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Ventilation system with water misting at the commercial slaughter plant on truck microclimate, physiological response and behavior of pigs

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Guimarães Rosa

RESUMO

PEREIRA, T. L. **Sistema de ventilação com nebulização no frigorífico sobre o microclima do veículo transportador, respostas fisiológicas e comportamentais de suínos**. 2018. 59f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2018.

Objetivou-se avaliar a efetividade da ventilação forçada associada à nebulização sobre as respostas comportamentais e fisiológicas de suínos mantidos dentro do veículo transportador antes do descarregamento na indústria frigorífica. Durante 6 semanas de transporte, 2 veículos pot-belly (PB) transportaram 191 suínos cada até a mesma planta frigorífica. Na chegada, ambos veículos permaneceram estacionados por 30 minutos antes da descarga. Um dos veículos PB foi posicionado ao lado do sistema de ventilação e nebulização (PBVM), enquanto o outro PB não teve acesso ao sistema de resfriamento (PBC). O tratamento (PBVM) consistiu em 10 minutos de ventilação (Espera 1); seguido de 10 minutos de ventilação e nebulização (Espera 2) e 10 minutos finais de ventilação (Espera 3). A temperatura do ar (T) e a umidade relativa (RH) foram monitorados. A frequência de suínos deitados e a frequência de acesso ao bebedouro foram calculadas. Temperaturas do trato gastrointestinal (GTT), lactato sanguíneo, hematócrito, creatina quinase e concentrações de cortisol foram avaliados. Durante a Espera 1, a ventilação forçada reduziu a temperatura do ar e o índice de temperatura e umidade no tratamento PBVM. Na Espera 3, a temperatura e o índice de temperatura e umidade foram menores ($P < 0.001$) no tratamento PBVM comparado ao PBC, contudo a umidade relativa do ar foi maior ($P < 0.001$). Suínos oriundos do compartimento 4 do PBVM apresentaram maior atividade durante o tempo de Espera 2 e 3, e reduzido tempo de latência para se deitar na baia do frigorífico ($P < 0.05$). Redução no GTT dos suínos oriundos do PBC foi observada após 1 hora de período de descanso comparada ao PBVM. O percentual de hematócrito tendeu a ser maior nos suínos PBC comparado ao PBVM. O sistema de ventilação e nebulização utilizado na indústria frigorifica antes do descarregamento melhora de forma eficiente as condições internas térmicas do caminhão e consequentemente o conforto térmico dos suínos mantidos no veículo transportador, com redução na temperatura dentro dos compartimento e baixo nível de desidratação no abate.

Palavras-chave: Comportamento. Metabólitos sanguíneos. Sistema de resfriamento. Suínos. Temperatura gastrointestinal. Transporte.

ABSTRACT

PEREIRA, T. L. **Ventilation system with water misting at the commercial slaughter plant on truck microclimate, physiological and behavior response of pigs**. 2018. 59f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2018.

The aim of this study was to evaluate the effectiveness of water misting with forced ventilation on a stationary trailer on internal vehicle ambient conditions, behavioral and physiological response of market pigs. During 6 weekly shipments, two identical tri-axle pot-belly (PB) trailers transported 191 pigs each (126 ± 5 kg BW) to the same slaughter plant. On arrival, both trailers were kept stationary in the yard for 30 min before unloading. One PB trailer was pulled over along a fan-misters bank (PBVM), while the other PB trailer had no access to this cooling system (PBC). The PBVM treatment consisted in 10 min of fan-assisted ventilation (wait 1) followed by 10 min of ventilation and water misting (wait 2) and final 10 min of ventilation (wait 3). The air temperature (T) and relative humidity (RH) were monitored. The frequency of lying pigs, the latency to rest and the frequency of drinking bouts were calculated. Gastrointestinal tract temperature (GTT) and exsanguination blood lactate, hematocrit, creatine kinase and cortisol concentrations were assessed. In wait 1, the application of the fan-assisted ventilation resulted in lower ($P < 0.05$) T and THI in the PBVM. In wait 3, T and THI were still lower ($P < 0.001$) in PBVM compared to PBC, but the RH was higher ($P < 0.001$). Pigs from compartment 4 of PBVM had a greater ($P < 0.001$) activity during waits 2 and 3, and a reduced latency to lie down in the lairage pen compared to the same compartment in the PBC ($P < 0.05$). A higher ($P < 0.05$) GTT drop was recorded until 1 h after lairage in pigs located in PBC compared to those transported in PBVM. At slaughter, hematocrit tended to be higher ($P = 0.08$) in blood of PBC pigs compared to PBVM. The fan-misters bank used on the PBVM vehicle sitting at the slaughter plant before unloading efficiently improves internal trailer thermal conditions and consequently thermal comfort of pigs kept in a stationary trailer, with reduced temperature within the compartments and lower dehydration condition at slaughter.

Keywords: Behavior. Blood metabolites. Cooling system. Gastrointestinal tract temperature. Pigs. Transport.

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SUMMARY

CHAPTER 1 – GENERAL CONSIDERATIONS

1. INTRODUCTION

Swine transport is a critical aspect of pork production, and is known to be a multi-factorial stressor for swine including novelty, physical exercise, unfamiliar mixing, and therefore a major concern from an animal welfare perspective and significant financial loss to the industry (RITTER et al., 2009). Within the effects of transport factors, road conditions, trailer microenvironment, traffic, transport time, waiting time at both the farm and slaughter plant, distance, and handling procedures have important role an effect on pig's welfare (SCHWARTZKOPF-GENSWEIN et al., 2012).

The occurrence of aspects listed above have impacts on welfare at different degrees but an important point has been thermal conditions during transport and the impact of weather conditions on the trailer thermal environment for on pigs thermoregulation. Previous research have reported the pigs has experienced thermal conditions of outdoor temperatures above 27 °C (80 °F), and therefore cooling methods are necessary to maintain core body temperature and homeostasis of market-weight pigs (10-21^{o}C) (MCGLONE et al., 2014).

Great challenges for maintaining thermally-acceptable conditions for pigs have been reported (BROWN et al., 2011; WESCHENFELDER et al., 2012) knowing that pigs do not sweat, being sensitive to heat stress, and therefore an appropriate recommendation of forced ventilation combined with a misting system may improve animal welfare, as reduce skin temperature by 10% (COLLEU; CHEVILLON, 1999), prevent increases in body temperature during short duration transport (FOX et al., 2014); lower lactate levels, reducing stress response of pigs transported in pot-bellied trailers (NANNONI et al., 2014).

An important critical point of pre slaughter period is the microclimate inside of pig´s trailer during the waiting period at the plant before slaughter (JIANG et al., 2016) where vehicle is stationary before unloading (FOX et al., 2014) and temperatures increase, what can be up to 6°C warmer than the external temperature (WESCHENFELDER et al., 2012) affected the percentage of dead pigs in the lorry on arrival (RITTER et al., 2009).

Thus, overall it is unknown about this combining (misting and ventilation) mainly in the wait period before unloading, therefore the objective of the present thesis was to evaluate the effect of water misting with forced ventilation in a stationary truck of pigs on trailer microclimate, behavioral and physiological response of market pigs.

2. LITERATURE REVIEW

2.1. Animal welfare

The question of animal welfare in pigs farming raises different opinions in technical, academic, and scientific groups. There is a marked disagreement between the behavioral needs of pigs and the living conditions offered to these animals, making swine management and breeding greatly debated and far from reaching consensus among the specialists (BRANDT; AASLYNG, 2015).

Since the domestication of pigs more than 9,000 years ago, there was a change in the human profile of hunter to animal livestock farmer, and consequently animals became dependent on humans (SORABJI, 1995).

With the confinement of these animals and the intensification of production systems, certain concerns about ethical care and the manner of creation were disclosed in the book "Animal Machines" by author Ruth Harrison in 1964, leading to an in-depth study with relation to animal welfare (FRASER, 2008).

Despite people's concern about the state of an individual during their attempts to adjust to the environment, as animal welfare concepts established by Broom (1991), few are willing to apply some rights that are described in the laws and policies of Animal welfare.

Society criticizes intensive animal production but is conniving with the existence of sterile or frustrating breeding environments, which provide smaller area of space per animal. This is opposed to aspects reported in the five freedoms of John Webster, namely: physiological (absence of hunger and thirst); environmental (adapted buildings); sanitary conditions (absence of diseases and fractures); behavioral (possibility of expressing normal behaviors); and psychological (absence of fear and anxiety) (FARM ANIMAL WELFARE COUNCIL, 1979).

In this perspective, there is an understanding of aspects, such as the physical and mental state of the animal, that animals have no motive, pain, or emotion, per concepts of Brambell Committee and in contradiction of the ideas of Descartes (1989) animals have not reason, pain and emotion, have presented increased in international and national panoramas. Based on that, Blokhuis (2008) - principles as good food, good health, expression of natural behavior, and good housing - refers to this to the livestock farming in non-stressful environments and free of suffering (VELARDE; DALMAU, 2012).

Therefore, it is necessary to know and measure certain behavioral, physiological, emotional, cognitive, and productive variables to determine the degree of animal welfare (FRASER, 2008).

2.2. Physiological state and thermal equilibrium of swine

Understanding the physiological interactions between animals and their thermal environment can be complex, but it is critical for effective production management. Adult swine present normal body temperature between 37.8 and 38.5°C (MADZIMURE et al., 2012). In addition, they are able to control their internal temperature when subjected to room temperature oscillations by means of thermoregulatory centers (such as the hypothalamus) and thermoreceptors in their skin and deeper tissues (RADOSTITS; MAYHEW; HOUSTON, 2002).

These individuals have a lower heat tolerance due to the reduced number of sweat glands that are keratinized, the great contribution of adipose tissue, and a high metabolism rate (LUDTKE et al., 2010).

The optimum temperature range can range from 12 to 18°C, in which body temperature remains constant with the minimum effort of the thermoregulatory system. The upper critical temperature zone is above 27°C (CURTIS, 1983). Upon reaching this temperature, the physiological mechanisms of the animals will be activated to minimize body heat production and dissipate the extra heat. On the other hand, the lower critical temperature is below 5°C, at which the heat production reaches its maximum value and begins to decline (SILVA, 1999).

When the ambient temperature is outside the range of lower and higher critical temperatures, the pigs adapt physiologically and/or behaviorally, depending on the degree of deviation from the critical level (ROBINSON, 2014).

When in cold stress situations, the pigs huddle and, thus, remain and seek warmer places. Moreover, in these situations, some physiological changes are observed, such as respiratory rate reduction, peripheral vasoconstriction, and piloerection, among others (RADOSTITS; MAYHEW; HOUSTON, 2002).

On the other hand, in heat-stress situations, pigs seek moist places, increase water intake, reduce food intake, and increase cardiorespiratory frequency with the aim of dissipating heat (WANG; LI; LEE, 2012). In addition, they promote behavioral changes, such as longer stay in lateral decubitus, a position that provides greater physical contact with the bay floor (SILVA, 1999).

In situations below the comfort zone, the pigs remain in sternal decubitus in order to reduce contact with the functional surface for less thermal exchange with the floor (SILVA, 1999).

From this perspective, outside the thermal comfort zone, swine heat exchange mechanisms with the environment are activated (CURTIS, 1983). Sensitive heat transfer is dependent on the temperature gradient between the swine and the environment. This transfer can occur through the processes of radiation, convection, and conduction (LOUW, 1993). Heat is acquired or lost through latent heat exchanges through evaporation or condensation of water, which depend on the vapor pressure gradient (CURTIS, 1983).

In the transfer by radiation, the heat is exchanged by means of electromagnetic waves. This transfer may depend on: 1) the temperature difference between the swine and the heat source; 2) the exposed body surface area; 3) the distance from the heat source; and/or 4) the color of the pig body skin. This mechanism occurs when the swine emits heat into a colder environment or absorbs the radiation under the waveform (LOUW, 1993).

In conduction transfer, heat exchange occurs by direct contact of the swine body with a surface and can vary in intensity according to: 1) the difference between surface temperatures; 2) the thermal conductivity; and 3) the area in contact with another surface (HUYNH et al., 2007).

Convective transfer can be defined as the heat exchange through air movement on the swine skin surface and may depend on both the relative air velocity and the temperature difference between the pig and the environment (RADOSTITS; MAYHEW; HOUSTON, 2002).

The main mechanism that causes heat loss for swine at elevated temperatures is evaporation - or respiratory evaporative cooling (panting) - in which heat exchange turns the liquid into gas. However, evaporation is dependent on some important variables, such as temperature and relative humidity (BRANDT; AASLYNG, 2015).

In this dynamic, it is ideal to understand that the sensible heat flow ceases to be effective in the homeothermic balance, as the ambient temperature approaches the body of the animal. When this occurs, pigs begin to use evaporative heat loss pathways in order to increase heat dissipation (MADZIMURE et al., 2012).

2.3. Impacts of thermal stress on pigs

The pigs present better zootechnical indexes in conditions of thermal comfort. Exposure of these individuals to elevated temperatures may lead to reduced metabolic rate, changes in cardiovascular and respiratory systems, altered vasomotor response, changes in behavior, and response in the general morphology of the animal (CURTIS, 1983).

In this context, considering that animals are exposed to thermal stress when the ambient temperature is above the thermal comfort zone and energy is expended to maintain body temperature, peripheral thermoreceptors coordinated by neural mechanisms will activate specific agents in order to promote vasodilation for increase of blood flow to the skin and limbs (BROWN-BRANDL; EIGENBERG; PURSWELL, 2013).

The elevation of the skin temperature and the projection of the central temperature towards the limbs increase the thermal gradient between the skin and the environment, which results in greater heat loss by irradiation and convection (LUDTKE et al., 2010). Studies have found that heat loss through the skin is also affected by aspects such as: skin and subcutaneous fat layer thickness, hair color, number of hairs per unit area, diameter, and angle of skin hairs (HUYNH et al., 2007).

If the vasodilatation response is insufficient to maintain the normal temperature, the evaporative cooling is increased. This cooling is the only heat loss process available when the ambient temperature exceeds the skin temperature (ROBINSON, 2014).

During transport, heat stress can aggravate other stressors, such as handling, animal density, and gathering of unfamiliar animals, among others. When pigs have open mouth breathing (panting) in an attempt to dissipate heat, effects such as muscle tremors, skin discoloration, abnormal vocalization, and fatigue - which in some cases makes them unable to stand up and walk– can classify them as "NANI animals", ie "non-ambulatory, not injured" (RITTER et al., 2009).

Such physiological changes trigger different responses, such as reduced food intake in order to decrease metabolic heat production and increased water intake in order to compensate for water loss due to respiratory evaporation (BROWN-BRANDL et al., 2001). If the pigs cannot recover and rest from the stress to which they have been subjected, the resulting metabolic changes can lead to death.

2.4. Heat stress

2.4.1. Behavior

Domesticated pigs have considerable backfat thickness, which gives them high insulation capacity and great heat sensitivity. Individuals are associated categorized as such by few and sparse hairs, the short snouts (SVC, 1997), ears with reduced vascularization, and circulatory systems with limited capacity (HÖRNING, 2007).

In this dynamic, the pigs produce high amounts of heat due to the muscular activity. Therefore, elevated temperatures produce behavioral changes such as, for example, longer stay of the pigs in the drinking fountain and lying laterally (KIEFER et al., 2009). A study with pigs performed in environments at 39.3 ± 0.1°C found that the pigs remained standing longer and agitated compared to the animals allocated at a temperature of 20.6 ± 0.1°C for 30 minutes (SAPKOTA et al., 2016).

When placed in facilities with water or mud, pigs tend to look for wet surfaces or lie in their own feces or urine in an attempt to dissipate heat by conduction (EFSA, 2012). This wallowing behavior can reduce the animal's body temperature by up to 2°C (HÖRNING, 2007).

In studies on thermal stress caused by transport, postural behavior may indicate if pigs are experiencing discomfort (CURTIS, 1983). In this situation, Fox et al. (2014) observed an average proportion of 47.75 ± 1.70% of time in which the pigs remained standing during the transit. They found that with the temperature increase, there was a reduction in the number of pigs that remained standing.

The presence of human observers alters the animal's natural behavior in a given environment, causing difficulties for observations and studies conducted in some circumstances, such as transport. Video assessments can provide accurate information over a given period of time (NANNONI et al., 2014).

Thus, it is natural for pigs in a high temperature environment to reduce their activity in order to minimize body production, which can reduce the time the animal stays in the standing position. This tends to aggravate possible urinary infections, as well as impair performance and compromise well-being, implying behavioral and physiological changes (BRACKE, 2011).

2.4.2. Body temperature

Although the recommended temperature range for pigs in the finishing stage is between 10 and 25 ° C, pigs often experience situations of thermal stress, resulting in increased respiratory rate and, consequently, higher temperature body (SAPKOTA et al., 2016).

Monitoring the pig body thermal status is important in assessing how they are adapting to environmental conditions. Different body parts can provide data for measuring body temperature, such as the ear, skin, rectum, and bladder. Some are more invasive, while others more susceptible to differences when located near the body surface (HANNEMAN et al., 2004).

The skin temperature has been measured by data loggers placed in either the ear or in the gastrointestinal tract using radiant thermometers or thermographic images, in addition to the rectum via probe, among other forms (BROWN-BRANDL et al., 2012).

The gastrointestinal tract temperature is closer to the central body temperature and has been used to assess the impact of thermal stress and thermoregulatory changes of pigs (FOX et al., 2014). Gastrointestinal tract temperature values for pigs transported during the summer for 12 hours (40.6 ± 0.1 ° C) were higher than when transported over 6 and 18 hours (39.8 ± 0.1; 40.0 ± 0.1°C, respectively) (GOUMON et al., 2013).

The gastrointestinal tract temperature (GTT) of the pigs observed during transportation in a potbelly vehicle was 39.73 ± 0.09ºC, showing an increase of GTT from loading to departure and a reduction after arrival to the cold plant (FOX et al., 2014). These temperature increases during loading were also reported by Weschenfelder et al. (2012) when comparing two types of vehicles, the potbelly and the flat deck, and the observed delta values were 0.18 ± 0.04 and 0.06 ± 0.04, respectively.

The thermal variation of the basal temperature (animal rest time) in a determined event has been reported by several authors as a delta value (ΔGTT). Delta temperature values were higher in summer than in winter for pigs observed during transport in potbelly type vehicles (0.31 ± 0.05; -0.007 ± 0.05), respectively. These values occur at high environmental temperatures, as well as other concomitant stressors, such as loading and increased human-animal interaction during unloading (CONTE et al., 2015).

Due to the high temperatures and absence of airflow when the vehicle is parked, such as loading moments, waiting time in the yard, and unloading, several authors reported an increase in the pig gastrointestinal tract temperature during periods of greater stress (CARR et al., 2008; TAMMINGA et al., 2009). There was also an evident decline when transport occurs with natural ventilation (WESCHENFELDER et al., 2012).

In fact, the sympathetic vasodilator can elevate blood flow in the skin, increasing convective heat transfer from the nucleus to the periphery. For this reason, the gastrointestinal tract temperature is important to indirectly indicate the variation of the metabolic rate of pigs and such changes with a physiological impact on the pigs (WESCHENFELDER et al., 2012).

2.5. Physiological blood changes

2.5.1. Creatine phosphokinase

Elevations in levels of lactate, creatine phosphokinase (CPK), and cortisol concentrations were verified in conditions of heat stress and management (FOX et al., 2014).

In order to understand the increase in creatine kinase (CK) activity in certain situations, it is necessary to brief explain the context of the subject. This specific muscle-skeletal enzyme has the primary function of reversible phosphorylation of creatine at the expense of ATP for the formation of creatine phosphate. When resting, creatine phosphate is produced by phosphorylation at the expense of transferring the phosphate group from ATP, and the reverse reaction is produced during muscle activity in ATP synthesis (GREGORY, 1998).

Creatine kinase values can be altered in situations of vigorous exertion or muscle damage, which makes it an indicator of adaptation to exercise, even with a minimum of cellular injury (SUTHERLAND; MCGLONE; BACKUS, 2014). In fact, CK measured in the pig serum or blood plasma is reported to be 2.4 to 22.5 UI/L (WEISS; WARDROP, 2010), 865 ± 220 UI (634-1148) (PREUS et al., 1989) or have values up to 1100 IU/L, which are considered normal (WARRISS et al., 1998).

The activity of creatine kinase is an important indicator of long-term stress, as it reaches its peak within 6 hours after physical stress and does not return to the basal level for 48 hours (CORREA et al., 2013).

Pig transport during the summer in potbelly vehicles may show elevations in the concentration of this enzyme with values of 4424 U/L (3324-5888) when compared to winter transport conditions 2734 U / L (2054-3639). This suggests that heat stress played a significant role, leading to an increase in muscular effort (SOMMAVILLA et al., 2017). On the other hand, Correa et al. (2014) reported lower levels of this enzyme concentration in the summer than in the winter with 4605 and 6865 U/L, respectively. This could be related to the greater number of falls and slips during the loading, that is, to a greater muscular fatigue during the winter.

No differences were observed in the CK enzyme concentration level when animals transported in the potbelly vehicle were housed in compartments 1, 2, 3, and 4. These presented values of 5695 U/L. In compartment 5, the CK values measured were 5892 U/L. In compartments 7 and 8, they were 4760 U/L. Finally, in compartments 9 and 10, the calculated values were 6327 U/L (CORREA et al., 2014).

Elevated CK blood levels are caused by an increased demand for mitochondrial phosphocreatine to replenish ATP for muscle activity. The disadvantage of measuring enzyme activity using serum instead of plasma is the greater occurrence of hemolysis in the serum, especially in stressed animals (GREGORY, 1998).

Work with forced ventilation and nebulization after transport is scarce. When evaluating broilers transported for 45 minutes to the plant unit and submitted to 15 (fifteen) minutes of nebulization and forced ventilation after transportation, Jiang et al. (2016) observed a reduction in creatine kinase level to 3.40 ± 0.67 U/mL. From this single study, these authors demonstrated that the application of this system during this period was beneficial to reduce transport stress at a temperature of 32°C with 56% relative humidity, being the conditions under which the birds were prepared.

2.5.2. Lactate dehydrogenase

Lactate dehydrogenase (LDH) is an enzyme of the Oxidoreductases class, having an active site formed by arginine 171 and histidine 195 residues. As it is present in various body tissues, its activity level is higher in the swine muscles. When muscle damage or vigorous physical exertion occurs, this enzyme is released from the sarcoplasm into the bloodstream (VOET; VOET, 1995). Since it is an indicator of physical stress, the blood lactate has a peak at 4 minutes and returns to the basal level 2 hours after exercise (ANDERSON, 2010) for values ranging from 160 to 425 U/L (BRANDT; AASLYNG, 2015).

When animals are in situations of intense physical exercise, danger, escape, or are exposed to temperature differences, epinephrine is released from the adrenal medulla. During glycogenolysis, glucose is produced as glucose-6-phosphate (G-6-P) by the breakdown of glycogen into skeletal muscles, and muscle cells receive ATP as a source of energy. In anaerobic conditions, production of lactate from pyruvate involves the conversion of NADH into NAD+ and is catalyzed by the enzyme lactate dehydrogenase (LDH) (EC 1.1.1.27) (LEHNINGER; NELSON; COX, 2006).

In conditions requiring intense physical activity (as in transportation), several authors reported a high level of blood lactate in the pigs that were transported in the summer in potbelly type vehicles when compared to those transported in the winter, being 17.8 vs 16.3 mmol L^{-1}, respectively (CORREA et al., 2013). On the other hand, in a later study by Sommavilla et al. (2017), the authors found higher lactate levels in swine transported during the winter when compared to those transported during the summer, being 20.72 vs 16.53 mM. The high levels detected in this study are due to the effects produced by low temperatures and shorter trips.

The increase in lactate concentration in pigs transported in different vehicle types was observed by Dalla Costa et al. (2016). Values close to 14.92 ± 0.52 mM were found, being above normal (4 mM), which evidenced a fatigued status (EDWARDS et al., 2010).

Lactate is commonly measured in plasma or serum by enzymatic analyses. The measurement process can be time consuming when harvesting during bleeding. Thus, a portable device called the Lactate Scout Analyzer allows for real time and quick results (15 sec) with a strong correlation (r = 0.97) between the blood lactate levels obtained by means of the enzymatic analytical method and this apparatus (EDWARDS et al., 2010).

Evidence of increased pig muscle activity during handling was described by Rocha et al. (2015). The authors found that lactate levels were 3.66 mM, varying from 3.50 to 3.83 mM during unloading; 2.88 mM after the waiting time; and 5.00 mM and 8.71 mM when conducted in group for desensitization and bleeding, respectively.

2.5.3. Cortisol

Produced in the fasciculate zone of the adrenal cortex, cortisol is the main glucocorticoid and is secreted as ACTH (adrenocorticotropic hormone) from the anterior pituitary, which is regulated by the corticotrophin-releasing hormone (CRH) in the hypothalamus. When the cortisol concentration rises, it is reduced to a normal level by an inhibitory effect of CRH in the hypothalamus and of ACTH in the anterior pituitary (MILLS et al., 2010).

The activation of the hypothalamic centers can occur in situations in which the individual perceives threats to homeostasis, that is, in situations of non-specific stress. This biological response to an event may trigger the activation of the sympathetic nervous system and the adrenal medulla. These promote the release of catecholamine, such as adrenaline and noradrenaline into the bloodstream, which accelerates the degradation of glycogen in the liver and, consequently, provides an increase in glucose supply (HEWAGALAMULAGE et al., 2016).

The stressor factor (such as the packing of pigs at elevated ambient temperatures) may raise the level of circulating cortisol, increase the amino acid content in the bloodstream, decrease glucose utilization, and elevate gluconeogenesis in the liver (WARRISS et al., 1998).

It is known that the interaction between animals and humans during management and stressful activities, such as pig transport, can raise the circulating cortisol level (DALLA COSTA et al., 2016; JAMA et al., 2016). The peak occurs approximately 20 minutes after an event and circulating ACTH levels decrease within 1 or 2 hours, varying according to duration (COOK et al., 1997).

Intensive secretions are indicators of stress due to transport (JAMA et al., 2016) and may cause vasoconstriction at elevated ambient temperatures. This can make it difficult to dissipate heat. Thermal stress, due to insufficient ventilation in the compartments where the pigs are transported, causes physiological reactivity of the nervous system, as well as making muscles become overactive. These responses may trigger glycolysis and block its uptake by tissues, which leads to undue catabolic activity, cause hyperthermia, and lead to the production of lethal metabolites in the blood (LEHNINGER; NELSON; COX, 2006).

2.6. Biometeorological indexes

The thermal environment interacts with factors related to the processes of heat flow between the animal and the environment. The most important climatic elements with direct action on domestic animals are temperature, solar radiation, wind, rain, and humidity. These cannot be individualized, and they constitute a climatic complex (BROWN-BRANDL; EIGENBERG; PURSWELL, 2013).

2.6.1. Ambient temperature

The thermal environment is a key point in livestock farmer due to the comfort and development of physiological processes of the animals (LUDTKE et al., 2010).

When referring to the pigs, individuals conditioned at elevated temperature and humidity present difficulties with heat dissipation (WANG; LI; LEE, 2012). Consequently, certain reactions have been observed within these organisms (COLLEU; CHEVILLON, 1999), such as increased respiratory rate and rectal temperature, changes in urinary excretion, which eventually result in dehydration, depletion of glycogen stores, alteration in acid-base balance, and even death (BROWN et al., 2011).

To better describe the thermal environment experienced by the animal, studies suggest the use of sensors, such as data loggers and digital thermometer (iButton® model), that can provide the information in intervals of seconds and should be located as close as possible to the animal (WESCHENFELDER et al., 2013).

Studies on air temperature evaluations performed using iButton® data loggers in the vehicle potbelly the observed the following thermal conditions: 16.92 ± 1.17ºC at loading; 24.01 ± 0.78ºC at the beginning of transport; 24.08 ± 1.15ºC on arrival; and 25.24 ± 1.31ºC during unloading (FOX et al., 2014). Minimum differences in temperatures within the potbelly vehicle are verified with these sensors, such as 22.9 ± 1.7ºC (12.5 to 40.1ºC) in the summer and 14.3 ± 1.7ºC (28.8 to 1.9ºC) in winter (SOMMAVILLA et al., 2017).

Undesirable temperature conditions during pig transport in the United States were reported by Xiong, Green e Gates (2015). The external temperature exceeded 27°C (80°F) during transport, and the pigs experienced various heat stress situations. It has been found, for example, that in more than 60% of the time, the measurements recorded extreme temperatures, above 35°C (95°F), in the vehicle during these trips.

Such situations are common in Brazil because most of the country is located in low latitude areas, where hot and humid climates prevail. As a result, animal transport care has been discussed by some authors such as Silveira (2010), Santiago et al. (2012), and Santos et al. (2013).

Due to its extensive territory and wide variety of climates, pork transport studies in Brazil, such as Dalla Costa et al. (2017) in Rio Grande do Sul, observed average temperatures of 14°C (averages from 13 to 19°C). Pereira; Corassa (2014) also recorded average temperatures of 24.70ºC in Mato Grosso.

In general, the air temperature in the compartments of a particular carrier is reflected in the balance between the heat produced by the pigs and the decrease of heat by the natural flow of air passing through the truck. Therefore, ventilation can adequately control internal temperatures, which will consequently impact the biological processes of animals (WARRISS, 1998).

2.6.2. Humidity

Humidity is the amount of water vapor present in the air, that plays an important role in air quality and climate control (BARCELLOS et al., 2008) can be expressed as relative humidity, which is the ratio of the current absolute humidity relative to the highest possible absolute humidity (which depends on the current air temperature) indicating the calculate from dew point and temperature measurements (HAHN et al., 2009).

Different authors recommend the ideal relative humidity level in pork production should range between 45 and 80% (BRANDT; AASLYNG, 2015), 60 to 80% for Barcellos *et al.* (2008); 70% for Madzimure et al. (2012); 45 to 75% for Brandt and Aaslyng (2015); or even 50 to 70%, in most practical circumstances, according to Bottcher, Matthis and Roberts (2001).

It is clear that the rates of humidity can affect the welfare of the pigs. For example, a higher the level of humidity in the air makes the evaporative cooling process the less effective (WOLP et al., 2012). In fact, the possibility of mortality increases when pigs are submitted to thermal stress, as observed by Fox et al. (2014), whom recorded deaths during transport in vehicles that did not have a sprinkler, as well as verified humidity data of 90.20 ± 2.10% at loading, 83.15 ± 2.50% at departure, 65.50 ± 4.00% at arrival, and 65.55 ± 4.30% at unloading.

Humidity variations from 62 to 90% were found to be stressful to swine females, leading to behavioral changes, such as longer stay in lateral decubitus and less frequent contact with the other individuals of the bay (MADZIMURE et al., 2012). Similarly, fewer animals were recorded as standing during transport and more pigs were laying down in order to allow heat exchange with cold surfaces, being the vehicle floor, when moisture levels ranged from 65 to 90% in carrier vehicles (FOX et al., 2014).

Within a potbelly vehicle, the moisture contents were measured at different times: beginning of loading, waiting at the farm, beginning of transport, and at the beginning and end of unloading. There registered values were 78.76; 75.69; 65.14; 71.50; and 71.90, respectively. From these data, it is assumed that the natural ventilation of the vehicle in transport reduced the moisture content (WESCHENFELDER et al., 2013).

Considering that the mass of air enters the carrier vehicle through the rear, which gradually becomes warmer and moister, the frontal compartments end up getting less cooled (BROWN et al., 2011). Cases of pig skin discoloration, mouth breathing, and even individuals who were unable to walk due to physical exhaustion can be found, being a consequence of the thermal stress (RITTER et al., 2009).

The environment humidity level determination is of the utmost importance, and there are numerous types of sensors applied to measure the relative air humidity, including the wet bulb thermometer, the acoustic surface wave sensor, the polymer-based sensor, lithium chloride based sensor, and thermal conductivity sensor, and the infrared absorption hygro-meter (BRANDT; AASLYNG, 2015).

2.6.3. Temperature and humidity index (THI)

The Temperature and Humidity Index (THI) combines several thermal parameters into a single quantifiable measurement to characterize environments. This approach relates air temperature and humidity through a simple linear adjustment of the dry bulb and wet bulb thermometer measurements as ITU = Ta + 0.36 Tpo + 41.5 (Ta, air temperature, and Tpo, Dew point temperature) (BACCARI JR., 2001; THOM, 1959).

The current development of this thermal index includes swine thermoregulation ability as its main focus. This is considered important in conditions of high temperatures in hot climates, which was well observed by Machado et al. (2014). During transport in Mato Grosso do Sul, the authors observed an index of 70.03, which is above the thermal comfort range between 59 and 65.

Regarding the THI applicability, there are other index classifications: normal is up to 70; critical is between 71 and 78; hazard is from 79 to 83; and emergency is above 83. According to these classifications, data obtained during transport recorded that pigs remained 46 minutes in dangerous situations and 34 minutes in conditions considered emergencies during long distance transport (VILLARROEL et al., 2011).

The THI measurement began with Thom (1959), as an attempt to measure the discomfort rate in humans, but it has been used extensively in animal production. It is stated that the critical THI values for the finishing pig may range from 38 to 80 (ROLLER; GOLDMAN, 1969).

With the use of the NRC formula (1971): THI = [({T - [0.55 - (0.0055 × RH)]} × [(1.8 × T) - 26])-32]*5/9, Fox et al. (2014) verified values at the beginning of transport, arrival, and unloading, being 22.4; 22.0; and 23.0, respectively. These data reflect the temperature data of each event. It can be concluded from these that the temperature never exceeded the alert value of 23.9ºC established by Haeussermann et al. (2007) during the evaluation periods.

The ambient temperature cannot be evaluated in isolation, and when indices combine with the air temperature measurement, they may represent the effect produced by the heat exchanges between the animals and the thermal environment (HAHN et al., 2009). There are several proposed equations, but pondering the results can help in determining a stressful environmental condition. While results of THI $\leq$ 74 are considered normal, alert situations occur for THI values equal to or above 75, dangerous conditions for THI values from 79 to 83. THI values equal to or above 84 indicate an emergency (NWSCR, 1976).

In determination of which indexes fit the experimental model, the evaluation of the cooling systems can be promoted. This is based on the analysis of the other parameters, such as animal behavior, to understand the efficiency of a system in function of the animal comfort (BRANDT; AASLYNG, 2015).

2.7. Use of the ventilation fan-misting bank to cool-off pigs

When pigs are subjected to warm area of more than 30^{o}C (e.g. in a transport vehicle), air renewal through artificial ventilation or nebulization is necessary (SCHWARTZKOPF-GENSWEIN et al., 2012). This should be done in order to control the ambient temperature, reduce thermal stress, improve welfare conditions, and, consequently, obtain corresponding productive responses in animals (HANNAS, 1999).

Evaporative or latent heat loss in individuals who do not transpire can be induced by sprinkling (pressure > 5MPa), a process in which the skin surface of the animal is wetted or by misting (pressure $\leq$ 5 MPa), systems of lower pressure with small drops of water that are sprayed in the air, causing cooling when they evaporate. This method, associated to mechanical ventilation, promotes greater air movement, and reduction in the internal ambient vehicle temperature and, consequently, in the animal. However, it should be used mainly in the hottest hours of the day, during which relative humidity is low and temperatures are higher (HAEUSSERMANN et al., 2007).

The heat load reduction can be achieved by means of a forced ventilation evaporative cooling system (mist), as used by Jiang et al. (2016) in an attempt to reduce stress after transportation of broilers. In their trial, the authors observed that the group that stayed 15 minutes under forced ventilation and nebulization had a lower level of corticosterone (120 ng/dl) after 45 minutes of transport as compared to the cases in which treatments did not alter the microclimate.

In fact, when the animal is submitted to transport, there is the action of a so-called natural ventilation, which promotes air renewal. However, when the vehicle stops or arrives in the refrigerator, the temperatures rise inside the vehicle, and there is no air exchange, which affects the welfare of the pigs (WARRISS et al., 1998).

When the pig arrives to plant, it remains inside the transport vehicle from minutes to hours. Upon arrival in the waiting yard, there is a need for artificial ventilation like misting, in which the evaporation of small droplets (mist) may increase the latent heat content of the environment and improve the sensitive heat loss of the swine caused by a higher temperature gradient (BRANDT et al., 2015).

In fact, spraying nozzles of water mist associated with artificial ventilation promote change in the microclimate, such as reduction in ambient temperature (JIANG et al., 2016; HAEUSSERMANN et al., 2007) and decrease in mortality during transport at high temperatures (SUTHERLAND; MCDONALD; MCGLONE, 2007), when applied at temperatures above 20°C for ventilation and at 25°C for nebulization (CHRISTENSEN; BARTON-GADE, 1999).

Studies are scarce with regard to forced ventilation and nebulization in pork transport (NIELSEN, 1982; CHEVILLON, 2000). When pigs are put in the carrier, regardless of the time of year, the microclimate of each compartment will depend on the natural ventilation created by the movement of the vehicle. Therefore, when using mechanical ventilation systems during the waiting time in the plant, the removal of extra heat and moisture will be promoted in an attempt to maintain the thermoneutral range (CURTIS, 1983).

2.8. Transport of pigs in potbelly vehicles

The carrier vehicle design directly affects the occurrence of impacts, vibrations, and displacement of the animals, which can affect mortality during transport (DALLA COSTA et al., 2016).

The potbelly vehicle design is used in both the United States and Canada. In this type of high-capacity vehicle, the potbelly truck (PB) can transport more than 200 pigs on three floors (two floors with four compartments each and a floor called the belly with two compartments) with 10 compartments each (WESCHENFELDER et al., 2013).

The main disadvantage of the potbelly vehicle is the internal ramps that can have angles of inclination up to 40 degrees. This makes handling difficult during loading and unloading, as well as exacerbates the use of electric shock, which causes changes in behavioral and physiological responses (SCHWARTZKOPF-GENSWEIN et al., 2012).

Respiratory distress in pigs transported in the lower compartments (belly) of potbelly vehicles was reported by Correa et al. (2013). Their study found that the physical exercise required on the ramps promoted muscular exhaustion, affecting the final product qualitatively and quantitatively, in regards to the mortality during transportation and the increased frequency of tired pigs, who were unable to walk on arrival to the plant industry.

The well-being of pigs in the potbelly vehicle may be affected by the following: elevated heart rate when induced to climb ramps (GOUMON et al., 2013); increase in blood cortisol and creatine kinase levels (SOMMAVILLA et al., 2017); and even high blood lactate concentration during bleeding, resulting from physical exertion (CORREA et al., 2013).

There are large and small side vents that are holes throughout the vehicle. On the small side, they allow a minimum air flow. The larger openings promote greater ventilation and promote improvement in thermal comfort during summer transport (XIONG; GREEN; GATES, 2015). McGlone et al. (2014), when evaluating 302 potbelly vehicles carrying 48,133 pigs, concluded that the lateral opening should increase as the ambient temperature increases, and they also concluded that in thermal environments from 5 to 26°C, the opening levels had no impact on pig mortality in transport.

However, airflows within the compartments may be distinct and some may have elevated temperatures and humidity, such as in the frontal, middle, and lower belly compartments (BROWN et al., 2011; WESCHENFELDER et al., 2012). In fact, Fox et al. (2014) and Nannoni et al. (2014) promoted water sprinkling for five minutes before leaving the farm, during the waiting time, and before unloading to improve the thermal environment at temperatures above 20°C. As a result, they observed a reduction in water intake during standby, physical fatigue, and blood lactate concentrations.

Obtaining an ideal towing design that meets a suitable thermal environment can be challenging (ELLIS et al., 2010). Changing vehicle design, as suggested by several authors, may be a goal that can drag on for the long term (XIONG et al., 2015). For this reason, options that promote adequate cooling in the vehicle environment with the use of mechanical ventilation and/or the use of nebulizers, especially in periods when there is no natural ventilation within the trailer, can become viable and positively affect the thermal comfort of the pigs.

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CHAPTER 2 - APPLICATION OF A VENTILATION FAN-MISTING BANK ON PIGS KEPT IN A STATIONARY TRAILER BEFORE UNLOADING: EFFECTS ON TRAILER MICROCLIMATE, AND PIG BEHAVIOR AND PHYSIOLOGICAL RESPONSE *

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ABSTRACT: The aim of this study was to evaluate the effectiveness of water misting with forced ventilation on a stationary trailer on internal vehicle ambient conditions, behavioral and physiological response of market pigs. During 6 weekly shipments, two identical tri-axle pot-belly (PB) trailers transported 191 pigs each (126 ± 5 kg BW) to the same slaughter plant. On arrival, both trailers were kept stationary in the yard for 30 min before unloading. One PB trailer was pulled over along a fan-misters bank (PBVM), while the other PB trailer had no access to this cooling system (PBC). The PBVM treatment consisted in 10 min of fan-assisted ventilation (wait 1) followed by 10 min of ventilation and water misting (wait 2) and final 10 min of ventilation (wait 3). Within each PB trailer, 4 out of the 10 compartments (rear top deck [C4], front middle deck [C5], center middle deck [C7], and front bottom deck [C9] compartments) were chosen for the data collection. The air temperature (T) and relative humidity (RH) were monitored using iButtons data loggers. The frequency of lying pigs, the latency to rest and the frequency of drinking bouts were calculated. Gastrointestinal tract temperature (GTT) and exsanguination blood lactate, hematocrit, creatine kinase and cortisol concentrations were assessed. In wait 1, the application of the fan-assisted ventilation resulted in lower ($P < 0.05$) T and THI in the PBVM compared to the PBC. A greater ($P \leq 0.05$) drop in RH was recorded in C5, C7, and C9 of the PBVM compared to the same compartments in the PBC. In wait 3, T and THI were still lower ($P < 0.001$) in PBVM compared to PBC, but the RH was higher ($P < 0.001$). Pigs from C4 in the PBVM had a greater ($P < 0.001$) activity during waits 2 and 3, and a reduced latency to lie down in the lairage pen compared to the same compartment in the PBC ($P < 0.05$). A higher ($P < 0.05$) GTT drop was recorded until 1 h after lairage in pigs located in PBC compared to those transported in PBVM. At slaughter, hematocrit tended to be higher ($P = 0.08$) in blood of PBC pigs compared to PBVM. Although the efficiency of the fan-misters bank varied by compartment location, overall this cooling system appears effective to improve the trailer internal thermal environment and thermal comfort of pigs kept in a stationary trailer.

Key words: behavior, blood metabolites, cooling system, gastrointestinal tract temperature, pigs, transport

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1. INTRODUCTION

In-transit losses, including dead and non-ambulatory pigs, have been reported to increase during the summer hauls (SCHWARTZKOPF-GENSWEIN et al., 2012; CORREA et al., 2013). This risk appears to be exacerbated by keeping pigs in a stationary vehicle during the wait before unloading (RITTER et al., 2006) as temperatures in some compartments of the trailer can be from 6 to 8°C warmer than the external temperature (WESCHENFELDER et al., 2012; FOX et al., 2014). As suggested by Brown et al. (2011), in summer, pigs in stationary trailers can be cooled by increasing the ventilation rate in combination or not with water misting. A previous study showed that the application of water sprinkling on a stationary trailer reduces the temperature, but also increases the relative humidity inside the vehicle (FOX et al., 2014). External fan banks used in alternative to a trailer model featuring in-built fans may be a useful and cheaper cooling technology. The use of external forced ventilation through fan banks combined with water misting may not only reduce the temperature of the trailer, but also help remove the excessive humidity produced by water misters increasing pigs' evaporative cooling and improving their thermal comfort. This cooling system was already shown to improve the welfare and meat quality of broilers kept in a stationary truck during the wait (45 to 60 min) before slaughter (SIMÕES et al., 2009; JIANG et al., 2016). However, its efficiency has never been studied in pigs as yet.

Therefore, the aim of this study was to evaluate the effectiveness of combining water misting with forced ventilation on a stationary trailer on internal vehicle ambient conditions, and behavioral and physiological response of market pigs.

2. MATERIALS AND METHODS

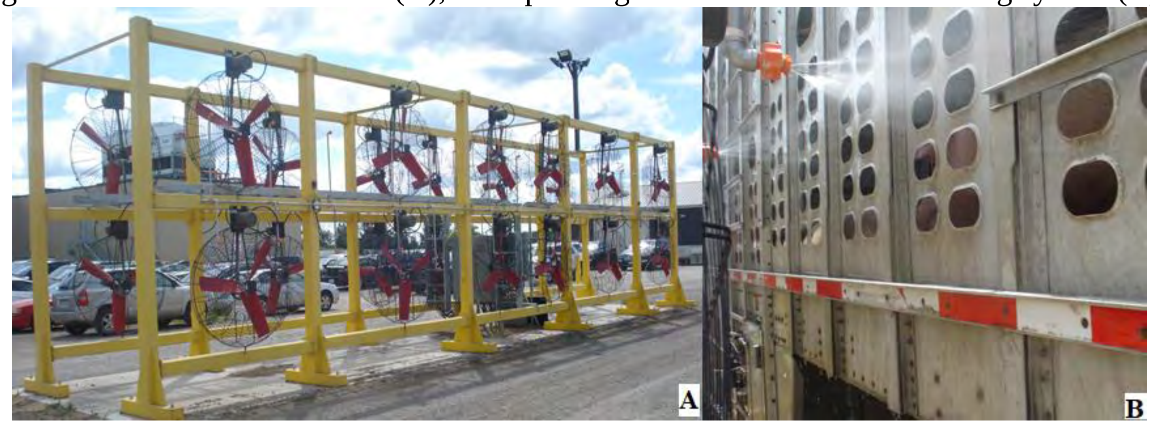
All experimental procedures performed in this study were approved by Agriculture and Agri-Food Canada (AAFC) Animal Care Committee in Sherbrooke (QC) based on the current guidelines of the Canadian Council on Animal Care (2009) (n° 507). The commercial slaughter plant is located 43°47'34"N (latitude) and 80°23'21.4"W (longitude) and at 325 m above sea level.

2.1. Animals and treatments

During the course of 6 weekly shipments (from July to August), a total of 2,292 pigs, evenly distributed into two identical naturally ventilated tri-axle pot-belly (PB) trailers of 191 pigs each (126 ± 5 kg BW), were transported by two drivers from the same commercial finishing farm to the same commercial slaughter plant (135 km, average travel time of 112 ± 6 min) in a randomized complete block design. To avoid the confounding effects of handling and driving skills on the results, drivers were rotated between PB trailers (or treatments) every week. On the day before each shipping, a total of 382 pigs were moved to shipping pens at the farm where they were withdrawn of feed for approximately 15 h before transport. Maximum total fasting time before slaughter was 21 h. Pigs were loaded in groups of 4-5 pigs on both PB trailers by the same loading crew and truck drivers between 0800 and 0930 h of the morning of each shipping day. Pigs were mostly handled with boards, while electric prods were only used when necessary. Average loading time was of 40 ± 5 min. The loading order of the two trailers was randomized from week to week to avoid the confounding effects of ambient conditions on the ease of handling. Loading order by deck within the trailer was also randomized each week to avoid the confounding effects of waiting time before departure with location in the trailer. Trailers departed from the farm immediately after loading.

On arrival at the slaughter plant, both trailers were kept stationary in the yard for 30 min before unloading. During the waiting time, the right side (as viewed from the rear) of one PB trailer was pulled over along a fan-misters bank (PBVM) and positioned at a 0.60 m distance from the bank side, while the other PB trailer was stationary in the yard with no access to the fan-misters bank (Control or PBC). The PBVM treatment consisted in 10 min of fan-assisted ventilation (wait 1) followed by 10 min of ventilation and water misting (wait 2) and final 10 min of ventilation (wait 3). The fan-misters bank (15 x 4.6 m) consisted of 12 large fans (1.2 m wide), distributed into two rows of 6 fans each (Fig. 1A), delivering 793 m^3/min each for a total 1,189 m^3/min. A total of 34 nozzles, each with a 180º spray pattern, were distributed along the bank and introduced water mist evenly across the compartments (Fig. 1B). Water was supplied by an external well water source through pipes.

Figure 1 – The fan-misters bank (A), and operating ventilation and water misting system (B)



Source: Own authorship.

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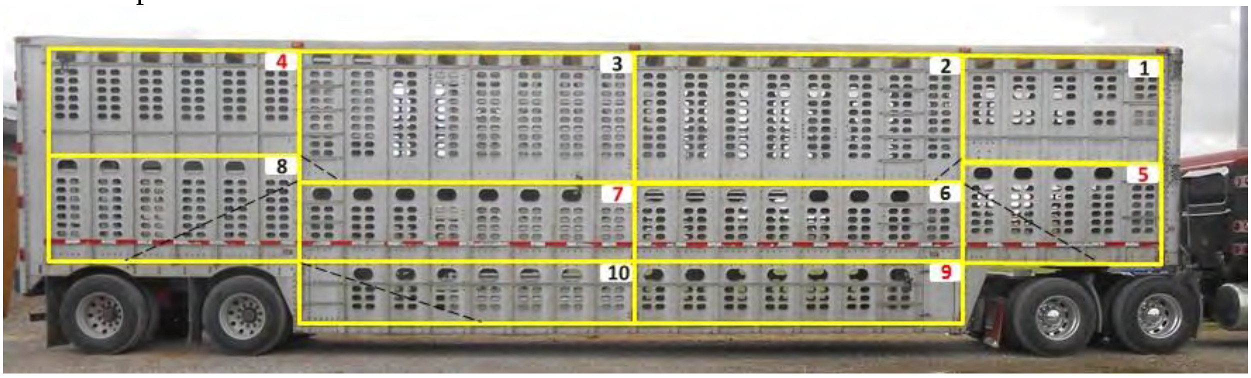
Within each PB trailer, 4 out of the 10 compartments (Fig. 2) were chosen for data collection based on previous results showing compartmental variations in microclimate, with warmer temperatures being reported in the front and bottom compartments (WESCHENFELDER et al., 2012; FOX et al., 2014). Test compartments were compartment 4 (C4, top deck, rear), compartment 5 (C5, middle deck, front), compartment 7 (C7, middle deck, middle), and compartment 9 (C9, bottom deck, front). Loading density ranged from 0.51 to 0.55 m^2/pig, resulting in 9 pigs in C4, 14 pigs in C5, 24 pigs in C7, and 20 pigs in C9. Pigs had access to the test compartments through 3 internal ramps: a 19° sloped ramp going to the upper deck, a 32° sloped ramp going to compartment 5 and a 22° sloped ramp feeding the bottom deck. Pigs transported in the middle deck only used the external ramp to go to the compartments. Trailer decks were evenly covered by 0.5-1.0 cm thick wood shavings bedding.

Figure 2 – Location of compartments within the pot-belly trailer. Selected compartments are presented in red



Source: Own authorship.

During unloading, pigs were moved using paddles and driven into separate lairage pens based on the transport compartment (no mixing of compartment groups) in each PB trailer. Pigs were kept in lairage at a stocking density of 0.64-0.67 m^2/pig with free access to water. After lairage (86.4 ± 19.6 min), pigs were driven to a CO_2 stunner (Combi 77, Butina, Denmark) using flags and paddles along the alley, and by an automatic pushing gate system at the entrance of the stunner. At the exit of the stunner, pigs were exsanguinated in the vertical position.

2.2. Data collection

2.2.1. Ambient climate and trailer microclimate conditions

Ambient temperature data were collected from a nearby Environment Canada Weather station. The average ambient temperature recorded on arrival at slaughter plant was 19.6 ± 1.6^{o}C, ranging from 16.9 to 21.7^{o}C.

The air temperature (T) and relative humidity (RH) were monitored within each selected compartment of the two trailers using 5 iButtons data loggers (DS1923 Hygrochron Temperature/Relative Humidity Logger, Maxim Integrated Products Inc., Sunnyvale, CA) suspended from the ceiling of each selected compartment, one in the center and 4 positioned at 15 cm from the midpoint of each wall. The data loggers were programmed to record T and RH data every min from loading to unloading. The data logger has a T range of −20 to +85°C with an accuracy and resolution of ± 0.5°C, and a RH range of 0 to 100%, with a resolution of ± 0.6%. Data from each data loggers were downloaded after each transport using the OneWireViewer software (Maxim Integrated Products Inc., Sunnyvale, CA).

2.2.2. Behavioral observations

All behaviors were recorded using cameras (HDRAS100V, Sony Corp., Tokyo, Japan). During the 30 min waiting period in both trailers at the slaughter plant, the posture of pigs (standing, lying, sitting) was recorded every min within each of the selected compartment. During lairage, a scan sampling every 2 min for 1h was used to record the number of lying pigs. The latency to rest was calculated as the time (min) from the start of lairage until the time when at least 45% of pigs were lying down. The frequency of drinking bouts (number of drinking bouts divided by the number of pigs) was recorded using continuous observations. A drinking bout was defined as any occurrence of a pig placing its mouth on a nipple drinker for any duration. A new bout was recorded if the pig's mouth was off the drinker for at least 5 s. Behavioral observations were performed by one trained observer and all data were standardized on a per pig basis or percentage to account for differences in group size.

2.2.3. Physiological data

A temperature data logger (DS1921 High Resolution Thermochron iButton; Dallas Semiconductor, Maxim Integrated Products, Sunnyvale, CA) for the gastrointestinal temperature (GTT) recording was orally administered to sentinel pigs (3 sentinel pigs / selected compartment / trailer; total of 24 sentinel pigs per shipment) the day before transport. To administer the GTT data logger, each sentinel pig was snared and a metal "pig gag" was inserted to keep the mouth open. A balling gun loaded with the ibutton data logger was then inserted into the mouth and the data logger was ejected. Each temperature data logger had a unique registration number and a built-in real time clock. Temperature was measured in 0.125°C increments in the range of +15°C to +46°C with ±1°C accuracy. The GTT data loggers were programmed to record temperature data every min from the time it was administered through slaughter. The GTT data loggers were later recovered from the viscera on the processing line. Data from each data loggers were downloaded after each transport using the OneWireViewer software (Maxim Integrated Products Inc., Sunnyvale, CA).

At exsanguination, blood was collected from the bleeding wound of the same sentinel pigs (144 in total, 12 pigs / treatment / week) in a plastic cup. Whole blood lactate values were immediately assessed in duplicate with a hand-held Lactate Scout Analyzer (Lactate Scout, EKF Diagnostic GmbH, Magdeburg, Germany) by dipping a test strip (two strips/animal) into the collected blood. Two blood samples were also collected in two serum tubes (BD Vacutainers®; VWR International Ltd., Montreal, Canada) for creatine kinase (CK) and cortisol analysis. Serum was collected after centrifugation at 1,400 *g* during 12 min at 4°C and then stored at -80°C until analysis. A third blood sample was collected into EDTA tube (BD Vacutainers®; VWR International Ltd., Montreal, Canada), refrigerated at 4°C and subsequently analyzed in duplicate for hematocrit determination according to the microhematocrit procedure described by Matte, Girard and Brisson (1986). Serum CK concentration was analyzed using a creatine kinase_SL kit (Creatine Kinase-SL Assay, SEKISUI Diagnostics, Charlottetown, Canada) and determined with a spectrophotometer. Serum cortisol concentration was measured by radioimmunoassay (ImmuChem™ Cortisol CT, MP Biomedicals, LLC Diagnostics Division, Orangeburg, SC). The intra-assay coefficients of variation for serum CK and cortisol were 5 and 6.7%, respectively.

2.3. Statistical analyses

All analyses were carried out using the SAS software (version 9.3; SAS Inst. Inc., Cary, NC). For technical reasons, the set of data related to pigs transported in C7 of the PBVM in one shipment was excluded from the analysis.

2.3.1. Trailer microclimate data

Four specific periods were considered (Table 1): arrival, wait 1, wait 2, and wait 3. The temperature humidity index (THI) was calculated according to the formula (1.8*T + 32) – [(0.55-0.0055*RH) x ((1.8*T- 26)] (WESCHENFELDER et al., 2013), where T is in °C and RH in %. Average values of T, RH, and THI were calculated for each of the 4 periods, and the calculated values from the 5 data loggers per compartment were averaged. The compartment was the experimental unit. The MIXED procedure was used with treatment and compartment in the model, in a randomized complete block (week) design. Data were analyzed using a homogeneous model for each period separately. The slice effect was used to analyze the interactive effect between treatment and compartment. A difference in RH was observed on arrival at the slaughter plant between the two trailers, despite the trailer arrival time randomization and the same PB trailer design. To limit this confounding effect on truck environment during the treatment, a delta RH (ΔRH) value was therefore calculated as the difference between the RH during the waiting periods and the RH value at arrival. Data are presented as Least Square Mean (LSM) ± SEM.

2.3.2. Physiological data

Average GTT values for each sentinel pigs were calculated for 7 specific periods: rest (at the farm), arrival, wait 1, wait 2, wait 3, unloading and lairage (Table 1). Delta GTT (ΔGTT) values were calculated for each sentinel pig as the difference between the averaged GTT value at any determined event and the averaged GTT value measured at rest (basal level). In order to maintain biological accuracy and to eliminate erroneous low readings considered indicative of drinking (GOUMON et al., 2013), GTT readings below 37°C were excluded from the calculations. The average data from sentinel pigs per compartment in a given week was the experimental unit. The MIXED procedure was used with treatment and compartment in the model, in a randomized complete block (week) design. Data were analyzed using a homogeneous model for each period separately. Blood data were analyzed using the MIXED procedure with treatment and compartment in the model, in a randomized complete block (week) design. The slice effect was used to analyze the interactive effect between treatment and compartment. Data are presented as LSM ± SEM.

Table 1 – Definition of events

Period	Definition
Rest	5-min period of undisturbed time before the arrival of workers in the barn (= basal level)
Arrival	5-min period before arrival at the plant
Wait 1	The last 5-min period of the 1st phase of treatment
Wait 2	The last 5-min period of the 2nd phase of treatment
Wait 3	The last 5-min period of the 3rd phase of treatment
Unloading	2-3 min period before entering the lairage pen
Lairage	5-min period 1 h after the start of lairage

2.3.3. Behavioral data

All analyses were performed for each compartment separately. The average percentage of pigs lying for each of the 3 specifics waiting periods was calculated. Except for the lairage behavior data of one week that could not be collected for technical reasons, data were analyzed using the MIXED procedure with treatment and period in the model, with period as repeated measure and week as random. The slice effect was used to analyze the interactive effect between treatment and period. Data are presented as LSM ± SEM. The frequency of drinking and the latency for 45% of pigs to lie down during lairage were analyzed using Wilcoxon text. Values are reported as median (lower and upper quartiles).

A probability level of $P \leq 0.05$ was chosen as the limit for statistical significance in all tests. Probability levels of $P \leq 0.10$ were considered as a tendency.

3. RESULTS AND DISCUSSION

3.1. Trailer microclimate conditions

On arrival at the slaughter plant, T and THI were similar inside both trailers ($P > 0.10$), but were different by compartment location, with values being higher ($P < 0.001$) in C9 (bottom front compartment) than in the other compartments in both PB trailers (Table 2). Higher T values and THI in this compartment were also reported on arrival in previous studies with similar PB trailers (BROWN et al., 2011; FOX et al., 2014). Either the higher temperature observed in C9 may be explained by the close proximity to external heat sources (i.e. truck engine, floor and drive wheels; BROWN et al., 2011) or by the lower air exchange rate resulting from the reduced pressure gradient along the trailer sides (GILKESON et al., 2016). In this study, the negative pressure that is created by the moving trailer might have been lower at the sides of C9 whose punch-type ventilation holes are rearward of C1 and C5. The lower air flow through compartments finally resulted in reduced heat loss rate from this trailer location.

In the first waiting period after arrival at the plant (wait 1), the application of the fan-assisted ventilation resulted in a lower ($P = 0.02$) T in the PBVM compared to the PBC (22.9°C vs. 23.7°C; SEM = 0.51; data not shown). The THI was also lower in this period in PBVM compared to the PBC (69.3 vs. 70.2 respectively; SEM = 0.56; $P = 0.02$; data not shown). These results confirm the efficiency of the external fan bank in rapidly reducing internal trailer temperature by increasing air flow inside the vehicle as observed in a preliminary study (BRADSHAW, 2013). However, during this initial interval, ΔRH was influenced by the interaction treatment x compartment ($P < 0.001$), with a greater drop in RH being recorded in C5, C7, and C9 of the PBVM compared to the same compartments in the PBC ($P \leq 0.05$). This RH drop shows the efficiency of fan-assisted ventilation in removing the excessive humidity from the environment of these compartments. However, the drop in RH was lower ($P \leq 0.05$) in C4 of the PBVM compared to C4 in the PBC in this study. A possible explanation for this result may be that, while the other compartments stretched along the whole width of the trailer allowing a free air flow through the compartment, C4 width corresponds to the half width of the trailer and its internal side is closed by a full metal gate. The presence of the full gate may have influenced the dynamics of air circulation inside this compartment, likely resulting in reduced removal of humidity from this location.

In the second waiting period, where the fan-mister bank operated simultaneously, there was an interaction treatment x compartment for the T, THI and ΔRH values ($P < 0.01$, $P < 0.001$ and $P = 0.01$, respectively). For each compartment, the T and THI values were lower in PBVM compared to PBC, with a higher ΔRH value in PBVM compared to PBC ($P < 0.001$). This result confirmed the trend observed in wait 1. Within the PBVM, there was a lower T and THI values, and increased ΔRH in C4 compared to the other compartments ($P < 0.001$). Fox et al. (2014) also reported a greater ΔRH after water sprinkling at this location in a similar stationary trailer model. The increased ΔRH in compartment C4 may be again explained by the design of this compartment in this study.

During the final forced ventilation period (wait 3), T was still lower ($P < 0.001$) in PBVM compared to PBC, regardless of the compartment location (20.8°C vs. 24.4°C, respectively; SEM = 0.66; data not shown). The THI was also still lower ($P < 0.001$) in PBVM compared to PBC, regardless of the compartment position (67.3 vs. 71.0, respectively; SEM = 0.76; data not shown). No difference in T and THI was observed between compartments within each trailer ($P > 0.10$). There was still an interaction between treatment and compartment for ΔRH ($P = 0.01$). In each compartment, the ΔRH was higher in the PBVM compared to the PBC ($P < 0.001$). However, while no difference in ΔRH was observed between compartments within the PBC ($P > 0.10$), in the PBVM a greater (= 0.01) RH decreased was found in C7 compared to other compartments. These results show that the fan-assisted ventilation system used in this study was not strong enough to completely remove the excessive humidity caused by water misting in the PB trailer. Jiang *et al.* (2016) also reported an increased RH percentage inside a stationary bird truck following a 15-min simultaneous application of forced ventilation and water misting.

Table 2 – The effects of treatment, compartment and their interaction on the trailer temperature, delta relative humidity (ΔRH), and the temperature–humidity index (THI) at the arrival, wait 1, wait 2, and wait 3 periods

	Treatment[1]									P-value		
	PBC				PBVM							
Compartment	C[3]4	C5	C7	C9	C4	C5	C7	C9	SEM	Treatment	Compartment	Treatment x Compartment
T°C												
Arrival	22.7	23.2	22.9	24.7	22.3	22.8	22.7	24.7	0.54	0.27	<0.001	0.96
Wait 1	24.0	23.4	23.1	24.4	22.0	23.2	22.9	23.4	0.65	0.02	0.19	0.23
Wait 2	23.9	23.9	23.5	25.1	18.5	20.8	20.7	20.8	0.54	<0.001	<0.01	<0.01
Wait 3	24.3	24.2	23.9	25.4	20.1	20.9	20.9	21.3	0.90	<0.001	0.41	0.74
ΔRH[2], %												
Wait 1	-4.1	-2.9	-1.0	-0.5	-2.2	-5.0	-3.3	-2.0	0.76	0.01	<0.001	<0.001
Wait 2	-4.8	-4.7	-2.6	-3.1	21.2	14.1	13.3	17.9	1.07	<0.001	<0.01	<0.001
Wait 3	-4.2	-4.7	-1.8	-2.8	11.2	10.8	6.6	10.5	1.57	<0.001	0.62	0.01
THI[3]												
Arrival	68.9	69.8	69.4	71.9	68.7	69.6	69.3	72.0	0.65	0.64	<0.001	0.93
Wait 1	70.2	69.8	69.6	71.3	68.1	69.7	69.3	70.1	0.72	0.02	0.03	0.25
Wait 2	70.2	70.3	70.0	72.0	64.4	67.6	67.4	67.7	0.73	<0.001	<0.001	0.01
Wait 3	70.4	70.6	70.4	72.4	66.3	67.4	67.3	68.1	1.05	<0.001	0.15	0.83

[1]PBC: pot-belly control; PBVM: pot-belly exposed to ventilation-misting.
[2]ΔRH: delta of relative humidity (RH) calculated as the difference between the RH during each waiting period and the RH at arrival.
[3]THI: Temperature-Humidity index, THI=(1.8*T+32)-[(0.55-0.0055*RH)x((1.8*T-26)] with T in ºC and RH in %.

3.2. Behavioral observations

As showed in Table 3, a lower ($P < 0.001$) percentage of pigs were lying in C5 of PBVM compared to the PBC, regardless of the waiting period (80.5 vs. 51.3% respectively, SEM = 9.28; data not shown). The same effect was observed in C4 in the PBVM, but during wait 2 and 3 only ($P < 0.001$). No difference in lying behavior was found between treatments in pigs located in C7 and C9 during any waiting periods ($P > 0.10$). The greater proportion of lying pigs in C5 exposed to the fan-misting bank operation is hard to explain based on the environmental parameters recorded in this study. However, the greater activity of pigs in C4 may reflect the discomfort in pigs at this location due to the more humid environment during the cooling procedure (greater RH) and the disturbance provoked by the blown air and water spraying. It has been, in fact, suggested that blowing air in close proximity to pigs kept in a truck may be a disturbance for them (KETTLEWELL et al., 2001), while water spraying has been shown to result in pigs' greater activity (WEEDING; GUISE; PENNY, 1993; FOX et al., 2014).

Table 3 – Effects of treatment and wait period on the percentage of pigs lying in the selected compartments

	Treatment[1]							*P*-value		
	PBC			PBVM						
Compartment	Wait 1	Wait 2	Wait 3	Wait 1	Wait 2	Wait 3	SEM	Treatment	Event	Treatment x Event
C4	86.2	87.0	83.1	86.9	37.4	31.7	8.77	<0.001	<0.001	<0.001
C5	76.7	80.1	84.6	56.7	50.5	46.7	13.98	<0.001	0.99	0.64
C7	62.5	73.5	70.1	60.8	67.6	65.5	7.93	0.25	0.11	0.87
C9	54.1	55.3	54.8	42.8	46.0	46.2	8.99	0.12	0.95	0.98

[1]PBC: pot-belly control; PBVM: pot-belly exposed to ventilation misting.

In lairage, pigs from C4 of the PBVM presented a shorter latency to rest compared to those in C4 of the PBC (*P* = 0.05; Table 4). Neither effect of the treatment was found on the latency to lie down in pigs from C5, C7 and C9 in both trailers nor on drinking behaviour in pigs from any compartment in this study (*P* > 0.10; Table 4). Fox et al. (2014) reported no effect of sprinkling on the latency to rest, but there was a lower frequency of drinking behavior in sprinkled pigs. In C4, as no difference in drinking behavior was found between treatments in this study, the fatigue resulting from the greater activity in the trailer during the wait before unloading may explain the need of C4 pigs to lie down more rapidly once entered the lairage pen.

Table 4 – Effects of the trailer compartment and treatment on drinking behaviour and latency to rest during lairage

	Treatment[1]		
Item	PBC	PBVM	*P*-value
Compartment 4			
Frequency of drinking, per pig	5.44 (4.56 - 7.78)	3.33 (1.11 – 8.00)	0.55
Latency to rest, min	42.0 (32.0 – 42.0)	26.0 (22.0 – 30.0)	0.05
Compartment 5			
Frequency of drinking, per pig	1.50 (1.14 - 4.71)	0.50 (0.36 – 2.86)	0.84
Latency to rest, min	16.0 (12.0 – 20.0)	18.0 (18.0 – 24.0)	0.54
Compartment 7			
Frequency of drinking, per pig	5.29 (5.17 - 7.54)	5.98 (5.63 – 8.35)	0.42
Latency to rest, min	18.0 (16.0 – 26.0)	23.0 (21.0 – 24.0)	0.72
Compartment 9			
Frequency of drinking, per pig	4.30 (1.55 - 6.50)	5.50 (5.42 – 8.30)	0.43
Latency to rest, min	12.0 (8.0 – 16.0)	18.0 (10.0 – 22.0)	0.42

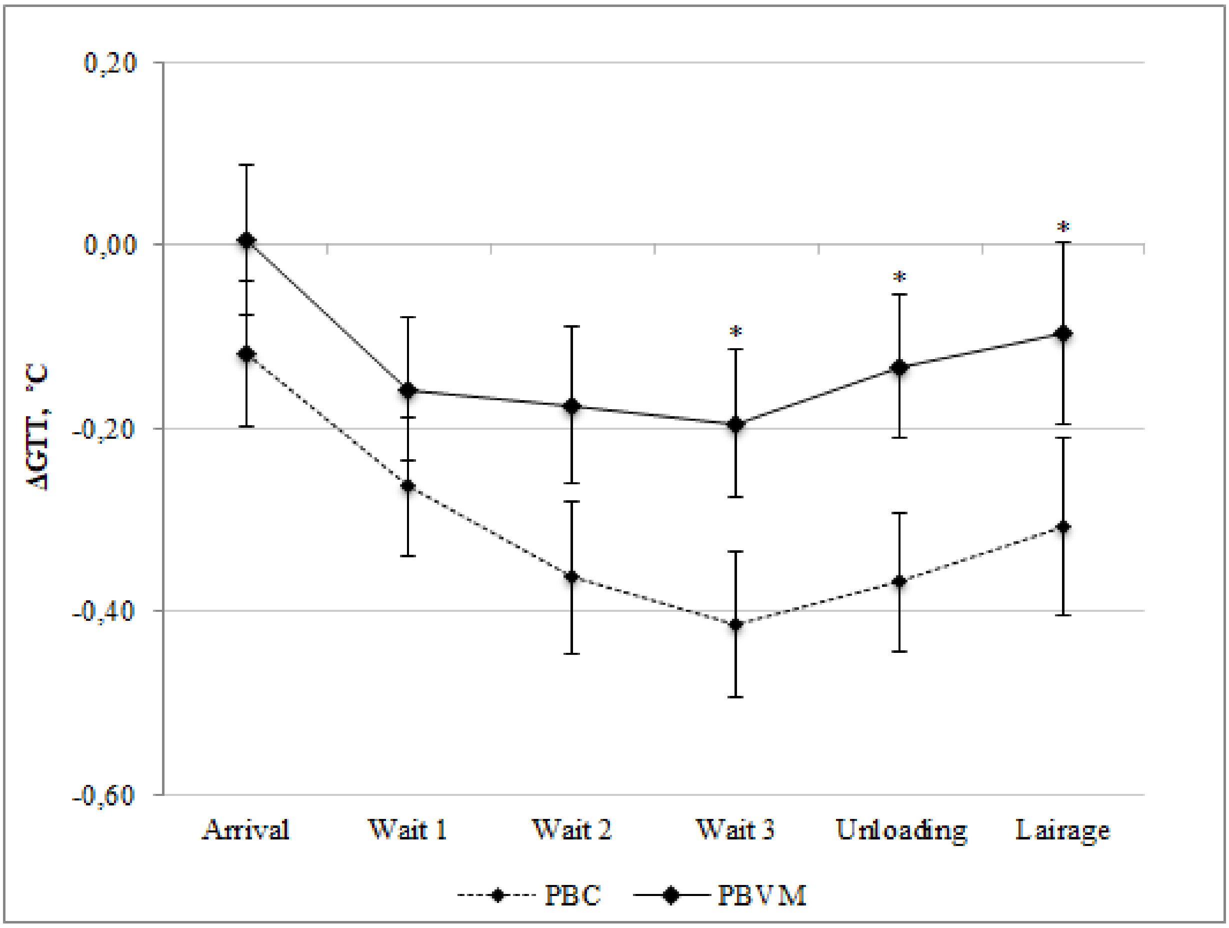
[1]PBC: pot-belly control; PBVM: pot-belly exposed to ventilation-misting

3.3. Physiological response

3.3.1. Gastrointestinal tract temperature (GTT)

Unlike Fox et al. (2014), neither the compartment location nor the interaction treatment x compartment influenced the pigs' GTT in any waiting period before unloading in this study (*P* > 0.10). Results are thus presented by treatment and waiting period (Fig. 3). There was no difference in the ΔGTT between trailers on arrival at the slaughter plant (-0.12°C and 0.01°C for PBC and PBVM, respectively; SEM = 0.08; *P* > 0.10) and during wait 1 (-0.26°C and -0.16°C for PBC and PBVM, respectively; SEM = 0.08; *P* > 0.10). A trend for a higher GTT drop was recorded during wait 2 in pigs located in PBC compared to those transported in PBVM (-0.36°C vs -0.18°C; SEM = 0.09; *P* = 0.10). This drop increased during wait 3 (-0.41°C vs. -0.19°C for PBC and PBVM, respectively; SEM = 0.08; *P* = 0.05) and continued at unloading (-0.37°C vs. -0.13°C for PBC and PBVM, respectively; SEM = 0.08; *P* = 0.02) and after 1 h of lairage (-0.31°C vs. -0.10°C for PBC and PBVM, respectively; SEM = 0.10, *P* = 0.05). At this time of lairage, the GTT of PBVM pigs almost returned to the rest level (Pr > |t| = 0.34). The lower ΔGTT values recorded in PBVM pigs in this study are indicator of their lower need to release core body heat as a result of the PBVM improved thermal environment (lower T and THI). The better thermal conditions during the wait before unloading also allowed PBVM pigs to recover their basal thermal status before slaughter in this study.

Figure 3 – Delta gastrointestinal tract temperature (ΔGTT) value (least squares means ± SEM) of pigs from the control pot-belly trailer (PBC) and the pot-belly trailer exposed to the fan-misting bank (PBVM) on arrival at the plant, and during waits 1, 2 and 3, unloading and lairage.



The ΔGTT corresponds to the difference between the measured gastrointestinal tract temperature at any determined event and the gastrointestinal tract temperature measured at rest (* = $P \leq 0.05$).

3.3.2. Blood parameters

The interaction treatment x compartment influenced the variation of blood cortisol and lactate concentrations at slaughter in this study ($P < 0.01$ for both; Table 5), with pigs from C7 of the PBVM presenting a greater concentration of cortisol and lactate than pigs from the same compartment in the PBC ($P < 0.001$ and $P < 0.01$, respectively). The lactate concentration was lower ($P = 0.03$) in blood of pigs from C9 of the PBVM than in pigs from the same compartment in PBC. Unfortunately, the environmental, behavior and GTT results obtained in this study do not allow a clear explanation for the variation in exsanguination blood cortisol and lactate levels observed in these specific compartments within the PBVM. Hematocrit tended to be higher in blood of PBC pigs compared to PBVM ones, regardless of the compartment location (46.4 vs.

45.3% respectively; SEM = 0.41; *P* = 0.08) and the observed drinking behavior, in this study. A lack of association between drinking behavior and exsanguination blood haematocrit has been already reported by Nannoni et al. (2014) and may indicate the importance of measuring water intake more than drinking behavior to explain the variation of blood hematocrit at slaughter. In this study, it appears that water intake of PBC pigs during lairage was not sufficient to reduce their dehydration status, likely due to exposure to heat in the stationary trailer, at slaughter. These pigs might have needed a longer rest/drinking time to recover their normal hydration conditions. No effect of treatments or compartment location was found on the serum CK concentration at slaughter (*P* > 0.10), which is not surprising as CK levels were not at their peak in blood (7 h; CORREA et al., 2013) at the time of slaughter in this study.

Table 5 – The effects of treatment, compartment and their interaction on cortisol, lactate, creatine kinase (CK) and hematocrit levels in exsanguination blood

	Treatment[1]											
	PBC				PBVM				SEM	*P*-value		
Compartment	C4	C5	C7	C9	C4	C5	C7	C9		Treatment	Compartment	Treatment x Compartment
Cortisol, nmol/L	124.8	121.5	88.1	116.0	93.7	130.2	170.8	101.3	21.09	0.28	0.36	<0.01
Lactate, mmol/L	12.0	11.1	11.2	13.7	11.7	13.5	15.3	10.9	0.99	0.19	0.51	<0.01
CK, log UI/L	3.7	3.8	3.8	3.7	3.7	3.8	3.9	3.8	0.78	0.13	0.13	0.85
Hematocrit, %	47.3	46.8	45.5	45.9	46.2	46.0	44.3	44.9	0.88	0.08	0.12	0.99

[1]PBC: pot-belly control; PBVM: pot-belly exposed to ventilation-misting.

4. CONCLUSIONS

Overall, the results of this study indicate that the fan-misters bank used on the PBVM vehicle sitting at the slaughter plant yard before unloading efficiently improves internal trailer thermal conditions as showed by the reduced temperature within the compartments, resulting in better pigs' thermal comfort (lower need to dissipate excessive heat) in the trailer, and lower dehydration condition at slaughter. However, the inter-compartmental variation in the effects of the fan-misters bank on the internal trailer environment and pig welfare observed in this study suggests the need for improvement in trailer design (pattern of side ventilation openings and internal gate type) to increase the efficiency of this cooling system at warm ambient conditions.

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5. FINAL CONSIDERATIONS

The global pork production have high production and according to the USDA is the most consumed protein in the world. Brazilian pork production became a consolidated activity that has shown an excellent way of expanding. As a result, be the world's fourth largest pork meat producer there is the huge numbers of animals transported for slaughter daily. The Brazilian pig sector has been under pressure from society and the requirements of importers for application of new welfare standards of swine from birth to slaughter.

The process of adaptation to standards and laws must be continuous with services and technical support from government initiatives as Ministério da Agricultura Pecuária e Abastecimento and Comissão Técnica Permanente de Bem-estar Animal - CTBEA. The concern with the transport conditions of the pigs on the welfare of pigs have been very studied as new height for trucks transporting livestock (4.70 m.) and developing projects that evaluate the transport of pigs across the country (MAPA and EMPRAPA).

Brazil has presented high temperatures and thermal oscillations, with locations with strong changes of temperature and humidity. In that environment, the transportation, a stressful experience for pigs, has been compromise the thermal comfort of the pigs and affected their physical and psychic conditions.

Although the design of the Brazilian trailer (double and triple-decked trucks) has open sides that allow greater air flow compared to the Canadian trailer, there are compartments that are differently affected in terms of air flow in relation to the others, which affects behavior and physiology response of pigs through ambient conditions (temperature, humidity, vibrations and noise) before unloading of truck at the slaughter plant.

This waiting period or the waiting time between arrival and unloading have impaired animal welfare, and according to the Brazilian legislation must it designated own area for await unloading process (Nº 711, 1995) with ventilation system activated within the area covered. The current reality of slaughterhouses does not reflect about this situation and the pigs remain from minutes to several hours on weather conditions of day.

The recommendation of unload pigs should be as soon as possible after arrival at the plant but logistic of plant and large number of trucks have hampered to execute this flow. Based on all information, in order to avoid an investment to redesign the truck, the implementation of nebulization and ventilation system became an obligation when thinks animal health care and welfare.