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Effect of Parenteral Supplementation with Liposoluble Vitamins and Trace Minerals on Growth, Stress, Antioxidant Status and Immunity Biomarkers in Feedlot Cattle

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GAM, LEF and AER designed the study. Authors LEF and DER assisted with data collection. Authors GAM and AER analyzed the data. Authors LEF and GAM coordinated the experiments and revised the manuscript. Authors GAM, LEF and AER writing–original draft. Authors GAM, LEF, DER, ET and AER writing–review and editing. All authors have read and agreed to the published version of the manuscript.

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ABSTRACT

Aims: weaning and transport are stressful events in receiving feedlot cattle. These events increased oxidative stress in cattle. For this reason, administration of vitamins and trace minerals associated with antioxidant properties is recommended at feedlot entry. The objective of this experiment was to evaluate the effects of parenteral supplementation with vitamins (A and E) and minerals (copper, zinc, selenium, and manganese) on days 1 and/or 7 after feedlot entry.
Methodology: heifers were assigned to one of four groups (n= 30); non- supplemented (CC), supplemented on day 1 and 7 (SS), and supplemented only on day 1 (SC) or 7 (CS). Blood parameters (cortisol, total antioxidant status -TAS-, thiobarbituric acid reactive substances -TBARS, haptoglobin -Hp- blood cell) and weight were recorded on day 1 and 21. Data were analyzed as a complete randomized design using a 2×2 factorial arrangement of treatments.
Results: there was an interaction ($P \leq 0.07$) for daily gain, and cortisol concentration. Heifers in the CC group had reduced daily gain compared with the other 3 treatments. Cortisol concentration was the lowest for heifers in the CC and the highest in the CS, with the other 2 treatments in between. Groups that received day 7 supplementation (CS and SS) increased the total antioxidant status (TAS; $P = 0.01$) and had lower lipid peroxidation (TBARS; $P = 0.01$).
Conclusion: supplementation with vitamins and minerals at feedlot entry improved daily gain. The antioxidant effect seems to be evident when considering day 7 supplementation since CS and SS groups showed greater TAS capacity and lower TBARS concentration.

Keywords: Ruminants; oxidative stress; average daily gain; beef calves.

1. INTRODUCTION

In beef cattle production, weaning, transportation, and feedlot entry are the most stressful stages leading to immunological and productive dysfunction in calves [1]. These consequences are caused by both inflammatory processes and high oxidative stress in the animals [2]. For this reason, administration of vitamins and trace minerals associated with antioxidant properties is recommended in rations on feedlot entry [3]. As dry matter intake is reduced during the first 15-21 feedlot days [4], parenteral supplementation is a possible option to reduce oxidative stress [5]. Benefits have been shown regarding supplementation with antioxidant trace minerals, such as copper (Cu), zinc (Zn), selenium (Se), and manganese (Mn) [6]. However, the results obtained on vitamins have been inconclusive. Although parenteral supplementation with vitamin C has decreased morbidity immediately after feedlot entry [2], no benefits have been obtained with liposoluble vitamins A, D and E [7]. Combined parental supplementation with liposoluble vitamins (A, D, E) and trace minerals (Cu, Zn, Mn, and Se) have had positive effects on calves' health at weaning either on beef or dairy cattle [5,8]. Likewise, subcutaneous supplementation with liposoluble vitamins (A, E) and trace minerals (Cu, Zn, Mn, and Se) in calves before feedlot entry reduced body weight loss due to transportation [9]. The effects of parenteral supplementation with liposoluble vitamins (A and E) and trace minerals (Cu, Zn, Mn, and Se) on calves after weaning, transportation, and feedlot entry have not been evaluated yet.

Identifying stress biomarkers is crucial to improve preventive actions [10]. Cortisol is the main glucocorticoid secreted by the adrenal glands as a response to adaptative changes [11]. Therefore, cortisol measurement has been used to assess stress in weaning calves [12]. Similarly, haptoglobin (Hp) is one of the most frequently used acute phase proteins reported in cattle experiments, since it is secreted under stressful physical and/or psychological conditions and so, its concentration is increased during weaning and transportation [13]. Leukocyte count is also an immune response marker that can be altered by stress during weaning and transportation [14].

Oxidative stress is determined through the balance between the antioxidant capacity and the oxidative damage of a cell/organism during adaptative processes [15]. There are several antioxidant mechanisms; thus, total antioxidant status (TAS) can be considered as an assessment option [16]. Oxidative damage occurs in different substrates, but lipid peroxidation, where soluble products can be measured with thiobarbituric acid reactive substances (TBARS), is mostly evaluated. Although the latter is an old technique, it

continues to be the preferred one when studying stressed animals [2].

The hypothesis for the current experiment was that supplementation with injectable vitamins (A and E) and minerals (Cu, Zn, Mn, and Se) improves growth and blood parameters related to oxidative stress in cattle entering the feedlot. The aim of this work was to evaluate the effects of supplementation with liposoluble vitamins (A and E) and trace minerals (Cu, Zn, Mn, and Se) on day 1 and/or 7 after feedlot entry on average daily gain, Hp, TBARS, TAS, cortisol, and leukocyte counts in weaned cattle.

2. MATERIALS AND METHODS

This experiment was conducted in May 2019 (autumn with an average temperature of 15ºC -8 to 22ºC- in a commercial feedlot located in Marcos Paz, Buenos Aires, Argentina (−34.7968, −58.9002). All experimental procedures were approved by the Committee for the Care and Use of Laboratory Animals, School of Veterinary Sciences, La Plata National University, Argentina (95-9-19P). None of the heifers included in this experiment showed any unusual behavior, such as depression, ataxia, or prostration, as a potential toxic sign induced by the treatment.

One hundred and twenty healthy crossbred Bos indicus × Bos Taurus heifers (recently weaned) were used in this experiment. Heifers were shipped 900 km from Esquina, Province of Corrientes, Argentina (−30.0173, −59.5496). Heifers were 9 to 11 months old and had an average body weight (BW) of 255 ± SD 26 kg. All heifers were kept in the same pen throughout the experiment.

Enrolled heifers were fed a common starting diet twice a day at 8.00 and 15.00 hours. The ingredients and composition of the diets fed to heifers through the study are shown in Table 1. Diets were formulated to meet all nutrient requirements for beef calves [3].

Heifers were randomly assigned to four groups (n = 30 each) in a 2×2 factorial arrangement of treatment. The main factors were supplementation (S) or not (C) on day 1 and supplementation (S) or not (C) on day 7. The parenteral vitamin supplement (subcutaneous 1 ml /50 kg BW; Adaptador Vit®, Biogénesis Bagó SA, Buenos Aires, Argentina) contained 59,500 IU/mL of vitamin A (as palmitate), and 50 IU/mL of vitamin E (as acetate). The mineral supplement (subcutaneous 1ml /50 kg BW; Adaptador Min®, Biogénesis Bagó SA, Buenos Aires, Argentina) contained 10 mg/mL Cu (as copper edetate), 10 mg/mL Mn (as manganese edetate), 5 mg/mL Se (as sodium selenite) and 40 mg/mL Zn (as zinc edetate).

The four treatment groups were SS for the animals that received subcutaneous vitamin and mineral supplementation on day 1 (arrival time: day 0) and on day 7; CC for the animals that did not receive supplementation on either day; and SC or CS for the groups that received one supplementation on day 1 or day 7, respectively. The subcutaneous supplementation was applied on the side of the neck in front of the shoulder. On day 7, all calves were treated with injectable moxidectin (1 mL/50 kg BW; Cydectin Alfa®, Fort Dodge, Argentina) and vaccinated against clostridial bacterin (Bioclostrigen®, Biogénesis Bagó, Buenos Aires, Argentina). All products were applied following the manufacturer's instructions.

Table 1. Ingredient and composition of the diet fed to beef heifers at feedlot entry

Ingredient	Value
Sorghum silage, % of DM	24
Corn grain, % of DM	33
Wheat middlings, % of DM	23.6
Soybean meal, % of DM	15.5
Premix MEAT®, % (minerals[2]; vitamins[3] and ionophores[4])	3.9
CP, % of DM[5]	16.66
NDF, % of DM[5]	29.01
NEm, Mcal/kg of DM[5]	1.72
NEg, Mcal/kg of DM[5]	1.12

[2]. Provided per kilogram of premix: 100.000IU of vitamin A; 20.000 IU of vitamin D3; 650 IU of vitamin E, and 650 IU Vitamin B1; [3] Provided per kilogram of premix: 1200 ppm of Zn; 600 ppm of Cu; 20 ppm of I; 6 ppm of Se; 4 ppm of Co; and 1200 ppm of Mn; [4] Provided per kilogram of premix: 1750 ppm of monensin; [5] Values estimated based on the NASEM (2016)

Each heifer was individually weighed before the morning feed on day 1 and 21. Blood samples were collected using jugular venipuncture on day 1 and 21 into tubes without (DVS-RED Argentina) and with heparin (DVS-GREEN Argentina) to obtain sera and plasma, respectively. Blood samples were kept at 4°C until processing within 4 hours.

In blood (whole heparinized blood) segmented neutrophils, banded, eosinophils, lymphocytes, macrophages, and total white blood cell count (n=12 per group) were carried out within six hours after extraction using a Neubauer counting chamber. Briefly, 20 µL of blood was mixed together with 380 µL Türk's solution (final dilution, 1:20) so that erythrocytes were lysed, and the leukocyte nucleus was stained. The total number of cells in four squares at the corners of the counting chamber was determined under a microscope (10×). The total number of cells per microliter of sample was calculated using the following formula: [(number of white cell count × 20 × 10)/4], where 20 is the dilution factor, 10 is the chamber depth, and 4 is the number of square counts [17]. Leukocytes analyses were conducted in the same animals each sampling.

Blood samples were centrifuged at 1,800 × g for 5 min. and plasma and sera were harvested and stored at −20°C in individual polypropylene microtubes until further analysis. Total antioxidant status, TBARS, cortisol, and Hp were conducted on the same animals, which they were randomly selected from each treatment group. Plasma concentration of TAS (n = 8 animals per group) and TBARS, (n = 8 animals per group) were determined through commercials kits (Cayman Assay Kit for TAS -709001- and TBARS -10009055-). Samples and reagents were prepared according to the manufacturer's recommendations. Plasma cortisol concentration (n = 8 animals per group) was assessed by using a chemiluminescence method (DIAB Private Laboratory). Haptoglobin was measured in sera (n = 8 animals per group) with a haptoglobin-turbitest (Wiener Laboratories, Rosario, Argentina). Total antioxidant status and TBARS assays were previously validated for bovine [9]. Haptoglobin was validated using a parallel displacement of serial sera dilution and recovery (97 ± 5%) of a known amount of Hp (haptoglobin turbitest AA Wiener Laboratories, Rosario, Argentina) in the bovine sera.

Data were analyzed as a complete randomized design with a 2×2 factorial arrangement of treatments using SAS mixed procedure (9.4). The model included supplementation or non-supplementation at day 1, day 7, and their interaction. Heifer was used as the experimental unit. All measurements on day 1 were used as a covariate and removed if not significant. Data were presented as least square means (LSM) and standard error of the means (SEM). Mean difference was discussed as a P value ≤ 0.05 for main effects and ≤ 0.1 for interaction [5,8,9]. The trends were considered as P value ≤ 0.1 for main effects and ≤ 0.15 for interactions. In the case of day 1 × day 7 interaction, the PDIFF option was used to separate means. Different numbers of animals (n = 30, n = 12, and n = 8) were selected based on a power analysis. The values used to estimate the power analysis were the LS means and variation based on the results of a previous report for body weight (n = 30), total leukocyte count (n = 12), TBARS (n = 8), cortisol (n = 8), and Hp (n = 8), respectively [5,9]; considering a significance at a P value of 0.05 and a power of 80%.

3. RESULTS

There was a day 1 × day 7 interaction ($P \leq 0.07$) for average daily gain (ADG), and plasma cortisol concentration (Table 2). Heifers that did not receive supplementation (CC) had the lesser ADG compared with the other groups (CS, SC, and SS). Plasma cortisol concentration was the least for heifers in the CC, intermediate in the CS and SS, and the greatest in the SC group.

There was no interaction in day 1 × day 7 ($P \geq 0.41$), nor for day 1 supplementation ($P \geq 0.11$) effects for plasma TAS and TBARS. Increased antioxidant capacity (TAS; $P = 0.01$) and lower lipid peroxidation (TBARS; $P = 0.01$) were observed on heifers supplemented on day 7 (CS and SS) compared with the non- supplemented (CC and SC) on day 7. No differences were observed for plasma Hp concentration ($P \geq 0.24$).

For leukograms, there was no observed effect (P = 0.51) of the interaction in day 1 × day 7, nor for day 7 supplementation. Leukogram results indicate that day 1 supplementation (SC and SS) increased total leukocyte count ($P = 0.05$) compared with CC and CS. Although neutrophils and lymphocytes were both numerically greater, but not significantly different, a trend was only observed for lymphocytes ($P = 0.08$).

Table 2. Effects of parenteral supplementation with liposoluble vitamins and trace minerals on weight, average daily gain, blood cortisol levels, thiobarbituric acid reactive substances, total antioxidant status, haptoglobin and leukocyte count in feedlot heifer calves

Day 1	C	C	S	S	SEM	*P-values*		
Day 7	C	S	C	S		Day 1	Day 7	Day 1 × Day 7
ADG[1]	1.86[a]	2.1[b]	2.2[b]	2.1[b]	0.07	<0.01	0.33	0.07
Cortisol[1] nM	115.9[a]	150.4[b]	181.1[c]	156.9[b]	4.87	<0.01	0.31	<0.01
TAS[1] mM	0.52	0.61	0.51	0.58	0.03	0.42	0.01	0.77
TBARS[1] µM	7.05	6.08	6.35	5.84	0.28	0.11	0.01	0.41
Hp[1] mg/dL	200.69	171.20	163.19	170.67	15.82	0.24	0.49	0.25
WBC								
Total[2] cell/µl	18894	17338	20463	20514	1200	0.05	0.53	0.51
Segmented[2] cell/µl	3894	4121	4677	4825	630	0.24	0.77	0.95
Banded[2] cell/µl	177	181	218	230	56	0.43	0.88	0.95
Eosinophils[2] cell/µl	94	26	118	130	64	0.33	0.66	0.53
Lymphocytes[2] cell/µl	14098	12501	15385	14957	1055	0.08	0.34	0.58
Macrophages[2] cell/µl	439	402	374	403	88	0.72	0.96	0.71

S = subcutaneous supplementation with trace minerals and vitamins (Adaptador Min® and Adaptador Vit®, Biogénesis Bagó SA, Buenos Aires, Argentina). C = without supplementation. ADG: average daily gain (kg). WBC: white blood cell. TAS: total antioxidant status. TBARS: thiobarbituric acid reactive substances. Hp: haptoglobin. nM: nanomolar. mM millimolar. µM: micromolar. mg/dL: milligram per deciliter. cell/µl= cell per microliter.

[1]The initial value was not used as a covariate. [2]The initial value was used as a covariate.

[a-b-c] different letters in the same row indicate significant differences between groups

4. DISCUSSION

In the present experiment, heifers supplemented with liposoluble vitamins and trace minerals improved ADG at feedlot entry. Weight changes due to adaptive stress can be considered a health indicator [5]. Considering that oxidative stress is a detrimental factor for cattle adaptation in the feedlot, it is possible to assume that a positive response to antioxidant vitamin and trace minerals supplementation can be produced [1]. However, the results obtained by other researchers are variables. Kegley et al. [18] attempted to explain such variability and suggested that the greater ADG achieved through parenteral trace minerals supplementation was reported only in deficient animals. Similarly, Genther and Hansen [6], who evaluated trace minerals (Cu, Zn, Mn, and Se) supplementation in steers that underwent long transportation to the feedlot, reported better ADG only in those steers that had mild Cu deficiency due to a previous antagonist supplementation with iron and molybdenum. However, greater ADG have been obtained in animals without trace minerals deficiency when the same vitamin and trace minerals dose were used as in the present experiment [8].

A decreased dry matter intake is an important factor associated with transportation and feedlot entry [19]. For this reason, NASEM [3] recommended a 50% increase of trace minerals levels at feedlot entry. Accordingly, a delayed return to voluntary dry matter intake has been reported in Cu and Se deficient steers after a 20-hour truck transportation [6].

Immune response in animals leads to metabolic alterations and energy utilization that may affect ADG [20]. Clark et al. [21] evaluated parenteral supplementation with 75 mg of Cu, 25 mg of Se, 50 mg of Mn, and 200 mg of Zn in preimmunized calves with low risk of bovine respiratory disease (BRD) vs. calves immunized after feedlot entry with high risk of BRD. Preimmunized calves showed less morbidity and required less treatments until recovery, but their ADG was subsequently lower during the first 28 feedlot days. In other studies, supplementation with trace minerals improved both ADG and immune response. For example, Berry et al. [22] injected 3 mL of a solution containing Zn (20 mg/mL), Mn (20 mg/mL), Se (5 mg/mL), and Cu (10 mg/mL) into weaned calves that had been kept in a sale barn and transported to a feedlot. Consequently, the calves showed a greater ADG during the first

42 days and a trend for a lower BRD incidence. Likewise, in another experiment, the same trace minerals were injected on arrival and then a greater ADG during the first 55 days and a reduced BRD incidence and treatment were reported [23].

Harris et al. [24] have recently shown an increased BW after parenteral vitamin A supplementation. Retinol doses of 150,000 UI or 300,000 UI administered in Angus calves at birth and one month of life has increased live weight until trial day 309 [24]. Vitamin A increases intramuscular lipogenesis in young animals [25] and mRNA expression of genes associated with muscle fiber differentiation [26]. As animals grow up, these effects disappear and even lipogenesis is facilitated through decreased vitamin A during the final stage [27]. The efficacy of parenteral vitamin E supplementation in increasing ADG was only observed when it was combined with vitamin A and trace minerals [5,8]. Based on the results of the present experiment, the parenteral supplementation at feedlot entry with vitamins and trace minerals improved the ADG when the supplementation was on day 1 (SC) and 7 (CS), without an extra benefit of supplementing on both days (SS).

Cortisol is considered a stress indicator [12]. It is assumed that its plasma cortisol concentration should undergo short-term variations since they constitute an alarm reaction to stressors [11]. In an experiment conducted in steers, plasma cortisol concentration was measured every 30 minutes within six hours of transport. It was observed that plasma cortisol concentrations were 100 times higher at truck loading, but they were reduced to baseline levels three hours later while traveling without changing until farm arrival and during the following days [12]. In a more recent experiment [9], changes in plasma cortisol concentration were not observed in calves that had been weaned and submitted to a five-hour truck journey. In contrast, Wernicki et al. [28] evaluated calves that had been transported for just two hours and observed greater plasma cortisol concentration in feedlot cattle at days 6 and 9. The concentration of plasma cortisol was correlated with greater TBARS concentration until day 6; thus, both parameters were considered adaptative stress indicators. Furthermore, Marques at al. [29] compared three groups exposed to different stressors: 24-hour transport group, 24-hour water and feed deprivation group, and non-transport or water and feed deprivation group. Cortisol concentration was found to be greater in the two stress-exposed groups on post-stress days 1, 4, and 7; but they were the same in the transported group and the control group on day 10. Moreover, Lippolis et al. [30] reported that cortisol concentration 45 days after transportation and feedlot entry was greater in un-supplemented calves than those orally supplemented with cobalt, Cu, Mn, and Zn on preconditioning. On the contrary, in the present experiment, all the groups supplemented with vitamins and trace minerals showed greater cortisol concentration on day 21 but associated with a greater ADG. Likewise, Montanholi et al. [31] observed that animals with better feed conversion showed greater fecal cortisol metabolite concentration. Nevertheless, cortisol concentration can be changed by several factors, which may make it a poor indicator of feed efficiency and ADG [32].

In the current experiment, the antioxidant effect of vitamins and trace minerals supplementation seems to be evident when considering day 7 supplementation. The CC and SC heifers showed lesser TAS capacity and greater TBARS concentration compared with CS and SS heifers. Transportation has been found to be the main contributor to oxidative stress [2]. Even in short trips of up to two hours, TBARS concentration increased; and it can take them three to six days to be restored to basal concentration [28,33]. Concentration of TBARS in the present experiment were still greater on the 21st post-transportation day. Greater TBARS concentration are produced by inflammatory reactions and oxidative damage is increased by leukocyte-derived O2-reactive species [34]. Vitamin E has reduced lipid peroxidation and proinflammatory processes [35]. Vitamin A and trace minerals have similar effect on reducing oxidative damage [36,37]. Day 1 supplementation with vitamin and trace minerals had no effect on TBARS concentration on day 21. However, such an effect was reported on day 7. It is difficult to explain this treatment difference and no cumulative effect of the two doses was observed since TBARS concentrations were similar in CS and SS groups. The same change was shown for TAS; whose antioxidant capacity was significantly increased after day 7 supplementation. These findings can be correlated with the one by Bordignon et al. [8] who detected the greater antioxidant capacity on the plasma of weaned calves 15 days after the second treatment using the same vitamins and trace minerals as in this trial.

In this experiment, no modifications were observed in the concentration of Hp between groups. Other authors have shown that this acute-phase protein can be increased by transport-derived stress [10]. In contrast, it has also been observed that it markedly decreases under such a stressing condition [38]. When there are no opportunistic infectious agents, Hp increases until day 4 after feedlot entry, but then reduced on day 28 [39]. Increased Hp concentration in calves challenged with Mannheimia haemolytica is associated with greater leukocyte count, lighter BW, and reduced dry matter intake [40]. In this experiment, the CC group showed lower BW and greater plasma TBARS concentration. However, the concentration of Hp was not different between treatments.

As to the leukocyte count, this experiment shows the same increasing trend after supplementation as in Bordignon et al. [8]; who supplemented calves with two doses of vitamins (A and E) and trace minerals (Cu, Zn, Mn, and Se) 15 days before and after weaning. Supplementation also produced an increase in ADG without clinical signs of disease, which led the authors to interpret increased leukocytes as an indicator of a stronger immune response. However, they found an increase in neutrophils and monocytes differing from the increased lymphocytes and a stronger response to day 1 supplementation was reported in the current experiment. Supplementation with Cu, Zn, Se, vitamin E, and beta-carotene (a vitamin A precursor) has improved innate and acquired immune response in beef cattle [40]; and it has been associated as a result of a greater mitogen-induced proliferative capacity of lymphocytes [41].

B-lymphocytes obtained from cows before delivery showed a stronger proliferative response after supplementation with vitamin E, Cu, and Zn [42]. This effect is comparable with the one reported in some in vivo trials. In an in vivo experiment, cattle with normal plasma concentration of these elements were parenterally supplemented with trace minerals (Cu, Zn, Mn, and Se) and had a stronger response to the vaccine against bovine viral diarrhea virus (BVDV) and bovine herpes virus type 1 [43]. In another experiment, the same supplementation also improved the response to the vaccine against BVDV in calves within the 14 days after feedlot entry [44]. In all these studies, the antioxidant vitamin and mineral supplementation was associated with a stronger immune response.

5. CONCLUSION

Heifers supplemented with vitamins and minerals improved ADG at feedlot entry. However, the supplementation with two doses (day 1 and 7) did not differ from a single dose (day 1 or 7) in the final ADG. In the same way, vitamin and mineral supplementation on day 7 showed greater antioxidant capacity and lower lipid peroxidation.

ETHICAL APPROVAL

All the experimental procedures performed in the current experiment were approved by the Committee for the Care and Use of Laboratory Animals (CICUAL, for its Spanish acronym), School of Veterinary Sciences, La Plata National University, Argentina, under Protocol N° (Protocol Number 95-9-19P).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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