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Effect of transport on blood selenium and glutathione status in feeder lambs¹

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ABSTRACT: Stress from transport may be linked to increased generation of reactive oxygen species, the removal of which requires reduced glutathione and selenium. The aim of this experiment was to examine the effect of transport on glutathione and Se status of feeder lambs. Recently weaned lambs ($n = 40$) were blocked by gender and BW on d 0 of the experiment and randomly assigned to 2 treatment groups: group 1, no transport and full access to feed and water (control), and group 2, 8-h road transport followed by another 16 h of feed deprivation (transport). After 24 h, both treatment groups were treated the same. All lambs were weighed, and blood samples were collected at 0, 8,

24, and 72 h and analyzed for whole-blood (WB) and serum Se concentrations, serum NEFA concentrations, and erythrocyte concentrations of glutathione. Transport of feeder lambs for 8 h followed by another 16 h of feed deprivation transiently (significant at 24 h but no longer different at 72 h) decreased BW and erythrocyte glutathione concentrations and increased serum NEFA and blood Se concentrations compared with control lambs. Our results suggest that 8 h of transport followed by another 16 h of feed deprivation results in fatty acid and Se mobilization from tissue stores with a coincident decrease in erythrocyte glutathione concentrations.

Key Words: glutathione; selenium; sheep; transport

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INTRODUCTION

Transport is a stressful event in the life cycle of lambs, resulting in increased plasma cortisol concentrations, loss of BW, elevated serum NEFA concentrations, and increased production of reactive oxygen species (Knowles et al., 1998; Parrott et al., 1998; Zhong et al., 2011). The tripeptide glutathione (**GSH**) is the most abundant antioxidant in aerobic cells (Owen and Butterfield, 2010). Reactive oxygen species are removed by oxidizing the free thiol group of GSH. This occurs most efficiently when catalyzed by the enzyme gluta-

thione peroxidase, a selenocysteine (**SeCys**)-containing enzyme (Rotruck et al., 1973). Thus, we hypothesized that transport would lead to Se and GSH losses in lambs.

Selenium is an essential micronutrient that is deficient in most U.S. soils in the Pacific Northwest, Great Lakes region, Atlantic Northeast, and Florida. Thus, livestock grazing or consuming crops raised on these soils may have inadequate intake of Se, resulting in various disorders, including nutritional myodegeneration (Muth et al., 1958). Selenium deficiency is an important nutritional disorder recognized in lamb feedlot management systems (Donoghue and Kronfeld, 1990). Although lambs may have low whole-blood (**WB**) Se concentrations, it is unknown whether Se loss occurred during transport or resulted from inadequate Se intake in the feedlot. To our knowledge, the effect of transport on blood Se concentrations and GSH status in livestock species, including sheep, has not been reported. In Chinook salmon smolts, up to 20% of tissue carcass selenium is lost during barge transport (Halver et al., 2004).

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Our objective was to evaluate the effect of transport for 8 h followed by food deprivation for another 16 h on WB and serum Se concentrations and on erythrocyte concentrations of GSH. We hypothesized that transport for 8 h followed by food deprivation for another 16 h would decrease WB and serum Se concentrations and decrease erythrocyte GSH concentrations.

MATERIALS AND METHODS

Experimental procedures used in this experiment were approved by the Oregon State University Animal Care and Use Committee.

Experimental Design and Treatments

This was an experiment involving 40 feeder lambs from Dorper/Polypay or Dorper/Suffolk crossbred ewes by Polypay rams. Lambs ranged in age and BW from 19 to 22 wk and 27.7 to 32.2 kg, respectively, and were weaned 3 wk before the start of the experiment. All lambs had been raised on the same pasture and had free-choice access to a custom-made mineral supplement (OSU Sheep Mineral Premix, Wilbur-Ellis Co., Clackamas, OR). The mineral supplement contained 8.0% to 9.5% calcium, 6.0% phosphorus, 33.5% to 37.5% salt (NaCl), 2.7% magnesium, 60 mg/kg cobalt, 1,700 mg/kg manganese, 210 mg/kg iodine, 1,350 mg/kg iron, 7,700 mg/kg zinc, 116,120 IU/kg vitamin A, 14,515 IU/kg vitamin D, 25.4 IU/kg vitamin E, and 200 mg/kg Se from sodium selenite. The measured Se concentrations of the pasture ranged from 0.12 to 0.14 mg/kg DM; other nutrients in the pasture were not measured. The experiment was conducted at the Oregon State University Sheep Center (Corvallis, OR) in July 2011.

Utilizing a randomized block design, lambs were blocked on d 0 by BW and sex and then were randomly assigned to 1 of 2 treatment groups: group 1, no transport and full access to feed and water (**control**), and group 2, 8-h road transport, followed by 16-h feed deprivation (**transport**). After 24 h, both treatment groups were treated the same. There were 10 Dorper/Polypay \times Polypay and 10 Dorper/Suffolk \times Polypay lambs in the control group and 18 Dorper/Polypay \times Polypay and 2 Dorper/Suffolk \times Polypay lambs in the transport group. Except during blood collection and weighing, control lambs were kept on the pasture adjacent to the barn, which they had been in before the experiment. Transport lambs joined the control lambs on the pasture after 24 h (8-h road transport followed by 16-h feed deprivation in the barn). Although this was not equivalent to the 30-h transport typical for long hauls of lambs to feedlots, it recreated the conditions of a shorter transport time followed by a period of time spent in a holding pen on arrival at the feedlot.

For blood collection, lambs were brought in the barn and manually restrained in a chute, and 20 mL of blood were collected from the jugular vein at 0 time (baseline) and at 8 h (immediately after transport), 24 h (end of feed deprivation), and 72 h (48 h after end of feed deprivation). Lambs were moved to a scale and weighed after blood collection. During the 72-h blood collection period, lambs had no access to a Se-containing salt-mineral mixture. Whole blood and serum were collected to measure WB and serum Se concentrations as described previously (Hall et al., 2012). Serum NEFA concentrations were measured using a commercial assay according to manufacturer's instructions (ACS ACOD method, Wako Diagnostics, Richmond, VA).

The 20 treatment lambs were loaded into a stock trailer for transport on public highways for a total of 8 h of driving time. The trailer was a 1.83 \times 4.88 m, aluminum, single-deck stock trailer with covered top and sides and air slots down both sides. Each lamb had 0.45 m² of floor space. All university, state, and federal regulations regarding transportation of livestock were followed, including length of continuous travel allowed before a rest stop was required. The total transportation time was 8 h, not including time spent for a lunch break (45 min), a rest stop (15 min), loading, and unloading. Additionally, American Sheep Industry Association animal welfare recommendations were followed. There was no overcrowding in the trailer (hence the reason for 20 lambs). Air temperature was approximately 25°C. Measures were taken to avoid injury, including no use of electric or nonelectric prods during loading and unloading and no sudden stops or accelerations during transport. After transport, lambs were unloaded at the OSU Sheep Center and put in a pen separate from the control lambs.

Glutathione Analysis in Erythrocyte Lysates

Total glutathione concentrations in erythrocyte lysates were measured using the OxiSelect Total Glutathione (GSSG/GSH) Assay Kit (Cell Biolabs Inc., San Diego, CA) according to the manufacturer's instructions. The assay is based on the method of Owen and Butterfield (2010): glutathione reacts in the presence of reduced form of nicotinamide adenine dinucleotide phosphate (**NADPH**), glutathione reductase, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase with Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid)) to produce 2-nitrobenzoic acid anion, a compound containing a chromophore that absorbs light at 412 nm.

In short, for this assay 9 mL of jugular venous blood were collected at each blood collection time into a heparinized Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ). The tube was placed on ice until processing began. The tubes were spun at 1,000 \times g in a Beckman

Table 1. Comparison of BW, serum NEFA concentrations, whole-blood Se concentrations, serum Se concentrations, and erythrocyte glutathione concentrations in weaned feeder lambs on pasture (control) or exposed to 8-h transport and 24-h feed deprivation (transport)¹

Indicator	Baseline-adjusted values ² (LSM ± SEM)			<i>P</i> -values ³		
	8 h	24 h	72 h	Transport main effect	Time main effect	Transport × time main effect
BW, kg						
Control	30.8 ± 0.3	31.4 ± 0.3	30.5 ± 0.3			
Transport	29.8 ± 0.3	29.0 ± 0.4	30.8 ± 0.4			
<i>P</i> -value ³	0.03	<0.001	0.60	0.02	0.02	<0.001
Serum NEFA, µEq/L						
Control	371 ± 53	360 ± 51	402 ± 50			
Transport	634 ± 57	953 ± 55	474 ± 54			
<i>P</i> -value ³	0.002	<0.001	0.34	<0.001	<0.001	<0.001
Whole-blood Se, ng/mL						
Control	139 ± 1	138 ± 2	132 ± 2			
Transport	142 ± 2	146 ± 2	136 ± 3			
<i>P</i> -value ³	0.14	0.01	0.27	0.06	<0.001	0.17
Serum Se, ng/mL						
Control	109 ± 2	108 ± 4	95 ± 3			
Transport	113 ± 2	119 ± 4	100 ± 3			
<i>P</i> -value ³	0.19	0.07	0.27	0.06	<0.001	0.40
Total glutathione, mM						
Control	2.74 ± 0.13	3.19 ± 0.14	3.04 ± 0.12			
Transport	2.20 ± 0.14	3.11 ± 0.15	3.02 ± 0.13			
<i>P</i> -value ³	0.008	0.70	0.89	0.12	<0.001	0.04

¹Measurements were taken at 0 h (baseline), 8 h (end of transport), 24 h (end of feed deprivation), and 72 h (48 h after end of feed deprivation).

²Baseline value covariate adjusted least squares means (LSM) ± SEM.

³Treatments were compared overall and at each sampling time.

TJ-6 (Beckman Coulter Inc., Indianapolis, IN) centrifuge with swinging bucket rotor for 15 min at 4°C. The plasma and buffy coat were aspirated and discarded; the erythrocyte pack was then vortexed. Next, 400 µL of erythrocytes were placed into duplicate 2-mL microcentrifuge tubes, and 1.6 mL of freshly prepared ice-cold 5% metaphosphoric acid (MPA; Sigma-Aldrich, St. Louis, MO) were added and vortexed. The tubes were stored on ice for 10 min and then centrifuged in a refrigerated microcentrifuge (Spectrafuge 24D; Labnet International Inc., Woodbridge, NJ) at 16,300 × *g* for 10 min. The MPA-treated deproteinated supernatant was transferred to a new microcentrifuge tube, quickly frozen in liquid nitrogen, and then stored at −80°C for future use.

The GSH assay was performed in triplicate using 96-well plates, and reactions were monitored at 412 nm with a plate reader (BioTek; Synergy MX, Winooski, VT). Briefly, 50 µL of MPA-treated deproteinated sample and 5 µL of 4 *M* triethanolamine (Sigma) were placed in each well of a 96-well plate together with 145 µL of assay cocktail. The assay cocktail was composed of 2-(*N*-morpholino)ethanesulfonic acid (MES; Sigma) buffer, NADPH (Sigma), glucose-6-phosphate (Sigma), glucose-6-phosphate dehydrogenase (Sigma), and glu-

tathione reductase (Sigma). Then 5 µL of Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid); Sigma) in MES buffer were added, and the plate was incubated in complete darkness in a 96-well plate reader. The reaction was monitored each minute for 15 min. A GSH standard was included on each plate to determine total GSH concentration (µM) by the kinetic method.

Statistical Analysis

Statistical analyses were performed using Statistical Analysis Software version 9.2 (SAS Inst. Inc., Cary, NC). After testing for normality, lamb BW, serum NEFA concentrations, WB and serum Se concentrations, and erythrocyte GSH concentrations were analyzed using a repeated-measures-in-time, randomized block design, using PROC MIXED and the Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Animal was considered the experiment unit, as all animals were equally exposed to treatment procedures. The variance-covariance structure of repeated measures of the same animal across time was modeled using an unstructured variance-covariance matrix. Fixed effects of the baseline-adjusted model

were values obtained at 0 h as the linear covariate, transport (control, transport), sampling time (8, 24, and 72 h after start of transport), genetic background (Dorper/Polypay \times Polypay; Dorper/Suffolk \times Polypay), gender (female, castrated male), and the interaction between transport and sampling time. Treatments were compared across time and at each sampling time. Finally, to evaluate whether dehydration alone (BW at 8 h – BW at 0 h) could explain the results, we added BW change as a linear covariate to the statistical model. To determine whether values at 8, 24, or 72 h differed from baseline values for the control group or for the transport group, we used *t* tests with the ESTIMATE function and the following fixed effects: transport (control, transport), sampling time (0, 8, 24, and 72 h after start of transport), genetic background (Dorper/Polypay \times Polypay, Dorper/Suffolk \times Polypay), gender (female, castrated male), and the interaction between transport and sampling time.

Data are reported as least squares means \pm SEM. All statistical tests were 2 sided. The *P*-values were not adjusted for multiple testing. Statistical significance was declared at $P \leq 0.05$ and a statistical tendency was at $0.05 < P \leq 0.10$. Gender and genetic background did not significantly affect serum NEFA concentrations, WB and serum Se concentrations, or erythrocyte GSH concentrations.

RESULTS AND DISCUSSION

The objective of this experiment was to evaluate the effect of transportation on blood Se and GSH status of feeder lambs. Transport of feeder lambs for 8 h followed by feed deprivation for another 16 h transiently (significant at 24 h but no longer different at 72 h) decreased baseline-adjusted BW compared with control lambs (-2.4 ± 0.5 kg at 24 h; $P < 0.001$; Table 1). The decrease in BW in transported lambs was minor (-1.0 ± 0.3 kg at 24 h; $P = 0.002$; Fig. 1A) and no longer different by 72 h (Table 1). Similar decreases in BW have been reported previously in transported sheep (Knowles et al., 1995; Broom et al., 1996; Knowles et al., 1996; Zhong et al., 2011) and probably resulted from water loss (1 urination weighs approximately 0.3 kg, or 1% of BW) and loss of gut fill during transport and subsequent feed deprivation, which together can account for up to 5% of BW losses (reviewed in Knowles, 1998).

Transport of feeder lambs for 8 h followed by feed deprivation for another 16 h increased baseline-adjusted serum NEFA concentrations compared with control lambs ($+593 \pm 76$ μ Eq/L at 24 h; $P < 0.001$; Table 1). In our experiment, serum NEFA concentrations increased 2-fold after 8 h transport and 3.1-fold after another 16 h of feed deprivation compared with baseline values (Fig. 1B). The increase in serum NEFA concentrations in transported lambs persisted after adjusting for loss

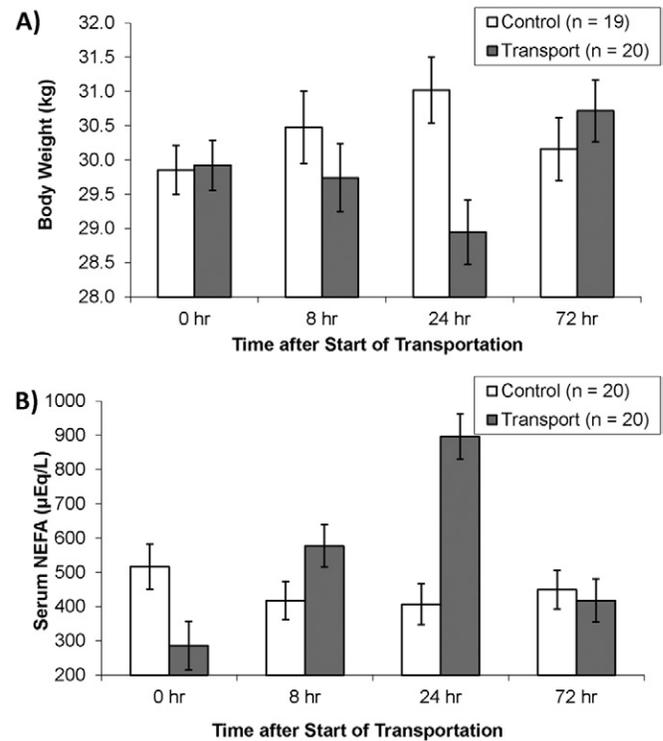


Figure 1. Comparison of (A) BW and (B) serum NEFA concentrations (least squares means \pm SEM) in weaned feeder lambs on pasture (control) or exposed to 8-h transport and 24-h feed deprivation (transport). Measurements were taken at 0 h (baseline), 8 h (end of transport), 24 h (end of feed deprivation), and 72 h (48 h after end of feed deprivation).

of BW (results not shown). A limitation of our experiment is that transported lambs had lower serum NEFA concentrations at baseline compared with control lambs (286 ± 71 vs. 517 ± 66 μ Eq/L, respectively; $P = 0.02$; Fig. 1B), even though they were randomly assigned to treatment groups after blocking for sex and BW. Nonetheless, transported lambs tended to have higher NEFA concentrations compared with control lambs at 8 h (577 ± 62 vs. 418 ± 56 μ Eq/L, respectively; $P = 0.06$) and had higher NEFA concentrations at 24 h (896 ± 66 vs. 407 ± 60 μ Eq/L, respectively; $P < 0.001$; Fig. 1B). Furthermore, similar increases in circulating NEFA concentrations have been previously reported in transported sheep (Knowles et al., 1995; Kent, 1997; Knowles et al., 1998) and likely result from depletion of liver glycogen stores with fasting and subsequent mobilization of body fat such that NEFA becomes the primary source of energy. At 72 h, serum NEFA concentrations did not differ ($P = 0.34$; Table 1); serum NEFA concentrations, however, remained elevated in transported lambs compared with baseline values ($+132 \pm 58$ μ Eq/L; $P = 0.03$; Fig. 1B).

Transport of feeder lambs for 8 h followed by feed deprivation for another 16 h increased baseline-adjusted WB Se concentrations compared with control lambs ($+7.7 \pm 3.0$ ng/mL at 24 h; $P = 0.01$; Table 1). The increase in WB Se concentrations in transported lambs was small

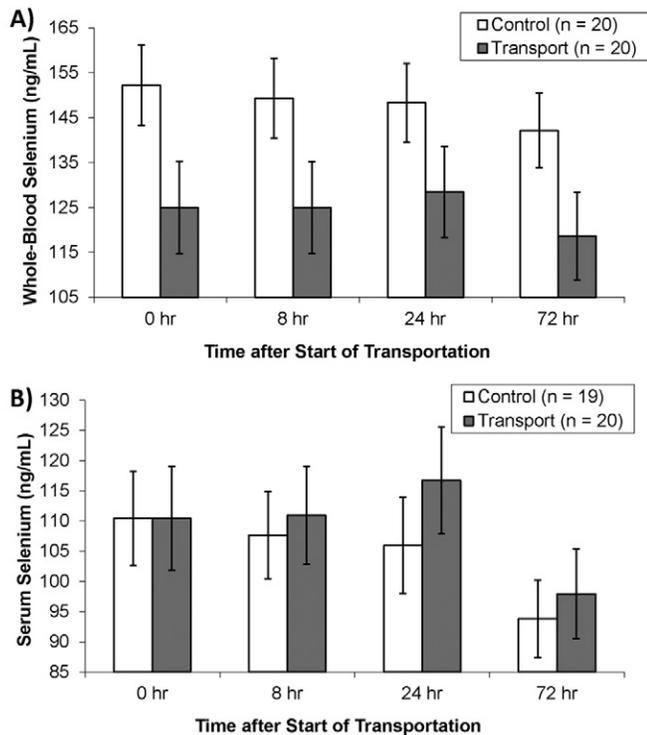


Figure 2. Comparison of (A) whole-blood Se and (B) serum Se concentrations (least squares means \pm SEM) in weaned feeder lambs on pasture (control) or exposed to 8-h transport and 24-h feed deprivation (transport). Measurements were taken at 0 h (baseline), 8 h (end of transport), 24 h (end of feed deprivation), and 72 h (48 h after end of feed deprivation).

and persisted after adjusting for loss of BW (results not shown). A limitation of our experiment is that transported lambs had lower WB Se concentrations at baseline compared with control lambs (125 ± 10 vs. 152 ± 9 ng/mL, respectively; $P = 0.05$; Fig. 2A), which we cannot explain because lambs were randomly assigned to treatment groups after blocking for sex and BW. With differences in baseline WB Se concentrations, it cannot be excluded that later differences were necessarily due to treatment. However, serum Se concentrations in control and transport lambs did not differ at baseline (110 ± 8 vs. 110 ± 9 ng/mL, respectively; $P = 1.00$; Fig. 2B), and parallel increases were noted in serum Se concentrations in response to transport and feed deprivation. Transport of feeder lambs for 8 h followed by feed deprivation for another 16 h tended to increase baseline-adjusted serum Se concentrations compared with those of control lambs ($+11.0 \pm 5.8$ ng/mL at 24 h; $P = 0.07$; Table 1); this small increase was similar in magnitude to that for WB Se concentrations. By 72 h, serum Se concentrations were decreased in transported and control lambs compared with baseline values (-12.5 ± 3.2 ng/mL [$P = 0.01$] and -16.6 ± 3.2 ng/mL [$P < 0.001$], respectively; Fig. 2B), which was also the case for WB Se concentrations (-6.3 ± 2.4 ng/mL [$P = 0.01$] and -10.1 ± 2.4 ng/mL [$P < 0.001$], respectively; Fig. 2A). In beef calves,

plasma Se declined with each successive sampling, from 7 d pretransit to 7 d posttransit (Burke et al., 2009). The absence of supplemental Se during the experiment was the explanation given for the gradual decline in plasma Se but is an unlikely explanation for our shorter-term findings.

The normal reference interval for serum Se concentration in lambs between 30 and 300 d of age at the Michigan State University diagnostic laboratory is 80 to 110 ng/mL (Stowe and Herdt, 1992), indicating that lambs in this experiment (average baseline serum Se concentration was 112 ng/mL) were Se replete. Whole-blood Se is found primarily in hemoglobin as selenomethionine (Se-Met) and in erythrocyte-bound glutathione peroxidase in the form of SeCys; plasma Se is found primarily in selenoprotein P as SeCys, in glutathione peroxidase as SeCys, and in albumin as SeMet (Deagen et al., 1993; Whanger et al., 1996; Finley, 1998). The distribution of Se in the serum or plasma of 21 healthy people showed that $53\% \pm 6\%$ was associated with selenoprotein P, $39\% \pm 6\%$ with glutathione peroxidase, and $9\% \pm 4\%$ with albumin (Harrison et al., 1996). Selenoprotein P is involved in maintaining Se homeostasis and transporting Se to tissues (Burk and Hill, 2009; Fairweather-Tait et al., 2010).

Under conditions of Se deficiency, most absorbed Se, regardless of chemical form, is used to maintain the concentrations of SeCys-containing selenoproteins (reviewed in Janghorbani et al., 1999). We have previously shown in sheep with Se depletion that the amount of nonserum Se, which is found primarily in hemoglobin as SeMet, decreases (Hall et al., 2012), resulting in a smaller ratio of WB Se to serum Se (1.3:1) compared with that in sheep fed Se yeast supplements at the FDA-permitted concentrations (WB Se to serum Se ratio of 2.5:1). The lambs in this experiment had a ratio of WB Se to serum Se of 1.3:1, suggesting they had only small amounts of SeMet-containing proteins in their blood.

We postulate that the transiently elevated and then decreased blood Se concentrations noted in transport lambs were the result of changes in selenoprotein P concentration. Under conditions of acute stress, Se may be mobilized from SeMet-containing proteins in peripheral tissues and transported by selenoprotein P to the liver to facilitate increased biosynthesis of glutathione peroxidases. Glutathione peroxidases are then used to remove the increased amounts of reactive oxygen species produced during stress or to maintain redox status in cells (Weber et al., 2010). In support, in Chinook salmon that were transported by barge for 30 h, tissue Se concentrations of their frozen eviscerated carcasses decreased by 20% after the barge trip (Halver et al., 2004). Liver glutathione peroxidase activity was increased 58%, indicative of mobilization of tissue Se to generate elevat-

ed glutathione peroxidase activity (Halver et al., 2004). Unfortunately, we could not measure muscle or liver Se concentrations or glutathione peroxidase activity in liver or erythrocytes in our lambs to determine if significant peripheral tissue Se mobilization occurred. Future studies are needed to confirm our hypothesis.

Baseline blood Se concentrations showed marked variability even though all lambs had been offered a Se-containing (200 mg/kg) mineral mix supplement ad libitum. At 200 mg/kg these salt-mineral mixtures contain more Se than what is considered the maximum allowable concentration (90 mg/kg for sheep; U.S. Food and Drug Administration regulations), but it is a common practice in our Se-deficient area to offer a premix containing 200 mg/kg Se as the sole mineral supplement. Baseline WB Se concentrations ranged from 37 to 221 ng/mL; serum Se concentrations ranged from 17 to 176 ng/mL, which suggests highly variable consumption of the mineral mix. This is consistent with previously reported rates of salt consumption in sheep. Langlands et al. (1990) showed that increases in blood Se content were highly variable in ewes and their lambs given access to selenized salt, resulting in only 67% to 93% flock efficacy (i.e., alleviation of Se deficiency), thus leaving 7% to 33% unprotected. B. Taylor (USDA Agricultural Research Service, Dubois, ID, personal communication; unpublished observations of production records) found that sheep intake of high NaCl (>95%) salt with sodium selenite (60 mg/kg) can vary from 0 to 45 g·sheep⁻¹·d⁻¹. These results highlight that sheep may become Se deficient even if they have access to selenized salt. This is also the most likely explanation for why WB Se concentrations differed at baseline in transport lambs vs. control lambs even though lambs were randomly assigned to treatment groups after blocking for sex and BW.

In erythrocytes, GSH functions as the most abundant antioxidant, regulating the activity of redox-sensitive enzymes, limiting lipid peroxidation of the plasma membrane in conjunction with glutathione peroxidase, and preventing oxidative denaturation of hemoglobin (reviewed in Ellison and Richie, 2012). The GSH concentration in erythrocytes of most species is between 2.0 and 4.0 mM (Srivastava and Beutler, 1969). The average baseline concentrations of GSH in our experiment were 3.8 mM (Fig. 3). Reduced GSH is the most prevalent form of GSH, constituting greater than 90% of the GSH pool in healthy cells (reviewed by Owen and Butterfield, 2010). During the first 8 h, erythrocyte GSH concentrations dramatically decreased in both transported and control lambs compared with baseline concentrations (-1.67 ± 0.15 mM at 8 h [$P < 0.001$] and -0.92 ± 0.15 mM at 8 h [$P < 0.001$], respectively; Fig. 3). One possible reason for the decrease is circadian changes in GSH concentrations. However, this explana-

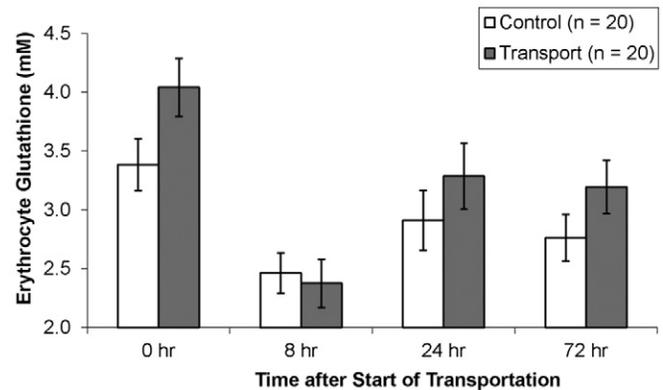


Figure 3. Comparison of erythrocyte glutathione concentrations (least squares means \pm SEM) in weaned feeder lambs on pasture (control) or exposed to 8-h transport and 24-h feed deprivation (transport). Measurements were taken at 0 h (baseline), 8 h (end of transport), 24 h (end of feed deprivation), and 72 h (48 h after end of feed deprivation).

tion is unlikely because erythrocyte GSH concentrations remain relatively constant (for months) within any particular healthy individual (Richie et al., 1996). Furthermore, erythrocyte GSH concentrations remained lower in control and transport lambs compared with baseline concentrations at 24 h (-0.48 ± 0.15 and -0.75 ± 0.15 mM, respectively) and 72 h (-0.62 ± 0.13 and -0.85 ± 0.13 mM, respectively; Fig. 3). Yardimci et al. (2013) recently showed in sheep that handling associated with weighing decreases reduced GSH concentrations in blood, which could explain the decrease in GSH concentrations in control lambs (Fig. 3). In addition, Mears et al. (1999) demonstrated that handling ewes before shearing increases plasma cortisol concentrations even in the absence of shearing and that the cortisol response was greater in ewes previously exposed to shearing. Thus, handling and blood collection were likely stressful events in our control lambs as well.

The decrease in GSH concentrations was greater in transported lambs compared with control lambs ($-40\% \pm 4\%$ vs. $-26\% \pm 3\%$, respectively; $P = 0.008$; Fig. 3). The greater decrease in GSH concentrations in transported lambs compared with control lambs suggests that transport adds to the handling-associated decrease in the erythrocyte GSH pool. A limitation of our experiment is that transported lambs had higher erythrocyte GSH concentrations at baseline compared with control lambs (4.04 ± 0.25 vs. 3.39 ± 0.22 mM, respectively; $P = 0.05$; Fig. 3), which we cannot explain. Thus, observed group differences at 8 h ($P = 0.008$; Table 1) could be due in part to differences in baseline concentrations. Although we cannot exclude this potential explanation, Zhong et al. (2011) reported that road transport of feeder lambs increased 2-thiobarbituric acid reactive substances in serum, indicating that road transport causes oxidative stress. Furthermore, Crookshank et al. (1979) showed that handling and transport both increased serum corti-

sol concentrations in weaned beef calves measured directly after transport, and Cooke et al. (2013) reported that transport increased serum cortisol and haptoglobin concentrations in weaned beef calves measured directly after transport.

A similar temporary decline in erythrocyte GSH concentrations has been reported in male humans during the first 24 h after running a half marathon (Duthie et al., 1990). Oxidative stress decreased reduced GSH concentrations in erythrocytes (Hayes and McLellan, 1999). To prevent accumulation of oxidized GSH in erythrocytes, oxidized GSH is either converted to reduced GSH by glutathione reductase, an NADPH-dependent enzyme whose activity is induced by oxidative stress (Rychlik et al., 2000), or exported out of the cell using an energy-dependent transport system, regulated in part by the multidrug resistance associated protein (**MRP1**; Ellison and Richie, 2012). Thus, the lower GSH concentrations in erythrocytes after 8 h of transport measured in our experiment (−40%) may reflect a greater efflux of oxidized GSH out of erythrocytes or a binding of oxidized glutathione to other proteins with free thiol groups (protein glutathionylation). Others have examined the effect of transport stress on erythrocyte (Sahin et al., 2009) and leukocyte (Burke et al., 2009) glutathione peroxidase activities in cattle and found no significant effects. In horses, erythrocyte glutathione reductase activities were decreased 24 h after road transport, suggesting disturbances in GSH metabolism during transport (Niedzwiedz et al., 2012). Future studies may require measurements of both enzymes and substrates. In addition, other markers confirming oxidative stress need to be measured, including urinary isoprostanes and plasma malondialdehyde concentrations.

In summary, transport of feeder lambs for 8 h followed by another 16 h of feed deprivation transiently (significant at 24 h but no longer different at 72 h) decreased BW and erythrocyte GSH concentrations and increased serum NEFA and blood Se concentrations. Our results suggest that 8 h of transport followed by another 16 h of feed deprivation results in fatty acid and Se mobilization with a coincident decrease in erythrocyte GSH concentrations. Additional studies utilizing tissue samples and Se-specific proteins are needed to further our understanding of the dynamics of Se metabolism with stress-related events.

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