# EFFECT OF DURATION OF TRANSPORT ON INDICATORS OF STRESS IN LAMBS

A Thesis

by

PETER DOWNS KRAWCZEL

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

May 2006

Major Subject: Animal Science

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Approved by:

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| Chair of Committee, | Theodore H. Friend |
| Committee Members, | Thomas H. Welsh, Jr. |
| | David J. Caldwell |
| Head of Department, | Gary Acuff |

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ABSTRACT

Effects of Duration of Transport on Indicators of Stress in Lambs.

(May 2006)

Peter Downs Krawczel, B.S., University of Maryland, College Park

Chair of Advisory Committee: Dr. Theodore H. Friend

Recommendations for the transportation of lambs from a European Commission, which required rest stops of 6 or 24 h, every 8 h, were evaluated for efficacy of reducing stress indicators using Rambouillet x Suffolk lambs (17.6 ± 0.5 kg). The lambs were randomly assigned to one of three groups: 1) transported for 22 h (Continuous; n = 15); 2) transported for 8 h, unloaded and rested for 6 h, transported for 8 h, unloaded and rested for 24 h, transported for 6 h (Rested, n = 15); and 3) Control, which remained in home pasture throughout the study (n = 16). The rest stops were off-trailer; a different pen was used for each; and, a limited amount of grain and ad lib hay and water were provided. Mean temperature in the trailer during the study was 28.4° C with a range of 18.2° C to 39.6° C. Food deprivation in the Continuous lambs was reflected by a decrease in plasma glucose ($P < 0.001$) and an increase in blood urea nitrogen ($P < 0.001$), creatinine ($P < 0.02$) and total bilirubin ($P < 0.001$) relative to the Rested or the Control lambs. Electrolytes varied within and between all three treatments ($P < 0.05$), but no distinct pattern indicating dehydration was evident. Serum concentrations of cortisol were greater ($P < 0.05$) in Continuous lambs than in the Control lambs at 14 h and both the Continuous and Rested lambs had higher concentrations of cortisol ($P < 0.05$) compared to the Control lambs at 22 h. Plasma IgG antibody response to ovalbumin was suppressed ($P < 0.05$) in the Continuous and Rested lambs compared to the Control lambs. Lambs in both transported treatments ate grain immediately upon release into the rest pens and drinking occurred following the food consumption. The Continuous lambs lost a greater ($P < 0.05$) amount of initial BW at the conclusion of transport compared to the Rested lambs and had a lower BW ($P < 0.05$) than the Rested and Control lambs 8 d after the start of transport. Rest stops improved welfare by reducing physical stress of food deprivation and eliminating BW loss during transport. However, rest stops failed to completely alleviate immunosuppression and 52 h were required to complete the otherwise 22 h long trip. The additional costs of providing the benefits of the rest stops should be examined before these regulations are widely implemented.

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# INTRODUCTION

Concern for the welfare of livestock in transit throughout Europe has been addressed through a series of directives and regulations starting with Council Directive 77/489/EEC, which was adopted in 1977. Another piece of legislation addressing the protection of animals was passed in 1991 (Council Directive 91/628/EEC) and then amended in 1995 (Council Directive 95/29/EC). The amending document (Council Directive 95/29/EC) mandated reports on how implementation of the directives was progressing as well as proposals on potential improvements that should be made. The Commission of the European Communities (2000) reported that Member States of the EU had difficulty fully applying Council Directives 91/628/EEC and 95/29/EC due to low priority, a lack of homogenized data resulted in limited applicability of inspection reports, and limited improvement has been found in horses, specifically, transported from Eastern and Central Europe. Additionally, the Commission of the European Communities (CEC, 2000) found, along with the general problems previously listed, there to be technical problems with the methods that livestock were being transported, such as violation of transport time limits, shipment of unfit animals, a lack of ventilation in trailers, and overcrowding. The Council Regulation (EC) No 1/2005 on the protection of animals during transport and related operations was the most recently passed regulation, which sought to address the limitations of previous directives. Council Regulation No 1/2005 established who was responsible for the health and safety of the animals at each stage of transport and the means of enforcement.

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This thesis follows the style of Journal of Animal Science.

This regulation did not address the more divisive issues incorporated in the report from the European Commission (2002), however a review of those issues must be completed prior to 2011 (EC No 1/2005). The most controversial recommendation was to limit trip duration to 8 h with alternating rest stops of 6 h and 24 h required until the final destination was reached for lambs, horses, calves, and pigs. The overall goal of this particular set of regulations may be to accomplish the goal of establishing “measures to encourage the slaughter of animals closer to their breeding place...” stated in the report issued by the European Commission (2002). Although this is a noble goal, it is most likely not practical due to the need for additional slaughter plants and the difficulty in finding acceptable locations for them.

The most common method used to study the effects of a treatment on the well-being of the animal was the evaluation of the stress response. W. B. Cannon (1929) was credited with describing the stress response with what is commonly referred to as “fight-or-flight”. He also determine that stress response to a specific stimulus could be highly variable. The definition of the stress-stessor response was later refined, by Selye (1955, 1973) and his theory of the “General Adaptation Syndrome” (GAS), to be standard with variation in response caused by the severity of the stressor. Friend (1991) summarized the GAS as having three components: the alarm reaction, which was characterized by an initial response from the adrenal system followed by depletion of stored hormones; the adaptation stage, characterized by replenishment of stored corticosteroids and a consistent rate of secretion determined by the specific stress during each response; and the exhaustion stage, which was characterized by the depletion of corticoids and the inability to generate more. Though research on the subject of stress has been conducted for close to a century there was a remaining uncertainty on how it should be specifically defined. Recently, negative stress, or "distress" has been defined as the inability to manage a stimulus (Fraser and Broom, 1990). More recently, stress, in general, has been defined more broadly as any stimulus that generates a response (Zulkifli and Siegel, 1995). The stress experienced by animals during transport has long been accepted as a cause of decreased welfare (CEC, 1984). The consistency that transport has been shown to have a detrimental effect on livestock makes the alleviation of transport stress a natural extension of the humane slaughter legislation that exist in this country as well as many others.

Several recent studies have investigated aspects of transport related to food deprivation (Horton et al., 1996), novelty of post-transport environments (Burritt and Provenza, 1997; Cockram et al., 2000), post-transport handling (Cockram et al., 1999), a single lairage during transport (Cockram et al., 1997; Parrott et al., 1998b), duration of transport (Knowles et al., 1998), the role of the driver and road conditions (Cockram et al., 2004), stationary periods (Fisher et al., 2005), and methods of loading or unloading (Parrott et al., 1998a).

The effects of fasting or fasting with transport were studied in 5 mo. old lambs (Horton et al., 1996). The fasted lambs were held for 3 d without food or water and the transported lambs were subjected to 1 h of transport on the first day with access to food and water following transport and then 8 h per d for the next 2 d without food or water (Horton et al., 1996). Food intake and overall BW gain were reduced in both fasted and transported lambs, however cortisol and nasal discharge increases were only evident in the transport lambs relative to the control and fasted lambs (Horton et al., 1996). Horton et al. (1996) concluded that fasting and transport had detrimental effects on performance during the post-treatment recovery period, but transport had a greater effect on overall health.

The response of sheep to novel and familiar foods when presented in a novel environment was recently investigated (Burritt and Provenza, 1997). Thirty-two, 5 mo. old lambs were divided evenly into two groups and half were moved to a novel location and provided access to familiar and unfamiliar foods (Burritt and Provenza, 1997). The lambs that remained in the familiar location consumed more of the unfamiliar foods than did those sheep moved to the novel location, however there was no difference in the consumption of familiar foods between the two groups of lambs (Burritt and Provenza, 1997). Burritt and Provenza (1997) concluded that lambs should be conditioned to new foods prior to moving them to novel locations to avoid decreased feed intake.

The effects of novel environments following 16 h of transport, using 16-wk-old lambs were recently studied (Cockram et al, 2000). Treatments varied in pre-transport environment (outside vs. inside), length of transport (16 h vs. 0 h), and post-transport environment (novel vs. familiar) (Cockram et al, 2000). During transport, lambs previously housed outside spent less time laying or ruminating than those kept indoors (Cockram et al, 2000). Following transport, lambs housed indoors did not differ in behavior regardless of the post-transport environment, however, lambs that were housed outdoors prior to transport and then moved indoors spent more time lying than those returned to the familiar environment (Cockram et al, 2000). Lambs initially housed outdoors that were placed in a novel outdoor pen post-transport spent less time grazing than non-transport controls (Cockram et al, 2000). Concentrations of cortisol increased during transport, but were not significantly affected by post-transport environment (Cockram et al, 2000). Cockram et al (2000) suggested that familiar foods be provided to lambs that are placed in novel post-transport environments and that rest periods of at least 12 h may be required to provide sufficient opportunity for adequate amounts of food and water to be consumed to replenish deficiencies that may arise during transport.

The effects of food deprivation for 12 h following 15 h of transport were studied using 16-wk-old lambs were divided into 4 groups: transported for 15 h then starved for 12 h with access to water, transported for 15 h then provided ad lib hay and water, kept in home pen then fasted for 12 h with access to water, or kept in home pen then provided ad libitum hay and water (Cockram et al., 1999). Transport resulted in greater plasma concentrations of cortisol relative to control, but did not effect concentrations of plasma creatine kinase or BW or result in any physiological indicators of dehydration (Cockram et al., 1999). Following transport, lambs without access to food had higher concentrations of plasma cortisol, but there were no differences between transported and non-transported groups (Cockram et al., 1999). Lambs with access to food and water laid down less, took longer to lay down, and had greater increases in BW compared to lambs without food, however transport did not effect any of these variables (Cockram et al., 1999). Transport followed by deprivation of food increased the time lambs spent foraging and exploring compared to those without access to food that had not been transported (Cockram et al., 1999). Cockram et al. (1999) concluded that the post-transport welfare of lambs could be increased by providing access to food and water if not immediately slaughtered.

The effects of 24 h of continuous transport compared to 12 h of transport, followed by 12 h or 3 h of off-trailer lairage with access to food and water or 3 h of access to food and water on the trailer, and then 12 h of additional transport were assessed using 16-wk-old lambs (Cockram et al., 1997). A large portion of the lairage periods was spent eating regardless of the duration of lairage, though lambs unloaded from the trailer spent more time eating than those that remained in it (Cockram et al., 1997). The percentage of lambs not observed drinking during lairage differed between the 3 h off-trailer group (8 %) and the 3 h on-trailer group (25 %) and there was mean latency of 66 min between unloading and first drink (Cockram et al., 1997). Only lambs provided lairage regained the BW returned to their initial BW within 48 h following the conclusion of transport (Cockram et al., 1997). Plasma concentrations of cortisol were greater in transported lambs compared to non-transported lambs and lambs transported continuously had greater concentrations than those provided lairage (Cockram et al., 1997). Cockram et al. (1997) concluded that lairage during transport provides a means to provide some benefit and that off-trailer lairage was preferable to on-trailer.

A study similar to the one previously done by Cockram et al. (1997) examined the effects of 31 h of transport with 1 h rest period (Parrott et al, 1998b). This study also compared the response of shorn and fleeced lambs to the treatment (Parrott et al., 1998b). Concentrations of glucose were not significantly effected by treatment, however cortisol and heart rate were increased in response to loading (Parrott et al., 1998b). During the lairage period, both groups of lambs readily consumed both hay and concentrates, but only a few fleeced lambs and no shorn lambs were observed to consume water (Parrott et al., 1998b). There was not a treatment effect on carcass quality (Parrott et al., 1998b).

The effects of 3, 9, 15, 18, or 24 h of transport on sheep and control groups with or without access to food and water were analyzed using sheep hauled at a density of 0.29 $m^2$ per animal, which was a commonly used density in commercial transport (Knowles et al., 1998). A consistent BW loss was observed for the first 15 h of transport; after which there was no BW loss (Knowles et al., 1998). Concentrations of cortisol and glucose increased during the initial 3 h of transport and then returned to pre-transport concentrations following 9 h (Knowles et al., 1998). Some differences, such as increased concentrations of glucose and decreased concentrations of urea, were observed in the sheep transported for 24 h compared to the sheep deprived of food and water for 24 h (Knowles et al., 1998). Additionally, only 3 d were needed for the sheep without food or water to regain the BW lost, while the transported lambs needed additional time to recover (Knowles et al., 1998). A considerable amount of feeding was observed for the first hour after transport, which was then followed by a return to previously observed feeding patterns (Knowles et al., 1998). Post-transport drinking behavior was approximately the same as the pre-transport observations, though a general increase existed for the 10 h immediately following transport (Knowles et al., 1998). Knowles et al. (1998) suggested that transport for up to 24 h should not be interrupted with a rest stop as the initial stages of transport appeared to be the most stressful, but also limited these conclusions to healthy sheep being transported in ideal circumstances.

The effect of 1) the driver, 2) events occurring during transport, and 3) road conditions on sheep in transit were determined using continuous behavioral observations (Cockram et al, 2004). The frequency that loss of balance occurred was affected by interactions of the driver and road type (Cockram et al., 2004). Additionally, interruptions of rumination and lying were caused by driver X road type X journey stage interactions (Cockram et al., 2004). Cockram et al. (2004) concluded that driver education was important to the welfare of animals during transport and responsible driving could minimize the disruptions caused by accelerations, maneuvering, and braking.

The role of stationary periods and external environmental conditions for potential heat stress in sheep during summer transport were determined using a temperature-humidity index calculated for two trips under commercial conditions in New Zealand (Fisher et al., 2005). Stationary periods, along with environmental conditions and initial temperature-humidity index, were responsible for increases in the temperature-humidity index and a mean increase of 0.16 in the temperature-humidity index was observed during the stationary periods (Fisher et al., 2005). Fisher et al. (2005) determined that stationary periods, even in mild conditions, could be detrimental to animal welfare and should be minimized.

Two methods of loading sheep for transport (tailgate ramp and hydraulic lift) were studied for implications on animal welfare (Parrott et al., 1998a). Increases in heart rate were observed during loading in either method and no significant treatment effects were evident in the concentrations of cortisol (Parrott et al., 1998a). Concentrations of cortisol increases during the first 2 h of ensuing transport led to the conclusion that transport resulted in a greater response than either loading procedure (Parrott et al., 1998a).

Transport stress can be divided into three categories: physical, physiological and psychological (Knowles, 1998). The welfare of animals during transport can be determined utilizing several measurements, which include degree of behavioral aversion exhibited, immunosuppression, prevalence of infectious diseases, prevalence of injury, and changes in physiology (Broom, 2003). The previously discussed studies utilized many of the same techniques to assess the condition of the sheep or lambs subjected to the treatments investigated. These techniques were blood chemistry (Cockram et al., 1997; Cockram et al., 1999; Knowles et al., 1995), cortisol (Parrott et al., 1998a; Knowles et al., 1995; Cockram et al., 1997), changes in BW (Knowles et al., 1995; Parrott et al., 1998b; Horton et al., 1996), and behavior (Cockram et al., 1997; Cockram et al., 2004; Burritt and Provenza, 1997). Additionally, immunosuppression (Coppinger et al., 1991) was used to access the stress resulting from restrain or isolation. The usage of a combination of the methods of assessment follows the model put forth by Moberg (1987) and Grandin (1997).

*Objectives*

The aim of this study was to determine the efficacy of the rest stops recommended to improve welfare during transport (European Commission, 2002) using lambs as a model. A multidisciplinary approach utilizing differences in plasma constituents, antibody response, behavior, and BW at major points during and after transport was used to compare continuous transport for 22 h, 22 h of transport with the prescribed rest stops, and remaining in the home pen. It was hypothesized that the rest stops would effectively reduce stress indicators by allowing for sufficient recovery following each transport as demonstrated by: eliminating differences in the plasma constituents and antibody response to vaccination with ovalbumin, consistency of behavior between the rest periods, and maintaining body weight throughout transport.

# MATERIALS AND METHODS

*Animals*

Forty-eight,14-wk-old, Rambouillet X Suffolk lambs (24 males and 24 females) were randomly selected from the breeding population of the Physiology of Reproduction lab at Texas A&M University. Prior to use in this study, the lambs were weaned at 8 wk of age and then housed as a group in outdoor pens. For 6 wk prior to transport, all lambs were moved from the adjoining pasture into a covered "home pen" where the group was fed 25 kg of 16%-protein pelleted feedstuff (Producers Cooperative, Bryan, TX 77801) at 0900 and 1700. During these feeding sessions, the lambs were also hand-fed grain and handled to habituate them to human contact. At the end of the 6 wk of handling, all lambs would readily approach the researchers. The lambs were housed as a group on Bermuda grass pasture when not in the home pen for feeding. Water was available continuously in a 1 m long water trough. At the start of transport, the lambs weighed 17.6 ± 0.5 kg.

*Treatments*

To ensure equal distribution of sexes in all treatments, lambs were blocked by sex and then randomly assigned to one of three treatments, Continuous, Rested, or Control. Prior to transport, one lamb from the Continuous and Rested treatments was removed from the group to reduce the group size to the correct number of lambs for transport at the density recommended by the European Commission (2002). Prior to the start of transport, the lambs were moved from the pasture into the home pen. The schedule of data collection and other related activities is summarized in Table 1. Transport occurred mainly on straight, flat

Table 1. Schedule of data collection and treatment specifics relative to the initiation of transport and time of day and location of sample collection. Transport began on July 29, 2003 and ended on July 30, 2003 for the Continuous Lambs and July 31, 2003 for the Rested lambs.

| Time relative to the start of transport | Time of day | Treatment | Activity | Location |
|---|---|---|---|---|
| -2 d | 1000 | All | Pre-vaccination blood sample and vaccination with ovalbumin. | Home pen |
| 0 h | 0800 | All | Blood sample and BW. Separation into treatments. | Home pen |
| | | Continuous and Rested | Loaded for transport. | Trailer |
| 8 h | 1600 | Continuous | Blood sample. | Trailer |
| | | Rested | Blood sample. Unloaded for 6-h rest period. Provided food and water. | Trailer |
| | | Control | Blood sample. | Home pen |
| 14 h | 2200 | Continuous | Blood sample. | Trailer |
| | | Rested | Blood sample. Loaded for transport. | Rest pen |
| | | Control | Blood sample. | Home pen |
| 22 h | 0600 | Continuous | Blood sample and BW. Conclusion of transport. Start of 24-h recovery period. Provided food and water. | Trailer |
| | | Rested | Blood sample. Unloaded for 24-h rest period. Provided food and water. | Trailer |
| | | Control | Blood sample and BW. | Home pen |
| 46 h | 0600 | Continuous | Blood sample and BW. Conclusion of 24-h recovery period. | Home pen |
| | | Rested | Blood sample. Loaded for transport. | Rest pen |
| | | Control | Blood sample and BW. | Home pen |
| 52 h | 1200 | Rested | Blood sample and BW. Conclusion of transport. Start of 24-h recovery period. Provided food and water. | Trailer |
| | | Control | Blood sample and BW. | Home pen |
| 78 h | 1200 | Rested | Blood sample and BW. Conclusion of 24-h recovery period. | Rest pen |
| | | Control | Blood sample and BW. | Home pen |
| 8 d | 1000 | All | Post-transport IgM and IgG sample and BW. | Home pen |

highways and with brief periods on rolling, paved county roads in the vicinity of College Station, Texas. All drivers avoided abrupt accelerations or decelerations. Transport began at 0800 on 29 July 2003 and ended at 0600 on 30 July 2003 for the Continuous and at 1200 on 31 July 2003. All procedures were approved by the Texas A&M University Agricultural Animal Care Committee.

*Continuous.* Lambs (n = 15) were transported for 22 h. Time spent on the trailer during blood sampling and while the Rested were loaded and unloaded was counted as transport.

*Rested.* Lambs (n = 15) were transported for 22 h following the timing of the recommended rest stops (European Commission, 2002). Each rest stop occurred in a pen (9.4 x 4.0 m) with wood shavings for bedding that was completely novel to the lambs to mimic conditions of commercial transport. Supplemental lighting was used at night to allow observation of behaviors. Sheep from another study were visible across the alleyway from the front of the rest pen. While in the rest pen, approximately 11 kg of 16%-protein pelleted feedstuff (Producers Cooperative, Bryan, TX 77801) and ad lib grass hay was provided in permanent feeders (6.5 m long x 0.5 m tall) with adequate space for all lambs to access feed at the same time. Water was provided in four buckets that averaged 2 m from the feeders. The lambs had no prior experience with the feeding and watering arrangement prior to the first rest stop. When the Rested lambs were reloaded on the trailer, no attempt was made to sort them into their original group of five in which they began transport.

*Control.* Lambs (n = 16) remained in the home pasture throughout the study, except when sampled. Eleven kg of 16%-protein pelleted feedstuff (Producers Cooperative, Bryan, TX 77801) was provided at the customary times of 0900 and 1700.

### *Trailer Arrangement*

The trailer (7.2 x 1.8 m) had a deck height of 0.4 m, which allowed the lambs to load or unload without a ramp. Slots running the length of the trailer beginning 0.4 m above the deck provided ventilation. Each lamb was allotted the recommended space of 0.23 $m^2$ (0.19 $m^2$ plus 20% due to high ambient temperature; European Commission, 2002) for lambs under 20 kg. The trailer was divided in half lengthwise and then into four pens on each side measuring 1.15 $m^2$. Three pens per side were used for transport of the lambs and the remaining pen was vacant to provide the extra space needed for on-board blood collection. One treatment was located on each side, which allowed for treatments to be loaded or unloaded without disrupting the other. Pens could be opened to allow access by researchers and permit loading and unloading of the entire length of each half of the trailer.

### *Blood Collection*

All blood samples were collected via jugular venipuncture using 20-g needles and 10-mL Vacutainer tubes containing Polymer Separator Gel and lithium heparin (VWR, West Chester, PA 19380). An assistant straddled the lamb and the head was held back and to the side to expose the jugular vein. Sample collection took an average of 1 min per lamb and the lambs displayed little reaction to the process. The treatments were sampled in a consistent order of Rested, Continuous, and Control, and lambs were randomly sampled within treatment. The samples were placed in an ice bath for no longer than 1 h, separated by centrifuge, and the plasma was frozen at –13° C until analyzed. Sampling occurred in the home pen, on the trailer, and (or) in the rest pen (Table 1).

*Plasma Constituents*

The Texas Veterinary Medical Diagnostic Laboratory used a Hitachi 911 (Hitachi, Ltd., 6-6, Marunouchi 1-chome, Chiyoda-ku, Tokyo, 100-8280 Japan) to determine the total plasma protein, albumin, calcium, phosphorus, glucose, blood urea nitrogen, creatinine, total bilirubin, creatine kinase, aspartate aminotransferase (AST), globulins, albumin/globulin ratio, gamma glutamyl transferase (GGT), magnesium, sodium, potassium, Na/K ratio, and chloride within 48 h of collection.

Concentrations of cortisol were determined by RIA (Coat-A-Count Cortisol, Diagnostic Products Corporation, Los Angeles, CA) using manufacturer specifications within 6 mo. of collection. Inter- and intra-assay coefficients of variation were 9.1%.

*Antibody Production.*

Effect of transport stress on primary immune response to the vaccination was determined using a modified version of the indirect ELISA described in Ameiss et al. (2004). The vaccination, a total of 0.5 mg ovalbumin (Sigma Chemical Company, St. Louis, MO) suspended in 0.125 mL of phosphate-buffered saline (PBS) and 0.125 mL incomplete Freund's adjuvant (Sigma Chemical Company, St. Louis, Missouri) was given intramuscularly in two locations on the upper thigh (Coppinger et al., 1991). Pre-vaccination and post-transport blood samples of 10 mL (Table 1) were separated by centrifuge (1000 X g) and the plasma was frozen at –13° C until analyzed within 6 mo. The modifications on the original were substituting ovalbumin for BSA and dilutions and secondary antibodies (FITC conjugated, Anti-Sheep IgG and IgM, Bethyl Laboratories, Inc., Montgomery, TX, 77356) specific to this study. Plasma samples were diluted to a concentration of 1 μL of plasma in 319 μL of PBS for IgG and 2 μL of plasma in 318 μL of PBS for IgM. Five serial dilutions of each sample and positive control (8 μL in 1.272 mL) were made to generate a response curve. Dilutions were based on the IgG and IgM titers of the positive controls, which were obtained from the highest responder of three ewes vaccinated with ovalbumin every three wk, for four mo. The negative control was plasma from an unvaccinated sheep at a dilution of 1 μL in 10.24 ML for IgG and IgM. Using the response curve, variation in IgG samples was corrected by dividing the response at 1:2550 μL by the response of the positive control at 1:10240 μL. Variations in IgM samples were corrected by dividing the response at 1:1272 μL by the response of the positive control at 1:2560 μL. All dilutions were duplicated and the mean was used for analysis of treatment effects.

*Behavior Analysis*

The time from unloading to first drink and first feeding for each lamb was recorded each time the lambs (Continuous and Rested) were unloaded. The number of lambs engaged in drinking, eating, lying, playing, or "other" was recorded at 15-min intervals throughout the rest periods. A lamb was recorded as drinking or eating when its body was oriented towards either a water or food source and the subject was actively ingesting. A lamb was lying when its weight was not supported by all four legs and it was not in motion. Play was recorded when a lamb was running, leapt into the air with rigid legs (Hafez et al., 1969), attempted to butt another lamb or butted another lamb. Due to the age of the lambs, all butting was assumed to be play. Other consisted of any behavior not previously mentioned.

*Weight Change*

The Continuous, Rested, and Control lambs were weighed using a portable scale following the plasma sampling (Table 1). To minimize handling and disturbance of the other lambs, a researcher held the lambs in his arms. The combined weight of the researcher holding the lamb and lamb were recorded. The known weight of the researcher was used to check the accuracy of the scale.

*Statistical Analysis*

Data were plotted and visually checked for normal distribution. Treatment effects for plasma constituents, IgG and IgM response to ovalbumin, and BW were determined using a repeated measures model within PROC GLM of SAS (SAS 8.02, SAS Institute Inc., Cary, NC). The main effects of the model were treatment, time, treatment interaction with time, and lamb nested within treatment. Lamb nested within treatment was the error term used to test for treatment effects. Mean separation was performed using the pdiff function ($P < 0.05$) when significant treatment by time interactions were found. The behavioral observations during the first rest period and the first 6 h of the second rest period and the latency between unloading and the first drink were analyzed using an ANOVA (SPSS for Windows, Version 11.0, SPSS Inc, Chicago, IL). The Continuous and Rested lambs were unloaded as a group following transport, however, the lambs were considered independent in regards to latency to drink due to the lack of an established social hierarchy and the close proximity of the food and water, which eliminated the need to separate from the flock to eat, drink, or lay down.

# RESULTS

## *General*

Mean temperature was 28.4° C with a range of 18.2° C to 39.6° C and mean relative humidity was 59.9% with a range of 29.1% to 92.4% during transport.

## *Plasma Constituents*

A treatment X time effect occurred in glucose ($P < 0.001$), creatinine ($P < 0.001$), total bilirubin ($P < 0.001$), blood urea nitrogen ($P < 0.001$), total plasma protein ($P < 0.001$), albumin ($P < 0.001$), calcium ($P < 0.001$), phosphorus ($P = 0.003$), aspartate aminotransferase (AST) ($P < 0.001$), globulins($P < 0.001$), albumin/globulin (a/g) ratio ($P < 0.001$), gamma glutamyl transferase (GGT) ($P < 0.001$), magnesium ($P < 0.001$), potassium ($P = 0.01$), Na/K ratio ($P < 0.001$), chloride ($P < 0.001$), and cortisol ($P < 0.001$).

Plasma concentration of glucose decreased ($P < 0.001$) in the Continuous lambs during transport (Figure 1A). Plasma concentrations of creatinine (Figure 1B), total bilirubin (Figure 2A), and blood urea nitrogen (Figure 2B) increased ($P < 0.001$) in the Continuous lambs after 14 h of transport. Though sporadic differences ($P < 0.05$) between treatments for the total plasma protein, albumin, calcium, phosphorus, aspartate aminotransferase, globulins, albumin/globulin ratio, gamma glutamyl transferase, magnesium, potassium, Na/K ratio, and chloride (Appendix figures 6-11) existed, the variation relative to the Control lambs suggested there was no meaningful treatment effect.

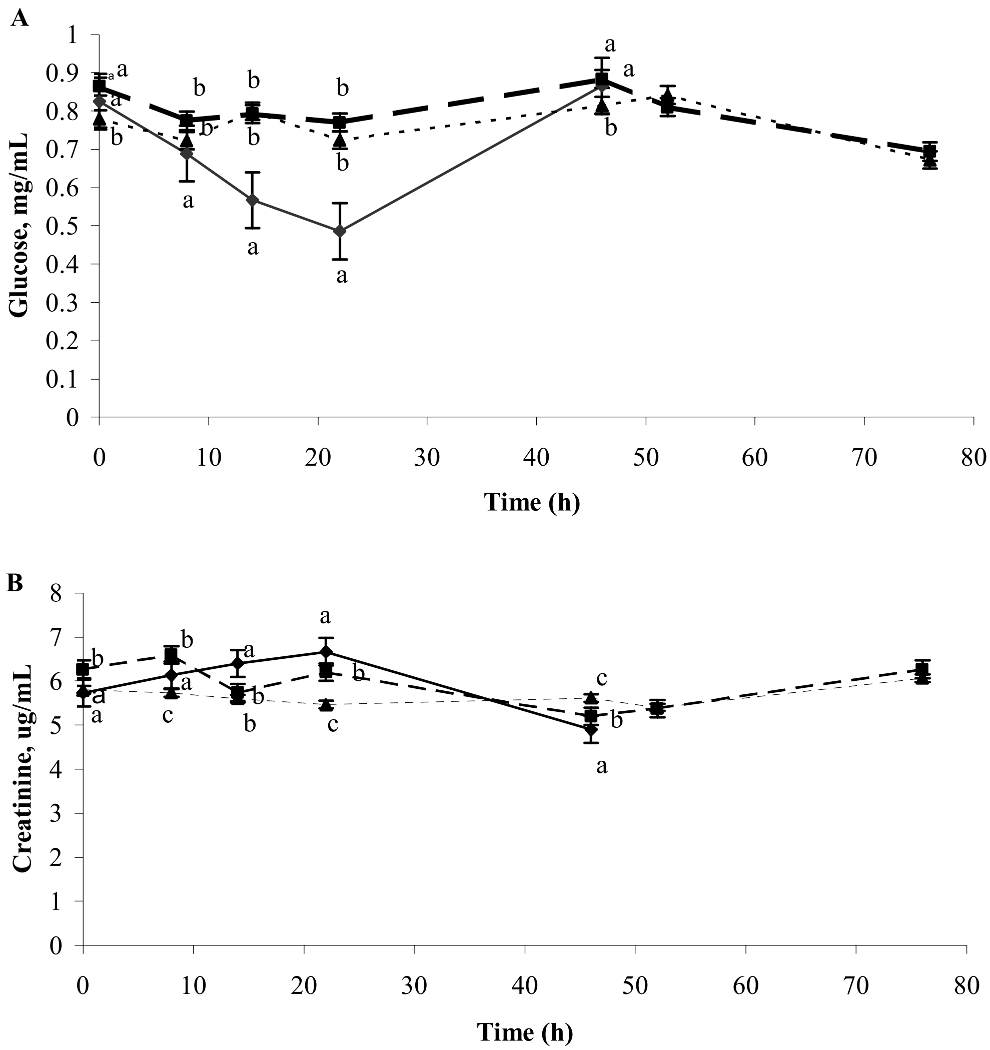


Figure 1. Mean concentrations with S.E. bars of glucose (A) and creatinine (B) for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. [abc]Means at each time with different superscripts differ ($P < 0.05$).

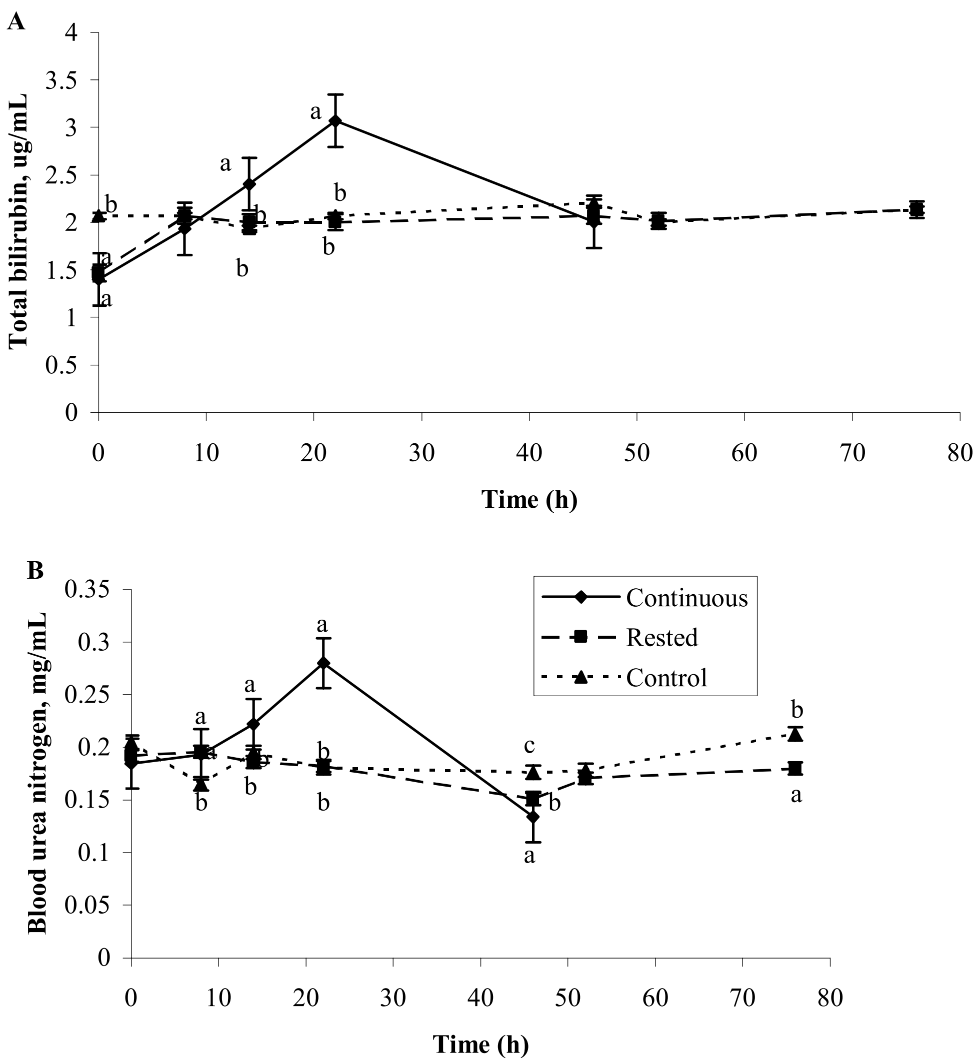


Figure 2. Mean concentrations with S.E. bars of total bilirubin (A) and blood urea nitrogen (B) for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. [abc]Means at each time with different superscripts differ ($P < 0.05$).

Plasma concentration of cortisol was influenced by treatment. Specifically, the plasma concentration of cortisol for the Continuous lambs was greater ($P < 0.05$) than for Control lambs at 14 h. The plasma concentration of cortisol for both the Continuous and Rested lambs was greater ($P < 0.001$) than for Control lambs at 22 h (Figure 3). A time effect ($P < 0.001$) was evident in sodium (Figure 4A). There was not a treatment, time, or treatment X time effect evident in creatine kinase (Figure 4B).

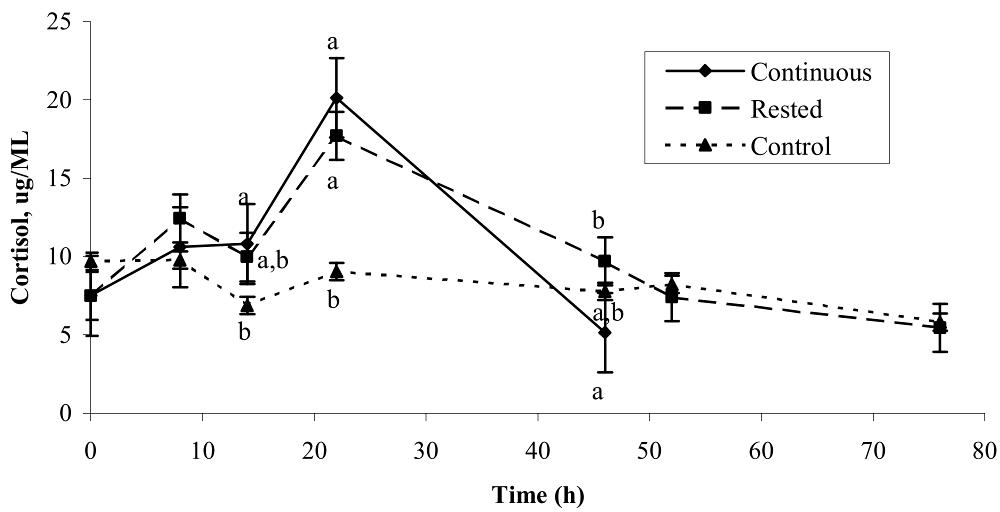


Figure 3. Mean concentrations with S.E. bars of cortisol for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. [ab]Means at each time with different superscripts differ ($P < 0.05$)

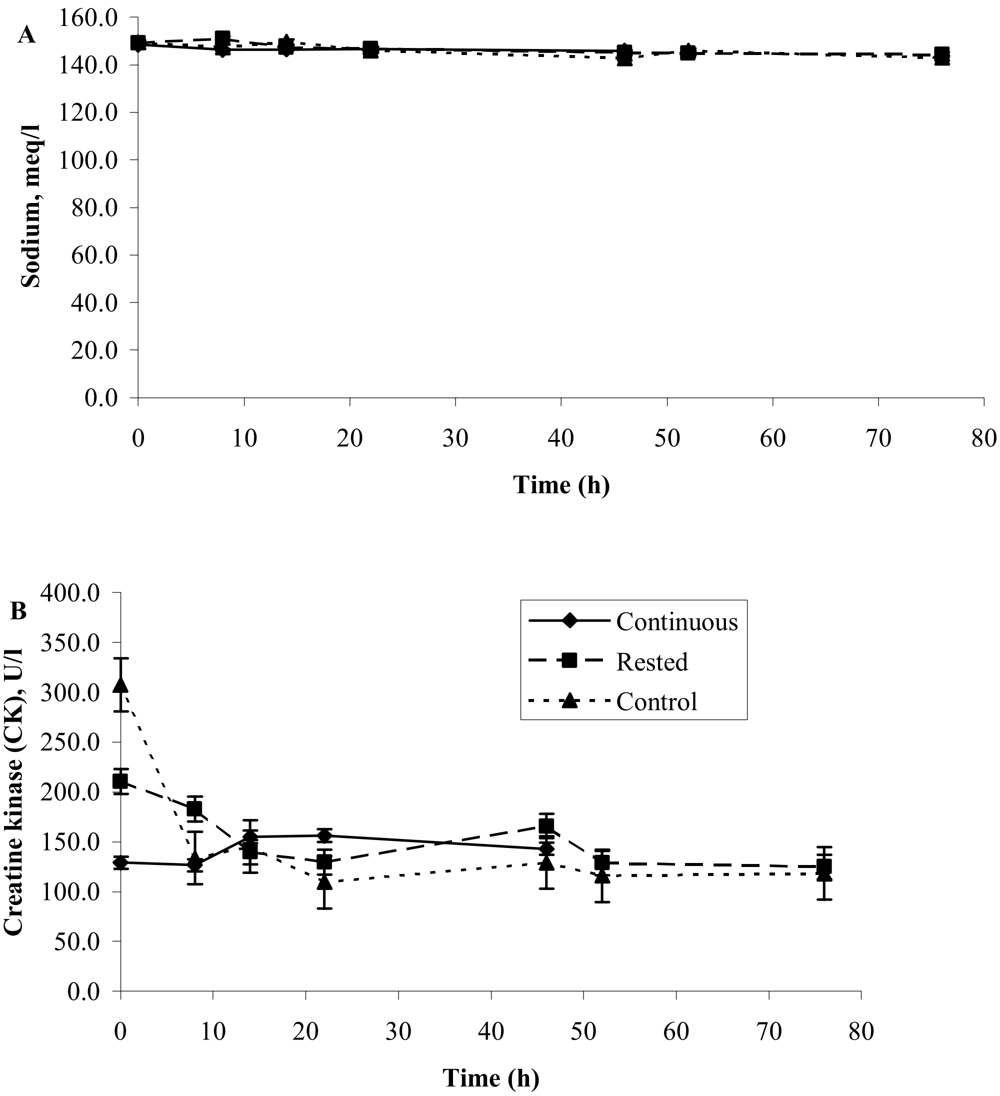


Figure 4. Mean concentrations with S.E. bars of sodium (A) and creatine kinase (B) for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. A time effect was evident in sodium. No treatment, time, or treatment X time effect evident in concentrations of creatine kinase.

*Antibody Response*

A treatment X time effect was evident in the IgG response (P = 0.01). The pre-transport (and prior to vaccination) plasma IgG responses did not differ between treatments (Figure 3). The post-transport IgG response was lower (P < 0.05) in the Continuous and Rested lambs compared to the Control lambs (Figure 5). A time effect was evident in the IgM response and the post-transport (8 d after the start of transport) values were greater (P < 0.001) than the pre-vaccination values (Figure 5)

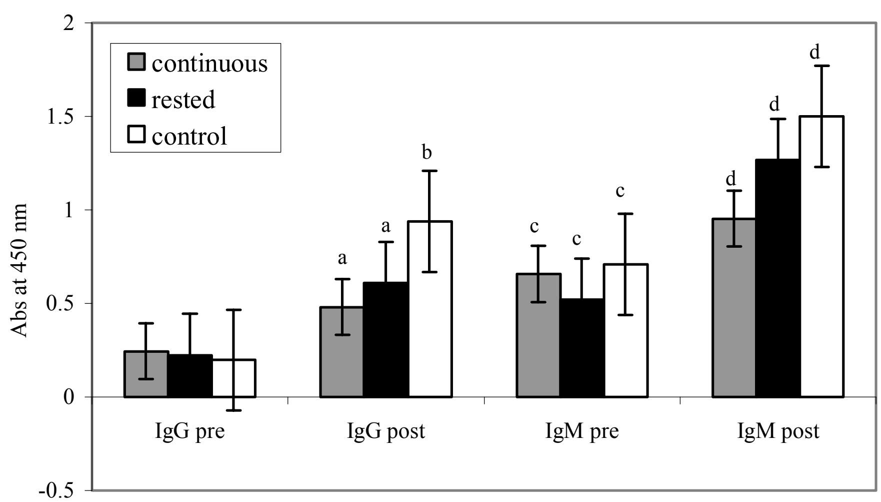


Figure 5. Mean pre- and post-transport absorbency reading determined from an indirect ELISA indicating antigen specific IgG and IgM response to vaccination with ovalbumin in the Continuous lambs (n = 15), transported for 22 h; Rested lambs, transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The pre-transport sample was collected 2 d before the start of transport and immediately prior to vaccination. The post-transport sample was collected 8 d after the start of transport. [ab]Means within a variable with pre and post with different superscripts differ (P < 0.05). [cd]Means with different superscripts differ between pre and post samples (P < 0.05).

*Behavior*

Most of the Continuous and Rested lambs were observed to be recumbent within the trailer upon returning from transport. The Rested lambs readily unloaded and loaded at the start and conclusion of each rest stop and no apparent injuries were observed. The Continuous lambs unloaded at the conclusion of transport without difficulty or visible injury. The Rested lambs went immediately to feed when released from the trailer for both rest periods. The percentage of time spent eating was greater ($P < 0.05$) during the 6-h rest stop than the first 6 h of the 24 h rest stop and lying was greater ($P < 0.05$) during the first 6 h of the 24 h rest stop than the 6 h rest stop (Table 2). At the conclusion of transport, the Continuous and the Rested lambs ignored the water and went immediately to the grain following unloading. The average time between first unloading and drinking was 23.9 ± 1.4 min for the Continuous and was 17.7 ± 4.5 min for the Rested, and was not effected by treatments.

Table 2. Percentage of observations of each behavior from lambs (n = 15) during the 6 h rest stop, the first 6 h of 24 h rest stop, and the total 24 h rest stop. Percentages calculated from the number of lambs engaged in each behavior during 15 min scan samples. The means for the 6-h rest stop and the first 6 h of 24 h rest stop were compared and means within a behavior with a different superscript differ ($P < 0.01$).

| | Behavior | | | | |
|---|---|---|---|---|---|
| Rest stop | Drinking | Eating | Lying | Playing | Other |
| Total 6 h | 6.4 ± 4.3 | 41.4 ± 6.7$^a$ | 18.8 ± 6.5$^a$ | 1.2 ± 0.8 | 29.6 ± 6.7 |
| First 6 h of 24 h | 2.9 ± 1.2 | 15.2 ± 4.0$^b$ | 63.7 ± 7.4$^b$ | 1.3 ± 0.9 | 16.8 ± 4.0 |
| Total 24 h | 1.9 ± 0.4 | 19.5 ± 2.4 | 61.0 ± 3.6 | 1.3 ± 0.4 | 16.3 ± 4.6 |

*Weight Change*

Prior to loading (0 h), the Continuous lambs weighed 17.8 ± 0.8 kg, the Rested lambs weighed 18.5 ± 0.6 kg, and the Control lambs weighed 17.0 ± 1.1 kg (Table 3). A treatment X time effect ($P < 0.001$) was evident in which the Continuous lambs lost a significant ($P < 0.001$) amount of BW at the end of transport compared to their initial BW and the BW of the Rested lambs at the end of their transport. The BW of the Rested lambs at the end of transport was not different from the initial BW. The BW of the Control lambs fluctuated over time compared to their initial BW and was both greater and less ($P < 0.01$) than the Continuous and the Rested lambs at various stages of the transport or recovery periods.

Table 3. The BW ± S.E. (kg) for the Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately before loading for the start of transport and each subsequent sample was relative to the start of transport.

| | Time | | | | | |
|---|---|---|---|---|---|---|
| Treatment | Prior to loading (0 h) | End of Transport for Continuous lambs (22 h) | 24 h Recovery for Continuous lambs (46 h) | End of transport for Rested lambs (52 h) | 24 h Recovery fo Rested lamb (76 h) | 8 d after start of transport |
| Continuous | $17.8 \pm 0.8^{a}$ | $15.2 \pm 0.7^{a}$ | $17.5 \pm 0.8^{a}$ | ND | ND | $20.8 \pm 0.9^{a}$ |
| Rested | $18.5 \pm 0.6^{b}$ | $21.9 \pm 0.6^{b}$ | $20.3 \pm 0.6^{b}$ | 18.4 ± 0.7 | $19.9 \pm 0.6^{a}$ | $23.7 \pm 0.7^{b}$ |
| Control | $17.0 \pm 1.1^{c}$ | $15.7 \pm 1.2^{a}$ | $18.2 \pm 1.1^{a}$ | 18.3 ± 1.1 | $18.6 \pm 1.2^{b}$ | $22.1 \pm 1.1^{c}$ |

[abc]Means within a column with different superscripts differ ($P < 0.05$).

[ND]Data not collected for Continuous lambs at these times.

# DISCUSSION

This study was one of the first to quantify the implications of providing multiple rest stops during long distance transport of lambs or other species of livestock. The rest stops benefited the lambs by reducing the observable effects of food deprivation and minimizing weight loss during transport resulting in greater post-transport BW during hot conditions (mean temperature of 28.4° C). However, the elevated concentrations of cortisol at 22 h and suppression of IgG response to immunization in the Continuous and Rested lambs implied the rest stops did not totally alleviate transport stress. Along with indicating that transport remained a stessor, the suppression of the IgG response to immunization indicates that pre-transport vaccinations could be rendered ineffective. Additionally, providing livestock with feed during transport to slaughter can be problematic due to the common recommendation that cattle be fasted for12 to 24 h prior to slaughter to reduce the possibility of carcass contamination (Savell and Smith, 2004). Livestock fed during transport to slaughter will likely have to be fasted at the slaughter plant prior to slaughter.

All transported lambs were hauled in the same trailer to ensure they were subjected to the same roads, drivers, and environmental stressors to the greatest extent possible. Stops for sampling and unloading of the Rested lambs resulted in the Continuous lambs experiencing stationary periods. However, during the commercial transport of lambs for 22 h the drivers will stop for food, water, traffic delays, and inspections.

The report published by the European Commission (2002) contained a discussion of the potential positive and negative effects from on- and off-trailer rest. On-trailer rest was concluded to be preferable to reduce the spread of disease and risk of injury assuming sufficient space for eating, drinking, and rest were provided. Although the space provided may be sufficient for lambs to rest (Buchenauer, 1997, and many of the lambs in this study were lying at the conclusion of periods of transport), the report (European Commission, 2002) provided no specific methods on how to deliver food and water for livestock receiving 6 or 24 h of on-trailer rest. Because slaughter horses are required to be unloaded for 6 h after 28 h of transport in the United States (USDA Veterinary Services, 2002) and the lack of technical information on how to supply food and water during on-trailer rest stops, the Rested lambs in this study were unloaded into pens to assess effects of rest stops.

Previous work with cattle (Kenny and Tarrant, 1987) and calves (Kent and Ewbank, 1983; Grigor et al., 2004) has shown that transport can potentially be a greater stressor than loading and unloading. However, crate-raised calves were found to be the most stressed from the loading and unloading process (Trunkfield and Broom, 1990). Grandin (1997) has hypothesized that animals that have been intensively handled, as the lambs in this study were, are more likely to respond negatively to the trip rather than the loading and unloading. The lambs in this study were habituated to handling to minimize the effects of the repeated handling that was necessary to obtain data. However it is important when interpreting these data to consider that commercial truck drivers in the United States report that sheep can be very difficult to load and unload under the conditions commonly found in large-scale lamb production. Hence, this study needs to be replicated using untamed lambs that would likely present more difficulties in loading and unloading before these results can be widely applied.

The reduced concentrations of glucose (Horton et al., 1996) and elevated concentrations of creatinine (Li et al., 2000), total bilirubin (Levin et al. 1993), and blood urea nitrogen (Kannan et al., 2000) that occurred in the Continuous Lambs are indicators of food deprivation. The concentrations of glucose responded similarly to what was reported by Knowles et al. (1995), which was a decrease in concentration during 24 h with food followed by a return to the baseline concentrations following access to food and water. The response of blood urea nitrogen was also similar to the response reported by Knowles et al. (1995) for sheep transported for 24 h followed by a 24 h recovery period. The increased concentrations of creatinine are similar to the response found in goats during 2.5 h of transport (Kannan et al., 2000). Unexplainable differences in creatinine between the Continuous and Rested lambs at 0 h also resulted in the same difference resulting at 8 h. The effect of transport on concentrations of total bilirubin were not previously reported. The access to food during the periodic rest stops of this study are one of the main benefits for the Rested lambs. The lack of meaningful differences in creatine kinase (CK) suggests that the 22-h trip was not physically strenuous. Previous work (Wilson et al, 1990) has shown physical exertion was a more important factor in changing CK concentrations then either transport or starvation. The relatively low density (0.23 $m^2$ per lamb) at which the lambs were transported is a likely explanation for the lack of physical exertion during transport. The majority of the Continuous lambs were observed to be lying down upon arrival after each period of transport. Calves provided sufficient space to lie down during transport were metabolically indistinguishable from non-transported calves (Todd et al., 2000). Also, transport over mainly flat, straight roadways may have also decreased the potential stressful effect of the transport. The importance of straight roadways and avoiding sudden stops and starts on welfare of sheep during transport has been previously established (Cockram et al, 2004). Based on the similarity between concentrations of CK in Continuous, Rested, and

Control lambs and the recumbent position the majority of lambs were in at the conclusion of a transport period, the maximum density recommended by the European Commission report (2002) appears sufficient for rest and avoidance of injuries.

While conducting this experiment (with peak temperature of 39.6° C), the researchers became concerned that the Continuous lambs were suffering from water deprivation. The Continuous lambs appeared to be much more lethargic than the Rested lambs at the 14-h sampling. As a result, water was offered from buckets following the 14-h sampling. The majority of the Continuous lambs, however, were not interested in the water offered on the trailer and the few that showed interest appeared to only investigate the novelty of the bucket rather than consume appreciable amounts of water. The results of the electrolyte analysis did not indicate dehydration had occurred during the 14 h of transport prior to the offering of water nor was there an indication that the Continuous lambs were dehydrated at the conclusion of transport 8 h later. Both Continuous and Rested lambs moved immediately to feed bunks and ignored water until all grain provided during rest stops or after their final unloading was consumed. There was no latency period between unloading and approaching feed following either unloading of the Rested lambs or the single unloading of the Continuous lambs. This was consistent with Parrott et al. (1998) and Cockram et al. (1999) who found sheep would not utilize water unless they had consumed food.

The elevated concentrations of plasma cortisol of the Continuous lambs at 14 h and the Continuous and Rested lambs at 22 h compared to the Control lambs most likely resulted from the stress of transport (Odore et al, 2004) and food deprivation (Cockram et al. 1999). The similarity of cortisol concentrations at the conclusion of transport (52 h) in the Rested and Control lambs may indicate habituation to transport (De Boer et al, 1990), rather than a depletion of stored glucocorticoids typical of situations of chronic stress (Selye, 1955, 1973). De Boer et al. (1990) found the duration and intensity of the physiological stress response was diminished in rats following application of the same stressor at regular intervals, which was similar to the transport stress the Rested lambs were subjected to in this study.

Both transported treatments had lower IgG responses to challenge against ovalbumin than the Control lambs, which indicates the transport periods had resulted in some immunosuppression. However, the lack of a significant treatment effect in IgM response indicated that transport stress did not suppress the immune response completely. The lack of suppression of the IgM response may be of greater importance than the suppression of the IgG response that did occur as IgM is the dominant antibody of the primary immune response (Abbas et al., 2000). Additionally, there was a trend of reduced IgM response in the treatment groups with the Continuous lambs having the lowest value. Transport has previously been found to reduce aspects of immune function of lambs (Horton et al, 1996) and goats (Kannan et al, 2000).

There was no latency period between unloading and approaching feed following either unloading of the Rested lambs or the single unloading of the Continuous lambs. The preference for food over water in sheep has previously been found after 15 h (Cockram et al, 1999) and 24 h (Cockram et al, 1997) of transport. Though it was not statistically significant, a 50% reduction of the percentage of lambs observed drinking occurred in the 24-h rest period. This may be explained from the 6-h rest period beginning at 16:30 with the temperature in excess of 35° C compared to the 24-h rest that begin at 06:00 with the temperature of approximately 25° C. Cockram et al. (1997) found that a 3-h on-trailer rest period did not provide sufficient access to water. The reduced water consumption indicated that dehydration was not occurring. The novelty of the rest pen, feeders, or watering buckets did not appear to affect the behavioral response as all lambs went immediately to the grain when released into the rest pen. Previous work demonstrated that novel environments do not negatively affect recovery in sheep, measured by plasma constituents and time spent feeding and drinking (Cockram et al., 2000). All lambs, either during the rest (Rested lambs) or recovery (Continuous and Rested lambs), were provided with the same grain that had been supplied throughout the acclimation period prior to the start of this study. This may have facilitated the immediate move to grain as the preference for familiar food in novel environments has been established (Burritt and Provenza, 1997).

The rest stops reduced BW loss to less than 1% of the initial BW in the Rested lambs. The 14.6% BW loss after 22 h of transport in the Continuous lambs was consistent with the 11% BW loss reported for lambs transported 24 h at 20° C (Knowles et al., 1998) and the 11% loss reported for goats transported and held for 18 h in temperatures approaching 40° C (Kannan et al., 2000). Loses in BW were 20% for lambs transported for 1 h during 72 h of fasting (Horton et al., 1996). Additionally, Horton et al. (1996) found that both fasted or fasted and transported lambs consumed less total food during a 28 d recovery period. A similar reduction in intake may be responsible for the lower BW in the Continuous lambs 8 d after the start of transport, however, this is speculation as post-transport feed intake was not measured in this study. Interpretation of the BW data was confounded by the unexplainable fluctuations of BW in the Control lambs, which had loses in BW that were equivalent to the Continuous lambs at 22 h without any food or water restrictions.

# CONCLUSIONS

The hypothesizes for plasma constituents were both accepted and rejected. The Rested treatment was found to reduce the indicators of food deprivation, which is associated with the stress of transport. However, concentrations of plasma cortisol were increased related to the Control lambs during portions of transport, which indicates that the stress of transport was not fully alleviated. This was further supported by the rejection of part of the hypothesis on the antibody response to ovalbumin vaccination. Significant suppression of the IgG antibody response was evident in both the Continuous and Rested lambs compared to the Control lambs. Conversely, production of IgM antibodies was not effected by treatment. The behavior of the Rested lambs was not consistent during the two rest periods, so that hypothesis was also rejected. Finally, the hypothesis on BW change was accepted due to the Rested lambs weighing significantly more at the end of transport compared to the Continuous lambs. Although the BW hypothesis was accepted, the treatment effect was confounded by significant BW differences prior to transport.

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# APPENDIX

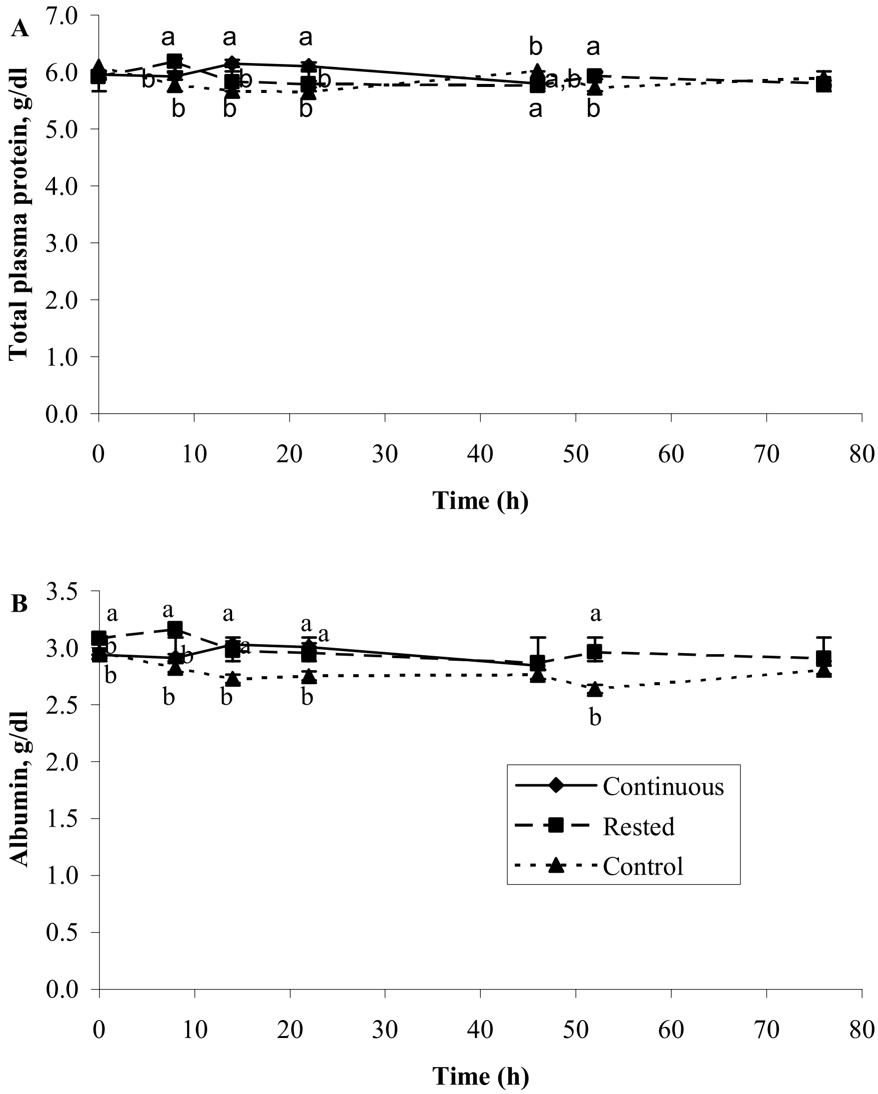


Figure 6. Mean concentrations with S.E. bars of total serum protein (A) and albumin (B) for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. [ab]Means at each time with different superscripts differ ($P < 0.05$).

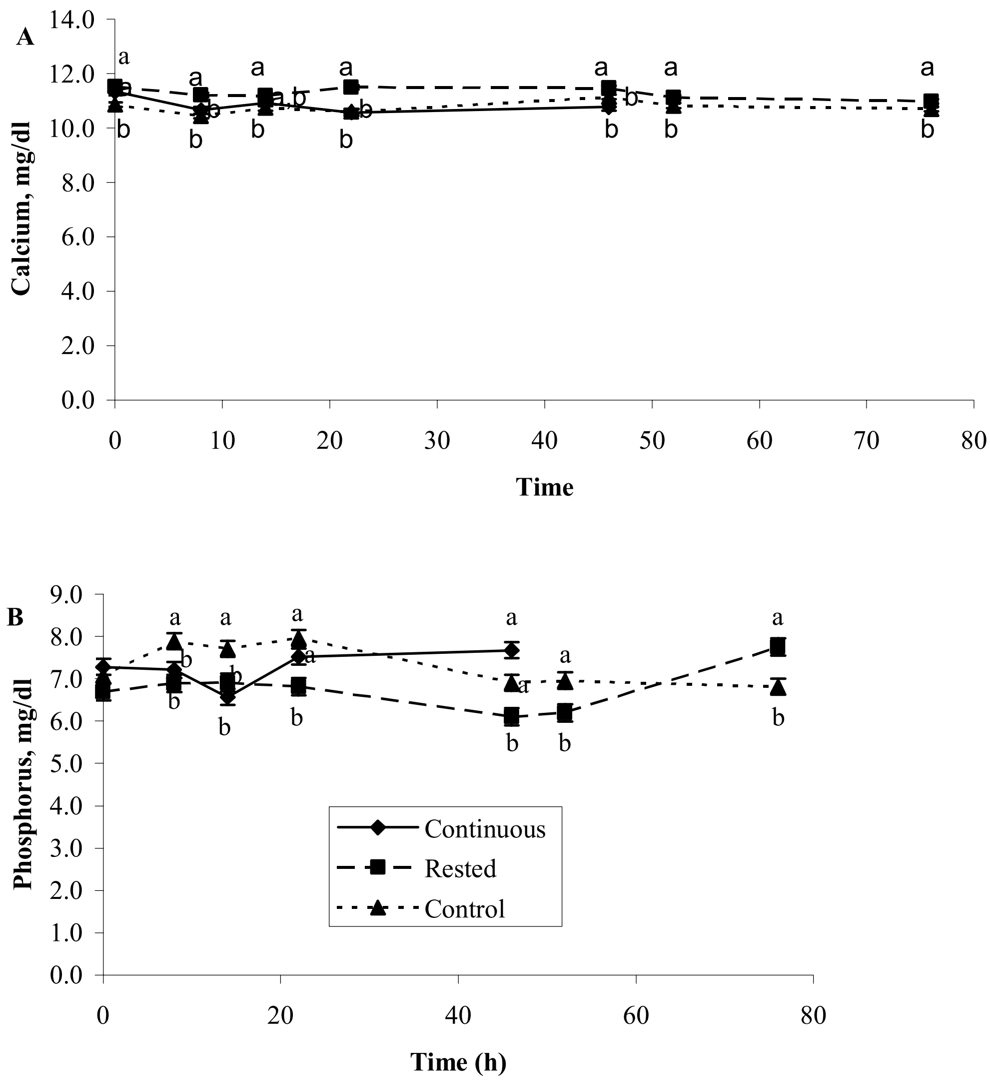


Figure 7. Mean concentrations with S.E. bars of calcium (A) and phosphorus (B) for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. [ab]Means at each time with different superscripts differ ($P < 0.05$).

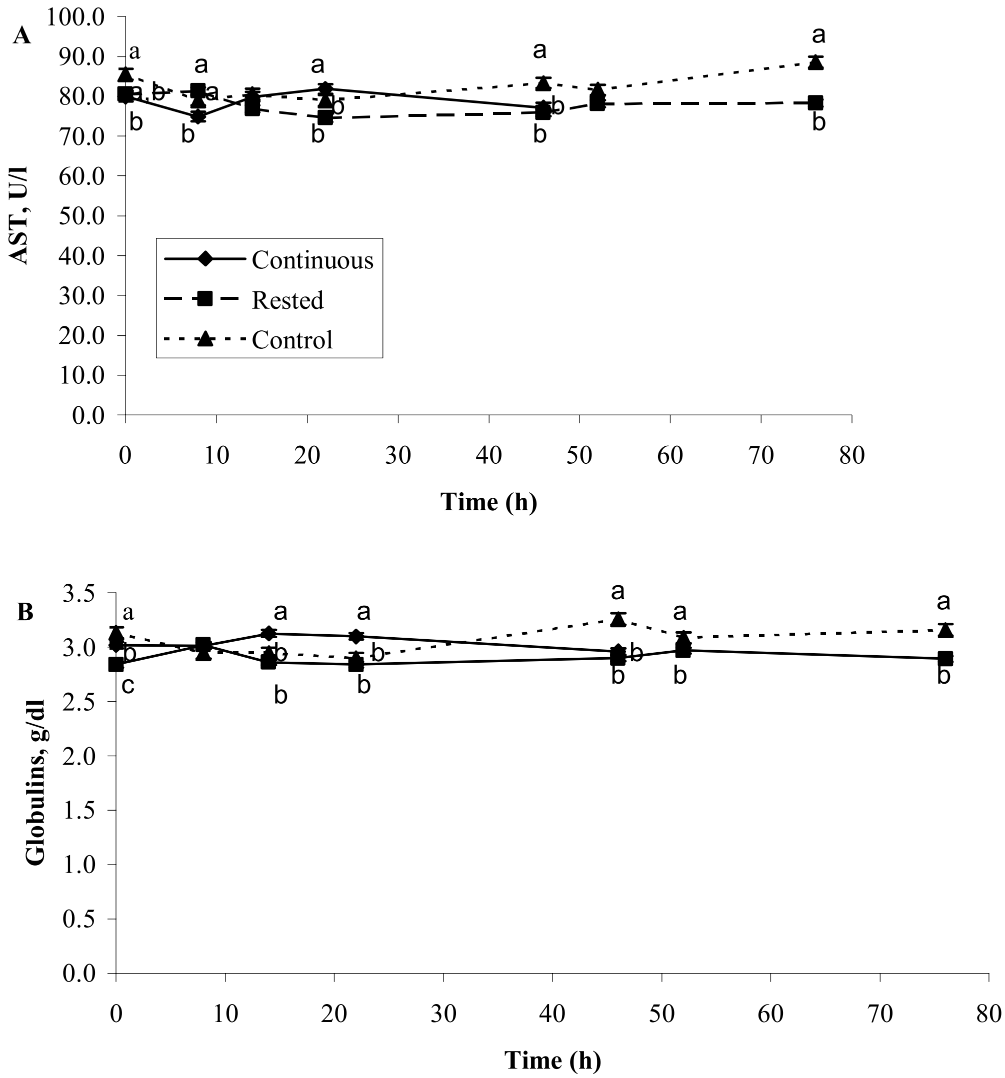


Figure 8. Mean concentrations with S.E. bars of aspartate aminotransferase (AST) (A) and globulins (B) for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. [ab]Means at each time with different superscripts differ ($P < 0.05$).

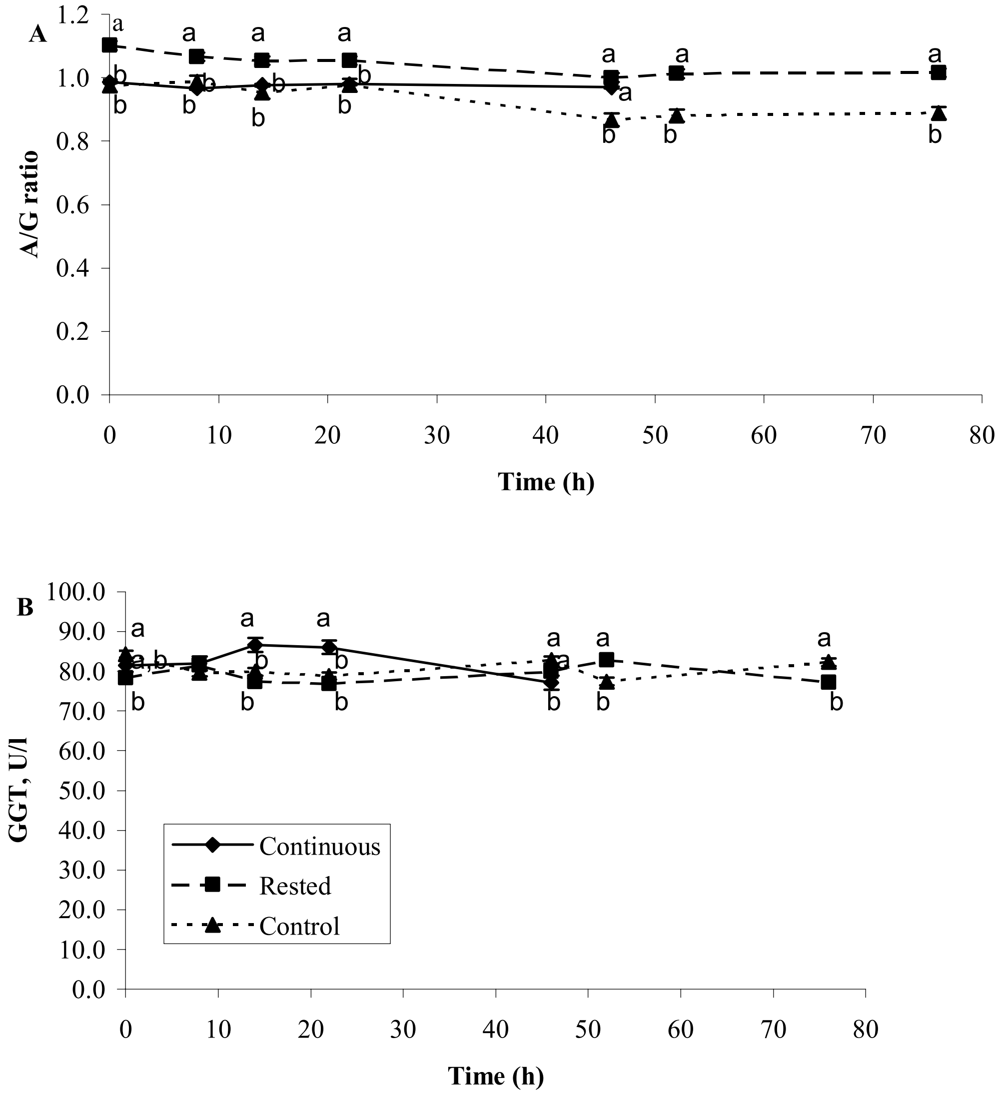


Figure 9. Mean concentrations with S.E. bars of albumin/globulin (A/G) ratio (A) and gamma glutamyl transferase (GGT) (B) for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. [ab]Means at each time with different superscripts differ ($P < 0.05$).

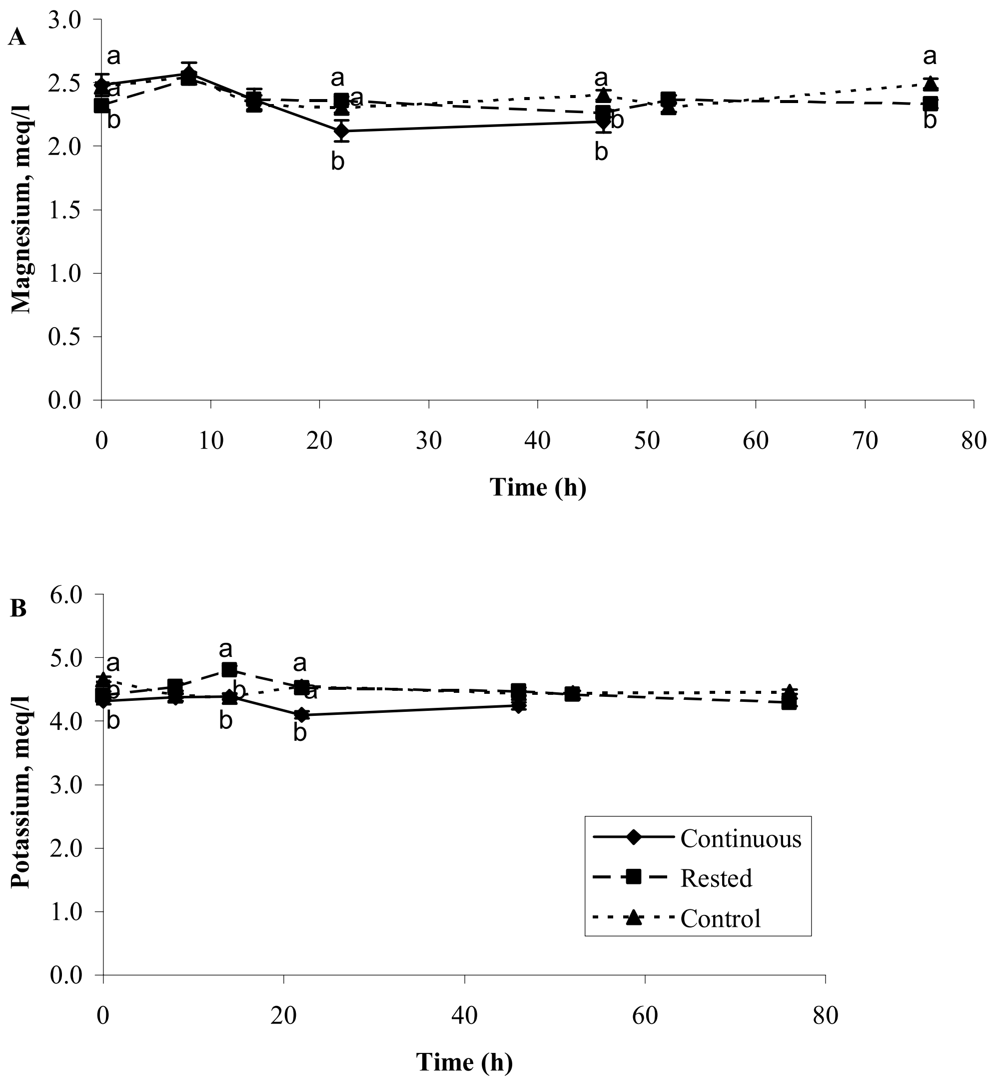


Figure 10. Mean concentrations with S.E. bars of magnesium (A) and potassium (B) for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. [ab]Means at each time with different superscripts differ ($P < 0.05$).

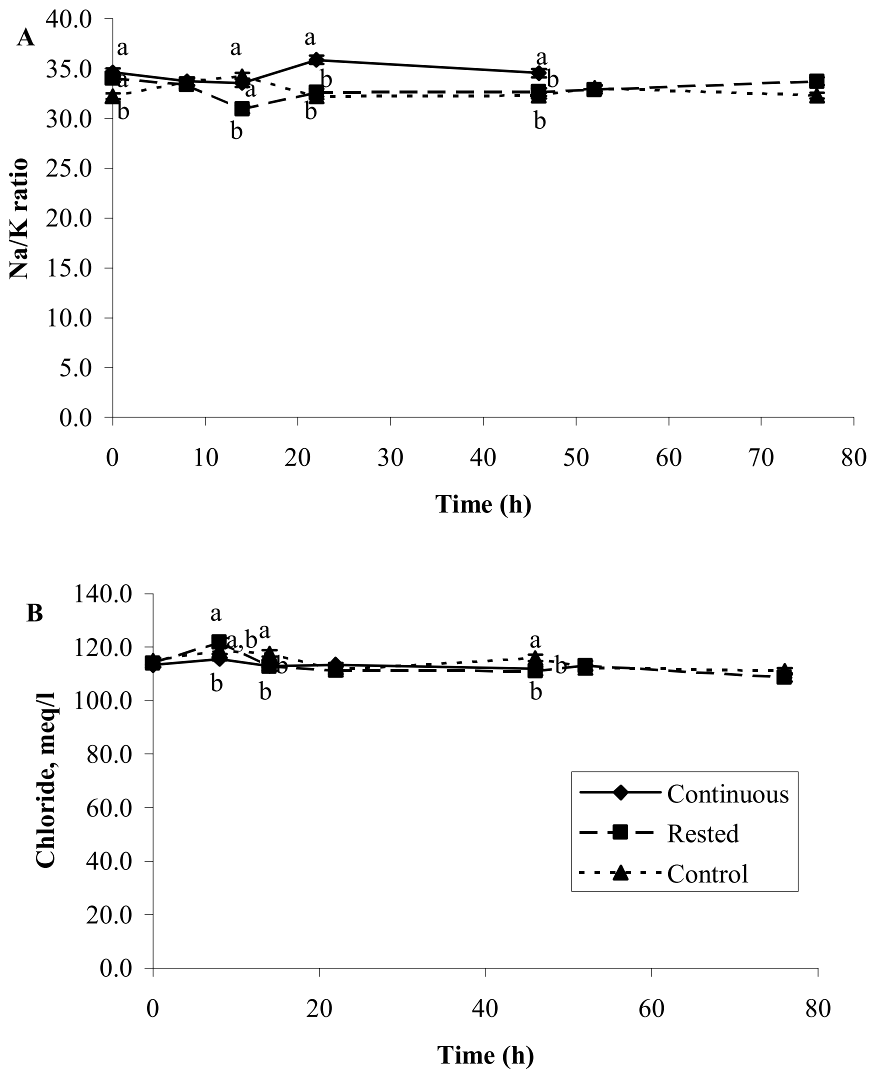


Figure 11. Mean concentrations with S.E. bars of sodium/potassium (Na/K) ratio (A) and chloride (B) for Continuous lambs (n = 15), transported for 22 h; Rested lambs (n = 15), transported for 22 h with a 6-h rest after 8 h transport and a 24-h rest stop after an additional 8 h of transport; and Control lambs (n = 16), who remained in home pasture. The initial sample (0 h) was collected immediately prior to the start of transport and the time of each subsequent sample was relative to the start of transport. [ab]Means at each time with different superscripts differ ($P < 0.05$).

# VITA

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Publications

Krawczel, P. D., T. H. Friend, and A. Windom. 2005. *Stereotypic behavior of circus tigers: Effects of performance*. Applied Animal Behavior Science. 95: 189-198.

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