Title: Weaned pig transport: animal welfare impact of duration of trip, and provision of feed and water - NPB# 13-061

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Industry Summary:

Weaned pigs are transported on many USA farms. Some are transported short distances and others many hours. During transport pigs can be exposed to several stressors prior to and during transport including handling during loading, exposure to a novel environment, mixing with unfamiliar pigs, food and water deprivation and temperature fluctuations. Weaning is also a very sensitive stage in the life of a pig and a time at which welfare may be at risk. Therefore, the combined stress of transport and weaning has the potential to compromise wean pig welfare. However, little research has been conducted to understand the effect of transport duration on the well-being of weaned pigs and the associated effect of feed and water withdrawal. Therefore, the purpose of this study was to evaluate the effect of feed and water withdrawal on weaned pigs during transport of different durations based on behavior, performance and physiological measures of welfare.

The objective of this study was to evaluate the effect of feed and water withdrawal on weaned pigs during transport of different durations. At 18-22 days of age (the average weaning age of pigs on commercial swine farms in the US) pigs were randomly assigned to one of five treatment groups. Both gilts and barrows were evenly presented in each treatment group (10 pigs/treatment; 5 gilts and 5 barrows). The study involved 5 treatment groups:

1) Not weaned: pigs remained on the sow (CON)
2) Weaned, not transported, but had access to feed and water: pigs removed from the sow and moved into pens that had feed and water (WEAN+)
3) Weaned but had no access to feed and water: pigs were removed from the sow and moved into pens that did not have feed and water (WEAN-)
4) Weaned and transported with access to feed and water: pigs were removed from the sow and transport for up to 32 hours with access to feed and water (TRANS+)
5) Weaned and transported (as normally done) without access to feed and water: pigs were removed from the sow and transport for up to 32 hours without access to feed and water (TRANS-)

This study was replicated with 2 sexes X 5 treatments = 10 pigs X 9 replications = 90 pigs. The transport involve placing pigs in pens and transporting them in a goose-neck trailer. The pigs were transported along the same route for each replicate. The route involved a 32-hour trip. Each 8 hours, the truck/trailer would return to the New Deal farm, all the pigs were blood sampled, and weighed, then the truck/trailer continued for another phase (sampling at times 0, 8, 16, 24 and 32 hours). During sampling periods, (approximately 15-25 minutes) travel time would continue; thus, all sampling periods would take place at the time markers listed above. At the end of the trip, all pigs were placed in weaning pens at the farm of origin and followed at 24 hours and 7 and 14 days after weaning.

Measures
To assess pig welfare, measures of performance (weights, health, injury), behavior (on the truck or at the farm and after unloading), were measured.

Performance. Pigs were weighed before, immediately after and at 7, and 14 days after transport. Percetn weight change was calculated. Before and after shipping, each pig was examined for injuries, wounds, abscesses, and lameness.

Behaviors. Pig behavior was recorded during and after transportation. Behaviors of interest during transportation included standing, lying, sitting, drinking, and eating. After transportation pigs were penned with the same pen mate they had while being transported and video was taken for 24 hours post transport for all groups and quantified to examine potential treatment effects.

Physiology. Only 2 mL of blood were collected before transport and 2 mL every 8 hours after the start of the study. Blood was examined for baseline total white blood cell (WBC) counts and differential counts for the different white blood cell populations (neutrophil:lymphocyte ratio goes up with stress), plasma cortisol concentrations (an indicator of stress), glucose, creatine phosphokinase (indicator of stress and fatigue), total protein and hematocrit (indicators of dehydration).

There was a significant loss in percent body weight within treatments. Control pigs had a 6.5 ± 0.45% increase in body weight by the end of the transport study. Weaning caused a 5.9 ± 0.45% loss in body weight. Weaning without feed and water caused and 7.8 ± 0.45% loss in body weight. Transport with feed and water caused a 6.5 ± 0.45% loss in body weight in addition to weaning loss in body weight. Not providing any feed and water during transport caused a 9.1 ± 0.46 % loss in body weight. While control pigs continued to increase in weight, all other groups lost weight and were significantly different from the control group. There was no difference in percent weight loss in treatment groups until after 16 hours of transport. Transporting without feed and water has a greater impact on percent loss in body weight, and becomes apparent by 24 hours of transport.

Neutrophil to lymphocyte ratio (N:L) had a significant increase by 8 hours of transport. All treatment groups were had significantly higher N:L ratios compared to control pigs. At 16 hours of transport only Wean+ pigs has similar N:L ratios than the control pigs. After 16 hours N:L ratios began to normalize, possibly suggesting piglet adaptation to their environment.

Blood glucose levels for all treatment groups were all significantly lower than the control group by 8 hours of transport. Blood glucose levels continued to drop until the end of the transport study. Although pigs were provided with feed, they may not consume enough to maintain blood glucose levels.

Creatine Kinase (CK) was significantly higher for Trans+ females than males. Typically at weaning age there is no sex difference.

Total plasma protein (TP) was significantly higher in Trans- pigs by 16 hours of transport and remained higher than all other treatment groups until 24 hours of transport. By 32 hours of transport all treatment groups has increased TP, except control pigs. This possibly indicates that dehydration and stress were increased at this point in time.

Significant changes in behavior were observed during and after transportation. Behavioral changes can be indicative of stress. Significant changes in behavior were observed during and post- transport, with lying behaviors and standing behaviors being predominantly high in both phases. It has also been previously reported that high levels of resting after weaning are common and can affect the level of lying during transport. Lying can also be a sign that pigs have habituated to their environment. Sitting behavior was significantly higher in Tran+ and Trans- and Wean- pigs compared to Wean+ and control pigs. It has been reported that sitting is a behavior identified as a stress indicator. This indicated that transported pigs and pigs weaned and not provided with feed and water were possibly more stressed than pigs weaned and provided feed and water. There was not a difference in eating behaviors between Trans+ and Wean+ pigs. There was a difference in water consumption in Trans+ and Wean+ pigs during the transport phase, where Trans+ pigs consumed significantly more water than Wean+ pigs by 32 hours of transport.

Transportation has an additive effect on weight loss, especially if not provided with feed and water. Additionally, transportation impacts physiological and behavioral changes over time. In conclusion, there is limited research pertaining to transportation of weaned pigs and comparison of studies is sometimes difficult due to the variation in methodology, pig age/weight and densities studied (Lewis 2008). The transportation process is a stressful situation for pigs as they are exposed to long periods of transport, in many cases high stocking density, fasting, environmental factors, noise, mixing with conspecifics, injuries, and even death. Transportation death is painful and by no means an easy death, characterized by heart failure, and suffocation that may last from 10 minutes to 2 hours (Van Putten 1982). Thus, transportation is important in an animal welfare point of view and economically. Further research is needed to provide more insight on how to improve weaned pig welfare during transport.

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Scientific Abstract:

Transportation is a complex stressor made up of many factors including weaning itself and withdrawal from food and water. All these factors have the potential to activate the hypothalamic-pituitary-adrenal axis (HPA) in pigs individually or in combination. Therefore, transportation has the potential to affect the health, especially in pigs already experiencing weaning stress. Piglets were transported for 32 hrs and measures of performance, physiological changes, and behavior were taken to assess piglet welfare. There were 5 treatment groups, including a control (Con), weaned pigs provided feed and water (Wean+), weaned pigs not provided with feed and water (Wean-), weaned and transported pigs provided with feed and water (Trans+), and weaned and transported pigs not provided with feed and water (Trans-). There was a significant loss in percent body weight within treatments ($P < 0.01$). Control pigs had a 6.5 ± 0.45% increase in body weight by the end of the transport study. Weaning caused a 5.9 ± 0.45% loss in body weight. Weaning without feed and water caused and 7.8 ± 0.45% loss in body weight. Transport with feed and water caused a 6.5 ± 0.45% loss in body weight in addition to weaning loss in body weight. Not providing any feed and water during transport caused a 9.1 ± 0.46 % loss in body weight. There was a significant treatment by time interaction for neutrophil to lymphocyte ratio (N:L) ($P < 0.01$). All treatment groups had a significant increase in N:L ratio by 8 hr compared to the Con group ($P < 0.05$). There was a significant treatment by time interaction for blood glucose levels ($P < 0.01$). By 24 and 32 hours of transport all treatment groups had significantly lower blood glucose levels compared to the Con group ($P < 0.05$). Creatine Kinase (CK) had a significant sex by treatment interaction ($P < 0.01$). Trans+ females had a significantly higher CK levels than males ($P < 0.05$). Total plasma protein (TP) has a significant treatment by time interaction ($P < 0.01$). By 16 hrs of transport Trans- pigs has significantly higher TP levels than all other treatment groups. Significant changes in behavior were observed during and after transportation. Overall, transportation has an additive effect in weight loss, especially if not provided with feed and water. Physiological and behavioral changes are similar for animals weaned and transported without feed.

Introduction:

Transportation is a complex stressor made up of many factors including fluctuating temperatures, stocking density, withdrawal from food and water, mixing with unfamiliar pigs and motion (Lambooij and van Putten, 1993). All these factors have the potential to activate the hypothalamic-pituitary-adrenal axis (HPA) in pigs individually or in combination. Therefore, transportation has the potential to affect the health and welfare of pigs, especially in pigs already experiencing weaning stress. The practice of transporting pigs' long distance via truck from farrowing to finishing units is becoming increasingly popular in the US. Most of the literature pertaining to the effect of transport on the stress response of pigs has been conducted on market weight pigs (Barton-Gade and Christensen, 1998; Warriss et al., 1998; Kim et al., 2004). Currently, there is limited knowledge regarding the effect of transport duration and the associated effect of feed and water deprivation on the welfare of weaned pigs.

Transport duration itself is one important factor that can potentially contribute to the overall stress experienced by pigs due to transport. In market weight pigs it has been reported that a percentage of pigs that were dead on arrival at the packing plant increased as travel time increased from 30 min to 4 h (Sutherland et al., 2009b) and a higher mortality risk was recorded in market weight pigs transported for 30 to 90 min in the U.S. (Rademacher and Davies, 2005). These findings suggest that market weight pigs may experience higher levels of stress during shorter transport periods and this could partially be attributed to pre-transport handling which in itself can cause an acute stress response in animals, including an increase in heart rate (Lewis and McGlone, 2007), glucose (Hemsworth et al., 2002) and lactate concentrations (Hamilton et al., 2004). Hamilton et al. (2004) found that it took at least 2 h for physiological parameters of stress in pigs to return to baseline levels after handling. Therefore, it would be useful for the industry to know the optimum time for transporting weaned pigs to reduce stress. Furthermore, it is important to understand other contributing factors associated with transport stress, particularly feed and water deprivation.

Transport time has been shown to effect morbidity and mortality in market weight pigs (Rademacher and Davies, 2005; Sutherland et al., 2009a). However, few studies have been conducted investigating the interaction between transport time and feed and water deprivation on the short and longer term physiological and behavioral responses of weaned pigs to transport. Once weaned, piglets are exposed to extreme social, environmental, and dietary stresses, they are mixed with piglets of different litters and sometimes require transportation from a breeding unit to a specialized nursery unit. The transition between nursing and eating solid foods may lead to a period of underfeeding, affecting growth, metabolism, and metabolic changes associated to endocrine adjustments while the animal adapts from milk to solid feed (Le Dividich and Seve 2000). Abrupt reduction in feed intake is also known as “weaning growth check” and is more severe in some piglets than others, as some piglets keep gaining weight after they are weaned (Tockach et al., and Azian 1993). Therefore, it is necessary for the industry to understand the impact of transport time on the welfare of weaned pigs so that standards can be put into place to reduce any detrimental effects to weaned pigs caused by transport.

In 2006, the USDA clarified that livestock conveyed by truck are subject to be off-loaded for feed, water, and rest after 28 consecutive hours in transport (National Pork Producers Council). This 28 hour law was enacted in 1873 and applied to rail transportation of cattle, sheep, swine, and other animals and was later amended to include transportation by express or common carriers involving confinement in a “vehicle or vessel” (National Pork Producers Council).

Transportation of weaning pigs is becoming more common in the U.S. but behavioral and physiological responses have not been well documented (Sutherland et al., 2009). Aside from the stress of transport, weaning pigs are even more susceptible because in addition they are dealing with weaning stress which may affect their immune system, performance and behavior (Hay et al., 2001 and Kanitz et al., 2002). Furthermore, weanling pigs may become dehydrated and experience muscle break down due to transport (Sutherland et al., 2009).

Thus, it is important to try and minimize stress at every point of the process. A particularly important stressor in pigs is heat shock. Cells in the pigs body will express heat shock proteins (Hsps) that are responsible for proper folding of newly formed proteins within the cell for cell survival and adaptation (Quingquing 2010), assist in repair of denatured protein and can promote degradation of proteins after stress or injury (Mogk et al., 2002; Dou et al., 2003) . HSP70 in pig hearts have been reported to increase after 2 hours of transportation (Yu et. al, 2007), but also have been reported to decrease after 6 hours of transport (Bao et al., 2008a) and therefore transport can change the expression of Hsps (Quingquing 2010). Quingquing et al., 2010 suggest Hsp27 may also play a protective role in response to transportation stress after 2 hours of transport. Creatine kinase (CK), lactate dehydrogenase (LDH), corticotrophin (ACTH), cortisol, insulin, T3 and T4 have been reported to change the response to stressors (Fàbrega et al., 2002; Fazio et al., 2005; Helmreich et al., 2006; Mansour et al., 1992) and can reflect stress coping mechanisms and metabolic status of pigs (Quingquing 2010). CK has been associated with heart damage and has been reported to increase during 1-2 hours of transport and decrease after 4 hours of transport, indicating that there may be more damage to myofibrils during the first 2 hours than at 4 hours of transport (Quingquing 2010). However, prolonged transport causes Hsps regulation to break down leading to pathological changes (Quingquing 2010). Hsps damage may not be such a big problem in recently weaned piglets because they are commonly kept in barns that are maintained at warm temperatures for them, however, those piglets being transported to a different facility may be affected because they are subjected to a different environment.

Individual and breed specific coping styles to stressful conditions, such as behavioral performance, physiological and biochemical responses have been associated with hypothalamic-pituitary-adrenal activity in pigs and mice (Quing Quing et. al, 2010). During transportation pigs may become habituated or exhausted and show more inactivity, characterized by lying or lying/huddling (Sutherland et al., 2009). However, it is difficult to determine whether inactive behaviors are due to exhaustion or are normal behaviors.

Therefore, the objectives of this were to evaluate the effect of feed and water withdrawal on weaned pigs during transport of different durations based on behavior, performance, and physiological measures of welfare.

Objectives:

Weaned pigs are transported on many USA farms. Some are transported short distances and others many hours.

During transport pigs can be exposed to several stressors prior to and during transport including handling during loading, exposure to a novel environment, mixing with unfamiliar pigs, food and water deprivation and temperature fluctuations. Weaning is also a very sensitive stage in the life of a pig and a time at which welfare may be at risk. Therefore, the combined stress of transport and weaning has the potential to compromise wean pig welfare. However, little research has been conducted to understand the effect of transport duration on the well-being of weaned pigs and the associated effect of feed and water withdrawal. Therefore, the objectives of this study are:

1) To evaluate the effect of feed and water withdrawal on weaned pigs during transport of different durations based on behavior, performance and physiological measures of welfare (controlled study).
2) To evaluate the effect of transport duration on the well-being of weaned pigs under commercial conditions (future study)

Materials & Methods:

Pigs were PIC USA genetics using the Camborough-22 sow line. All animals were fed a diet to meet or exceed NRC nutrient requirements. Feed and water were provided ad libitum. All animal procedures were approved by the Texas Tech University Animal Care and Use Committee.

At 18-22 days of age (the average weaning age of pigs on commercial swine farms in the US) pigs were randomly assigned to one of five treatment groups. Both gilts and barrows were evenly presented in each treatment group (10 pigs/treatment; 5 gilts and 5 barrows). The study involved 5 treatment groups:

1) Not weaned: pigs remained on the sow (CON)
2) Weaned, not transported, but had access to feed and water: pigs removed from the sow and moved into pens that had feed and water (WEAN+)
3) Weaned but had no access to feed and water: pigs were removed from the sow and moved into pens that did not have feed and water (WEAN-)
4) Weaned and transported with access to feed and water: pigs were removed from the sow and transport for up to 32 hours with access to feed and water (TRANS+)
5) Weaned and transported (as normally done) without access to feed and water: pigs were removed from the sow and transport for up to 32 hours without access to feed and water (TRANS-)

The study was replicated with 2 sexes X 5 treatments = 10 pigs X 9 replications = 90 pigs. The piglets were selected by date of birth ± 2 d in order to keep them in the same age range. They were further randomly selected from their litter and assigned a group. Each treatment group weighed approximately the same ± 0.25 kg.

The study began at 0700 and ended by 1600 the following day. At weaning, blood samples were taken from each individual pig. Control pigs were blood sampled, weighed, checked for lesions or lameness and placed back in the farrowing pen. The same procedures were conducted on all other treatment groups, except that they were either placed in randomly assigned weaning pens or pens in the trailer were they would be transported. Blood samples were taken by placing pigs in a supine position and collecting 4 ml of blood from the jugular vein into vacutainers.

Transport involved placing piglets in pens (0.6 m x 0.6 m) with sawdust shavings, approximately 10 cm in depth. HOBO (Onset Computer Corporation, Bourne, MA) data loggers to record temperature and humidity were placed in the trailer on each experimental pen. Temperatures in the trailer ranged from 2.4 ° C to 32.6 ° C and relative humidity inside the trailer ranged from 15.1% - 76.9%. The piglets were then transported in a goose-neck trailer (6 m X 2 m). The route involved a 32-hour trip, broken down into 8 hour phases in which the truck/trailer would return to the New Deal farm. Once the animals were back at the farm, blood samples were collected. During sampling periods, (approximately 15-25 minutes) travel time would continue; thus, all sampling periods would take place at 0, 8, 16, 24 and 32 hours. During sampling times in addition to being weighed, and blood samples, piglets were checked for injuries and feed and water consumption was recorded. Piglets were then returned to the truck/trailer to continue another phase of transport. At the end of the trip, all pigs were placed in weaning pens at the farm of origin with their currently assigned pen mates to avoid further stress caused by mixing of unfamiliar pigs.

To assess piglet welfare, measures of performance (weights, health, injury), behavior (during transport, at the farm, and post-transport), and physiological changes were measured.

Performance. Piglets were weighed before, immediately after, and at 7, and 14 days post-transport. Percent weight change was calculated. Before and after shipping, each pig was examined for injuries, wounds, abscesses, and lameness.

Physiology. Only 2 mL of blood were collected in BD Vacutainers ® containing 5.4 mg of K2 EDTA and 2 ml were collected in BD Vacutainers ® without additives for blood analysis. Only 4 ml in total were collected from every piglet every 8 hours after the start of the study. Whole blood was examined for baseline total white blood cell (WBC) counts and differential leukocyte counts for the different white blood cell populations, hematocrit (HCT) and neutrophil to lymphocyte ratio (N:L) ratio. Blood samples were all examined manually at Texas Tech University, Department of Animal and Food Sciences within one hr of sample collection. Blood samples were centrifuged and serum and plasma was collected for analysis. Serum and plasma were extracted from the samples and frozen at (Temp) until analysis.. Blood chemistry measures were conducted by (lab name) and obtained for glucose, total protein, and creatine kinase.

Behaviors. Piglet behavior was recorded during and after transportation. Wild life cameras (Brand) were placed across form each experimental pen and were used to record transported pigs behaviors. Four pigs were recorded per frame. The cameras were motion activated and instantaneous shots were taken upon activation. Digital video recorders (DVRs) (Brand) were used to record behaviors of control pigs in the nursery and behaviors of weaned pigs in the weaning barn during the transport study. 10 min scan samples were used to record the frequency of lying, standing, sitting, drinking, and eating in 2 hr intervals for the 32 hr transportation period. Post-transport pigs were penned with the same pen mate they had while being transported and video was taken for 24 hours post-transport for all groups and quantified to examine potential treatment effects. DVRs were used to record the same behaviors post-transport that were recorded during transportation for weaned pigs.

Statistical Analysis

The study used a Complete Randomized Design (2 sexes X 5 treatments = 10 pigs X 9 replications = 90 pigs). A general linear model was used and the data were analyzed using analysis of variance procedures in SAS. The statistical model included the effects of sex, treatment, pen, time, and all possible interactions. Behavior was analyzed in a general linear model using analysis of variance procedure in SAS. The behavior observation periods were in 2 hour intervals. All data were tested for homogenous variances and normal distributions. The experimental unit was a group of 12 pigs. All data were analyzed using SAS 9.3 General Linear Models procedure (SAS, 2010 SAS Inst., Inc., Cary, NC).

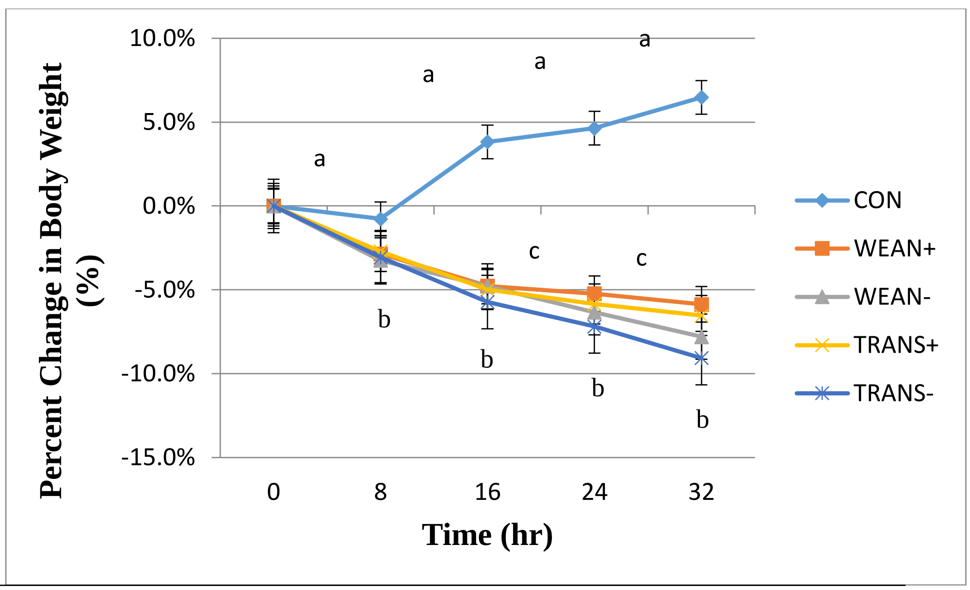
Results:

There were significant changes in performance, physiology, and behaviors. Main effects will be discussed first, followed by interactions.

3.1.1 Weight

There was a treatment by time interaction for percent change in body weights ($P < 0.01$). All treatments were different from the Control (Con) group throughout the entire transportation period starting at 8 hr (Figure 1). Aside from the Con group and the Transport pigs transported without feed and water (Trans-), all groups has a similar percent change in body weights. Trans- pigs were significantly different at 24 h from Wean + pigs ($P < 0.05$), but did not differ from the other groups aside from the Con group. At 32 hr Trans- pigs were significantly different than all treatment groups ($P < 0.05$). The additional loss in body weight that Trans- pigs experience was likely due to the lack of feed and water. These findings have also been documented in slaughter weight hogs that have been transported and fasted for 25-48 hrs (Lambooy et al., 1985; Jones et al., 1985; Brumm et at., 1987; Lambooy, 1988).

Figure 1. Least Squares means for treatment effects 32 h after weaning or transportation ($P < 0.01$). Treatment groups are abbreviated by Wean + = animals weaned and not transported, provided with feed and water Wean - = animals weaned, not transported, and not provided with feed and water, Trans + = animals weaned and transported, provided with feed and water, Trans - = animals weaned and transported, not provided with feed and water, Con = not wean or transported. (N= 90 pigs).



Superscripts without a common letter differ at $P < 0.05$.

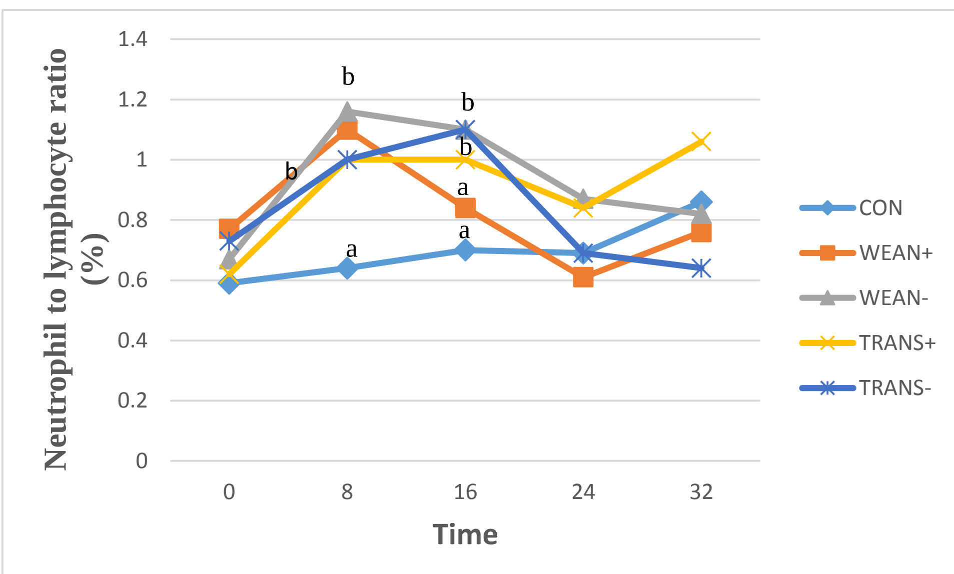
Control pigs had a 6.5 ± 0.45% increase in body weight by the end of the transport study. Weaning caused a 5.9 ± 0.45% loss in body weight. Weaning without feed and water caused an additional 1.9 ± 0.45% loss in body weight. Transport with feed and water added a 0.6 ± 0.45% loss in body weight in addition to weaning loss in body weight. Not providing any feed and water during transport added an additional 3.2 ± 0.46 % loss in body weight to weaning loss. There was an apparent additive effect in weight loss with weaning and transport without feed and water. Slaughter weight hogs that have been transported and fasted for 25-48 hrs have been reported to lose 4-12% in body weight (Lambooy et al., 1985; Jones et al., 1985; Brumm et at., 1987; Lambooy, 1988).

3.2.1 Neutrophil to lymphocyte ratio interaction

There was a significant treatment by time interaction for neutrophil to lymphocyte ratio (N:L) ($P < 0.01$). When comparing treatment groups to the control group, all treatment groups were different to the Con group by 8 hrs (Figure 2). By 16 hrs only Wean+ pigs were similar to the Con group, and all other groups significantly differed from Wean+ and Con pigs in their N:L ratio ($P < 0.05$). By 24 hr until the end of the study, there were no significant differences in N:L ratio among all study groups. This can possibly be due to adaptation of the piglets to long distance transport. Studies conducted in cattle have also shown that transport causes an increase in N:L ratio (Dunn 1989; Averos et al., 2008b; Riondato et al., 2008). Additionally, N:L ratio in pigs weighing 27 kg and transported for 4 hrs have been reported (McGlone et al., 1993).

There was no difference in N:L in the control pigs from 0 hr to 24hr, however, there was a significant increase ($P < 0.05$) in N:L ratio when comparing 0 hr and 32. The increase in N:L ratio at 32 hr possibly indicates that the stress duration intensified lymphocyte suppression towards the last 8 hrs of the transport transport. Wean + pigs experience a significant ($P < 0.05$) increase in N:L ratio at 8hrs of transportation compared to baseline, but all other time points were similar. The Wean – pigs, similarly to the Wean + pigs experienced a significant increase compared to baseline between 8 hrs and 16 hrs of transport. Trans + pigs experience a significant increase ($P < 0.05$) in N:L ration compared to baseline from 8-16 hrs. At 24 hrs of transport N:L ratio values for Trans+ pigs came down, but increased again at 32 hrs of transport. Trans – pigs similar to Trans + pigs experience a significant increase ($P < 0.05$) at 8-16 hrs of transportation compared to baseline but by 24 and 32 hours they were no longer had a significant difference from baseline. All treatment groups besides the control pigs has a significant increase in N:L ratio between 8-16 hrs. Indicating that immunosuppression between these timeframes is more predominant.

Figure 2. Least Squares means for neutrophil to lymphocyte ratio (N:L) for treatment by time interaction ($P < 0.01$). Treatment groups are abbreviated by Wean + = animals weaned and not transported, provided with feed and water Wean - = animals weaned, not transported, and not provided with feed and water, Trans + = animals weaned and transported, provided with feed and water, Trans - = animals weaned and transported, not provided with feed and water, Con = not wean or transported. (N= 18 pen observations).



Superscripts are different at ($P < 0.05$).

3.2.2 Blood Glucose interaction

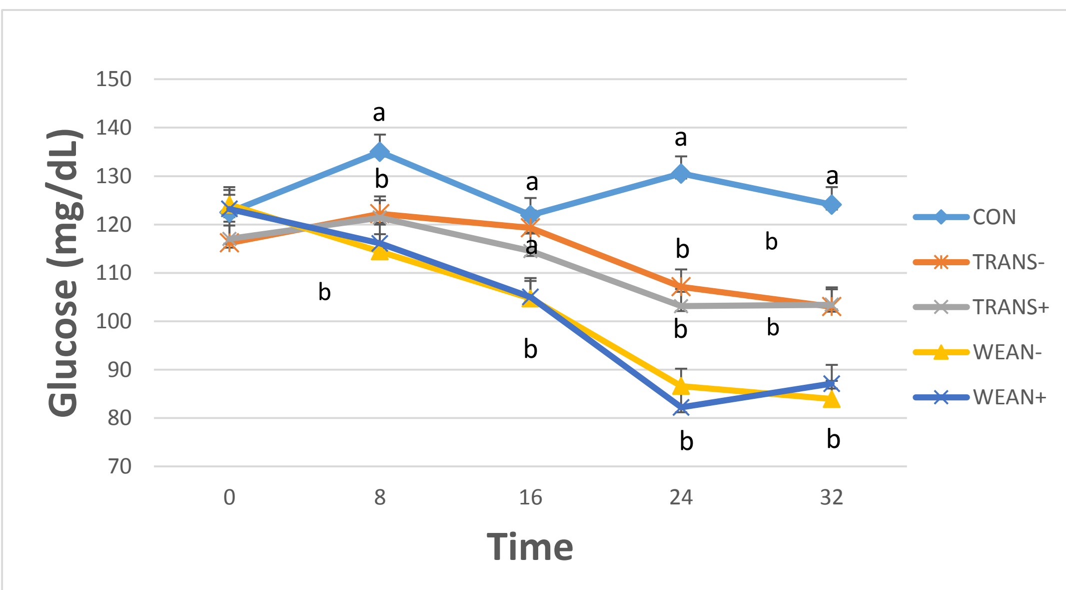
There was a significant treatment by time interaction for blood glucose ($P < 0.01$). When comparing treatment groups to the Con group, all treatment groups were significantly different from the Con group ($P < 0.05$) by 8 hrs

(Figure 3). By 16 hrs Trans + and Trans – groups had similar blood glucose levels to Con group, while Wean + and Wean – groups still had significantly lower blood glucose levels than the Con group. At 24 hrs and up to 32 hrs of being weaned or transported, all treatment groups had significantly lower blood glucose levels than the Con group. Blood glucose levels continued to decrease over time for weaned and transported pigs.

The only significant change in blood glucose for control pigs was at 8 hrs, in which blood glucose increased significantly from baseline ($P < 0.05$). The Wean+ treatment group maintained their blood glucose up until 8 hrs. By 16 hrs blood glucose levels in the Wean+ treatment group dropped significantly compared to baseline ($P < 0.05$) and continued to drop until 24 hrs. By 32 hrs blood glucose levels for the Wean+ treatment group increased slightly but were still significantly lower than baseline levels. The Wean – treatment group had a significant decrease in blood glucose levels by 16 hr compared to baseline levels ($P < 0.05$). Blood glucose levels in Wean- treatment group continued to decrease for the rest of the study. The Trans + treatment group maintain their blood glucose levels up until 16hrs of transport. By 24 hrs of transport Trans+ treatment group blood glucose levels dropped significantly ($P < 0.05$) compared to baseline. Blood glucose increased slightly by hour 32 of transport but was still significantly low compared to baseline levels. The Trans- treatment group maintained their blood glucose up 24 hrs of transport. At 32 hrs of transport their blood glucose levels dropped significantly from baseline ($P < 0.05$).

Higher levels of blood glucose at 8 hrs of transport have been documented in finishing pigs and were reported to be due to a shorter fasting period compared to 16 hr transported pigs (Becerril-Herrera et al., 2010). Blood glucose levels may be maintained during short-term fasting, partially due to hepatic gluconeogenesis (Castillo, et al., 1991). However, stress induced hyperglycemia has been suppressed in 24 hr fasting pigs (Fernandez et al., 1995).

Figure 3. Least Squares means for treatment by time interaction for blood glucose ($P < 0.01$). Treatment groups are abbreviated by Wean + = animals weaned and not transported, provided with feed and water Wean - = animals weaned, not transported, and not provided with feed and water, Trans + = animals weaned and transported, provided with feed and water, Trans - = animals weaned and transported, not provided with feed and water, Con = not wean or transported. (N= 18).



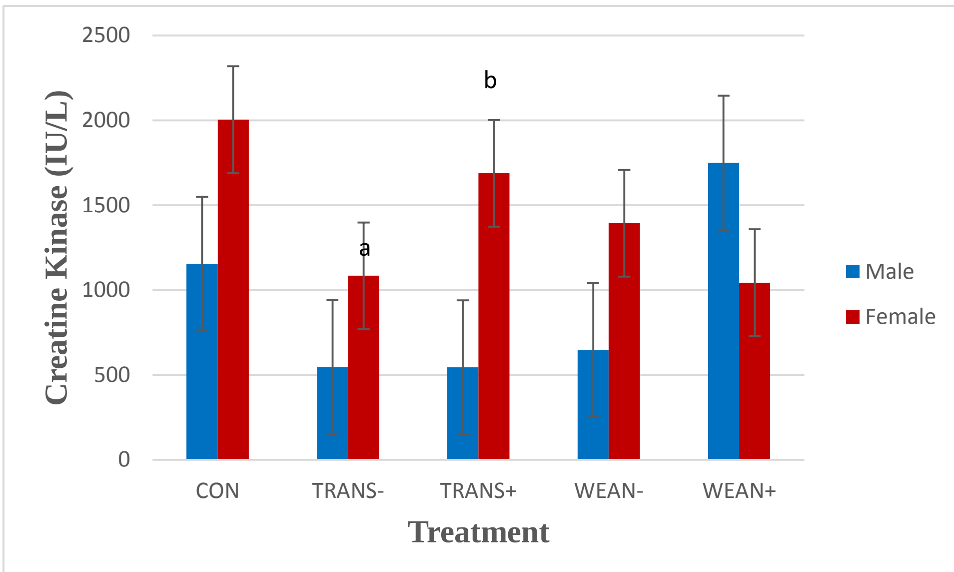
Superscripts are different at $P < 0.05$.

<u>3.2.3 Creatine Kinase interaction</u>

There was a significant sex by treatment interaction for creatine kinase (CK) ($P < 0.01$). Trans+ female pigs had significantly higher creatine kinase levels than Trans+ males ($P < 0.05$), respectively 1687.3 ± 253.2 IU/L and 543.8 ± 370.4 IU/L (Figure 4).

Creatine kinase is released from muscle fibers into circulation in response to muscular activity, tissue damage (Van der Meulen et al., 1991; Fabrega et al, 2002; Yu et al., 2007) and can indicate physical fatigue (Averos et al., 2009). In a study by Elizondo et al. (1976) guilts exhibited a significantly higher level of CK than barrows. Sex has been shown to affect stress responses of piglets during transport, however, most studies have shown this response in older male and female pigs transported to slaughter (Warriss 1996; Guardia et al., 2004)

Figure 4. Least Squares means for treatment by sex interaction for creatine kinase ($P < 0.01$). Treatment groups are abbreviated by Wean + = animals weaned and not transported, provided with feed and water Wean - = animals weaned, not transported, and not provided with feed and water, Trans + = animals weaned and transported, provided with feed and water, Trans - = animals weaned and transported, not provided with feed and water, Con = not wean or transported. (N= 90 pig observations).

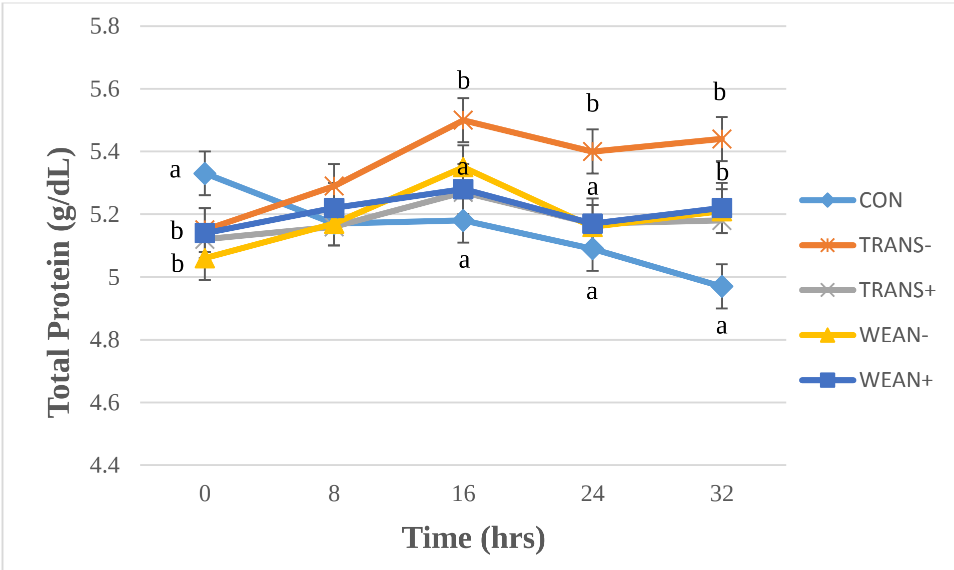


3.2.4 Total Plasma Protein interaction

There was a significant total plasma protein (TP) treatment by time interaction ($P < 0.01$). Total plasma protein levels differed significantly by 16 hr for Trans- pigs compared to other treatment groups and remained different from other groups until 32 hrs of transport (Figure 5). By 32 hrs of transport all treatment groups had significantly higher total protein levels than the control group.

Increased total protein may suggest dehydration as a result of transport and has been reported in transport durations as short as 60 min (Sutherland et al., 2009).

Figure 5. Least Squares means for total protein and time interaction over a 32 hr transportation period (P < 0.01). Treatment groups are abbreviated by Wean + = animals weaned and not transported, provided with feed and water Wean - = animals weaned, not transported, and not provided with feed and water, Trans + = animals weaned and transported, provided with feed and water, Trans - = animals weaned and transported, not provided with feed and water, Con = not wean or transported. N = 18.



Superscripts without a common letter differ at P < 0.05.

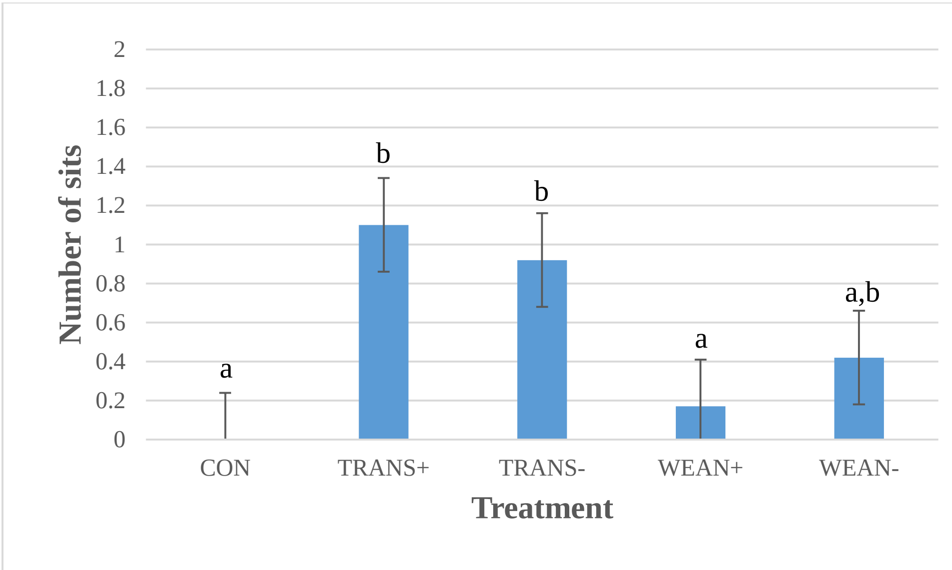
<u>3.3 Behavior during transport</u>

Overall, the Con group spent 65% of their time lying during the 32 hr study. The Trans + group spent 77% of their time lying, Trans- and Wean+ treatment groups spent the same amount of time lying, 81% of their time. The Wean- treatment group spent 87% of their time lying.

The Con group and Wean- treatment group pigs spent 11% of time standing during the 32 hr study. Trans-, Trans+, and Wean+ treatment groups stood respectively 14%,16%, and 17% of the time during the 32 hr study.

There was a significant treatment effect for sitting behavior (P < 0.01). Both Trans + and Trans – treatment groups sat significantly more (P < 0.05) than the Con group (Figure 6). The Weaned + treatment group had similar sitting behaviors as the Con group. The Weaned – treatment group sitting behaviors did not differ from Con or Trans+ or Trans- treatment groups. The Con group did not spend any time sitting during the study. The Wean+ treatment group spent 1% of their time sitting, while Wean-, Trans- and Trans+ treatment groups spent respectively 2%, 4% and 5% of their time sitting.

Figure 6. Least Squares ± 0.24 means for number of sitting behaviors for treatment groups during a 32 h transport study (P< 0.01). Treatment groups are abbreviated by Wean + = animals weaned and not transported, provided with feed and water Wean - = animals weaned, not transported, and not provided with feed and water, Trans + = animals weaned and transported, provided with feed and water, Trans - = animals weaned and transported, not provided with feed and water, Con = not wean or transported. N = 90 pigs.

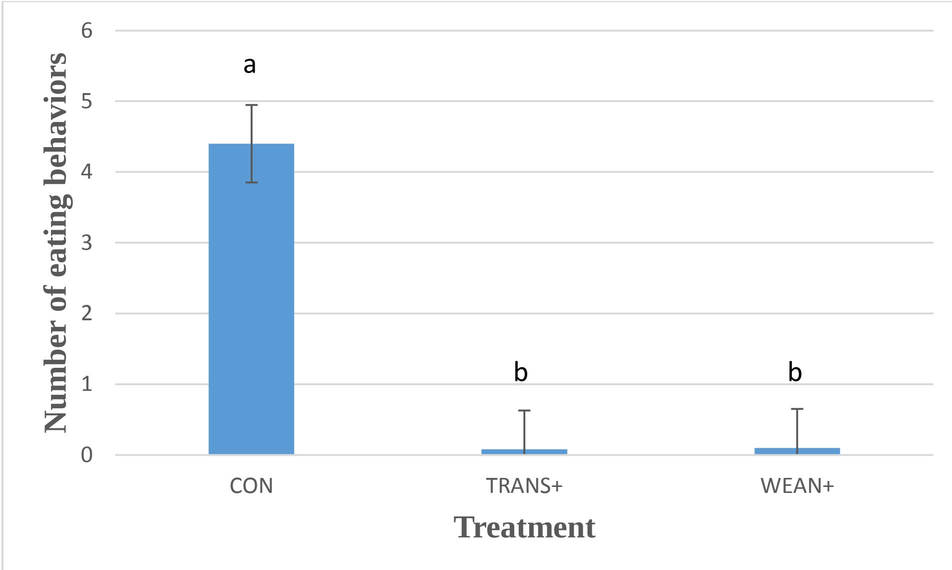


Superscripts differ P < 0.05.

There was a significant treatment effect on eating behaviors (P < 0.01). Control pigs had significantly more eating behaviors than both Trans+ and Wean+ pigs (P < 0.05). Trans+ and Wean+ pigs did not differ in their eating behaviors (Figure 7). Control pigs spent 18 % of their time eating during the 32 hr study. Wean+ pigs spent 0.43% and Trans+ pigs spent 0.35% of their time eating during the 32 hr study.

Experimental transport of segregated early weaned pigs showed lying and standing are the most prevalent behaviors during transport (Lewis et al., 2006). Lewis et al. (2006) reported that 75.6% of time in transit was occupied by lying and 21.6% by standing. It has also been reported that high levels of resting after weaning are common and can affect the level of lying during transport (Metz and Gonyou 1990). Sitting has been a behavior identified as a stress indicator (Dybkjaer 1992) and has been reported to be more common during the first 12 hrs of transport (2.8%) than in the second 12 hrs (0.3%) (Lewis and Berry 2006). Increased resting and decrease in sitting behavior later in transport may indicate that piglets have become habituated to some of the elements of transport (Lewis 2008).

Figure 7. Least Squares means ± 0.55 Least Squares means for number of eating behaviors for treatment groups during a 32 h transport study (P < 0.01). Treatment groups are abbreviated by Con = control, Wean + = animals weaned and not transported, provided with feed and water, Trans + = animals weaned and transported, provided with feed and water. N = 90 pigs.



Superscripts differ at P < 0.05.

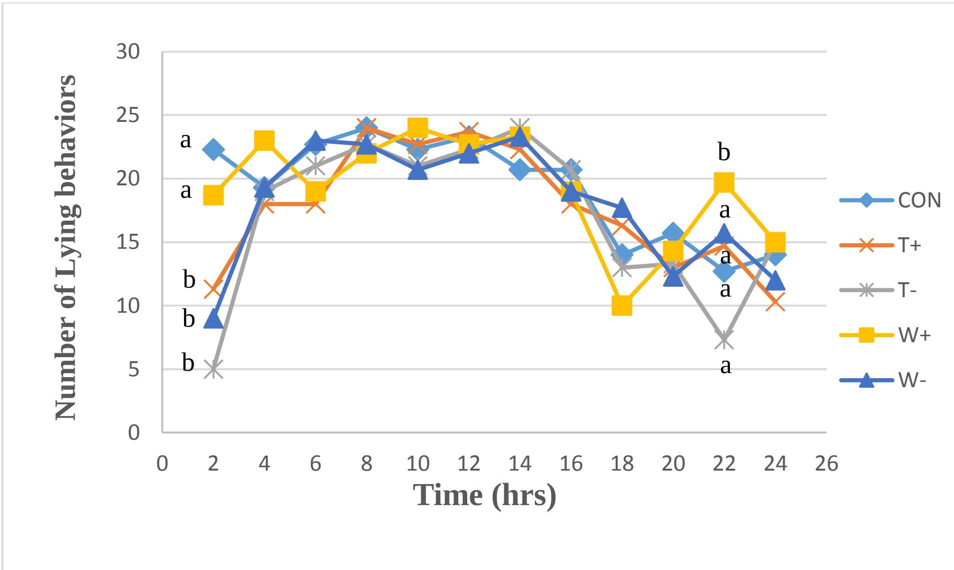
It is typical to see limited feeding in recently weaned pigs within the first day of weaning. Lewis et al. (2006) reported that feeding was infrequently observed (0.5%) on the first day of weaning whether pigs were transported or not, and has also been corroborated in other studies (Metz et al., 1990; Gonyou et al., 1998; Worobec et al., 1999).

3.3 Post-transport behavior

Overall, the Con group spent 81% of their time lying during the 24 hr post-transport period. The Wean+ treatment group spent 80% time lying, almost the same amount of time as the Con group. The Wean- treatment group spent 75% of their time lying. The Trans+ and Trans – treatment groups spent repectively 74% and 71% of their time lying,

There was a significant treatment by time interaction for lying behavior during the 24 hr post-transport period (P < 0.01). Lying behaviors were different by treatment at 2 hrs post transport and 22 hrs post-transport. At 2 hrs post-transport Wean+ pigs did not differ in lying behaviors from Con pigs, their number of lying behaviors were higher than the other treatment groups (Figure 8). Trans +, Trans-, and Wean- pigs differed significantly in lying behaviors compared to control pigs at 2 hrs post-transport, their lying behaviors were lower than Con and Wean+ pigs (P < 0.05). At 22 hrs post-transport all treatment groups were similar in lying behaviors to Con pigs except for Wean + pigs. Wean + pigs had a significantly higher number of lying behaviors than did the all other groups at 22 hrs post-transport (P < 0.05). From 4hrs – 20hrs and at 32 hr post-transport lying behaviors were similar among treatments.

Figure 8. Least Squares means ±1.96 for time by treatment interaction for lying behaviors in 2 hr intervals ($P < 0.05$). Treatment groups are abbreviated by Wean + = animals weaned and not transported, provided with feed and water Wean - = animals weaned, not transported, and not provided with feed and water, Trans + = animals weaned and transported, provided with feed and water, Trans - = animals weaned and transported, not provided with feed and water, Con = not wean or transported. N = 3.

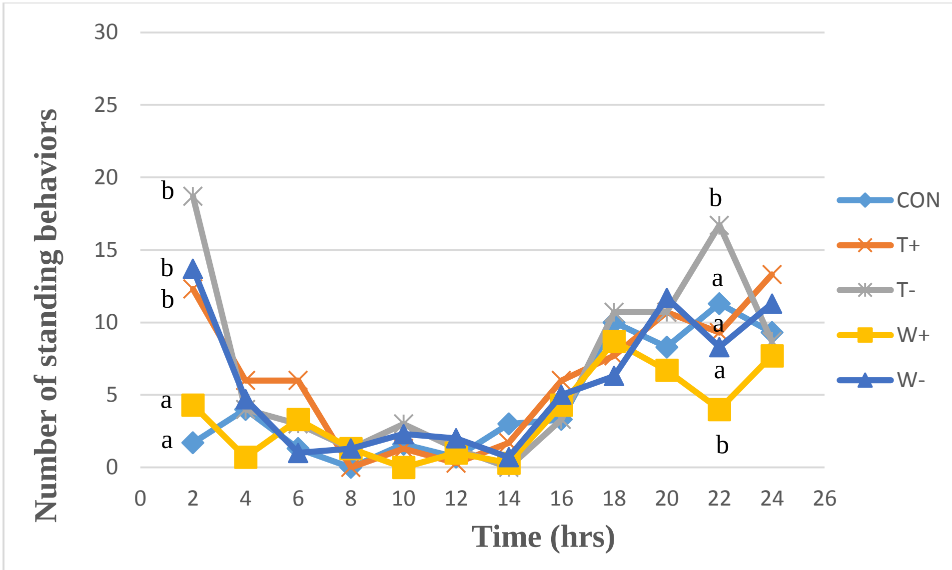


Superscripts differ at $P < 0.05$.

The Con group spent 19% of time standing after the 32 hr study. Trans- and Trans+ treatment groups spent respectively 28% and 26% of their time standing. The Wean- and Wean+ treatment groups spent respectively, 24%, and 15% of the time standing after the 32 hr study.

There was a significant treatment by time interaction for standing behavior during the 24 hr post-transport period ($P < 0.05$). Standing behaviors were different by treatment at 2 hrs post transport and 22 hrs post-transport. At 2 hrs post-transport Wean+ pigs did not differ in standing behaviors from Con pigs, their number of standing behaviors were lower than the other treatment groups (Figure 9). Trans +, Trans-, and Wean- pigs differed significantly in lying behaviors compared to control pigs at 2 hrs post-transport, their standing behaviors were significantly higher than Con and Wean+ pigs ($P < 0.05$). At 22 hrs post-transport all treatment groups were similar in standing behaviors to Con pigs except for Wean + and Trans- pigs. Wean + pigs had a significantly lower number of standing behaviors than did the all other groups at 22 hrs post-transport ($P < 0.05$). From 4hrs – 20hrs post-transport standing behaviors were similar among treatments.

Figure 9. Least Squares means ± 1.99 for time by treatment interaction for standing behaviors in 2 hr intervals ($P < 0.01$). Treatment groups are abbreviated by Wean + = animals weaned and not transported, provided with feed and water Wean - = animals weaned, not transported, and not provided with feed and water, Trans + = animals weaned and transported, provided with feed and water, Trans - = animals weaned and transported, not provided with feed and water, Con = not wean or transported. N = 3.



Superscripts differ at $P < 0.05$.

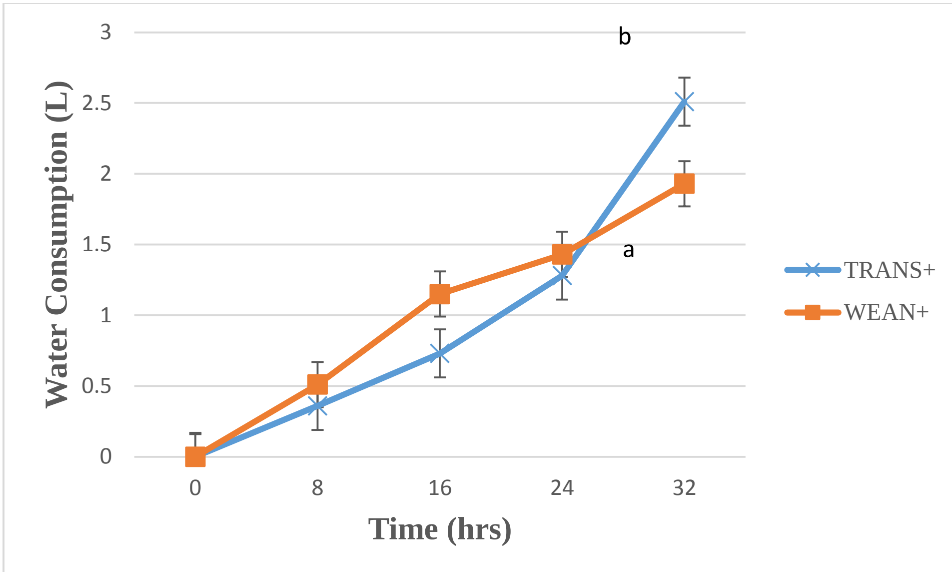
Only the Trans- treatment group displayed sitting behaviors post transport, but these behaviors were fairly low, at 1%.

After transport, standing frequency has been seen to drop and lying behavior frequency increases (Lewis 2008). Similar patterns have also been reported by Lambooy et al. (1985), attributing these behaviors to muscle fatigue. Siting behavior in the Trans- treatment group was minimal but it has been reported as stress measure (Dybkjaer 1992).

<u>3.4.1 Feed and water consumption during and after transportation</u>

There was a significant treatment by time interaction for water consumption during the 32 hr transport study ($P < 0.01$). Water consumption did not differ between Trans+ and Wean+ treatment groups from 0 hr to 24 hr (Figure 10). However, after 24 hrs the Trans+ treatment group drank significantly more water than the Wean+ treatment group ($P < 0.05$), respectively 2.51 ± 0.17 L and 1.93 ± 0.16 L by 32 hrs of transport.

Figure 10. Least Squares means for time by treatment interaction for water consumption during a 32 hr transport study (P < 0.01). Treatment groups are abbreviated by Wean + = animals weaned and not transported, provided with feed and water, Trans + = animals weaned and transported, provided with feed and water. N = 36 pen observations.



Superscripts without a common letter differ at P < 0.05.

Post-transport feed and water consumption was recorded at 7, and 14 days. No significant findings were found among treatments for feed and water consumption. Post-transport weights were taken at 1, 7, and 14 days. There were also no significant difference in weight among treatment groups.

Discussion:

Literature pertaining to effect of transportation on welfare of weaned pigs is limited (Villé et al., 1993, Geers et al., 1994, Hicks et al., 1998, Berry and Lewis, 2001, Lewis et al., 2005, Lewis and Berry, 2006 and Bench et al., 2008). Stress during transportation is inevitable and is generally aggravated by loading and unloading, vibration, coping with a new environment, restricted space, mixing of conspecifics, lack of ventilation, and deprivation of feed and water (Pineiro et al., 2007). The swine industry in North America has adopted a segregated early weaning (SEW) management system in which piglets are transported to facilities separate from housing of older pigs at an age (14-19 d) in which maternal antibodies are high (Lewis 2008). Adding weaning stress to transport stress has a higher impact on welfare of weaned pigs.

In the current study, weight performance was compromised with duration of transport and lack of feed and water. Physiological measures, such as blood glucose, N:L ratio, CK, and TP increased in response to prolonged stress. The degree of stress may vary in respect to duration of transportation, and the amount of time animals are deprived from feed and water. Water consumption in this study started as early as 8 hours of transport and water consumption was significantly higher by 32 hrs of transport, indicating that water can possibly be the reason for weaned and transported pigs provided feed and water has a lower percent loss in body weight than weaned and transported animals without feed and water.

Behavioral changes can also be indicative of stress. In the current study, significant changes in behavior were observed during and post- transport, with lying behaviors and standing behaviors being predominantly high in both phases. Theses finding can be substantiated by experimental transport of SEW piglets showing that lying and standing behaviors were the most prevalent during transport (Lewis and Berry 2006). Metz et al. (1990) reported that high levels of resting (79.6%) are common in recently weaned pigs, and may affect the level of lying.

Further studies are needed to determine whether feed or water can help maintain body weight. It has been reported that pigs provided with access to water on a 2-day journey did not consume adequate quantities of water and lost as much weight as pigs without water and therefore, providing water during transport was not an effective solution. (Lambooy et al., 1985).

Studies have shown that supplementing pigs with magnesium mica (MM) can aid in reducing blood cortisol levels as well as catecholamine concentrations (Kietzman et al., 1985, D'Souza 1998, 1999) and results in calmer pigs after long distance transportation (Kuhn et al., 1981). Additionaly, acute phase proteins (APP), including pig major acute phase protiens (Pig-MAP), hepatoglobin, serum amyloid A (SAA), and C-reactive protein (CRP) have recently been suggested as a good means of measuring animal welfare (Eckersall 2000; Murata et al., 2004), as these increase in response to inflammation due to tissue damage, infections, immunological disorders, or stress (Gaby et al., 1999; Murata et al., 2004).

In conclusion, there is limited research pertaining to transportation of weaned pigs and comparison of studies is sometimes difficult due to the variation in methodology, pig age/weight and densities studied (Lewis 2008). The transportation process is a stressful situation for pigs as they are exposed to long periods of transport, in many cases high stocking density, fasting, environmental factors, noise, mixing with conspecifics, injuries, and even death. Transportation death is painful and by no means an easy death, characterized by heart failure, and suffocation that may last from 10 minutes to 2 hours (Van Putten 1982). Thus, transportation is important in an animal welfare point of view and economically. Therefore, further research is needed to provide more insight on how to improve weaned pig welfare during transport.

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