Effect of preshipment preconditioning and injectable antioxidant trace elements (Cu, Mn, Se, Zn) and vitamins (A, E) on plasma metabolite and hormone concentrations and growth in weaned beef cattle

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ABSTRACT: Weaning and transport represent a high stress time for calves. Preconditioning (**PC**) by weaning before the transport separate these stressors. The stressors generate oxidative stress, which can be reduced by mineral and vitamin supplementation (**MVS**) with an antioxidant capacity. Our objective was to evaluate the effect of PC and MVS on performance of steers. The experiment used a 2 × 2 factorial arrangement design, considering a 26-d PC treatment from weaning to transport to the feedlot (day 0), and injectable MVS on days −45, −26, and 0. The MVS consisted of Cu, Zn, Mn, Se, vitamin E (0.2, 0.8, 0.2, 0.1, and 1 mg/kg body weight [**BW**], respectively), and vitamin A (1,190 IU/kg). Sixty Angus-crossbred steers (186.4 ± 27.6 kg) were randomly assigned to the four treatments (**MVS+PC**; **N+PC**; **MVS+N**; **N+N**; $n = 15$ per treatment). BW was recorded on days −45, −26, 0, 8, 15, and 29. On day 0, an additional BW was taken 30 min after the 5-h transportation (day 0.5). Between days 0 and 29, dry matter intake (**DMI**) and average daily gain (**ADG**) to DMI ratio (**G:F**) were measured. Between days −26 and 29 plasma concentrations of glucose, nonesterified fatty acids (**NEFA**), cortisol, insulin, total antioxidant status (**TAS**), and thiobarbituric acid-reactive substances were evaluated. Data were analyzed using the MIXED procedure of SAS with repeated measures, using treatment, time, and treatment × time as fixed effects and steer as a random effect. Between days −26 and 0, there was an interaction of MVS × PC ($P < 0.01$) for ADG. From days −26 to 0, N+N and N+PC had the greatest and lesser ADG, respectively. On day 0.5, no-PC steers tended to lose BW, whereas the PC steers tended to gain BW ($P = 0.09$). In the period days 0 to 8, there were no differences ($P \geq 0.27$) in DMI, but the PC steers had greater G:F and ADG ($P < 0.01$) compared with no-PC steers. Plasma NEFA concentration on day 0 was affected by MVS × PC ($P < 0.01$) because MVS decreased plasma NEFA concentration in no-PC steers, but it increased in the PC steers. Plasma concentrations of glucose, insulin, and cortisol did not differ among treatments ($P \geq 0.23$). There was an MVS × PC interaction ($P = 0.09$) for TAS on day 0; N+N had the greatest TAS concentrations and MVS+N had the lowest TAS concentrations. In conclusion, a 26-d PC decreased steers BW compared with no-PC steers. The BW loss during PC was not recovered 29 d after feedlot entry. Despite this BW loss, MVS treatment decreased BW loss in the steers allocated to PC treatment on the day of transport.

Key words: antioxidant, minerals, preconditioning, steers, vitamins

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INTRODUCTION

In beef cattle production systems, calves are usually weaned between 6 and 7 mo of age to improve the cow's body weight (**BW**) and condition score before the next calving (Rasby, 2007; Enríquez et al., 2011). Traditionally, the weaning process subjects the calf to a multitude of different stressors. The stressors at weaning include separating calves from their dams, loss of access to milk, and social and environmental changes (Enríquez et al., 2011). Furthermore, it is common for calves to be transported directly to a feedlot or sale barn at weaning, adding further stress to the calf (Chirase et al., 2004). Preconditioning programs can help cattle producers to minimize the detrimental effects of stress on calves at weaning and later in their development. One important part of a preconditioning program consists of delaying the transportation of calves directly to a feedlot for 20 to 45 d after weaning (Wieringa et al., 1974; Herrick, 1979). Exposure to stressors will activate the hypothalamic–pituitary–adrenal axis with a consequent release of adrenocorticotropic hormone (**ACTH**). Increased concentration of ACTH in the blood will cause an increase in cortisol concentration (Brown and Vosloo, 2017). Increased cortisol concentration is known to suppress the immune system. In addition to the immunosuppression, cortisol concentration in the blood creates metabolic changes within the calf such as an increase in protein and lipid catabolism (Reece, 2015). The hypothalamic–pituitary–adrenal axis may also cause an imbalance between the antioxidant defense of the body and reactive oxygen species (**ROS**) production, generating a net oxidative stress in the body (Sies, 1997). Greater ROS in the blood relative to antioxidant defense mechanisms may cause lipoperoxidation of fatty acid membranes, leading to an alteration in cellular function and an increased energy cost associated with cellular repair (Halliwell and Gutteridge, 2015).

Copper (**Cu**), manganese (**Mn**), selenium (**Se**), and zinc (**Zn**) are cofactors of enzymes that metabolize the ROS in the body to water. In addition, vitamins A and E function as antioxidants for cells in the body (Celi, 2001). These vitamins and trace minerals are important to prevent (directly or as cofactors) cells from ROS damage; however, a decrease in mineral and vitamin intake and/or a decrease in absorption and transport can result in an increased production of ROS in the body, and therefore, predispose the animal to oxidative stress (Celi, 2001). Weaning, transport, and handling at a feedlot arrival are associated with a reduction in feed intake, decreasing the consumption of minerals and vitamins (Noffsinger et al., 2015), and therefore, increasing the production of ROS within the animal (Chirase et al., 2004).

The hypothesis tested in the current experiment was that preweaning parenteral treatment with the minerals Cu, Mn, Se, and Zn and the vitamins A and E will improve antioxidant capacity at weaning and increase the subsequent BW of calves compared with calves that were not treated with the mineral and vitamin supplement. We also hypothesized that the mineral and vitamin treatment would have the greatest effect on calf BW gain when given to steers that were preconditioned. Therefore, the aim of this study was to evaluate the effect of parenteral supplementation of the trace minerals, Cu, Mn, Se, Zn, and the vitamins A and E, and preconditioning after weaning on BW gain in steers during the feedlot phase of their growth.

MATERIALS AND METHODS

This study was conducted at the Eastern Agricultural Research Station and at the Beef Center of the Ohio Agricultural Research and Development Center, Ohio (IACUC 2017A00000049).

Animals and Treatments

Sixty Angus-crossbred steers (186.4 ± 27.6 kg of BW and 181.8 ± 17.5 d of age) were used in this experiment. The treatments were arranged in a 2 × 2 factorial arrangement design. The main factors of the model were mineral and vitamin supplementation (**MVS**) and preconditioning (**PC**). For MVS, calves were given their treatment 45 and 26 d before and immediately prior to transportation to a feedlot, or no MVS (calves were given subcutaneous isotonic saline the same d that the MVS received their supplement). For PC, calves were preconditioned (weaned and housed in a fescue-based pasture and received ad libitum soybean hulls) or no preconditioned (calves remained with their dams on a fescue pasture and weaned on the d of transportation to a feedlot). The steers were randomly assigned to four treatments: 1) MVS+PC, steers with MVS and preconditioned, n = 15; 2) N+PC, steers with no MVS and preconditioned, n = 15; 3) MVS+N, steers with MVS and not preconditioned, n = 15; and 4) N+N, steers with no MVS and preconditioned, n = 15. The number of animals was selected based on a power analysis, using results from a

previous research with the use of a similar MVS (Mattioli et al., 2020). The mineral and vitamin treatment was applied subcutaneously (s.c.) at a dose of 1 mL/50 kg of BW. Each injection provided 10 mg/mL Cu (as cooper edetate), 10 mg/mL Mn (as manganese edetate), 5 mg/mL Se (as sodium selenite), 40 mg/mL Zn (as zinc edetate; Adaptador Min, Biogénesis Bago, Argentina), 59,500 IU/mL vitamin A (as palmitate), and 50 IU/mL vitamin E (as acetate; Adaptador Vit, Biogénesis Bago, Argentina).

All steers were vaccinated against Clostridial bacterin (Ultrachoice 7, Zoetis, Parsippany, NJ) and Pinkeye (Moraxella Bovoculi Bacterin, Addison Biological Laboratory, Fayette, MO) on day 117 before transport. On day 56 before transport, steers received a Clostridial bacterin booster (Ultrachoice 7, Zoetis) for infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza-3, bovine respiratory syncytial virus, 5 *Leptospira* serovars (Bovi-Shield Gold 5, Zoetis), and Pinkeye (Addison Biological Laboratory). On day 34 before transport, steers received their second vaccines (Ultrachoice 7 and Bovi-Shield Gold 5, Zoetis; Pinkeye, Addison Biological Laboratory). In addition, the calves received their first vaccine against bovine respiratory disease (Bovi-Shield Gold One, Zoetis) and were treated with pour-on solution doramectin (Dectomax, Zoetis). At arrival to feedlot, all steers were treated with ivermectin pour-on (1 mL/10 kg BW; Vetrimec, VetONE, Boise, ID) and vaccinated against bovine respiratory disease (Bovi-Shield Gold One. Zoetis). All vaccines were applied following manufacturer instructions.

Experimental Design

Steers transportation to the feedlot was considered day 0. On day −45, all steers were weighed and randomly assigned to treatments. Treatment MVS+N and MVS+PC received a s.c. injection of the mineral solution at a dose of 1 mL/50 kg BW and another s.c. injection of vitamins solution at the same dose. The dose of minerals and vitamins used has been evaluated in two supplementation experiments with calves around weaning (Bordignon et al., 2019; Mattioli et al., 2020). In both experiments, applications of treatments were separated by 7 to 30 d, and improvements in average daily gain (**ADG**), immune response, and antioxidant capacity in dairy and beef calves were reported. On day −26, all steers were weighed and blood sampled. On day −26, a second dose of MVS was applied to the steers in the MVS+N and the MVS+PC treatments. Saline solution was applied to the N+PC and N+N treatments. After the blood sampling and application of the mineral and vitamin treatments, the steers allocated to the MVS+PC and N+PC treatments were weaned and moved to a different pasture to start the preconditioning. On day 0, all steers were weighed and the MVS+N and the MVS+PC steers received a third dose of the mineral and vitamin treatment. After the applications of the treatments, all calves were weighed and transported for 5 h from the Eastern Agricultural Experimental Station (Caldwell, OH) to the feedlot (Wooster, OH). The BW recorded at the start of transport was considered the day 0 BW for all the growth performance data analysis. The day of transport was the day of weaning for the MVS+N and N+N steers. At arrival to the feedlot, the animals were unloaded and left for 30 min to free access to water and alfalfa hay in a dry lot. After 30 min of rest, the steers were weighed (day 0.5 BW) and blood was sampled. The difference in BW between the two measurements collected on day 0.5 and day 0 was used to assess the variation in BW on the shipment day. After day 0.5 BW, all the steers were housed and fed in individuals pens. Pens (2.6 × 1.5 m) had concrete slatted floors, with a 1.5-m-long concrete feed bunk, and ad libitum access to clean and fresh water. On the day of transport, all animals were given ad libitum access to alfalfa hay. Throughout the finishing period of the study, water was available on an ad libitum basis. From day 1 until the end of the experiment, steers were fed with a mixed ration consisting of 60% corn silage, 15% dried distiller's grains with solubles (**DDGS**), 15% ground corn, and a 10% mineral and vitamin supplement (on dry matter [**DM**] basis; Table 1), which exceed NASEM (2016) recommendation for maintenance in beef cattle. The first 3 d after transport, feed was offered at 1.8% of BW (DM basis). After the initial 3 d, feed offered was increased by 5% (DM basis) from previous dry matter intake (**DMI**) every other d until reaching ad libitum feed intake. If there were feed orts in the bunk, the orts were collected and weighed 30 min before feeding time to estimate DMI. A weekly feed sample was taken for the determination of DM. Steers' BW was measured 1 h before feeding time on day 8, day 15, and at the conclusion of the study on day 29. On day 15, all steers were blood sampled.

Sampling and Sample Analysis

Blood samples (20 mL) were collected in the morning (8:00 to 10:00) by jugular venipuncture in two plastic tubes, 10 mL were placed in tubes with sodium heparin (158 USP units; Becton Drive,

Table 1. Ingredient and nutrient composition of the feedlot diet

Ingredient	% DM[1]
Cracked corn	15
DDGS[2]	15
Corn silage	60
Supplement	10
Ground corn	23.1
Urea	4.4
Soybean meal	56.3
Limestone	6.4
Dicalcium phosphate	0.9
White salt (ClNa)	1.4
Calcium sulfate	3.5
Potassium chloride	2.6
Vitamin A	0.06
Vitamin D (D-3)	0.06
Vitamin E	0.2
Selenium	0.13
Cobalt carbonate	0.001
Cooper sulfate	0.07
Zinc sulfate	0.2
Manganese sulfate	0.09
Rumensin 90[3]	0.09
Analyzed composition[4]	
Crude protein, %	14.88
ADF, %	19.04
NDF, %	28.47
EE, %	2.41
Ca, %	0.39
P, %	0.36
Mg, %	0.3
K, %	1
S, %	0.25
Ash, %	5.79

[1]DM = dry matter.

[2]DDGS = dried distiller's grains with soluble.

[3]Elanco Animal Health (Greenfield, IN).

[4]NDF = neutral detergent fiber; Ca = calcium; P = phosphorus; Mg = magnesium; K = potassium; S = sulphur.

Franklin Lakes, NJ), and 10 mL were placed in tubes (Sarstedt, Nümbrecht, Germany), which contained solutions of disodium EDTA and benzamidine HCL (1.6 mg and 4.6 mg/mL of blood, respectively); tubes were kept on ice during the sampling. Within 2 h of extraction, blood samples were centrifuged at 1,800 × *g* at 4°C for 25 min, and plasma was harvested and stored at −80°C in individual polypropylene tubes until further analysis.

Plasma from sodium heparin tubes was used to measure concentration of total antioxidant status (**TAS**) and thiobarbituric acid-reactive substances (**TBARS**). Plasma from disodium EDTA and benzamidine HCL tubes was used to measure glucose, nonesterified fatty acids (**NEFA**), insulin, and cortisol concentration. TAS and TBARS were determined as antioxidant defense and oxidative stress parameters, respectively. Plasma glucose, NEFA, and insulin concentrations were determined as metabolism parameters. Cortisol concentration was determined as an indicator of stress.

Plasma TAS and TBARS concentrations were determined with commercials kits (Antioxidant Assay Kit, catalog no. 709001 and TBARS Assay Kit, catalog no. 10009055, respectively, Cayman Chemical Company, Ann Arbor, MI). Samples and reagents were prepared according to the manufacturer's recommendations.

Plasma glucose concentration was measured via a colorimetric assay with a commercial kit (Stanbio Laboratory, Boerne, TX). Plasma NEFA concentration was determined using an enzymatic assay by a commercial kit (Wako Diagnostics, Mountain View, CA). Plasma insulin concentration was analyzed using an RIA (Porcine RIA #PI-12K; EMD Millipore Corporation, Billerica, MA), as previously validated for bovine plasma (Miqueo et al., 2019). Plasma cortisol concentration was measured with a commercially available RIA kit (MP Biomedicals, LLC., Solon, OH). All samples for plasma insulin and cortisol concentrations were analyzed in the same run. The intra-assay coefficient in variation was 5.3% and 7.5% for insulin and cortisol assays, respectively.

Statistical Analysis

Data were analyzed as completely randomized design, using the PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc.), with a 2 × 2 factorial arrangement of treatments. Because the calves were individually supplemented with the mineral and vitamin mix before transportation and individually housed at the feedlot, calf was considered the experimental unit for all data. The statistical model included the fixed effect MVS, preconditioning, day, their interaction, and the random effect of steer within treatment. All models, except the model to assess the BW variation on shipping day, were run as repeated measurements on time. Compound symmetry covariance structure was used for all models because it provides the lowest AICC. Least square means and standard error were obtained using LSMEANS procedure of SAS (9.4). Because of the three-way interactions, the time × any treatment interaction was not presented, but the differences in means among main factors (MVS, preconditioning, and MVS × preconditioning) were determined

using the SLICE statement of SAS. BW on day −45 was included as a covariate in the models for the performance data analysis. As described previously, BW variation on the day of shipping was determined by the difference in BW on day 0.5 and day 0. Mean differences for main effects were declared at $P \leq 0.05$ and for interactions $P \leq 0.10$.

RESULTS

Growth Performance

An interaction between MVS and PC was demonstrated ($P < 0.01$) for BW on days 0, 8, 15, and 29. On these 4 d, N+PC were the lighter steers. On days 0 and 8, the MVS+N and N+N treatment steers had similar BWs and they were heavier than the MVS+PC steers. On day 15, the MVS+N treated steers continued to be heavier than the MVS+PC; and the N+N steers had an intermediate BW compared with MVS+N and MVS+PC. On day 29, N+N, MVS+N, and MVS+PC had similar BW (Figure 1).

There was an ADG interaction MVS × preconditioning ($P < 0.01$) from day −26 to 0. During this period, N+N had the greatest ADG; however, N+PC has the lowest ADG. MVS increased the ADG in the preconditioned animals, but decreased it for the control weaned animals (Figure 2). There was a PC effect ($P < 0.01$) from days 0 to 8, where preconditioned steers had greater ADG than nonpreconditioned steers. There were no effects of MVS or its interaction ($P \geq 0.44$; Figure 2) on ADG from days 0 to 8, nor PC, MVS, or their interaction ($P \geq 0.11$ for main effects and $P \geq 0.40$ for the interactions) from days 8 to 15 and days 15 to 29 (Figure 2).

There were no effects ($P \geq 0.27$) of PC, MVS, or their interaction for DMI on the first 8 d. There was an interaction of MVS × PC ($P = 0.08$) from days 8 to 15, where N+PC had a lesser DMI compared with N+N, MVS+N, and MVS+PC (Figure 3). There was no effect of PC, MVS, or any interaction present ($P \geq 0.27$) for DMI from days 15 to 29.

Regarding feed efficiency (ADG/DMI; **G:F**), a PC effect was observed ($P < 0.01$) from days 0

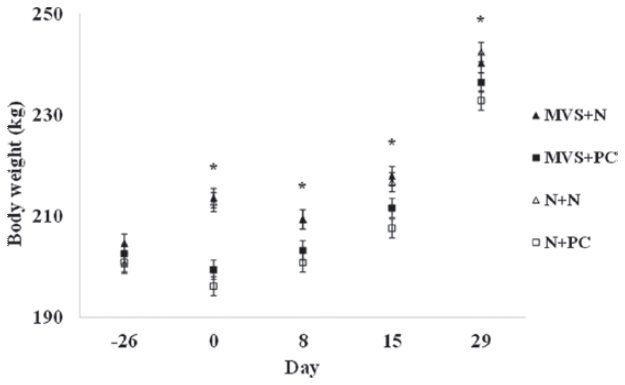

Figure 1. Effect of preconditioning and mineral and vitamin supplementation preshipping to feedlot on body weight in steers. Treatments were as follows: 1) mineral and vitamin supplementation and no preconditioning period (MVS+N); 2) mineral and vitamin supplementation and a 26-d preconditioning period (MVS+PC); 3) no mineral and vitamin supplementation and no preconditioning period (N+N); and 4) no mineral and vitamin supplementation and a 26-d preconditioning period (N+PC). Vitamin and mineral supplementation (Adaptador Min-Vit, Biogénesis Bago, Argentina). Preconditioning x mineral and vitamin supplementation, $P < 0.10$; $n = 15$ steers per treatment.

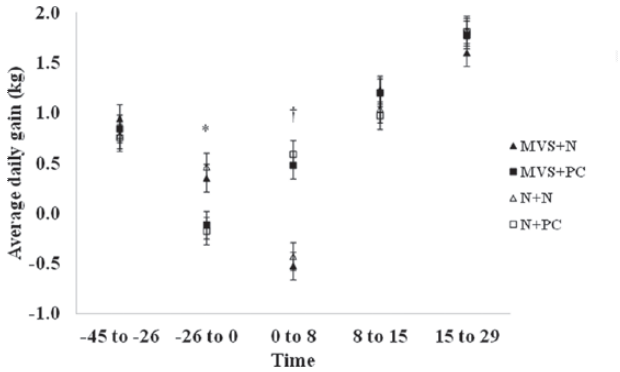

Figure 2. Effect of preconditioning and mineral and vitamin supplementation preshipping to feedlot on average daily gain in steers. Treatments were as follows: 1) mineral and vitamin supplementation and no preconditioning period (MVS+N); 2) mineral and vitamin supplementation and a 26-d preconditioning period (MVS+PC); 3) no mineral and vitamin supplementation and no preconditioning period (N+N); and 4) no mineral and vitamin supplementation and a 26-d preconditioning period (N+PC). Vitamin and mineral supplementation (Adaptador Min-Vit, Biogénesis Bago, Argentina). *Preconditioning x mineral and vitamin supplementation, $P < 0.1$; †Preconditioning, $P < 0.05$; $n = 15$ steers per treatment.

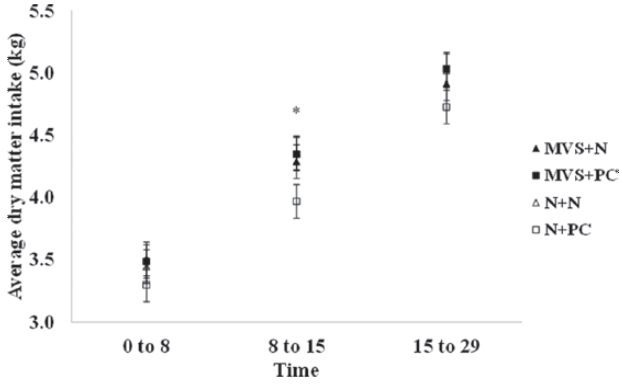

Figure 3. Effect of preconditioning and mineral and vitamin supplementation preshipping to feedlot on dry matter intake in steers. Treatments were as follows: 1) mineral and vitamin supplementation and no preconditioning period (MVS+N); 2) mineral and vitamin supplementation and a 26-d preconditioning period (MVS+PC); 3) no mineral and vitamin supplementation and no preconditioning period (N+N); and 4) no mineral and vitamin supplementation and a 26-d preconditioning period (N+PC). Vitamin and mineral supplementation (Adaptador Min-Vit, Biogénesis Bago, Argentina). *Preconditioning x mineral and vitamin supplementation, $P < 0.10$; $n = 15$ steers per treatment.

to 8. In this period, preconditioned steers had a greater G:F compared with nonpreconditioned steers. There was no MVS or its interaction effect ($P \geq 0.42$) on G:F. From days 8 to 15 and days 15 to 29, there were no effects ($P \geq 0.39$) of PC, MVS, or their interaction on G:F (Figure 4).

There was an MVS × PC interaction ($P = 0.09$) for BW difference on the day of shipping. The preconditioned steers (MVS+PC and N+PC) demonstrated a similar weight gain after transport during the rest period, which was 4.05 and 4.85 kg, respectively. The nonpreconditioned steers had a decrease in their BW

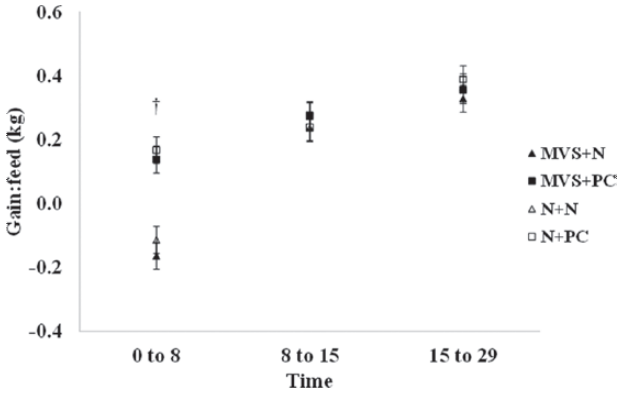

Figure 4. Effect of preconditioning and mineral and vitamin supplementation preshipping to feedlot on feed efficiency (ADG/DMI; gain:feed) in steers. Treatments were as follows: 1) vitamin and mineral supplementation and no preconditioning period (MVS+N); 2) vitamin and mineral supplementation and a 26-d preconditioning period (MVS+PC); 3) no vitamin and mineral supplementation and no preconditioning period (N+N); and 4) vitamin and mineral supplementation and a 26-d preconditioning period (N+PC). Vitamin and mineral supplementation (Adaptador Min-Vit, Biogénesis Bago, Argentina). †Preconditioning, $P < 0.05$; $n = 15$ steers per treatment.

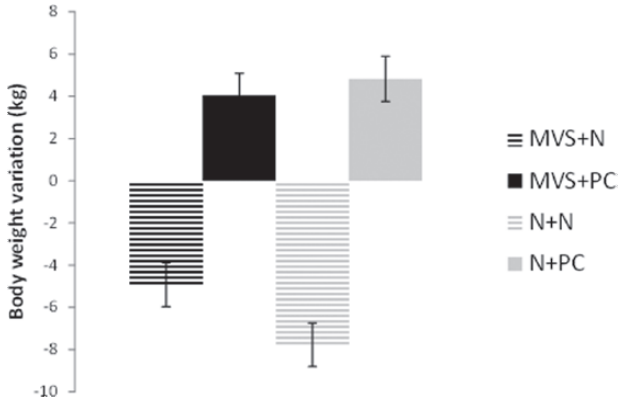

Figure 5. The mean calf body weight gain or loss (kg) after transportation for 5 h and allowed to rest for 30 min with ad libitum access to alfalfa hay and water. Treatments were as follows: 1) mineral and vitamin supplementation and no preconditioning period (MVS+N); 2) mineral and vitamin supplementation and a 26-d preconditioning period (MVS+PC); 3) no mineral and vitamin supplementation and no preconditioning period (N+N); and 4) no mineral and vitamin supplementation and a 26-d preconditioning period (N+PC). Vitamin and mineral supplementation (Adaptador Min-Vit, Biogénesis Bago, Argentina). $n = 15$ steers per treatment.

after arrival at the finishing facility, but N+N had the greatest BW loss; which was −7.6 vs. −4.9 kg, for N+N and MVS+N, respectively (Figure 5).

Plasma Metabolites, Hormones, Antioxidant Capacity, and Lipid Peroxidation Biomarkers

There were no effects of either the MVS or the PC treatment, nor their interaction ($P \geq 0.23$) on plasma glucose, insulin, and cortisol concentration (Table 2). Regarding the plasma NEFA concentration, there was an interaction of MVS × PC on day 0 ($P < 0.01$). The interaction occurred because the nonsupplemented steers had the greatest and least plasma NEFA concentration at day 0 (459.6 and 258.2 µEq/L for N+N and N+PC, respectively), whereas the MVS steers had the intermediate values (435.7 and 297.6 µEq/L for MVS+N and MVS+PC, respectively; Figure 6).

Regarding TAS, there was an interaction of MVS × PC on day 0 ($P = 0.09$). Steers from the N+N steers had the greatest plasma TAS concentration and MVS+N had the lesser plasma TAS concentration (0.83 vs. 0.74 mM, respectively; Figure 7). There were no effects of the main factors or their interaction on plasma TBARS concentration ($P \geq 0.23$; Table 2).

DISCUSSION

The main objective of preconditioning is to minimize the calf's stress response to stressors at arrival to a finishing system (Wieringa et al., 1976; Cole, 1985; Enríquez et al., 2011). Donnell et al. (2007) reported that during the preconditioning process, calves gain additional weight, being an important factor in the system economic balance. In our study, the preconditioned animals (MVS+PC and N+PC) decreased their BW during the preconditioning when compared with the nonpreconditioned calves on day −26 to 0. The effect of preconditioning on calf BW in the present study may have been limited because of the treatment time of 26 d or the type of diet and the DMI. Other authors have recommended that calves remain in a preconditioning program for a minimum of 45 d (Donnell et al., 2007; Ward et al., 2019). Cole et al. (1979) observed an additional weight gain of 5 kg in preconditioned steer calves compared with nonpreconditioned steer calves, which had a 30 d preconditioning period (similar to ours), but were fed a 50% concentrate diet. In the current experiment, however, the decreased BW during the preconditioning treatment was reduced by the MVS

Table 2. Effect of preconditioning (PC) and vitamin and mineral (MVS) treatments preshipping to feedlot on mean plasma glucose, insulin, cortisol, and thiobarbituric acid-reactive substances (TBARS) a biomarker of lipid peroxidation in steers

	Treatments				*P*-values		
	N + N[1]	N + PC[2]	MVS + N[3]	MVS + PC[4]	MVS	PC	MVS × PC
n (steers)	15	15	15	15			
Glucose, mg/dL							
Day −26	87.5	96.0	89.0	92.9	0.87	0.23	0.65
Day 0	85.7	88.3	84.1	80.7	0.37	0.94	0.84
Day 15	98.9	95.2	92.9	95.1	0.55	0.88	0.65
SEM	3.4	6.6	3.4	6.4			
Insulin, ng/mL							
Day −26	6.09	7.18	6.17	6.77	0.86	0.34	0.79
Day 0	8.45	7.66	8.12	8.79	0.65	0.95	0.84
SEM	0.84	0.91	0.83	0.94			
Cortisol, µg/dL							
Day −26	8.22	7.99	6.98	6.52	0.28	0.78	0.73
Day 0	2.13	2.61	3.21	3.06	0.54	0.89	0.93
SEM	1.26	1.28	1.28	1.23			
TBARS[5], µM MDA							
Day 0	7.39	6.7	6.67	6.4	0.27	0.29	0.23
Day 15	7.28	7.36	6.58	7.4	0.47	0.33	0.27
SEM	0.28	0.58	0.28	0.57			

[1]N+N = no mineral and vitamin supplementation and no preconditioning period.

[2]N+PC = no mineral and vitamin supplementation and a 26-d preconditioning period.

[3]MVS+N = mineral and vitamin supplementation and no preconditioning period.

[4]MVS+PC = mineral and vitamin supplementation and a 26-d preconditioning period.

[5]TBARS = thiobarbituric acid-reactive substances.

Mineral and vitamin supplementation (Adaptador Min-Vit, Biogénesis Bago, Argentina).

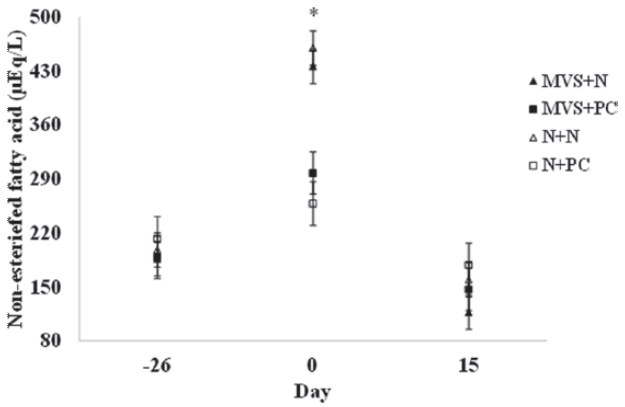

Figure 6. Effect of preconditioning and vitamin and mineral supplementation preshipping to a feedlot on plasma nonesterified fatty acid concentration in steers. Treatments were as follows: 1) mineral and vitamin supplementation and no preconditioning period (MVS+N); 2) mineral and vitamin supplementation and a 26-d preconditioning period (MVS+PC); 3) no mineral and vitamin supplementation and no preconditioning period (N+N); and 4) no mineral and vitamin supplementation and a 26-d preconditioning period (N+PC). Vitamin and mineral supplementation (Adaptador Min-Vit, Biogénesis Bago, Argentina). *Preconditioning × mineral and vitamin supplementation, $P < 0.10$; $n = 15$ steers per treatment.

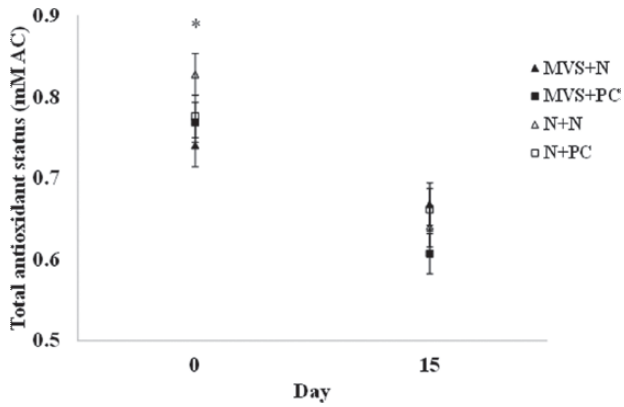

Figure 7. Effect of preconditioning and mineral and vitamin supplementation preshipping to feedlot on plasma total antioxidant status in steers. Treatments were as follows: 1) mineral and vitamin supplementation and no preconditioning period (MVS+N); 2) mineral and vitamin supplementation and a 26-d preconditioning period (MVS+PC); 3) no mineral and vitamin supplementation and no preconditioning period (N+N); and 4) no mineral and vitamin supplementation and a 26-d preconditioning period (N+PC). Vitamin and mineral supplementation (Adaptador Min-Vit, Biogénesis Bago, Argentina). *Preconditioning × mineral and vitamin supplementation, $P < 0.10$; $n = 15$ steers per treatment.

treatment. Holstein calves of 45 d of age were supplemented with the same product (Adaptador Min and Adaptador Vit) and demonstrated greater BW gain when compared with nonsupplemented calves. This greater calf BW was associated with an increase in total antioxidant capacity and a decrease in ROS in serum (Bordignon et al., 2019). The imbalance generated by a ROS increase or an antioxidant defenses decrease leads to oxidative stress. This oxidative stress generates cell and tissue damage, leading to an extra energy cost to repair the damage, which may produce a lesser growth (Celi, 2001; Cusack et al., 2009). On the other hand, the negative impact that MVS had on animals under conventional weaning on ADG in this period (day −26 to 0) could be associated with an increase in plasma acute phase protein production. Although plasma acute phase proteins were not measured in this work, Arthington et al. (2014) observed that heifers, injected with the same minerals and at a similar dose as in the present experiment, had an increase in plasma haptoglobin, ceruloplasmin, and acid proteins soluble concentration, in association with a lower ADG.

Preconditioning improved performance upon arrival, as well as, during the first 8 d (days 0 to 8). Shipping causes BW loss, explained by excretion of feces and urine, and loss of water in the body tissues (Phillips et al., 1982; González et al., 2012). In the current experiment, the preconditioned steers (MVS+PC and N+PC) gained BW from loading to the truck until processed in the feedlot. This BW gain may indicate gut fill and body rehydration due to the consumption of hay and/or water during the rest period after arriving at the feedlot. Although the nonpreconditioned steers (MVS+N and N+N) lost weight during transport and arrival at the feedlot, the MVS+N steers lost less weight compared with the N+N steers. This finding coincides with the results of Genther-Schroeder and Hansen (2014), which showed that steers fed a diet supplemented with Cu, Mn, Se, and Zn before transport had a lower weight loss in the transport period (taken 4 d before and 2 d after a 20-h transport), which was also associated with a greater DMI. Although we did not measure DMI in the rest period upon arrival at feedlot, the lower weight loss in the MVS+N steers may reflect a greater consumption of water and/or hay of these animals.

In the period between days 0 and 8, the positive effect of the preconditioning treatment on ADG was evident. The preconditioned steers demonstrated a greater ADG when compared with the nonpreconditioned treated steers. In addition, the nonpreconditioned steers demonstrated a negative ADG during days 0 and 8. Moreover, MVS-treated steers had no effect on ADG during days 0 and 8. Weaning calves and immediately placing them in a feedlot is known to be detrimental to the animal's ADG, especially during the first 21 d introductory period. Calves weaned and immediately placed in a feedlot yard had a lesser ADG, but the same DMI compared with calves weaned 14 or 77 d earlier (Smith et al., 2003). Although preconditioning had a positive effect on ADG from days 0 to 8, the BW of the nonpreconditioned steers continued to be heavier than the preconditioned treated steers on day 8. The MVS+PC steers maintained the BW difference with the N+PC steers until the end of the experiment on day 29. On days 15 to 29, ADG was similar for the four steers.

There was no difference in DMI between treatments from days 0 to 8, but the preconditioned treated steers had a greater G:F. The greater G:F demonstrated by the preconditioned steers was the result of the greater ADG seen by the preconditioning treatment. MVS did not affect these parameters in the current experiment. This coincides with Genther-Schroeder and Hansen (2015), who reported no effect of trace mineral injection on DMI or G:F in calves that were transported to a feedlot. In the period days 8 to 15, there was a decrease in DMI for the N+PC steers. This was similar to the data observed by Rauch et al. (2019), who evaluated the effect of preconditioning and trace minerals injection in calves and reported that preconditioned calves that did not receive a trace mineral injection had decreased DMI for the first 42 d in a feedlot.

Among the parameters that evaluated the energy metabolism, only plasma NEFA concentration varied between treatments. Plasma NEFA concentration was affected by a MVS × PC interaction on day 0, where MVS decreased plasma NEFA concentration in nonpreconditioned steers, but increased NEFA concentration in preconditioned steers. There are no previous results that support this change in plasma NEFA concentration, and we do not know the physiological mechanism that can explain this change. The effect of injectable MVS on lipid metabolism has been evaluated in dairy cows. Omur et al. (2016) injected prepartum dairy cows with minerals (Cu, Zn, Se, and Mn) and vitamins (A, D, and E), and reported a decrease in plasma NEFA concentration in the treated cattle 3-wk postpartum compared with the untreated control cattle. Furthermore, prepartum parenteral supplementation of dairy cows with vitamin E and Se also reduced plasma NEFA concentration (Abuelo et al., 2016). This association

was seen in the current experiment in nonpreconditioned steers, where the MVS+N steers had less plasma NEFA concentration at day 0 compared with the N+N steers. In the preconditioned steers, the data reflect an opposite trend to the nonpreconditioned steers, where MVS increased plasma NEFA concentration. As mentioned previously, we do not have a physiological explanation for this result. On the other hand, in consideration of the preconditioning treatment, the lesser plasma NEFA concentration at day 0 for the preconditioned steers compared with the nonpreconditioned steers could be caused by the decreased BW gain for the preconditioned treated calves from days −26 to 0. This may reflect the reduced body fat reserves in the preconditioned treated calves and their ability to mobilize NEFA may have been reduced. The stress generated by abrupt weaning increases cortisol concentration in the blood (Enríquez et al., 2011). Furthermore, the preconditioned treated steers may have had a reduced response to the stressors imposed by transportation, handling, and the new environment on day 0. A lesser response to the stressors by the preconditioned treated steers would decrease fat mobilization compared with the nonpreconditioned treated steers (Boyles et al., 2007). However, in the present experiment, plasma cortisol concentration did not vary between steers on day 0. The lack of difference in plasma cortisol concentration between steers may due to the timing of the sampling and transport. Steers transported for 6 h demonstrated plasma cortisol concentration to peak at 30 m after the start of transport and returned to pretransport values within 3 h after the start of transport (Pettiford et al., 2008). The duration of transport in our study was 5 h, and it is possible that our sampling frequency for plasma cortisol concentration was inadequate to capture differences because of treatment but reflected a return to a basal concentration.

Plasma glucose concentration was similar between treatments at the three collection times (days −26, 0, and 15). This coincides with the plasma insulin concentration that was also similar between treatments on days −26 and 0. However, there are conflicting results with previous studies. Cole et al. (1982) found that 14 d after entering the feedlot, preconditioned steers had greater plasma glucose concentration than nonpreconditioned steers (81.9 vs. 74.5 mg/dL), which coincides with greater DMI by the preconditioned steers in that period. In our experiment, we did not measure the DMI between days −45 and 0 to be able to associate DMI with plasma glucose concentration. Between days 8 and 15, the N+PC steers had the lowest DMI, but plasma glucose concentration was not different than the other treatments. On the other hand, Crookshank et al. (1979) conducted a study in which they did not measure the DMI and did not observe changes in plasma glucose concentration in the first 16 d of the experiment. In that experiment, calves were preconditioned for 14 d and compared with calves weaned on the same d at the start of the trial, and a third group of calves weaned and transported for 12 h at the start of the trial.

The MVS treatment did not have the expected response on plasma TAS and TBARS. Plasma TAS was greater in the N+N steers compared with the MVS+N steers at day 0. Although plasma TAS has been effective in evaluating the antioxidant capacity in dairy cows (Castillo et al., 2006; Gong and Xiao, 2016), in trials with calf transport plasma TAS response has been inconsistent. In a trial in calves that received similar supplementation to the one evaluated in the current experiment, weaned on pasture with a fence-line system and without subsequent transport, plasma TAS was greater in supplemented calves compared with nonsupplemented calves (Mattioli et al., 2020). On the other hand, Chirase et al. (2004) observed that plasma TAS decreased by only 10% in weaned and transported calves for 1,930 km and 20 h in transit. In the Chirase et al. (2004) experiment, plasma TBARS concentration tripled after transport. A similar increase in the post-transport TBARS concentration was also reported by Wernicki et al. (2006), who observed that in calves transported for 2 h, plasma TBARS increased during the first 6 d post-transport and then decreased after day 9 post-transport. Although we did not measure pretransport plasma TBARS, the values observed immediately after transport were not greater than those observed 15 d later.

CONCLUSIONS

Preconditioning calves for 26-d decreased BW at the time of transport to the feedlot compared with nonpreconditioned calves. In the preconditioned calves, MVS treatment decreased the BW loss during these 26 d compared with not supplemented with mineral and vitamins. The BW loss during the preconditioning period was not recovered 29 d after feedlot entry. Mineral and vitamins supplementation reduced weight loss in nonpreconditioned steers due to 5 h of transportation, measured by the difference of BW from before loading the steers and the BW 30 min after unloading the steers. These effects on BW growth were not associated with changes in plasma insulin, cortisol, glucose, nonesterified fatty

acids, or thiobarbituric acid-reactive substances concentrations, nor plasma TAS.

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