Nutritional Strategies to Overcome Transit Stress

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Lay Summary

Transportation is a necessary event that cattle are subjected to at least once in their lives. With transit comes shrink, decreased performance, stress, and overall decreased animal welfare. Our studies investigated cost effective management strategies that may help cattle transition after a transit event. Injectable Vitamin C has application in combatting reactive oxygen species produced by transit stress and improved performance measures of cattle treated with vitamin C. Zinc is essential to various biological functions and has a role in protein synthesis; its supplementation may improve immune system mediation in response to muscle damage from transit and improve intake and subsequent growth following transit.

Introduction

The cattle industry has a segmented structure; cattle will be transported at some point in their lives (Schuetze et al., 2017). Due to variety and relative abundance of feed resources in different regions, producers opt to move cattle to feed. Transit events subject cattle to standing for prolonged periods, inducing muscle fatigue, inflammation, oxidative stress, and behavioral differences. Effects from transit are detrimental to production, BW shrink, immunosuppression, and decreased feed efficiency are costly and should be managed proactively.

Oxidative stress results from oxygen reduction due to leaked electron transport chain electrons. Resulting reactive oxygen species **(ROS)** cause cellular damage. The body's defense against ROS comes from antioxidants, including water-soluble Vitamin C **(VC)**, which cattle can produce endogenously. There needs to be more research on the requirements by cattle for VC, especially during stressful events, such as transportation. In previous studies, our findings support using an injectable VC supplement before transit, aiding in performance recovery following transit (Deters and Hansen, 2020).

During a transit event, cattle are subjected to vibrations transmitted through trailer floors, which change in response to driving conditions (Gebresenbet et al., 2011). Cattle use their muscles to correct against vibrations and swaying, resulting in fatigued muscles (Park et al., 2020). Fatigued muscles make a shift from oxidative phosphorylation as its main source of ATP production to anaerobic glycolysis, a less efficient means of energy production. Poor efficiency of ATP production results in mobilization of energy sources, such as amino acids, triglycerides, and non-esterified fatty acids **(NEFA)** into the blood stream to be metabolized in muscle cells. Lactate resulting from anaerobic glucose metabolism is shuttled to the liver where it is converted to glucose by Zn-dependent enzyme lactate dehydrogenase B (Price, 1962).

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It is hypothesized by supplementing Zn prior to transit, more enzymatic conversion of lactate to glucose would result in decreased muscle fatigue. In this review of our recent transit studies, we outline cost-effective management strategies that may help mitigate the effects of transit stress.

Oxidative Stress Resulting From Transit

Within muscle cells, mitochondria convert glucose to ATP through glycolysis, the citric acid cycle, and oxidative phosphorylation. Oxidative respiration is the most efficient means of energy production within cells (36 to 38 ATP/glucose) due to highly reactive oxygen (Vella, 1993). During ATP production, the electron transport chain leaks electrons, and oxygen molecules acquire these electrons and form ROS. ROS are highly unstable molecules that will cause damage to proteins, DNA, and other crucial cellular components (Deters and Hansen, 2020). When under stress, ROS form at greater concentrations, leading to an imbalance between oxidants and the molecules that combat them, antioxidants. Extent of oxidative damage depends on several factors, such as stress intensity, duration, and ability to mount an antioxidant response (Lushchak, 2014). Under severe oxidative stress, called oxidative distress, cells may undergo apoptosis and/or necrosis (Deters, 2020). While apoptosis is controlled cell death, necrosis is uncontrolled cell death; cellular contents are spilled into intracellular space upon rupturing. Effects of necrosis elicit an inflammatory response in which macrophages are recruited to "clean up" ruptured cells and their contents. Cattle utilize antioxidants to counter ROS and oxidative stress.

Antioxidants can be acquired exogenously through feedstuffs and supplements or produced endogenously. Antioxidants act on ROS and reduce them, donating an electron to lower their reactivity. VC is an antioxidant of interest due to cattle's ability to produce VC in the liver. The enzyme L-gulonolactone oxidase converts glucose to VC and synthesizes enough VC for normal conditions (Chatterjee, 1973). Increased ROS production in the mitochondria and other enzymes may push balance in favor of ROS and increase requirements for VC that must come from an exogenous source.

Vitamin C on oxidative stress

In our transit study (Deters and Hansen, 2020), steers were treated with a VC injectable supplement prior to an 18 h transit event, immediately upon return from transit, or received saline. At midway point of the trial, steers given a VC injection pre-transit had higher BW compared to post-transit and control groups. From days 7 to 31, pre-transit VC steers had greater ADG over post and control (Table 1). Both groups of steers who received a VC treatment (pre- and post-transit) exhibited higher DMI than control steers during d7 to 31. In blood metabolite analysis, plasma ascorbate concentrations immediately after transit d1 were highest in the pre-transit injection group. In addition, plasma ferric reducing antioxidant power **(FRAP)** analysis of antioxidant concentrations was lowest d1 before returning to pre-transit level on d2. Serum NEFA increased d1 following transit and decreased to pre-transit levels by d2. Serum haptoglobin **(HP)** concentrations also showed a day effect and were highest following transit. In addition, serum HP concentration also showed a day effect and was highest immediately following transit before returning to pre-transit levels by d7. Analysis of ascorbate potentially illustrates increased antioxidant demand in which it is used more readily during and immediately after transit. NEFA concentrations following transit increase to support metabolic processes during stress in response to increased cortisol and no food access. Serum HP acted as a biomarker for an inflammatory response to transit in the study, and increased concentration following our stressor shows evidence that steers had heightened inflammation that may be due to oxidative stress. Aside from performance findings, results from blood metabolite analysis illustrate an increased strain on antioxidant supplies during transit. VC is a crucial antioxidant, and while cattle can synthesize VC endogenously, their own VC production may not meet the increased demand created by transit.

In a follow-up study (Beenken et al., 2021), steers were given a saline **(CON)** or VC injection prior to an 8- or 18-hour transit event. Based on performance metrics, VC steers were heavier day-of-transit and day-after-transit. As expected, duration adversely affected BW; 18 h duration steers had lower BW than 8 h. VC-8 steers displayed heavier BW as compared to other groups following transit. Performance measures after d1 were similar when comparing VC to CON. Plasma ascorbate concentrations were similar between CON and VC treatment groups prior to injection and transit and at concentrations near the recommended range of 17.1 to 28.2 μM/L (Matsui, 2012) but increased in steers given VC (Figure 1). It is believed that beginning antioxidant status may have limited the improvement expressed through performance following transit. Initial VC status may be decisive in following performance; treatment of cattle deficient in VC is predicted to result in more substantial performance improvements. However, FRAP concentrations d1 and d2 were higher in both VC treatment groups; increased antioxidant capacity may result in greater inhibition of ROS produced by transit.

Cattle with low VC concentration prior to transit due to diet deficiencies in other areas may be at increased risk for oxidative stress. Oxidative stress takes resources away from performance and partitions amino acids towards synthesizing acute-phase proteins and antioxidants, as well as ATP to repair oxidatively damaged molecules (Ullrich et al., 1997; Griffith, 1999). As a cofactor in many adrenal gland enzymes, VC uptake by the adrenal gland may be upregulated while synthesizing catecholamine in response to transit stress (Kipp and Rivers, 1987). The cattle that received VC before transit demonstrated enhanced performance metrics compared to other groups, such as higher ADG and BW. The response to this treatment supports that cattle may benefit from exogenous sources of VC to combat oxidative damage. Increased performance of VC steers compared to control suggests supplemented VC mitigated some harmful effects of oxidative stress and inflammation induced by transit. Supplementing cattle with injectable VC before a transit event may be a cost-effective way of preserving post-transit performance by proactively supplying cattle with additional antioxidants. With treatment costs at $1 per animal, a VC injection prior to transit could reduce detrimental factors decreasing subsequent performance in VC deficient cattle. The potential benefits demonstrated in these results highlight the need for additional research concerning VC requirements.

Muscle Fatigue and Effects on Energy Production

Muscle fatigue is defined as "reversible loss of muscle force due to work over time" (Gosker and Schols, 2008). Cattle are typically trailered at high density with a relatively small space allotted per animal for efficiency reasons and to prevent an animal from laying and being injured by other animals. When standing for long periods, cattle rely heavily on their muscles to make adjustments to trailer movements to remain upright. When cattle are given adequate space to orient themselves in a trailer, they will stand either parallel or perpendicular and avoid standing diagonally to the trailer direction. Drivers commonly assess cattle orientation to determine if their stocking density is too high for cattle to stand to their preference (Schuetze et al., 2017).

During transport, vibrations are transmitted through the trailer floor to animals, altering center of gravity and creating uncomfortable conditions. The severity of vibrations can be influenced by multiple factors, including road conditions, driving performance, and poor suspension (María et al., 2003). Gebresenbet et al. (2011) found that standing orientation affected vibration exposure; cattle standing perpendicular to trailer direction experienced milder vibrations. Cattle respond to the conditions by bracing and self-correcting to maintain stability, utilizing muscles. At times of high usage, such as physical exercise, muscles require increased amounts of energy substrates to produce ATP. Muscle cells produce most of their ATP needs within the mitochondria via oxidative phosphorylation. Oxidative phosphorylation converts pyruvate into ATP through the Kreb's Cycle, resulting in a net gain of 36 to 38 ATP molecules per glucose molecule. When under stress, exercise, or otherwise high activity, cells partition some glucose to anaerobic glycolysis in the cytoplasm. While anaerobic glycolysis does not require oxygen to proceed, it is far less efficient, resulting in a net gain of only 2 ATP per glucose molecule. Lactate is the resulting product of glycolytic metabolism and is a biomarker for muscle fatigue (Van Hall, 2010).

Decreasing efficiency of ATP production increases energy substrate requirements, resulting in mobilization of triglycerides and NEFA from fat stores for ATP production. Decreased blood glucose concentration decreases blood insulin and increases glucagon, the hormone responsible for signaling glucose mobilization. Lipases then break down stored triacylglycerols, with glycerol and NEFA as products of the breakdown. Animals are restricted from food and water during transit, reducing readily available glucose. Feed restriction also acts on muscle glycogen stores, an important storage form of glucose, and mobilizes stores through glycogenolysis. Cattle also utilize gluconeogenesis to increase blood glucose for ATP production, catabolizing the carbon skeleton of some amino acids to support new glucose production. Gluconeogenesis accounts for 90% of cattle glucose production due largely to carbohydrate conversion from VFA in the rumen. Feed restriction, stress, and muscle damage create a higher glucose requirement for ATP production, depleting blood glucose and drawing on tissues to mobilize energy stores to meet the new requirement.

Muscle damage and immune response

In study on muscle fatigue in military drivers (Park et al., 2020), it was hypothesized that extent of muscle fatigue is a function of the extent of vibrations exposed. Soldiers are subjected to vibrations when driving military trucks in the field due to poor road conditions. Fatigue was noticeably worse in the shoulder region because of usage during steering vehicles. Vibrations were measured using a 6-degree-of-freedom exciter, and electromyography **(EMG)** signals measured fatigue before and after driving. Before and after vibration exposure, Maximal Voluntary Contraction **(MVC)** state was determined. Interestingly, EMG signals at MVC following vibration exposure decreased. The change of median frequency value after vibration exposure was reduced, illustrating that muscles fatigued more rapidly when under the same load.

In a study on transit-related stress in turkey pullets (Wein et al., 2017), creatine kinase **(CK)** was measured around a transit event. CK is a commonly used biomarker to assess muscle damage; CK produces creatine phosphate, a shuttler of ATP production within mitochondria (Wallimann et al., 2011). CK's enzymatic production of creatine phosphate reduces electron transport chain **(ETC)** uncoupling and has been suggested as a direct/indirect antioxidant. In another study on stress in turkeys, Huff and colleagues observed a 6- to 19-fold increase during transit (Huff et al., 2008; Wein et al., 2017). Findings have shown CK concentration as a telling marker for stress and muscle damage. In other studies, it is theorized that inorganic phosphate produced by CK causes muscle fatigue (Dahlstedt et al., 2001). In the study, CK knockout mice fast-twitch muscle reached 30% force slower than mice with CK fast-twitch fibers. Phosphate produced by CK may have an effect on fatigue resistance in muscles. CK's function is thought to be more effective in scenarios with high-intensity exercise as opposed to long-duration exercises that cattle experience during transit (Dahlstedt et al., 2001). Observation of CK concentrations has proved to be an effective method of gauging muscle damage in transit studies.

Furthermore, muscle damage elicits an immune response to repair or degrade faulty proteins (Gallucci and Matzinger, 2001). According to the Danger Model (Matzinger, 2002), the body can distinguish itself from damage and pathogens, explaining the absence of an immune response to a growing fetus and some tumorous growths. Damage associated molecular patterns **(DAMPs)** are produced surrounding a stressor or cellular damage, signaling to initiate an immune response (Wein et al., 2017). The innate immune system recognizes cells damaged by transit relatively quickly and initiates a complement system response. The complement system comprises 30 different circulating plasma proteins, mainly produced in the liver, a major site of Zn action. In presence of damaged cells, proteins interact and activate the complement system. Through various combinations, targeted cells will be destroyed or phagocyted. After identifying damaged cells, protease expression is amplified, resulting in 3 main complement system responses, inflammation, phagocytosis, and cell membrane attack (Murphy, 2022). In an innate immune response, pro-inflammatory cytokine expression is upregulated from cytokine-producing T helper cells and macrophages. Phagocytes can kill targets using ROS and use ROS to signal for an inflammatory response, perhaps exacerbating oxidative stress (Jay Forman, 2001).

Zinc Role and Impact on Performance Following Transit

Zinc is an essential micronutrient necessary for many biological functions. Over 300 enzymes require Zn as a cofactor; it is implicated in numerous transcription factors and is heavily involved as a transcription factor (Suttle, 2010; Messersmith, 2021). Zinc enzymatic involvement in protein synthesis creates a positive balance in favor of muscle anabolism, resulting in increased protein accretion in cattle supplemented with Zn compared to control. Steers supplemented with Zn show improvement in ADG and feed efficiency, perhaps due to increased enzymatic activity (Heiderscheit and Hansen, 2022). In addition to growth and performance implications, both innate and adaptive immune cells are affected by Zn. The role of Zn is highlighted by Zn deficiency- reduced feed intake and immune dysfunction are common symptoms that significantly impact cattle performance. University studies in dairy and beef cattle have shown promise in improving performance via immune and cellular function through Zn supplementation.

Zinc role as antioxidant and in immune function

Zinc is often supplemented to cattle diets since ingredients, especially forages, may be inadequate sources of micronutrients (Cao et al., 2002). The current recommendation is 30 mg Zn/kg DM (Nutrient Requirements of Beef Cattle, 2016), but consulting nutritionists commonly supplement at concentrations of 109 and 87.3 mg Zn/kg DM for receiving and finishing diets, respectively (Samuelson et al., 2016). Zinc fed over current recommendations does not negatively affect cattle performance. It needs to be clarified if requirements have changed for current high-performance beef cattle since foundational work used to determine requirements for Zn was completed decades ago (Nutrient Requirements of Beef Cattle, 1984). Zinc bioavailability may differ between organic and inorganic sources, and organic Zn sources have grown in popularity due to improved availability.

A university study examined effects of a zinc-methionine compound (Zn-met) on immunoglobulin and antioxidant concentrations in blood, somatic cell counts **(SCC)** and milk production of high-producing dairy cows (Oconitrillo Hidalgo and María, 2021). In the study, researchers compared cows fed Zn-met at 76 mg Zn/kg DM and 96 mg Zn/kg DM. High-producing dairy cows experience high metabolic demands and health issues, increasing oxidative stress (Abuelo et al., 2016). Decreased oxidative stress and a fast, efficient immune response are important factors in maintaining production levels. In the second half of the study (DIM 102 to 137), milk production wanes because of fewer secretory cells in mammary glands. Zinc-met fed cattle produced 2.8 lb/day (1.25 kg/day) more milk than CON, perhaps due to Zn-met cattle having increased ability to sustain production during mid-lactation because of Zn involvement in cellular signaling. Zinc-met cattle also had lower SCC concentrations than CON, especially true during the final five weeks of the study. SCC comprises white blood cells and sloughed epithelial cells; Zn has been shown to improve epithelium integrity, perhaps explaining decreased SCC in Zn-met cows (Sobhanirad et al., 2009; Oconitrillo Hidalgo, 2021).

Zn impact on intake and growth implications

Lactate dehydrogenase, a Zn-dependent enzyme, is heavily concentrated in liver and muscle tissue and converts lactate produced by glycolysis to pyruvate for energy production (Price, 1962). Zinc deficiency can result from inadequate Zn intake, increased Zn loss, or increased body requirement for Zn. Chao et al. (2018a) examined the effects of a Zn supplement given to children between ages 2 to 10 who visited a clinic and suffered poor weight gain and a weight-for-age <15 on growth charts. It was determined that improved Zn status improved growth and appetite, further highlighting its importance in growth processes (Chao et al., 2018). Ohinata et al. (2009) demonstrated a Zn effect on intake where rats supplemented with Zn showed increased intake compared with CON. Control rats exhibited cyclic decreased intake patterns aligned with anorexia resulting from Zn deficiency. When CON rats were fed a Zn-sufficient diet, intake recovered to pre-deficiency levels. When the vagal nerve was severed, intake between Zn sufficient and CON rats did not differ, suggesting Zn improvement of intake is due to stimulation of the vagal nerve (Ohinata et al., 2009).

During transit, cattle are feed-restricted and experience muscle fatigue, and following transit, exhibit decreased DMI, immune function, and growth performance. Upon arrival to a feed yard, cattle with low trace mineral **(TM)** status display decreased intake and subsequently, poor performance (Genther-Schroeder and Hansen, 2015). Cattle in 2007 had a 44% increase in growth compared to cattle in 1977 (Capper, 2011). Accompanying, huge strides in growth are perhaps increased requirements of cofactors and transcription factors implicated in growth, namely Zn (Messersmith et al., 2022). Zinc was identified as a potential treatment for transit-related symptoms due to documented growth and appetite improvements with supplemented Zn in human and animal models (Ohinata et al., 2009; Chao et al., 2018; Messersmith, 2021).

Heiderscheit and Hansen (2022) supplemented Zn to steers before and after an 18h transit event; cattle were fed one of three diets: 54 mg Zn/kg DM from basal diet **(CON)**, CON+70 mg Zn/kg DM **(IND)**, and CON+120 mg Zn/kg DM **(SUPZN)**. From d 6 to 28 post-transit, ADG linearly increased with increasing Zn (Table 2). There was a difference in DMI recovery on d2 and 4 post-transit with IND and SUPZN recovering before CON steers. Serum lactate increased by 20% in CON and SUPZN, produced by cells favoring glycolysis in cytosol to metabolize glucose molecules. Serum lactate is shuttled out of cells and can be converted by Zn-dependent lactate dehydrogenase into pyruvate that can reenter the Kreb's Cycle. Muscle glucose was also increased in CON steers compared to Zn-supplemented steers. Increased glucose concentration may be due to an increased need for energy substrates compared to Zn-supplemented treatments. Plasma Zn concentration increased with increasing dietary Zn, perhaps illustrating improved Zn status with increased dietary Zn.

Genther-Schroeder et al. (2016) found in steers supplemented with ractopamine **(RAC)** and increasing dietary Zn-amino acid complex **(ZnAA)** concentration, there was a linear increase in both ADG and feed efficiency. This further highlights the significance of Zn in relation to maximizing performance and muscle growth (Genther-Schroeder et al., 2016). Shrink resulting from transit stress and feed restriction is regained; compensatory gain following transit may require additional TM. The rapid advancement in cattle performance coupled with muscle fatigue and oxidative stress encountered during transit may heighten the need for Zn in cattle diets. Zinc supplementation is a low-cost management tool that may effectively mitigate discomfort caused by muscle fatigue and increase DMI following a transit event.

Conclusion

Transportation of cattle is a significant and costly event that can be managed to reduce the effects of stress and fatigue. Oxidative stress is detrimental to animal performance and can cause acute inflammation in response to ROS-related cellular damage. Vitamin C is a crucial antioxidant of interest produced endogenously in cattle, but requirements during transit are poorly understood. Additional VC supplemented via injection has improved steer VC status and subsequent performance, suggesting that requirements may increase during oxidative stress (Deters and Hansen, 2020). The application of supplemental VC has proven practical; treatment costs are around $1 and can be administered as needed rather than treating an entire pen.

The importance of Zn is well-documented; it is involved in over 300 enzymes as a cofactor. Its role in lactate conversion to pyruvate via Zn-dependent lactate dehydrogenase and in protein synthesis is particularly relevant to animal performance post-transit. Zinc has an inhibitory effect on several proinflammatory cytokines which would be upregulated during transit in response to muscular damage and oxidative stress, leading to further inflammation when Zn is deficient (Olechnowicz et al., 2018). Approximately 77% of consulting nutritionists report using a blend of inorganic and organic trace mineral sources in receiving diets (Samuelson et al., 2016). The ratio of inorganic to organic sources of Zn and the concentration of Zn supplemented should be examined to determine optimal supplementation in receiving diets.

Additional research on VC and Zn requirements around a stress event is necessary to determine how much the event depletes VC and Zn. In addition, research guided toward assessing current needs for high-producing animals is necessary to correctly feed animals for optimal performance and efficiency. Transit is necessary for the industry; producers should seek means to mitigate its effects and aim to resume pre-transit performance promptly following a transit event.

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Table 1. Effect of injectable vitamin C treatment on feedlot performance by beef steers (adapted from Deters and Hansen, 2020).

	Treatment[1]				Treatment
	CON	PRE	POST	SEM[2]	*P*-value
Steers (n)	24	23	24		
d 0 BW, kg	354	357	355	3.7	0.83
d 1 BW, kg	334	335	334	3.5	0.97
Shrink[3], %	5.7	6.2	6.0	0.23	0.28
Mid BW[4], kg	390[b]	395[a]	389[b]	1.6	0.03
Final BW[4], kg	429[y]	436[x]	430[y]	2.4	0.07
Average daily gain, kg/d					
d 1 to 7	1.43	1.78	1.34	0.198	0.25
d 7 to 31	1.94[b]	2.10[a]	1.94[b]	0.054	0.05
d 31 to 57	1.48	1.61	1.59	0.059	0.25
d 1 to 57	1.67[b]	1.84[a]	1.72[b]	0.042	0.02

[1]Treatments were saline before and after trucking (CON); 5 g sodium ascorbate (20 mL) given IM before trucking, saline given after trucking (PRE); saline prior to trucking and 5 g sodium ascorbate 920 mL) give IM after trucking.

[2]Highest SEM of any treatment shown.

[3]Steers were transported for 18 hours by commercial semi, shrink was the percent BW loss during trucking.

[4]Final BW is the average of d 56 and 57 weights, post-trucking.

Table 2. Treatment averages of average daily gain (ADG) of feedlot steers fed increasing concentrations of supplemental Zn (adapted from Heiderscheit and Hansen, 2022).

	Treatment[1]				Contrasts		
	CON n = 18 steers	IND n = 18 steers	SUPZN n = 18 steers	SEM	Linear	Quadratic	No Zn vs Zn
ADG, kg/d							
d -25 to -1	2.40	2.31	2.47	0.10	0.69	0.29	0.94
d 1 to 6	6.22	6.84	6.05	0.38	0.86	0.13	0.63
d 6 to 28	1.93	2.31	2.34	0.13	0.02	0.39	0.02
d 1 to 28	2.73	3.15	3.03	0.14	0.10	0.16	0.04

[1]Steers received a corn-silage based growing diet for 25 days prior to an 18 hour transit event on a commercial semi, then remained on trial for an additional 28 d post transit. Steers received their respective dietary treatment for the entire period (pre and post trucking); which included: CON (no supplemental Zn, diet analyzed 53 mg Zn/kg DM); IND (70 mg Zn/kg DM supplemented as bis-glycinate Zn); SUPZN (120 mg Zn/kg DM supplemented as bis-glycinate Zn).

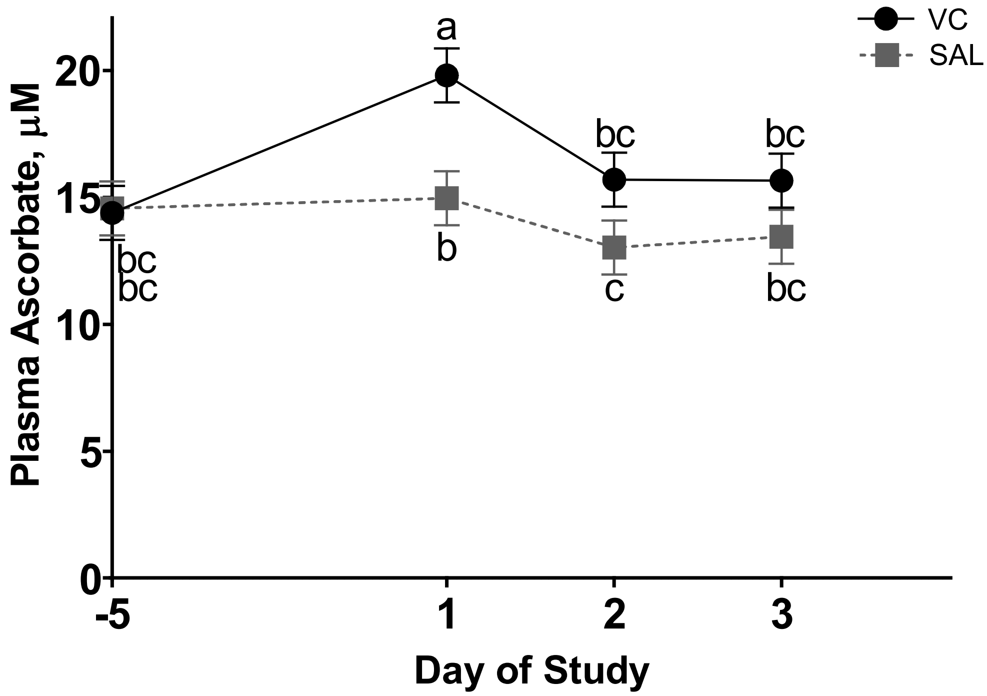

Figure 1. Effect of injectable VC and day relative to transit event on d 0 (INJ × Day $P < 0.01$) on plasma ascorbate concentrations based upon repeated measures analysis of samples collected on d -5, d 1 (post-transit), d 2 and 3 (24 and 48 h post-transit); values with unlike superscripts differ ($P \leq 0.05$) across treatments and sampling days. VC = 20 mL of sodium ascorbate (250 mg/mL) administered intramuscularly **(IM)** immediately prior to transit. SAL = 20 mL of saline administered IM immediately prior to transit. (From Beenken et al. 2022).