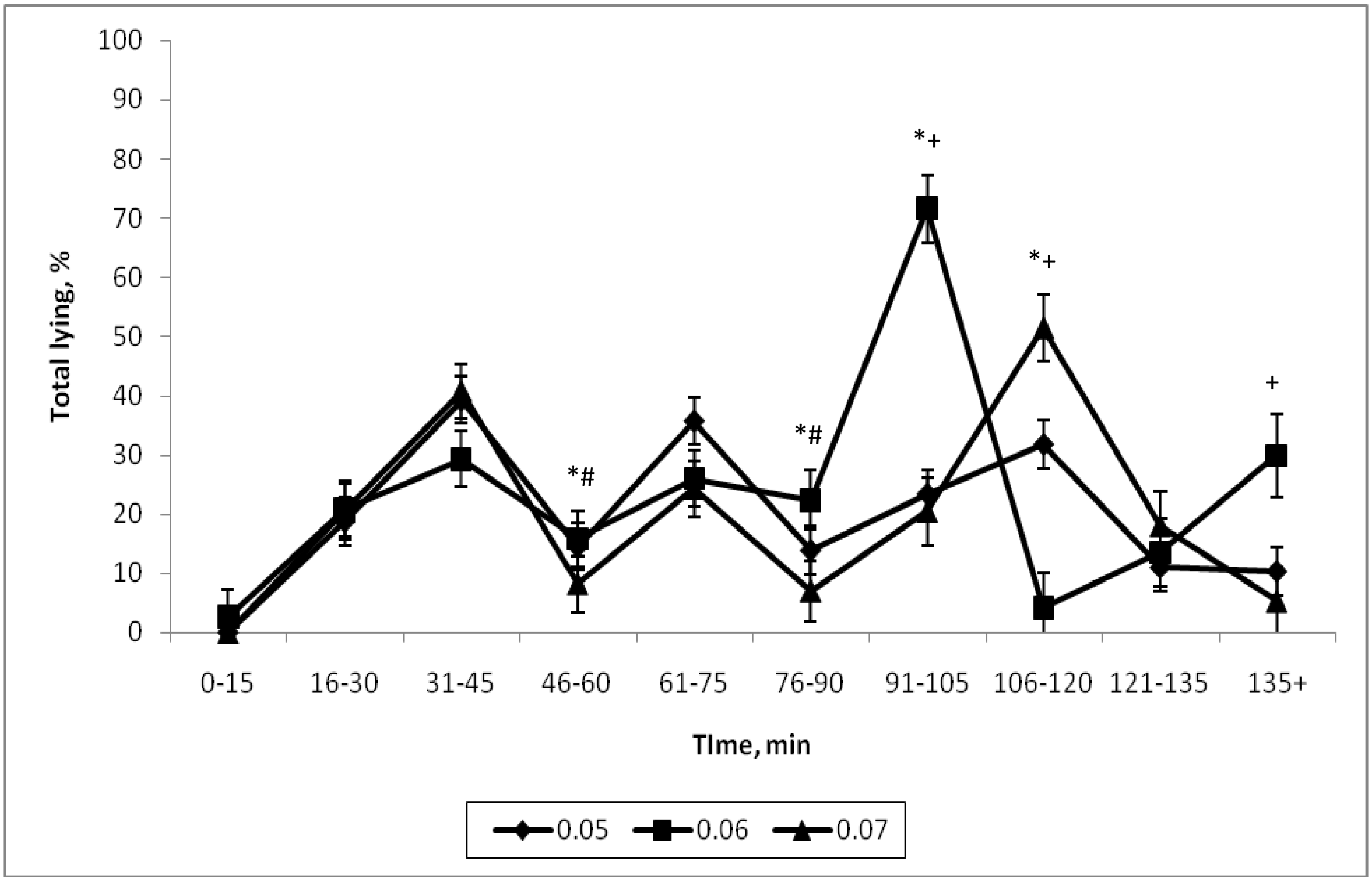
20
10
0
Total standing, %
0-15
16-30
31-45
46-60
61-75
76-90
91-105
106-120
121-135
135+
Time, min
0.05
0.06
0.07

Figure 8.


100
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Total lying, %
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16-30
31-45
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61-75
76-90
91-105
106-120
121-135
135+
TIme, min
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0.06
0.07