INFLAMMATORY GENE EXPRESSION IN GOATS IN RESPONSE TO TRANSPORT

A Thesis

by

MARK J. CARTER, JR.

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2012

Major Subject: Animal Science

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Approved by:

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Committee Members,	Penny Riggs
	Thomas H. Welsh
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ABSTRACT

Inflammatory Gene Expression in Goats in Response to Transport. (August 2012)

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Chair of Advisory Committee: Dr. Ted H. Friend

Transport, a common cause of stress in livestock, has been documented to increase cortisol, and epinephrine in goats. However, little is known about the timing of changes in the immune system in these stressed animals. The objective of this study was to determine whether expression of immune-related genes changes in goats that are exposed to transport stress. In this study, 15 Spanish-Boer goats ranging from 3 to 4 yrs of age were transported for 12 h. Goats were divided into 5 groups of 3 and placed in 1.219 m x 1.219 m pens. Blood samples were collected via jugular veni-puncture from each animal at 0 h, 3 h, 6 h, 9 h, and 12 h of transport, plasma and leukocytes were harvested for cortisol analysis and PCR analysis for gene expression. Data was analyzed using trailer location (group) as the experimental unit in a mixed model, repeated measures analysis of variance with compound symmetry and autoregressive covariance structures, depending on the best fit for each model. Percent weight losses were analyzed using a diagonal covariance mixed model. Hourly temperature humidity index (THI) values inside the trailer and from the shade were analyzed using a two-independent sample T-test. Cortisol concentrations were significantly elevated during transport ($P<.049$), indicating that goats experienced stressful events during hours of transport. Cortisol concentrations peaked after 6 hours, and returned

to near basal concentrations after 12 h of transport. There was an overall trend for greater expression of many of the genes of interest to increase expression after 12 h of transport, but none were significantly different from pre-transport expression values. Overall, the data suggests that the goats transported during this study experienced transport stress, as indicated by the elevation in cortisol concentrations, but did not have significant changes in expression of the immune-related genes after 12 h of transport.

DEDICATION

This has been a long and eventful 2 years. I couldn't have done it without the support of my family and friends and for that I dedicate this to you, for always giving me little words of encouragement that kept me focused on the goal at hand.

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that I have people in my corner as support for the last 2 years has made this experience a little less stressful and for that I would like to thank all of you.

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NOMENCLATURE

m	Meter
wks	Weeks
h	Hours
min	Minutes
sec	Seconds
kg	Kilogram
cm	Centimeters
ng	Nanogram
µl	Microliter
nm	Nanometer
RQ	Relative Quality
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction

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INTRODUCTION

History of Animal Transport

Since ancient times (antiquity) animals have been transported for two major reasons, when they could not physically cross a barrier without causing major risk to themselves or when they were being moved across water, either overseas or to cross large bodies of water. Romans valued animals, like elephants that were imported for beast shows, and prized race horses. When animals were transported by sea when traveling long distances, Romans tried to keep the animals in good condition and prevent them from suffering exhaustion (Blancou and Parsonson, 2008).

Until the Middle Ages (Medieval period), the earliest documented animal transported was an elephant sent as a gift to form an alliance with the Turkish. As transportation techniques improved, most goods were transported on water-ways, i.e., rivers or by sea during the 13th century to the end of the Middle Ages. The shipments usually contained a variety of large animals, such as cattle and horses. During times of war in the Middle Ages, a large number of horses were in great demand to help transport goods, supplies, and carry soldiers long distances. These animals usually embarked on long journeys on specially designed ships to wherever they were needed. The boarding, sailing, and landing of these vessels were often difficult tasks.

This thesis follows the style of Journal of Animal Science.

Approaching the end of the Middle Ages and entering the early 15th century, a time of discovery and exploration of new lands, many European colonists embarked on long voyages looking for a new start in the New World (America). Before European colonists were present in America, no domesticated animals were present apart from the dog, and some avian species. On the second voyage to America by Christopher Columbus, horses were transported for the first time. The horses were lifted onto boats by ropes and placed in specially designed stalls to help avoid injury. Following the Christopher Columbus expedition, many other explorers brought numerous varieties of animals to developing colonies around the world.

When steamships started transporting animals in larger numbers, cattle and horses were shipped to places like Africa for civil or military purposes (Blancou and Parsonson, 2008). During these long transports, animals interacted with other animals from different herds and animals from different parts of the world, causing the spread of diseases, like rinderpest. Rinderpest was an infections viral disease of cattle that was introduced to East Africa and spread to South Africa, killing millions of wild and domesticated ruminants.

At the turn of the century, new means of transport proved to be more convenient for animals. Among these were the railroads. The first documented shipment of cattle by rail was in 1852, from Lexington, Kentucky, to New York after being driven overland from Cincinnati (East, 1942). Following the transport of the first cattle by rail, many more forms of transport were implemented, from ships, air, and truck or still by walking long distances in some places. The basic techniques used to load and unload animals

have been modified over the years due to the development of new means of transporting animals. The improvement of these techniques all come from a similar background and are based on a thorough knowledge of the anatomy, physiology and psychology of different species.

Animals and humans have depended on each other over the centuries. Humans needed animals to transport goods, and when they started expanding to new lands, people had to organize new ways to transport horses, cattle, sheep, goats and pigs over long distances because the animals were essential for the new settlements. Due to the ever increasing amount of animals being transported daily, today, rules and regulations are being developed to ensure the welfare and well-being of animals in transport. Transport is a common cause of stress in livestock, and substantial research has examined the effects of handling and transport in cattle, pigs and poultry (Rajion et al., 2001). Little research has been done to examine the impact of stress on goats during transportation.

History of the Goat

The goat is one of the most widespread species found on all the continents and in the far corners of the world, and goats may have been one of the earliest hoofed animals domesticated (Kilgour and Dalton, 1984). Early settlers brought goats to the Americas on long voyages as a source of milk and meat. They are able to adapt to very rough and challenging conditions due to the environments they inhabit, which may be either hot and dry, or cold and barren. From their wild ancestors, goats have inherited two major traits; they are very agile and sure–footed. These small ruminants are herbivores that are

capable of consuming vegetation types like tree bark and other undesirable vegetation not normally eaten by other ruminants and are able to digest poor-quality roughages (Louca et al., 1982).

One of the breeds brought to America in the mid 1990s was the Boer goat, this hardy animal evolved in South Africa from indigenous Africans (Casey and Van Niekerk, 1988). The Boer goat is a compact animal with high growth rate, high fertility, and has a high resistance to diseases, making it a hardy adaptable animal. Boer goats are considered to be a dual purpose breed; they are known for their ability to produce an abundance of milk and for their superior meat and hide quality. This breed of goat is often cross bred with dairy breeds to increase milk and meat production. Boer goats have been referred to as the key to upgrading rural goats for meat production. This highly sought out breed of goat is transported and shipped across all corners of the world. The *2010 Nontraditional Lamb Market in the United States review* (Shiflett et al., 2010) stated that 17% of goat meat is consumed by ethnic groups in observance of many religious holidays. The demand for goat meat is expanding worldwide due to the increased demand for meat and skin in the global markets (Ayo et al., 2009).

Stress Indicators

One study conducted on transportation of goats was done in a temperate region of the world (Rajion et al., 2001) and aimed at alleviating road transportation stress in goats. Rajion et al. (2001) transported goats through tough and eventful terrains for 1.5 h at ambient temperatures 25°C and 29°C in the morning and afternoon and noticed

significant elevation in neutrophil/lymphocyte ratios (NLR) and serum glucose concentrations. High and low temperatures and relative humidity are stressors to animals in transport. The nature of the journey, the experience, the condition of the animals, and the duration of the transport can impact livestock.

For many centuries livestock have been driven across land on foot receiving food and water en route to slaughter. Recent production systems require that animals be transported by road, air or rail, and these journeys cover thousands of kilometers and many take several days (Hogan et al., 2007). The majority of goats are kept in rural environments where the land is rugged and dry. The process of gathering these goats on a farm and placing them in a holding pen, followed by loading and unloading, can subject them to multiple stressors. These stressors are apparent by an increase in corticosteroids, especially cortisol, and an increase in epinephrine (Kannan et al., 2000).

Goats are exposed to numerous conditions associated with being transported to the location where they are being loaded, including handling, loading, and feed and water deprivation prior to and during transportation. Animals can be stressed by both physiological stress, like restraint and handling, or physical stressors like hunger, thirst, fatigue and injury (Grandin, 1997). Reducing stress, during handling and loading may improve the health of the animal and prevent physiological changes that may reduce productivity (Grandin, 1997). Kannan et al. (2002) demonstrated that goat's behavioral changes can indicate discomfort. Goats indicate these discomforts by vocalization, kicking, backing- off, and attempt to escape during transport. Such changes in behavior

are the usual signs of distress. It is useful to distinguish between situations in which animals are affected by their environments.

When environmental conditions begin having effects on an individual, the event is defined as stress. Studies have been conducted in cattle and ostriches that suggest methods to scoring the stresses imposed by handling, loading and unloading of animals (Minka and Ayo, 2008). The environmental factors which lead to stress often cause an animal to exhibit abnormal concentrations of plasma corticosteroids and other hormonal changes (Fraser and Broom, 1990). This can be due to exposure of a variety of environmental events that cause a broad range of physiological changes. These stressors can be the cause of many adverse consequences, ranging from discomfort to death.

The release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland was described by Selye (1936) as the general adaptation syndrome. This activates the release of corticosteroids from the adrenal cortex. The general adaptation syndrome model in 1936 was broken down into three phases; the alarm stage, resistance stage and the exhaustion stage. The alarm stage is the flight or fight response, which is the first reaction to danger and how the threat is dealt with. The resistance stage is when the body begins to restore balance and is a period of recovery and stress hormone levels return to normal. The final phase is the exhaustion stage; this is when the body has not had the ability to recover. This is where stress levels are elevated and remains elevated; this stage of the general adaptation syndrome is the most dangerous to health.

Cortisol

Several chemical and hormonal parameters like packed cell volume and cortisol have been used as indicators in species like sheep to identify inadequate welfare. Garey et al. (2010) found that horses subjected to short transport times of approximately 6 hours had increased cortisol concentrations compared to those that were not transported. Cortisol is the primary corticosteroid secreted by the adrenal cortex and released by the adrenocorticotropic hormone (ACTH). Cortisol is a marker of stress that is commonly used to assess the effects of non-cognitive and cognitive stimuli.

Welfare and Transportation Regulations

The 2012 Canadian *Health of Animals Act* states that causing any form of suffering to any animal during transport, loading, or unloading is a federal offense. This act addresses diseases and toxic substances that could possibly affect the well-being of animals or be transmitted from animal to person. “Animal welfare includes the physical and mental state of animals; this implies both fitness and a sense of well-being without suffering under the care of man” (Farm Animal Welfare Council, 2009). The care of animals, on the farm, in transit, at market, or at a place of slaughter, should be considered in terms of the “five freedoms”: (1) the freedom from hunger and thirst, (2) the freedom from discomfort, pain, injury or disease, (3) the freedom from fear and distress, and (4) the freedom to express normal behavior while in the care of man (Farm Animal Welfare Council, 2009). When animals are no longer able to display these freedoms they develop

different coping mechanisms or display stereotypic behaviors that release endorphins that helps the animal deal with their environments.

The 2005 Council of the European Union developed regulations for the protection of animals during transport and related operations. The regulations proposed methods to improve welfare of livestock during various stages of transport that exceed eight hours (Krawczel et al., 2007). The basic principle was that animals being transported must not be hauled in any way that will cause injury or undue suffering. In order to guarantee consistent and successful compliance of regulations, detailed requirements for various types of transport for livestock were implemented in Europe (Council of the European Union, 2005).

The European regulations have specific guidelines for the transport of animals by road, rail, sea, air, and containers. All means of transportation should prevent animals from escaping, protect them from rough weather, be sanitized and provide animals with clean bedding and proper ventilation during transport (Council of the European Union, 2005). Sufficient space should be provided for goats inside compartments of transport vehicles. Goats less than 35 kg need 0.20 - 0.30 m^2, goats 35 - 55 kg need 0.30 - 0.40 m^2, and goats over 55 kg need 0.40 - 0.75 m^2 of space per goat for satisfactory transport with quality air ventilation (Council of the European Union, 2005). During transport, goats are exposed to a number of factors not related to vehicle motion, which may result in animal welfare issues including hunger and thirst, along with thermal and physical discomfort. These issues can be stressors that can cause expression of certain inflammation and immunity-related genes.

Preliminary Study

Data collected in previous equine and bovine studies in our lab indicate significant differences in gene expression of certain inflammation and immunity related genes between non- stressed animals and those experiencing a stressful event. In these studies, differences in the expression of selected primers, including prostaglandin F receptor, multiple interleukins, C-X-C motif chemokines, cathelicidins, and tumor necrosis factor genes, have been observed in market cattle, freshly weaned foals, and stall isolated horses. This data suggests that these genes may also be up- or down-regulated in goats during periods of stress.

Due to the recent completion of the Human Genome Project (HGP) and bovine genome sequence, it is now possible to analyze and study gene expression that is critical in the immune defense response in cattle. A study conducted by Terrill et al. (2011) analyzed gene expression of 93 bovine target genes important in the inflammatory and innate immune responses. In that study 2 trials were conducted on recently weaned calves. During the study, calves were subjected to either a period of acute stress where the calves were separated from their dams and castrated, or a period of chronic stress in which the calves were weaned from their dams and immediately shipped to auction and the eventual transport to a feed yard, arriving after 3 or 4 days of transport. Gene analysis from the 2 trials produced quality amplification; Terrill et al. (2011) found 30 to 36 of the 93 genes increased gene expression. In these 2 experiments, 15 common genes displayed significant expression. The results of that study suggest the expression of these genes can be used to identify chronically stressed animals.

Examining of comparative literature, Maddox and Cockett (2007) reported a 60% success rate and concluded that the sequence and chromosomes of cattle are highly conserved in goats, suggesting that PCR analysis of samples collected from goats using bovine primers may be highly successful, which is consistent with Pepin et. al. (1995) who has reported a 40% success rate.

Our study was a preliminary experiment conducted to validate using bovine primers for goat RNA analysis. Samples were added to Low Density Array cards (LDA), identical to those used in the Terrill et al. (2011) and sealed using a TaqMan low density array sealer (Applied Biosystems, Foster City, CA). The focus of the preliminary study was specific to the 15 genes of interest identified by Terrill et al. (2011). Results from the preliminary study presented quality amplification of 64 genes (Appendix Table 11) out of the total 93 target genes. These results indicate a 67% success, which was 7% higher than what was reported in the study performed by Maddox and Cockett (2007). Only 13 of the 15 immune-related genes of interest seen in the Terrill et al. (2011) study (Table1) were useful for analysis of goat mRNA expression. With quality amplification of these 13 genes, the use of bovine primers in this study was validated. These results support similar findings by Maddox and Cockett (2007) and Pepin et al. (1995) in identifying genetic expression though cross-species comparisons.

Table 1. Descriptions of the 15 inflammatory genes of interest

Gene ID	Gene name	Pathway
18s (Reference gene)	Ribosomal RNA biogenesis protein	
LSP-1	Lymphocyte- specific protein	Protein coding
IL4R	Interleukin- 4 receptor	Cytokine-Cytokine receptor interaction
HSP90AA1	Heat shock protein 90, alpha (cytosolic), class A member 1	Heat shock
IL10RB	Interleukin- 10 receptor β	Cytokine-Cytokine receptor interaction
SERP1	Stress- associated endoplasmic reticulum protein 1	Protein coding
HSF2	Heat shock factor 2	Heat shock
CCRL1	Chemokine (C-C motif) receptor – like 1	Chemokine signaling
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1a	TNF superfamily
CXCR2	Chemokine (C-X-C motif) receptor 2	Chemokine signaling
IL1RN	Interleukin- 1receptor antagonist	Cytokine-Cytokine receptor interaction
IFIT5	Interferon-induced protein with tetra-tricopeptide repeats 5	Protein coding
IL12B	Interleukin- 12B	Cytokine-Cytokine receptor interaction
IK	(Cytokine), down regulator of HLA II	Function Unknown
CXCR5	Chemokine (C-X-C motif) receptor 5	Cytokine-Cytokine receptor interaction
CCR5	Chemokine (C-C motif) receptor 5	Cytokine-Cytokine receptor interaction

Goat Genome

The lack of a sequenced genome in goats makes the determination of gene expression in the species a challenge. One of the most powerful ways to discover these elements is through cross-species comparisons with other mammalian genomes. The closest species to the goat are cattle and sheep having only a relatively short evolutionary separation, which makes it possible to use microsatellites to successfully genotype goats (Fontanesi et al.,2010). The goat genome is still a work in progress, with only 226 sequences recorded in GenBank in 1994 (Arevalo et al. 1994; Bhebhe et al. 1994). Up to the late 80's, the genetic mapping of the goat was limited to only one linkage group between casein genes (*CASA1* and *CASA2*, Grosclaude et al. 1987). In 1996 and 1998 a genetic and cytogenetic maps in the goat was published (Vaiman et al., 1996; Schibler et al., 1998). The most recent comparative cytogenetic map of the goat genome (2009) has been developed using cattle and sheep bacterial artificial chromosome (BAC) clones. This map is complied of 268 genes and 144 microsatellites, including 65% of the goat chromosome bands (Fontanesi et al.,2010).

When compared to other species the goat genome has not made much improvements. In a recent preliminary study, conducted by Du et al. (2012) developed a fundamental tool for genomic research in the goat. In that study, a 5000 rad goat–hamster panel of 121 whole genome radiation hybrids. A male Boer goat was fused with a recipient thymidine kinase deficient hamster cell line. The 121 radiation hybrids were grown and produced an average of 8.4 mg of DNA per radiation hybrid. The retention frequencies of the 121 hybrids were preliminarily estimated using a collection of 42

unlinked molecular markers. The development of this panel will provide a fundamental tool for advanced goat genome mapping studies and for mammalian comparative mapping.

Gene Expression

Over the past few years, many hopes have been raised due to the genetic improvement in the human and bovine genome using marker-assisted selection schemes. The ability to map genetic pathways and identify genetic expression should allow for the differentiation between stressed and unstressed animals. Gene expression data offers the ability to identify multiple genetic pathways of interest, including the chemokine signaling pathway and cytokine-cytokine receptor pathway.

The cytokine-cytokine receptor pathway is made up of multiple ligands important for signaling that bind to specific receptors on the cell surface of target cells. Cytokines are crucial intercellular regulator of cells in innate and adaptive inflammatory defenses, cell growth, differentiation, cell death, angiogenesis, and development processes aimed at the restoration of homeostasis (Leonard et al., 2000). In response to an activating stimulus, various cells in the body release by soluble extracellular proteins. They can be grouped by structure into different families, and their receptors can likewise be grouped eg. *IL4R, IL10RB, IL1RN, IL12B*. A cytokine *IL-10RB* (Inerleukin-10 receptor β) is part of a two accessory signaling subunit. It derives from *IL-10* which is a cytokine with potent anti-inflammatory properties, which represents the expression of inflammatory cytokines with important immune-regulatory functions (SABiosciences, 2010).

The chemokines signaling pathway is made up of structurally related proteins that mediate a wide range of biological activities. Chemokines are a significant component of the basal leukocyte processing which is essential to immune system development. Four classes of chemokines have been identified (*CCR5, CXCR5, CXCR2, CCRL1*). One of the genes from the four classes of chemokines, chemokine (C-C motif) receptor 1 (*CCRL1*) is a member of the interleukin 1 cytokine family. This protein inhibits the activities of a variety of interleukin 1 related immune and inflammatory responses. Chemokines also participate in the growth, differentiation, and activation of leukocytes and stimulate other functions (SABiosciences, 2010).

Heat shock protein gene (*HSP90AA1*) is found in all major cellular components. It is present in all the cells of living organisms and is essential for cellular viability and major physiological roles in protein homeostasis (Ellis and Van der vies, 1991). HSPs are detectable in unstressed cells, and increase in abundance during stress. Various stressors are dependent on the activation of specific transcription factors. HSPs expression acts as a potential indicator of animal adaptation to harsh environmental stress (Valenzuela 2000; Hansen 2004). Earlier studies conducted by Kristensen et al. (2004) and Lacetera et al. (2006) found this protein in different breeds of cattle, but work on goats is lacking.

Tumor necrosis family (TNF) family members play important roles in various physiological and pathological processes, including cell proliferation, apoptosis, and modulation of immune responses and induction of inflammation. One gene that stood out was Tumor Necrosis factor receptor superfamily, member 1A (*TNFRSF1A*) and members

of this TNF superfamily are expressed in different cell types and have a range of affinities for various intracellular adaptors, which provide tremendous signaling and biological specificities that promote both survival and death signaling cells. TNF is a pro-inflammatory cytokine that is expressed though the binding of both TNF-α and TNF-β, which is produced by many cell types in response to inflammation, infection and other environmental, stresses (SABiosciences, 2010).

Hypothesis and Objective

Stress negatively impacts the immune system and certain genes are responsible for different aspects of the immune system response. Once it is determined when the genes begin to change in expression due to that stress, researchers can identify which among those are most active, which remain active for the longest duration, and which genes are responsible for controlling the stress response. Once identified, those genes can be targeted to prevent suppression of the immune system. This may also be able to determine which aspects of transport stimulate the greatest response in the animal.

The objective of this study was to determine when immune-related genes begin to change in expression in goats that are exposed to transport stress. This will allow researchers to evaluate transport stress in goats in relation to proposed European regulations on livestock transport using behavioral and physiological indicators of stress.

MATERIALS AND METHODS

This study took place at the Thomsen Center, which is located at the Texas A&M University Animal Science Teaching Research Center (ASTRC). The 25 goats used in this study were provided by a private goat producer from Burnet, TX. The goats used were female Spanish-Boer goats ranging from 3 to 4 years of age. The goats were given 2 weeks to habituate to the Thomsen Center pasture. The pasture contained a holding pen in which the goats were fed for the 2 wks. The goats could freely enter and exit the pen, habituating the goats to being held in the pen. The holding pen was made out of four 3.6 m wide cattle panels. The exit gate used for loading the goats onto the trailer was located in front of the holding pen. Before the goats were placed on the trailer they were placed on a scale (Paul Hog and Sheep Basic Scale, W-W Manufacturing, Thomas, OK) that was located between the exit gate and the back of the trailer. After initial body weights were recorded, the goats were loading on the trailer in their assigned holding pens. Holding the goats in the familiar area was used to eliminate any extra stress to the animals other than being placed on the trailer on the morning of the study.

The day before the study, one HOBO (Onset Computer Corporation, Bourne, MA) temperature and humidity data logger was placed strategically in the pasture and another was placed on the trailer. The HOBO devices were placed against on an 11.4 X 8.5 cm piece of styrofoam and held in place with duck tape that was placed across the center of the device leaving the sensors exposed. The HOBO devices were programmed to collect data every 30 min over a 24 h time point. The pasture HOBO was hung from a

tree branch out of the sun to collect the environmental changes of the pasture. The trailer HOBO was placed on the aisle side of the panel forming the forward holding pen at eye level of the goats. The HOBO was secured to the panel using zip ties. It recorded temperature and humidity that the goats were experiencing while on the trailer during transport.

The morning of the study, the 15 most tame and healthy goats, out of the 25 goats, were selected to be transported and the remaining goats were left in the pasture until the study was complete. Gathering of the goats started around 0700 h the morning of the study, to facilitate weighing and sampling at 0800 h. Goats were brought from the pasture to the holding pen where they were fed during their habituation phase. Placing the goats in the holding pen provided better access for catching the goats; this reduced the amount of stress that would have been applied to the goats if they were chased around the pasture. Once in the holding pen, the 15 selected goats for the study were divided from the other goats that were going to be released back into the pasture. After the remaining goats were returned to the pasture, the selected goats were caught one by one and placed on a scale to record their beginning weight, and also at this time a pre-transport blood sample was collected before placing each goat on the trailer in their designated pen

The 15 goats were divided into 5 groups of 3 goats each. Assignment to the groups was blocked by weight class so that the average total weight of each group was similar. The goats were placed in 1.219 m x 1.219 m pens (4 X 4 foot) made from metal panels that were installed in a steel livestock trailer (6.096 m x 1.829 m,

Gooseneck Trailer Mfg. Co., Inc., Bryan, TX). These panels were tied with zip ties, which allowed for quick release if researchers needed to cut open a gate to access an animal in any of the pens. All pens were lightly bedded with wood shaving to provide some comfort for animals that are lying down during transport. The shavings also helped to absorb and retain urine and feces in the trailer. The trailer design can be seen in Figure 1 and in Figure 2.

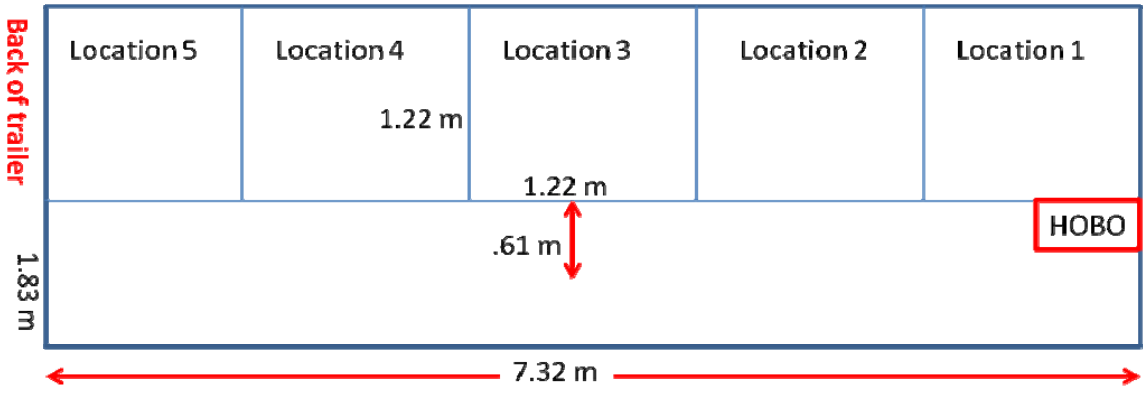

Figure 1. Floor plan of the trailer showing the 5 separate pens with the adjoining alley

Figure 2. Inside of the trailer during loading of the goats

The trailer was connected to a 2004 Chevrolet 4-door ¾ ton truck. The timing of the transport sessions and blood sampling are summarized in Table 2.

Table 2. Time, location of blood sampling, and other events in relation to hours of transport

Times	Blood samples
8:00AM	0 h; pre-loading, load trailer
11:15AM	3 h; on trailer, after 3 h of transport
2:15 PM	6 h; on trailer, after 6 h of transport
5:15 PM	9 h; on trailer, after 9 h of transport
8:15PM	12 h; on trailer, after 12 h of transport, then unloaded and returned to home pen

After the last goat was loaded, transport began at 0815 h, handling prior to 0815 h was not considered to be transport because the goats were very accustomed to being handled. The truck and trailer was driven in a circular route on paved roads at posted

highway speeds in the vicinity of ASTRC for one hour. After one hour the truck briefly stopped, and the goats were visually inspected. The goats were checked for panting, increased respiration rate, depression or not being capable of standing. Any goat showing the above symptoms was to be unloaded and no longer transported. After inspecting the goats transport resumed immediately in the opposite direction, the inspection process was repeated every hour, until the goats had been transported 3 h. The trailer then returned to the Thomsen Center for blood sampling of the goats.

Blood samples were collected from the transported groups via jugular venipuncture using a 20 gauge x 1 inch needle with holder (Vacutainer® Becton, Dickinson and Company, Franklin Lakes, NJ) and 9 ml plastic evacuated collection tubes containing sodium heparin (Vacuette, Greiner Bio-One, New York, NY). Each sample was then inverted repeatedly for approximately 15 sec and then placed in ice until sample collection was complete. Samples during transport were collected on the trailer. An assistant straddled each goat holding its head back and to the side to expose the jugular vein, a process that took less than 2 minutes. This process was repeated for the collection of a blood sample from each animal after 3 h, 6 h, 9 h, and 12 h of transport.

Transportation concluded after the collection of the 12 h sample. The repeated transport and handling is fairly typical of the handling and transport that most U.S. goats experience in the market system. The arrangement of the pens in the trailer allowed the researchers to safely and quickly obtain jugular blood samples from each goat. Once returned to the holding pens after the last blood sample, the goats were

given ad libitum access to hay and water for 30 min, and then fed one-third of the portion that they receive twice daily of the concentrate ration. The goats were then returned to their pasture. The goats were carefully monitored for the first 2 h after transport to insure that there were no symptoms of illness. The symptoms assessed included labored breathing, lethargy, isolation, lowered head, droopy ears, nasal mucus, coughing or wheezing, extreme movement or violence, and lack of eating or drinking. The goats used in this study were accustomed to transport, and no illness was anticipated.

Once blood collection was completed for each time point, the blood samples were processed through a leukocyte capturing filtration system which isolated leukocytes from whole blood and stabilized the cells on the filter. Exclusion of red blood cells allowed purified RNA from the captured leukocytes to be free of globin mRNA, which improved sample utility for expression profiling and other applications (LeukoLOCK^{TM} Total RNA Isolation System, Ambion, Inc., Austin, TX). A RNA preservative (RNAlater, Ambion Inc., Austin, TX), was added to each of the filters by attaching a Leukolock syringe that contained 3 mL of RNA preservative to stabilize leukocyte RNA. Leukocytes that were captured by filtration were flushed with 3 mL of Phosphate Buffered Saline (PBS) to remove the RNA preservative that was added during sample filtering. Immediately after the filters were flushed with PBS they were placed in a plastic Ziploc baggie and placed on dry ice for fast freeze to prevent RNA degradation. When the study was complete RNA samples were placed in a freezer at -80°C until RNA extraction could take place.

The filtered blood was collected in a 10 mL glass evacuated collection tube containing no chemical additives (Vacutainer, Becton, Dickinson and Company, Franklin Lakes, and NJ). The filtered blood was spun down at a speed of 3300 ± 5% RPMs for 10 minutes using a LW Scientific-Ultra-8F centrifuge (LW Scientific Inc., Lawrenceville, GA) to separate the red blood cells and blood plasma. After the red blood cells settled at the bottom of the tube, the plasma was then collected from the upper layer and transferred into 5 mL Falcon tubes (BD, Bedford, Massachusetts) and stored at -80°C until the blood plasma samples could be used for quantitative analysis of cortisol concentrations.

Cortisol samples were processed following a modified ELISA cortisol protocol (Stressgen, Assay Design, USA) that was developed in collaboration with Dr. Luc R. Berghman. The micro-well ELISA plates were coated with goat anti-mouse IgG (Sigma Aldrich, St. Louis, MO) at a concentration of 5 µg/mL coating buffer. Samples collected from the study were diluted to a 1:20 dilution ratio then added 100 µl of each sample into three wells for triplicate testing, allowing for the binding of the primary antibody. Following the addition of the triplicate samples, 50 µl of a commercially produced conjugate was added to each well except total activity (TA) and Blank wells, and 50 µl of antibody was added to each well except Blank, TA and non-specific binding (NSB), and incubated to allow for competition for binding sites. During the washing and decanting step all unbound materials were removed. After the removal of the unbound material a para-Nitrophenyl phosphate (pNpp) substrate solution was added to all wells, binding the enzyme attached to the antibody. The bound hormone conjugate is detected by the

addition of the substrate which generates an optimal color. This allowed for colorimetric detection on a photometric multi-label plate reader at an optical density of 405 nm (Wallac Victor II 1420, Perkin Elmer, Waltham, MA). The samples were compared to the known standards used on every plate which allowed comparisons between plates to be optimized. Data obtained from the plate reader were then inputed into a curve-fitting software program (StatLIA®, Brendan Technologies, Inc., Carlsbad, CA) that calculated total concentrations of each hormone assay based on averages of the triplicate samples in comparison to the generated logarithmic curve of the known standards.

Extraction of the RNA from the leukocytes followed the protocol included with the filtration and extraction kit (LeukoLOCKTM Total RNA Isolation System, Ambion, Austin, TX). Extracted RNA was evaluated for quantity by spectrophotometry (Nanodrop, Thermo Scientific, Wilmington, DE); samples containing a minimum of 80 ng/µl were considered to have sufficient quantity of RNA. The quality of RNA was measured by flow capillary electrophoresis (2100 Bioanalyzer, Agilent Technologies, Foster City, CA). Samples meeting a minimum of 350 ng/µl RNA or more were diluted with RNase free water to prevent clogging of the pins in the Bioanalyzer. RNA samples meeting the minimum quantity and quality standards were converted to cDNA using a reverse transcription kit (High Capacity cDNA RT Kit, Applied Biosystems, Foster City, CA). The converted RNA samples were amplified on custom Taqman array 96-well microtiter fast plates containing custom bovine primer assays for the previously identified inflammation and immunity related genes. The quantity of the mRNA for genes of interest was measured in all samples from

the study by quantitative reverse transcriptase (qRT-PCR). Plates were processed using the Applied Bio-system 7900HT Fast Real-Time PCR system. The system allows for continuous wavelength detection from 500-660 nm and the use of multiple fluorophores in a single reaction. This device measures gene expression levels among the bovine primers. Gene amplification was analyzed using Sequence Detection System (SDS) Software v2.4.1 (Livak et al., 2001**)** and measured using relative quantitification method (ΔΔ C(T)).

In this study, plasma cortisol concentrations were analyzed two different ways. For the first analysis trailer location (mean for each group of 3 goats) was the experimental unit (n = 25) for cortisol because of the possibility the animals within these small groups could influence each other. The second analysis for plasma cortisol used the individual animal as the experimental unit (n = 75) to see if there was an interaction difference between locations on the trailer during hours of transport. Body weight, however, is less likely to be influenced by other group members, so individual animals served as the experimental unit for those data. The gene expression data were analyzed using trailer location (mean for each group of 3 goats) was the experimental unit (n = 25) because RQ values were missing at certain time points (hours of transport) for some of the goats. Cortisol concentrations and gene expression data for each location and hour of transport were analyzed (SAS 9.3, SAS Institute, Inc., Cary, NC) using a mixed model, repeated measures analysis of variance with compound symmetry and autoregressive covariance structures, depending on the best fit for each model. Each model included hours of transport as an independent variable. A model was generated for each gene and

for cortisol concentrations. Weight loss data was in terms of percentages for each animal. Percent weight loss was analyzed using a diagonal covariance mixed model that included trailer location. The temperature humidity index (THI) values inside the trailer and from the pasture were recorded in degrees Celsius and calculated using the formula of (Collier and Zimbelman, 2007): THI = [Temp°C + (.36 * Dew point°C)] + 41.2 and was analyzed using a two-independent sample T-test.

RESULTS

Cortisol

Cortisol concentrations were first analyzed using location on the trailer as the experimental unit. Using that analysis, plasma cortisol concentrations in the goats significantly differed ($P < .05$) between hours of transport (Table 3). Hour 6 was greater than hours 0, 3, 9 and 12. Cortisol concentrations peaked after 6 hours, and returned to near basal concentrations after 12 h of transport.

Table 3. Mean plasma cortisol concentrations (ng/ml) calculated using location on the trailer as the experimental unit, in relation to hours of transport

	Duration of transport					
Variable	0 h	3 h	6 h	9 h	12 h	*P* value
Cortisol	46.15±10.53[a]	68.20±8.15[b]	86.99±9.12[c]	53.68±8.15[b]	49.74±9.12[a]	*P* < .05

[a,b,c]All durations of transport with different superscripts differ

Plasma concentrations of cortisol were also analyzed using individual animals as the experimental unit. Using this analysis, cortisol concentrations (ng/ml) in goats displayed significant differences ($P < .0002$), during hours of transport (Table 4). Location on trailer was not significant ($P = .856$) between hours of transport. There were no significant interaction ($P = .287$), between location on the trailer and hours of transport. Hour 6 differed from hours 0, 3, 9 and 12. Cortisol concentrations peaked at 6 h and returned to near basal levels at 12 h.

Table 4. Mean plasma cortisol concentrations (ng/ml) calculated using individual animals as the experimental unit, in relation to hours of transport

	Duration of transport					
Variable	0 h	3 h	6 h	9 h	12 h	*P* value
Cortisol	38.84±8.35[a]	68.20±8.35[b]	92.57±8.71[c]	53.65±8.35[b]	50.17±8.35[a]	*P* <.0002

[a,b,c]All durations of transport with different superscripts differ

Weight Loss

Location on the trailer did not influence (*P* = 0.199) the proportion of an animal's weight that was lost during transport (Shrink). There was a tendency for location 5 (Table 5) to have a higher percent weight loss (7.10 ± .77), compared to location 3 which tended to have the least weight loss (4.33 ± .77) overall.

Table 5. Mean percent weight loss (kg) from 0 h to 12 h of transport for each location within the trailer

	Location within trailer					
Variable	Location 1	Location 2	Location 3	Location 4	Location 5	*P* value
Weight loss	5.00 ±.77	5.67 ±.77	4.33 ±.77	6.00 ±.77	7.10 ± .77	*P* = .199

Gene Expression

During the RNA extraction process 2 samples were combined into one 15 mL tube, and the wash solution 1 during this particular extraction was not mixed with isopropanol, making the samples unusable. A second problem developed after RNA extractions. RNA samples were measured for quantity using Nanodrop, but during the process of measuring the quantity of RNA, the Nanodrop analyzer, not all of the results were saved to the analysis report. This problem was noticed while running the samples through the Bioanalyzer to determine quality of RNA and it was too late to retrieve the data. The missing samples were omitted from the study, and only samples with viable RNA were used for genetic evaluation through PCR.

The quantity of mRNA for genes of interest was measured in all samples using quantitative reverse transcriptase PCR (qRT-PCR). For qRT-PCR, 750 ng of RNA was reverse transcribed into cDNA. Samples were then applied, 3 samples per custom Taqman array 96-well microtiter fast plates containing custom bovine primer assays. Data was normalized for the amount of RNA in the reaction, to an endogenous control included on the card. Eighteen S was used as our control gene for these assays. The selected calibrator was an untreated control. Relative quantities (RQ) of gene expression were calculated from the raw data, using the $2^{-\Delta\Delta}$ Ct method (Livak and Schmittgen, 2001). Relative quantification describes the changes in expression of the target gene relative to a reference group.

The expression of the 15 genes was calculated by relative quantification after (qRT-PCR) produced C_t values for where the amplification curve crossed the threshold.

While there was no statistically significant (P <.05) expression of differences between hours of transport, however, 3 genes approached significance.

The amplification of genes *CCR5* (P = .084), and *CXCR5* (P = .068) which are part of the chemokine receptor pathway and gene *TNFRSF1A* (P = .103), a member of the TNF superfamily pathway tended (Table 6) to express differences between hours of transport.

Table 6. Mean RQ values (±SE) for genes that tended to express differences between hours of transport

	Duration of transport					
Variable	0 h	3 h	6 h	9 h	12 h	*P* value
CCR5	1.05 ±0.64	1.63 ±0.51	0.99 ±0.56	1.10 ±0.51	3.18 ±0.64	P = .084
TNFRSF1A	0.77 ±1.18	2.91 ±0.96	1.69 ±1.05	3.22 ±0.95	4.46 ±1.05	P = .103
CXCR5	0.88 ±0.63	0.98 ±0.49	0.71 ±0.63	0.92 ±0.49	3.14 ± 0.63	P = .068

There were no significant differences in expression (Table 7) found between hours of transport in the cytokine-cytokine signaling pathway for genes *IL4R* (P = .225), *IL10RB* (P = .339), and *IL12B* (P = .339).

Table 7. Mean RQ values (±SE) for genes found in the cytokine-cytokine signaling pathway that expressed no differences between hours of transport

	Duration of transport					
Variable	0 h	3 h	6 h	9 h	12 h	*P* value
IL10RB	0.89 ±1.47	1.88 ±1.14	1.00 ±1.27	2.19 ± 1.14	4.45 ±1.27	P = .339
IL12B	2.51 ± 2.57	7.97 ± 2.57	6.78 ± 2.23	7.30 ± 1.99	2.15 ± 2.57	P = .339
IL4R	0.87 ± 0.71	1.84 ± 0.59	1.02 ± 0.64	1.44 ± 0.59	2.33 ± 0.64	P = .225

Gene *CXCR2* (*P* = .328), in the chemokine receptor pathway had no significant difference in expression (Table 8) between hours of transport.

Table 8. Mean RQ values (±SE) for genes found in the chemokine receptor pathway that expressed no differences between hours of transport

	Duration of transport					
Variable	0 h	3 h	6 h	9 h	12 h	*P* value
CXCR2	0.94 ± 1.35	2.67 ± 1.05	1.53 ± 1.17	1.59 ± 1.05	4.15 ± 1.18	*P* = .328

The expression of heat shock factor protein genes *HSF2* (*P* = .259) and *HSP90AA1* (*P* = .345) was not influenced by duration of transport (Table 9).

Table 9. Mean RQ values (±SE) for heat shock factor protein genes that were not influenced by duration of transport

	Duration of transport					
Variable	0 h	3 h	6 h	9 h	12 h	*P* value
HSF2	0.80 ±1.29	1.51 ±1.01	1.23 ±1.12	1.05 ±1.01	3.10 ±1.12	*P* = .259
HSP90AA1	0.97 ±0.97	1.53 ±0.79	1.21 ±0.86	1.33 ± 0.79	3.03 ± 0.86	*P* = .345

There were no significant differences in expression (Table 10) found between hours of transport for protein coding sequence genes *LSP1* (*P* = .177), *IL1RN* (*P* = .245), *IK* (*P* =.455), *IFIT5* (*P* = .151), *CCRL1* (*P* = .238), and *SERP1* (*P* = .146). The RQ values varied as much as 4-fold at times, resulting in the very high standard errors.

Table 10. Mean RQ values (±SE) for protein coding sequence genes that were not influenced by duration of transport

	Duration of transport					
Variable	0 h	3 h	6 h	9 h	12 h	*P* value
LSP1	0.61 ± 1.05	2.35 ± 0.84	1.13 ± 0.92	2.24 ± 0.83	3.51 ± 0.92	*P* = .177
IL1RN	0.63 ± 0.98	2.27 ± 0.86	1.72 ± 0.91	1.97 ± 0.91	2.99 ± 0.98	*P* = .245
IK	0.85 ± 3.64	1.70 ± 2.28	8.44 ± 3.15	1.53 ± 2.82	3.05 ± 3.15	*P* =.455
IFIT5	1.29 ± 0.69	2.16 ± 0.54	0.95 ± 0.60	1.19 ± 0.54	2.77 ± 0.60	*P* = .151
CCRL1	1.50 ± 3.41	8.67 ± 2.70	5.74 ± 2.99	10.03 ± 2.70	4.24 ± 2.99	*P* = .238
SERP1	0.83 ± 1.06	1.20 ± 0.83	1.03 ± 0.92	1.04 ± 0.83	3.83 ± 0.92	*P* = .146

After running a Two-Independent sample T-test comparing THI (calculated every 30 minutes) for the two different locations (Pasture and Trailer), THI was not statistically difference ($P = 0.291$) between the pasture and trailer locations. In relation to cortisol and gene expression, THI also had no significant effects.

DISCUSSION

Prior to starting the study, during the habitation phase, 2 out of the 25 animals had to be isolated due to possibly infectious abscesses, and to avoid infecting any of the other animals. All animals in the study had previous exposure to being handled, loaded and unloaded into a trailer.

Leukocyte RNA extraction was selected for this study due to availability of animals. While RNA may be extracted from a variety of tissues and organs, our goats were on loan so blood samples were the best practical choice. However, other tissues like the liver may display a more time sensitive alteration of expression values due to differences in cell turnover rate. Leukocyte cell extraction is still the most practical for the use of live animals.

Blood samples were collected in the morning starting at 0800 h. The pre-transport sample was collected from the goats while each goat was still on the scale; this sample was collected 30 to 60 sec after the initial body weight was taken to obtain a resting plasma cortisol level. The initial spike in cortisol begins within 3 to 5 min of a stressor being applied and peaks within 10 to 20 min (Grandin 1997). Therefore, the cortisol concentrations in this study were not influenced by the sampling procedure because each sample was obtained within 30 to 60 sec of restraining each subject.

The immune system of farm animals can be affected due to stress, because immunity is reduced by the release of cortisol (Nwe et al., 1996). Plasma cortisol concentrations in goats in our study showed significant changes during the course of the

transport. There was a rapid increase in plasma cortisol concentrations from the pre-transport sample, 0 h, to the 3 h transport sample. This increase in cortisol could be due to factors like previous experience of being handled (Fell et al., 1985). A rapid increase in plasma cortisol after the start of transportation is consistent with Nwe et al. (1996). Three adult male Japanese Tokara goats were used by Nwe, and transported approximately 140 km to a farm, which took 6 h. There was no food, water or unloading for rest. Nwe et al. (1996) took blood samples before the start of the transport at 0800 h, at 30 and 60 min, and then at 1 h intervals. Plasma cortisol concentrations averaged 42 ng/ml before transport, and rose immediately after the start to a peak of 166 ng/ml within 1h.

In another study conducted by Buckham et al. (2007), transportation of young beef cattle for 9 h by road was used to determine changes in expression of candidate genes known to be important in neutrophil mediated defense and inflammation in the lung. To explore gene expression changes, blood were collected, plasma harvested, and neutrophils isolated from 6 Belgian Blue x Friesian bulls at -24, 0, 4.5, 9.75, 14.25, 24, and 48 h relative to commencement of 9 h road transportation by truck. Plasma cortisol concentrations were elevated at 4.5 and 9.75 h peaking at 50.64 ng/ml. The Buckham study is consistent with this study in regards to the increase in cortisol concentrations.

Percent weight loss showed no significant differences between locations on the trailer in our study, although that is confounded with group. The same group of goats stayed in the same location throughout the trial. Live weight loss depends on a series of factors such as: means of transportation, transport duration, livestock density, and

availability of food and water. The Council of the European Union (2005) states that goats less than 35 kg need 0.20 - 0.30 m^2, goats 35 - 55 kg need 0.30 - 0.40 m^2, and goats over 55 kg need 0.40 - 0.75 m^2 of space per goat for satisfactory transport with quality air ventilation. Kannan et al. (2000) investigated live weight loss (shrink) in goats due to differences in stocking densities. In that study, a total of 150 Spanish does were transported on two different days and held overnight without feed in low (25 does, .18 m^2/animals) or high (50 does, .37m^2/animals) density groups. Blood samples in that study were taken in the pens at 0, 1, 2, 3, 4 or 18 h. Individual animals were weighed just before loading and after overnight holding. Results suggest that live weight loss was not influenced by the high and low density groups. Those results are similar to this study in that weight loss did not have a significant effect on goats when placed in groups (3 goats, .495 m^2/animal) during transport. From an economic stand point weight loss is important and is correlated with species, transport distance and conditions.

To determine effects on the immune system, expression of 13 genes of particular interest involved in two immune pathways and 2 reference genes were observed in our study. These pathways include the chemokine signaling pathway and the cytokine-cytokine receptor pathway. The missing data from errors during the laboratory analysis probably contributed to the variation in the gene expression data. In addition, reference gene 18S expressed variation in raw Ct values that ranged from 9 to 13. However, even though the primer for LSP-1 did not express any significant differences, it showed quality amplification in this study and in the Terrill et al. (2011), and would make a great reference gene in future studies.

Genes *CCR5* (P = .084), *TNFRSF1A* (P = .103), and *CXCR5* (P = .068) expressed a trend for being influenced by 12 h of transport. These genes started rising in expression between hours 0 to 3, then started to decline around hour 6 of transport, and rose again at hour 9 and continued to rise during hour 12 of transport.

Genes *CCR5* and *CXCR5*, which are a part of the chemokine receptor pathway and are important signaling molecules found on leukocytes that influence both innate and adaptive immunity, control leukocyte migration into lymphoid tissues and localization to sites of infection and inflammation (Blumerman et al., 2007). *CXCR5* is known to be expressed on CD4+ follicular T helper cells in lymph nodes. *CXCR5* is also known to be highly inducible following activation of CD4+ T cells, but thereafter only temporarily expressed. *CCR5* is a major chemokine receptor expressed by T cells involved in inflammatory responses (Blumerman et al., 2007). Gene *TNFRSF1A* plays an important roles in various physiological and pathological processes, including cell proliferation, apoptosis, and modulation of immune responses and induction of inflammation. These genes that tended to express differences between 12 h of transport regulate immune cell trafficking, promoting inflammation and stimulate leukocyte migration in domestic animal species.

Even though there were no significant differences found between hours of transport for gene expression, the trends that we did see show promise and provides a reference for future studies. After reviewing the literature, there were no specific studies that looked at the rate of change in gene expression. Because our results generally showed an increase in expression after 12 h of transport, it may be appropriate for future

studies to conduct a study longer than 12 h. A study over a course of five or more days may be very useful.

The inability to replicate this study made it difficult to compare location on the trailer, cortisol and hours of transport. However, these data are still the first of their kind using goats. The data from our preliminary study and the data from the Du et al. (2012) indicate that studying the goat genome can be very useful. The data from this study also indicate that it may take more than 12 h from the onset of a stressor for the expression of certain genes to be altered by stress.

CONCLUSION

Goat plasma cortisol concentrations were significantly elevated (*P<.049)* indicating that goats experienced stressful events during transport. Cortisol concentrations peaked after 6 hours, and returned to near basal concentrations after 12 h of transport. However, while the goats had increased cortisol concentrations they did not have statistically significant increased expression of immune inflammatory response genes.

There was a consistent trend for three genes to increase in expression during transport, with the greatest expression being after 12 h of transport. These genes show promise in evaluating stress related physiological reactions in goats, especially if this response is investigated over a longer time period with replications. Future analysis should look at determining at what point these genes begin to up-regulate to help determine a more accurate effect of stress on the immune system of goats in transport. This information may help determine at what point goats are most susceptible to infection. Although it shows promise, gene expression data may not be as useful a tool at this point in determining stress in goats as cortisol hormone assays.

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APPENDIX

Table 11. Description of the 64 goat genes that amplified out the 93 target bovine genes

Genes	Gene names
CCL25	Chemokine (C-C motif) ligand 25
CCL28	Chemokine (C-C motif) ligand 28
CCR5	Chemokine (C-C motif) receptor 5
CCRL1	Chemokine (C-C motif) receptor-like 1
CD40	CD40 molecule, TNF receptor superfamily member 5
CD40LG	CD40 ligand
CRH	Corticotropin releasing hormone
CSF-1	Colony stimulating factor 1 (macrophage)
CSF1R	Colony stimulating factor 1 receptor
CXCL12	Chemokine (C-X-C motif) ligand 12
CXCL5	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
CXCL9	Chemokine (C-X-C motif) ligand 9
CXCR3	Chemokine (C-X-C motif) receptor 3
CXCR5	Chemokine (C-X-C motif) receptor 5
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HSF2	Heat shock transcription factor 2
HSP90AB1	Heat shock protein 90kDa alpha (cytosolic), class B member 1
HSPA14	Heat shock 70kDa protein 14
HSPA1A	Heat shock 70kDa protein 1A
HSPA8	Heat shock 70kDa protein 8
HSPB1	Heat shock 27kDa protein 1
HSPB6	Heat shock protein, alpha-crystallin-related, B6
HSPB8	Heat shock 22kDa protein 8
HSPCA	Heat shock protein 90kDa alpha (cytosolic), class A member 1
IFIT5	Interferon-induced protein with tetratricopeptide repeats 5
IFNAR2	Interferon (alpha, beta and omega) receptor 2
IFNG	Interferon, gamma
IK	IK cytokine, down-regulator of HLA II
IL10	Interleukin 10
IL10RB	Interleukin 10 receptor, beta
IL11RA	Interleukin 11 receptor, alpha

IL12B	Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic Lymphocyte maturation factor 2, p40)
IL12RB2	Interleukin 12 receptor, beta 2
IL13	Interleukin 13
IL16	Interleukin 16
IL18	Interleukin 18 (interferon-gamma-inducing factor)
IL1B	Interleukin 1, beta
IL1F5	Interleukin 36 receptor antagonist
IL1RN	Interleukin 1 receptor antagonist
IL2	Interleukin 2
IL23R	Interleukin 23 receptor
IL4	Interleukin 4
IL4R	Interleukin 4 receptor
IL6R	Interleukin 6 receptor
IL8RB	Interleukin 8 receptor, beta
IRAK2	Interleukin-1 receptor-associated kinase 2
KLRA1	Killer cell lectin-like receptor subfamily A, member 1
KLRK1	Killer cell lectin-like receptor subfamily K, member 1
LBP	Lipopolysaccharide binding protein
LOC529196	C-C chemokine receptor type 1-like
LSP1	Lymphocyte-specific protein 1
NKG7	Natural killer cell group 7 sequence
PTGDR	Prostaglandin D2 receptor (DP)
PTGER4	Prostaglandin E receptor 4 (subtype EP4)
SERP1	Stress-associated endoplasmic reticulum protein 1
SFTPA1B	Surfactant protein A1
TLR10	Toll-like receptor 10
TLR6	Toll-like receptor 6
TLR9	Toll-like receptor 9
TNF	Tumor necrosis factor
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A
TNFRSF4	Tumor necrosis factor receptor superfamily, member 4
TNFRSF9	Tumor necrosis factor receptor superfamily, member 9
TNFRSF8	Tumor necrosis factor receptor superfamily, member 8

Table 12. RQ values for all goat expression of gene *CCR5* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.329791
A- B SHO	1	3	2	0.821456
B-B SHO	1	3	3	.
C-B SHO	1	3	4	0.78968
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	0.493687
A- GRN BCK	2	3	2	1.317015
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	0.636813
B-R SHO	3	4	3	0.3883
C-R SHO	3	4	4	0.633597
D-R SHO	3	4	5	2.374411
P-RED BCK	4	2	1	1.729316
A- RED BCK	4	2	2	1.929562
B- RED BCK	4	2	3	1.874589
C- RED BCK	4	2	4	1.979801
D- RED BCK	4	2	5	1.267928
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	1.399093
B-BLK BCK	5	1	3	0.756873
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	0.604251
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.806383
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	1.785156
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	1.673282
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.589866

A-BLU X	9	2	2	1.140675
B-BLU X	9	2	3	0.201081
C-BLU X	9	2	4	0.876305
D-BLU X	9	2	5	0.801407
P-G BCK	10	5	1	.
A-G BCK	10	5	2	3.227042
B-G BCK	10	5	3	.
C-G BCK	10	5	4	1.176501
D-G BCK	10	5	5	6.264977
P-G 14	11	4	1	.
A-G14	11	4	2	1.316869
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	3.350997
A-GRN SHO	12	2	2	1.355033
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	1.125287
D- GRN SHO	12	2	5	1.422882
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	1.260918
D- ORG BCK	13	1	5	.
P-ORG SHO	14	5	1	0.349905
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.706228
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	0.459799
C-RED HIP	15	3	4	0.815951
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 13. RQ values for all goat expression of gene *CCRL1* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.08288
A- B SHO	1	3	2	0.641407
B-B SHO	1	3	3	.
C-B SHO	1	3	4	10.47846
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	4.800988
A- GRN BCK	2	3	2	38.72801
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	2.947923
B-R SHO	3	4	3	8.386191
C-R SHO	3	4	4	1.920686
D-R SHO	3	4	5	1.906609
P-RED BCK	4	2	1	0.277439
A- RED BCK	4	2	2	1.036139
B- RED BCK	4	2	3	2.090236
C- RED BCK	4	2	4	0.812431
D- RED BCK	4	2	5	1.472349
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	6.700076
B-BLK BCK	5	1	3	1.12206
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	1.093702
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	5.90475
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	1.511591
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	1.550556
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	3.553263

A-BLU X	9	2	2	26.95938
B-BLU X	9	2	3	4.901169
C-BLU X	9	2	4	6.68781
D-BLU X	9	2	5	3.544588
P-G BCK	10	5	1	.
A-G BCK	10	5	2	1.964708
B-G BCK	10	5	3	.
C-G BCK	10	5	4	45.23319
D-G BCK	10	5	5	6.695805
P-G 14	11	4	1	.
A-G14	11	4	2	6.912837
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	0.404966
A-GRN SHO	12	2	2	2.226957
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	0.951059
D- GRN SHO	12	2	5	0.468687
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	8.37803
D- ORG BCK	13	1	5	3.695197
P-ORG SHO	14	5	1	2.946608
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.674293
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	11.76529
C-RED HIP	15	3	4	16.88734
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 14. RQ values for all goat expression of gene *CXCR2* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.120955
A- B SHO	1	3	2	1.684954
B-B SHO	1	3	3	.
C-B SHO	1	3	4	0.70203
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.850079
A- GRN BCK	2	3	2	3.609261
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	1.488841
B-R SHO	3	4	3	1.067635
C-R SHO	3	4	4	0.921067
D-R SHO	3	4	5	2.047837
P-RED BCK	4	2	1	0.981134
A- RED BCK	4	2	2	1.820772
B- RED BCK	4	2	3	1.65555
C- RED BCK	4	2	4	1.498395
D- RED BCK	4	2	5	1.082557
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	1.576633
B-BLK BCK	5	1	3	1.364058
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	1.275879
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	2.057668
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	3.019427
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	2.591613
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.437338

A-BLU X	9	2	2	5.650964
B-BLU X	9	2	3	1.449692
C-BLU X	9	2	4	1.37732
D-BLU X	9	2	5	1.128551
P-G BCK	10	5	1	.
A-G BCK	10	5	2	4.321395
B-G BCK	10	5	3	.
C-G BCK	10	5	4	2.563841
D-G BCK	10	5	5	11.98967
P-G 14	11	4	1	.
A-G14	11	4	2	2.49409
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.86686
A-GRN SHO	12	2	2	1.404892
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	2.003088
D- GRN SHO	12	2	5	1.89198
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	2.227553
D- ORG BCK	13	1	5	1.375637
P-ORG SHO	14	5	1	1.140795
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.507536
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	1.006717
C-RED HIP	15	3	4	0.515923
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 15. RQ values for all goat expression of gene *CXCR5* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.199425
A- B SHO	1	3	2	0.563223
B-B SHO	1	3	3	.
C-B SHO	1	3	4	1.391164
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.129279
A- GRN BCK	2	3	2	1.486486
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	0.517472
B-R SHO	3	4	3	0.412864
C-R SHO	3	4	4	0.629972
D-R SHO	3	4	5	2.453025
P-RED BCK	4	2	1	1.394903
A- RED BCK	4	2	2	0.700385
B- RED BCK	4	2	3	0.891092
C- RED BCK	4	2	4	0.760516
D- RED BCK	4	2	5	0.770376
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	1.226052
B-BLK BCK	5	1	3	0.761252
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	0.316668
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.121461
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	0.330948
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	0.741379
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.097924

A-BLU X	9	2	2	0.734377
B-BLU X	9	2	3	0.294392
C-BLU X	9	2	4	0.462092
D-BLU X	9	2	5	0.660697
P-G BCK	10	5	1	.
A-G BCK	10	5	2	1.480531
B-G BCK	10	5	3	.
C-G BCK	10	5	4	1.373379
D-G BCK	10	5	5	6.179787
P-G 14	11	4	1	.
A-G14	11	4	2	0.476264
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	0.970661
A-GRN SHO	12	2	2	0.584076
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	0.705289
D- GRN SHO	12	2	5	0.565564
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	1.333427
D- ORG BCK	13	1	5	2052.896
P-ORG SHO	14	5	1	1.132289
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	1.052844
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	.
C-RED HIP	15	3	4	0.843574
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 16. RQ values for all goat expression of gene *HSF2* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.146951
A- B SHO	1	3	2	1.04204
B-B SHO	1	3	3	.
C-B SHO	1	3	4	0.747709
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	0.925704
A- GRN BCK	2	3	2	1.508965
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	0.920695
B-R SHO	3	4	3	0.820936
C-R SHO	3	4	4	0.772857
D-R SHO	3	4	5	2.541204
P-RED BCK	4	2	1	1.274198
A- RED BCK	4	2	2	1.064991
B- RED BCK	4	2	3	1.727887
C- RED BCK	4	2	4	1.433386
D- RED BCK	4	2	5	0.741449
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	0.904505
B-BLK BCK	5	1	3	1.077214
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	1.126934
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.045352
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	1.008031
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	1.080651
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.281382

A-BLU X	9	2	2	0.901985
B-BLU X	9	2	3	0.432127
C-BLU X	9	2	4	0.51108
D-BLU X	9	2	5	0.712175
P-G BCK	10	5	1	.
A-G BCK	10	5	2	3.215595
B-G BCK	10	5	3	.
C-G BCK	10	5	4	1.167809
D-G BCK	10	5	5	11.54633
P-G 14	11	4	1	.
A-G14	11	4	2	1.28985
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.314817
A-GRN SHO	12	2	2	1.158477
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	1.469108
D- GRN SHO	12	2	5	1.196373
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	1.685155
D- ORG BCK	13	1	5	1.155441
P-ORG SHO	14	5	1	1.598958
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	1.033031
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	0.710935
C-RED HIP	15	3	4	0.427505
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 17. RQ values for all goat expression of gene *HSP90AA1* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.192255476
A- B SHO	1	3	2	0.882781526
B-B SHO	1	3	3	.
C-B SHO	1	3	4	1.266422545
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.456657645
A- GRN BCK	2	3	2	2.10454425
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	0.742443859
B-R SHO	3	4	3	0.53983918
C-R SHO	3	4	4	0.72100009
D-R SHO	3	4	5	1.668424432
P-RED BCK	4	2	1	0.63086265
A- RED BCK	4	2	2	0.864923741
B- RED BCK	4	2	3	1.002113101
C- RED BCK	4	2	4	0.882526707
D- RED BCK	4	2	5	0.541895978
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	1.324864066
B-BLK BCK	5	1	3	0.981716823
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	0.684936118
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.631246542
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	1.268532378
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	1.009671752
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.320692461

A-BLU X	9	2	2	3.168213027
B-BLU X	9	2	3	0.781953023
C-BLU X	9	2	4	1.124457663
D-BLU X	9	2	5	0.983093707
P-G BCK	10	5	1	.
A-G BCK	10	5	2	2.12112162
B-G BCK	10	5	3	.
C-G BCK	10	5	4	3.540872372
D-G BCK	10	5	5	8.783992629
P-G 14	11	4	1	.
A-G14	11	4	2	1.066412683
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	0.95472017
A-GRN SHO	12	2	2	1.323212569
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	1.205001835
D- GRN SHO	12	2	5	0.878111073
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	1.349615403
D- ORG BCK	13	1	5	1.013365307
P-ORG SHO	14	5	1	3.312170279
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.657679152
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	0.786113671
C-RED HIP	15	3	4	0.962952035
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 18. RQ values for all goat expression of gene *IFIT5* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.164509
A- B SHO	1	3	2	1.052772
B-B SHO	1	3	3	.
C-B SHO	1	3	4	0.956927
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	3.617974
A- GRN BCK	2	3	2	8.038252
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	0.887843
B-R SHO	3	4	3	0.540258
C-R SHO	3	4	4	0.487671
D-R SHO	3	4	5	1.481522
P-RED BCK	4	2	1	0.613382
A- RED BCK	4	2	2	1.647491
B- RED BCK	4	2	3	0.885081
C- RED BCK	4	2	4	0.719516
D- RED BCK	4	2	5	0.367864
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	0.68401
B-BLK BCK	5	1	3	0.470995
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	1.076195
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.953239
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	1.061953
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	2.270818
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.255815

A-BLU X	9	2	2	2.61822
B-BLU X	9	2	3	1.136062
C-BLU X	9	2	4	0.866761
D-BLU X	9	2	5	0.794272
P-G BCK	10	5	1	.
A-G BCK	10	5	2	2.395563
B-G BCK	10	5	3	.
C-G BCK	10	5	4	1.64374
D-G BCK	10	5	5	5.138566
P-G 14	11	4	1	.
A-G14	11	4	2	1.831078
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.188571
A-GRN SHO	12	2	2	1.189025
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	1.244441
D- GRN SHO	12	2	5	1.126932
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	2.240193
D- ORG BCK	13	1	5	3.72397
P-ORG SHO	14	5	1	1.673503
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.475881
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	0.5478
C-RED HIP	15	3	4	1.059151
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 19. RQ values for all goat expression of gene *IK* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.282595
A- B SHO	1	3	2	0.823538
B-B SHO	1	3	3	.
C-B SHO	1	3	4	1.259019
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.40711
A- GRN BCK	2	3	2	2.117168
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	0.918718
B-R SHO	3	4	3	0.996594
C-R SHO	3	4	4	0.702426
D-R SHO	3	4	5	1.537493
P-RED BCK	4	2	1	0.733808
A- RED BCK	4	2	2	1.636063
B- RED BCK	4	2	3	1.80485
C- RED BCK	4	2	4	1.544532
D- RED BCK	4	2	5	0.739856
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	1.602114
B-BLK BCK	5	1	3	1.455273
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	1.213377
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.176377
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	1.34462
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	1.419413
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.468446

A-BLU X	9	2	2	3.267064
B-BLU X	9	2	3	0.733041
C-BLU X	9	2	4	1.327543
D-BLU X	9	2	5	0.695648
P-G BCK	10	5	1	.
A-G BCK	10	5	2	1.997022
B-G BCK	10	5	3	.
C-G BCK	10	5	4	3.701681
D-G BCK	10	5	5	7.138612
P-G 14	11	4	1	.
A-G14	11	4	2	1.392959
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.36866
A-GRN SHO	12	2	2	1.841271
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	2.485752
D- GRN SHO	12	2	5	1.184581
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	1.486258
D- ORG BCK	13	1	5	1.997061
P-ORG SHO	14	5	1	1.701926
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.813447
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	29.75559
C-RED HIP	15	3	4	1.00027
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 20. RQ values for all goat expression of gene *IL10RB* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.218746
A- B SHO	1	3	2	0.777041
B-B SHO	1	3	3	.
C-B SHO	1	3	4	1.897309
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.491092
A- GRN BCK	2	3	2	3.095702
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	1.012107
B-R SHO	3	4	3	0.810912
C-R SHO	3	4	4	1.335298
D-R SHO	3	4	5	1.563057
P-RED BCK	4	2	1	0.710324
A- RED BCK	4	2	2	1.173259
B- RED BCK	4	2	3	1.34225
C- RED BCK	4	2	4	1.506674
D- RED BCK	4	2	5	0.776439
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	2.09454
B-BLK BCK	5	1	3	1.155301
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	0.624605
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.783943
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	1.823109
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	0.998299
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.254889

A-BLU X	9	2	2	3.913701
B-BLU X	9	2	3	0.830322
C-BLU X	9	2	4	1.881118
D-BLU X	9	2	5	0.978984
P-G BCK	10	5	1	.
A-G BCK	10	5	2	1.737858
B-G BCK	10	5	3	.
C-G BCK	10	5	4	3.362449
D-G BCK	10	5	5	13.1848
P-G 14	11	4	1	.
A-G14	11	4	2	1.811173
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.490593
A-GRN SHO	12	2	2	1.613915
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	3.098661
D- GRN SHO	12	2	5	1.702771
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	2.689831
D- ORG BCK	13	1	5	1.887338
P-ORG SHO	14	5	1	1.135053
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.914889
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	1.106837
C-RED HIP	15	3	4	2.401842
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 21.RQ values for all goat expression of gene *IL12B* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.187707
A- B SHO	1	3	2	5.585827
B-B SHO	1	3	3	.
C-B SHO	1	3	4	1.633121
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	9.839069
A- GRN BCK	2	3	2	110.9515
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	8.075423
B-R SHO	3	4	3	6.430028
C-R SHO	3	4	4	2.750891
D-R SHO	3	4	5	1.31936
P-RED BCK	4	2	1	0.308228
A- RED BCK	4	2	2	8.203547
B- RED BCK	4	2	3	4.519411
C- RED BCK	4	2	4	3.119857
D- RED BCK	4	2	5	0.616029
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	2.469745
B-BLK BCK	5	1	3	0.636887
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	3.321578
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.9881
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	7.345602
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	22.44318
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	5.905545

A-BLU X	9	2	2	23.21482
B-BLU X	9	2	3	3.70244
C-BLU X	9	2	4	5.284926
D-BLU X	9	2	5	0.841509
P-G BCK	10	5	1	.
A-G BCK	10	5	2	11.36636
B-G BCK	10	5	3	.
C-G BCK	10	5	4	30.30744
D-G BCK	10	5	5	25.29472
P-G 14	11	4	1	.
A-G14	11	4	2	12.38827
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.157348
A-GRN SHO	12	2	2	35.36234
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	5.953313
D- GRN SHO	12	2	5	3.044507
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	3.646745
D- ORG BCK	13	1	5	3.252333
P-ORG SHO	14	5	1	1.689693
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.342469
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	6.200288
C-RED HIP	15	3	4	11.32727
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 22.RQ values for all goat expression of gene *IL1RN* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.262451
A- B SHO	1	3	2	0.862981
B-B SHO	1	3	3	.
C-B SHO	1	3	4	0.732539
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.677476
A- GRN BCK	2	3	2	1.266823
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	1.146679
B-R SHO	3	4	3	0.58993
C-R SHO	3	4	4	0.732841
D-R SHO	3	4	5	1.351423
P-RED BCK	4	2	1	1.059918
A- RED BCK	4	2	2	1.882844
B- RED BCK	4	2	3	2.630873
C- RED BCK	4	2	4	2.107257
D- RED BCK	4	2	5	1.00074
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	1.59936
B-BLK BCK	5	1	3	0.871214
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	1.44632
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	2.765979
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	3.901129
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	0.001491
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.751526

A-BLU X	9	2	2	4.14611
B-BLU X	9	2	3	1.762082
C-BLU X	9	2	4	3.053976
D-BLU X	9	2	5	1.096503
P-G BCK	10	5	1	.
A-G BCK	10	5	2	4.872462
B-G BCK	10	5	3	.
C-G BCK	10	5	4	5.305929
D-G BCK	10	5	5	7.850673
P-G 14	11	4	1	.
A-G14	11	4	2	.
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.737405
A-GRN SHO	12	2	2	2.004544
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	1.783731
D- GRN SHO	12	2	5	1.378984
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	.
D- ORG BCK	13	1	5	.
P-ORG SHO	14	5	1	0.625837
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.527254
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	0.794224
C-RED HIP	15	3	4	1.27382
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 23.RQ values for all goat expression of gene *IL4R* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.215444
A- B SHO	1	3	2	0.633191
B-B SHO	1	3	3	.
C-B SHO	1	3	4	1.005053
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.513631
A- GRN BCK	2	3	2	2.773089
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	0.898651
B-R SHO	3	4	3	0.543509
C-R SHO	3	4	4	0.627286
D-R SHO	3	4	5	1.330406
P-RED BCK	4	2	1	0.577901
A- RED BCK	4	2	2	1.198213
B- RED BCK	4	2	3	0.821799
C- RED BCK	4	2	4	0.777247
D- RED BCK	4	2	5	0.459854
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	1.306992
B-BLK BCK	5	1	3	0.817583
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	0.603548
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	2.725189
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	1.756887
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	0.746236
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.410654

A-BLU X	9	2	2	7.06249
B-BLU X	9	2	3	1.544692
C-BLU X	9	2	4	1.975288
D-BLU X	9	2	5	1.546043
P-G BCK	10	5	1	.
A-G BCK	10	5	2	2.124786
B-G BCK	10	5	3	.
C-G BCK	10	5	4	3.997297
D-G BCK	10	5	5	6.303444
P-G 14	11	4	1	.
A-G14	11	4	2	1.370349
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.131703
A-GRN SHO	12	2	2	1.931355
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	1.408036
D- GRN SHO	12	2	5	0.738737
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	0.778185
D- ORG BCK	13	1	5	0.739711
P-ORG SHO	14	5	1	2.250469
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.574653
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	0.925165
C-RED HIP	15	3	4	1.639626
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 24.RQ values for all goat expression of gene *LSP1* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.293981
A- B SHO	1	3	2	0.803415
B-B SHO	1	3	3	.
C-B SHO	1	3	4	3.352909
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.651792
A- GRN BCK	2	3	2	5.933662
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	1.002196
B-R SHO	3	4	3	0.640153
C-R SHO	3	4	4	0.561908
D-R SHO	3	4	5	1.626427
P-RED BCK	4	2	1	0.709392
A- RED BCK	4	2	2	1.424796
B- RED BCK	4	2	3	0.960829
C- RED BCK	4	2	4	0.846703
D- RED BCK	4	2	5	0.498641
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	2.408557
B-BLK BCK	5	1	3	0.814683
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	0.824933
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.854432
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	0.884655
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	0.858591
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.641035

A-BLU X	9	2	2	5.705855
B-BLU X	9	2	3	1.236566
C-BLU X	9	2	4	1.335999
D-BLU X	9	2	5	0.971121
P-G BCK	10	5	1	.
A-G BCK	10	5	2	1.79675
B-G BCK	10	5	3	.
C-G BCK	10	5	4	6.999989
D-G BCK	10	5	5	9.326053
P-G 14	11	4	1	.
A-G14	11	4	2	1.98466
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	0.822185
A-GRN SHO	12	2	2	2.417225
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	2.367294
D- GRN SHO	12	2	5	0.808179
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	1.636998
D- ORG BCK	13	1	5	1.888974
P-ORG SHO	14	5	1	1.320992
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.886182
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	1.544653
C-RED HIP	15	3	4	3.159677
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 25.RQ values for all goat expression of gene *SERP1* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.212397
A- B SHO	1	3	2	1.043634
B-B SHO	1	3	3	.
C-B SHO	1	3	4	0.855542
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.107104
A- GRN BCK	2	3	2	1.57457
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	0.975531
B-R SHO	3	4	3	0.638229
C-R SHO	3	4	4	0.74006
D-R SHO	3	4	5	2.753723
P-RED BCK	4	2	1	1.043769
A- RED BCK	4	2	2	0.798327
B- RED BCK	4	2	3	1.224563
C- RED BCK	4	2	4	1.321376
D- RED BCK	4	2	5	0.804214
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	0.755259
B-BLK BCK	5	1	3	0.789864
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	1.112692
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	1.020411
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	1.207971
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	1.38503
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.236576

A-BLU X	9	2	2	0.932708
B-BLU X	9	2	3	0.385062
C-BLU X	9	2	4	0.56832
D-BLU X	9	2	5	0.61413
P-G BCK	10	5	1	.
A-G BCK	10	5	2	1.885798
B-G BCK	10	5	3	.
C-G BCK	10	5	4	1.387771
D-G BCK	10	5	5	10.05468
P-G 14	11	4	1	.
A-G14	11	4	2	1.381308
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.045168
A-GRN SHO	12	2	2	0.911806
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	1.058481
D- GRN SHO	12	2	5	1.136694
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	1.550581
D- ORG BCK	13	1	5	1.75223
P-ORG SHO	14	5	1	1.679684
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.825942
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	0.602368
C-RED HIP	15	3	4	0.430133
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 26.RQ values for all goat expression of gene *TNFRSF1A* during time point 0 h, 3 h, 6 h, and 12 h in all 5 locations in the trailer

Animal[a]	ID #	Location in the trailer	Time points (h)	RQ values
P-B SHO	1	3	1	0.224158
A- B SHO	1	3	2	0.759635
B-B SHO	1	3	3	.
C-B SHO	1	3	4	2.827138
D-B SHO	1	3	5	.
P-GRN BCK	2	3	1	1.985824
A- GRN BCK	2	3	2	6.124894
B-GRN BCK	2	3	3	.
C-GRN BCK	2	3	4	.
D-GRN BCK	2	3	5	.
P-R SHO	3	4	1	.
A- R SHO	3	4	2	1.166636
B-R SHO	3	4	3	0.706074
C-R SHO	3	4	4	0.725813
D-R SHO	3	4	5	1.747062
P-RED BCK	4	2	1	0.603525
A- RED BCK	4	2	2	1.370757
B- RED BCK	4	2	3	1.032075
C- RED BCK	4	2	4	0.823053
D- RED BCK	4	2	5	0.487443
P-BLK BCK	5	1	1	.
A-BLK GOAT	5	1	2	2.784225
B-BLK BCK	5	1	3	0.68463
C-BLK BCK	5	1	4	.
D-BLK BCK	5	1	5	.
P-BLK HIP	6	4	1	.
A-BLK HIP	6	4	2	.
B-BLK HIP	6	4	3	0.550681
C-BLK HIP	6	4	4	.
D-BLK HIP	6	4	5	.
P-BLK SHO	7	5	1	3.550445
A-BLK SHO	7	5	2	.
B-BLK SHO	7	5	3	.
C-BLK SHO	7	5	4	2.451076
D-BLK SHO	7	5	5	.
P-BLU VERT	8	1	1	.
A-BLU VERT	8	1	2	.
B-BLU VERT	8	1	3	1.263315
C-BLU VERT	8	1	4	.
D-BLU VERT	8	1	5	.
P-BLU X	9	2	1	0.578289

A-BLU X	9	2	2	10.00471
B-BLU X	9	2	3	3.560236
C-BLU X	9	2	4	4.015694
D-BLU X	9	2	5	1.018159
P-G BCK	10	5	1	.
A-G BCK	10	5	2	2.183161
B-G BCK	10	5	3	.
C-G BCK	10	5	4	8.051647
D-G BCK	10	5	5	10.19973
P-G 14	11	4	1	.
A-G14	11	4	2	3.106901
B-G 14	11	4	3	.
C-G 14	11	4	4	.
D-G 14	11	4	5	.
P-GRN SHO	12	2	1	1.130208
A-GRN SHO	12	2	2	2.586689
B-GRN SHO	12	2	3	
C-GRN SHO	12	2	4	2.680707
D- GRN SHO	12	2	5	0.96879
P-ORG BCK	13	1	1	.
A-ORG BCK	13	1	2	.
B-ORG BCK	13	1	3	.
C-ORG BCK	13	1	4	1.822189
D- ORG BCK	13	1	5	4.281505
P-ORG SHO	14	5	1	1.048329
A-ORG SHO	14	5	2	.
B-ORG SHO	14	5	3	.
C-ORG SHO	14	5	4	.
D-ORG SHO	14	5	5	.
P-RED HIP	15	3	1	0.506481
A-RED HIP	15	3	2	.
B-RED HIP	15	3	3	2.405413
C-RED HIP	15	3	4	7.39384
D-RED HIP	15	3	5	.

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 27. Plasma cortisol concentrations (ng/ml)

Animal[a]	ID #	Location in the trailer	Time points (h)	Cortisol
P-B SHO	1	3	1	18.004
A- B SHO	1	3	2	45.067
B-B SHO	1	3	3	25.422
C-B SHO	1	3	4	57.571
D-B SHO	1	3	5	38.346
P-GRN BCK	2	3	1	78.655
A- GRN BCK	2	3	2	43.825
B-GRN BCK	2	3	3	121.226
C-GRN BCK	2	3	4	81.530
D-GRN BCK	2	3	5	63.199
P-R SHO	3	4	1	18.543
A- R SHO	3	4	2	94.173
B-R SHO	3	4	3	57.203
C-R SHO	3	4	4	26.788
D-R SHO	3	4	5	29.459
P-RED BCK	4	2	1	5.157
A- RED BCK	4	2	2	42.939
B- RED BCK	4	2	3	67.207
C- RED BCK	4	2	4	23.720
D- RED BCK	4	2	5	33.327
P-BLK BCK	5	1	1	2.448
A-BLK GOAT	5	1	2	112.076
B-BLK BCK	5	1	3	108.937
C-BLK BCK	5	1	4	115.038
D-BLK BCK	5	1	5	83.020
P-BLK HIP	6	4	1	23.824
A-BLK HIP	6	4	2	117.419
B-BLK HIP	6	4	3	66.131
C-BLK HIP	6	4	4	54.026
D-BLK HIP	6	4	5	28.764
P-BLK SHO	7	5	1	14.172
A-BLK SHO	7	5	2	50.390
B-BLK SHO	7	5	3	61.952
C-BLK SHO	7	5	4	45.147
D-BLK SHO	7	5	5	46.278
P-BLU VERT	8	1	1	45.864
A-BLU VERT	8	1	2	107.194
B-BLU VERT	8	1	3	95.917
C-BLU VERT	8	1	4	77.780
D-BLU VERT	8	1	5	82.440
P-BLU X	9	2	1	35.372
A-BLU X	9	2	2	139.681

B-BLU X	9	2	3	105.929
C-BLU X	9	2	4	16.488
D-BLU X	9	2	5	46.139
P-G BCK	10	5	1	20.976
A-G BCK	10	5	2	47.630
B-G BCK	10	5	3	113.656
C-G BCK	10	5	4	73.444
D-G BCK	10	5	5	81.783
P-G 14	11	4	1	18.263
A-G14	11	4	2	51.717
B-G 14	11	4	3	109.303
C-G 14	11	4	4	69.228
D-G 14	11	4	5	38.055
P-GRN SHO	12	2	1	129.320
A-GRN SHO	12	2	2	68.361
B-GRN SHO	12	2	3	.
C-GRN SHO	12	2	4	42.939
D- GRN SHO	12	2	5	40.101
P-ORG BCK	13	1	1	58.270
A-ORG BCK	13	1	2	45.674
B-ORG BCK	13	1	3	50.187
C-ORG BCK	13	1	4	28.983
D- ORG BCK	13	1	5	50.682
P-ORG SHO	14	5	1	76.000
A-ORG SHO	14	5	2	56.245
B-ORG SHO	14	5	3	154.239
C-ORG SHO	14	5	4	43.757
D-ORG SHO	14	5	5	36.619
P-RED HIP	15	3	1	37.754
A-RED HIP	15	3	2	45.674
B-RED HIP	15	3	3	150.272
C-RED HIP	15	3	4	48.269
D-RED HIP	15	3	5	54.334

[a]Legend: A = 3 h, B = 6 h, C = 9 h, D = 12 h, P = 0 h

Table 28. Percent weight loss

Animal	ID #	Location in the trailer	% Weight loss
D-B SHO	1	3	5.8
D-GRN BCK	2	3	3.8
D-R SHO	3	4	5.6
D- RED BCK	4	2	7.2
D-BLK BCK	5	1	4.1
D-BLK HIP	6	4	7.2
D-BLK SHO	7	5	8.9
D-BLU VERT	8	1	5.9
D-BLU X	9	2	4
D-G BCK	10	5	6.8
D-G 14	11	4	5.2
D- GRN SHO	12	2	5.8
D- ORG BCK	13	1	5
D-ORG SHO	14	5	5.6
D-RED HIP	15	3	3.4

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