Final Technical Report

FCEB Project #60

UF UNIVERSITY of FLORIDA

College of Veterinary Medicine
Department of Large Animal Clinical Sciences

2015 SW 16th Avenue
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352-294-4349
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Florida Cattle Enhancement Board Funding
FL Cattleman's Association, P.O. Box 421929,
Kissimmee, Florida 34742-1929.

Dear Mr. Handley:

This is the FINAL REPORT with preliminary results for the following project:

Agency Award Numbers: Subcontract Number: 60
Principal Investigator name: João Jabur Bittar, DVM, MSc, PhD
Project title: **Effect of injectable trace minerals use at two different times on stress responses to weaning and transportation stress in beef calves**
UF award number: AWD15782
Award period: 10/30/23 – 05/31/2024

We conducted the research study at Wynne Ranch (Ft. Pierce, FL) between April and July of 2024, after approval of the IACUC-UF protocol (ID: IACUC202300000584).

We enrolled 76 Brangus heifers and bull calves in the study, and they were naïve (never been vaccinated) and averaged 4 months old on the study enrollment day (Day minus 7, **A -7**).

Calves were randomized and allocated into one of the three groups:
(1) ITM$_{pre\text{-}weaning}$ = 30 calves were injected with ITM (1 mL/100 Lb BW; Multimin 90, Multimin USA Inc, Fort Collins, CO) 7 days before weaning (day minus 7; **A -7**);
(2) ITM$_{weaning}$ = 30 calves were injected with ITM treatment following the protocol similar to the previous group, but on the day of weaning and transportation (day zero; **B 0**);
(3) Control (CONT) = 16 calves were injected with 5 ml of saline solution SQ on the day of weaning (day zero; **B 0**).

On the day of weaning (Day zero; **B 0**), after the sample and data collection were done (late in the morning), all calves were transported in a single trailer for approximately 7 hours and returned to the source ranch by 5 PM. The 76 calves were kept as one single group in a large corral with free access to water and hay and were fed dry ration daily for 50 days.
On the morning of Day 1 (one day after the weaning day), all calves were dewormed, received an ear tag with insecticide, and were vaccinated for common BRD pathogens according to the ranch SOP.

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Samples and information on the calves were always collected in the morning (7 AM to 11 AM) and were done on the following days: -7 (Day minus 7, **A -7**), 0 (Day zero, day of weaning, **B 0**), 7 (Day 7, **C 7**), 16 (Day 16, **D 16**), 30 (Day 30, **E 30**) and 50 (Day 50, **F 50**).

Important note: As provided in the Interim report, unfortunately, the proposed research study was delayed in its start. The delay in releasing the funds in 2023 impacted the initial plan with the initial ranch that would allow for using their calves, and the source ranch could not wait, so they sold the calves committed to the study. However, we were able to perform the study with a private ranch in Okeechobee, FL, but the study and sample collection were only finalized in late July 2024. Therefore, not all the analyses were performed, and we apologize for the delay.

Preliminary results are presented below:

All samples collected (hair, serum, and saliva) and planned to be analyzed for cortisol are stored and will be analyzed within the next months as soon as our analyzer becomes available. Therefore, at this point, there are no results available for cortisol to be presented yet.

Table 1. Mean ± SD of Biochemical parameter concentrations in control-treated calves (CONT), calves treated with injectable trace minerals (ITM) 7 days before weaning (ITM pre-weaning), and calves treated with ITM at weaning (ITM weaning).

	CONT	ITM pre-weaning	ITM weaning	*P-value*		
				Group	***Day***	***Group*Day***
TP (g/dL)	6.7 (0.5)	6.6 (0.5)	6.6 (0.5)	0.298	**<0.001**	0.883
ALB (g/dL)	181.9 (75.5)	169.0 (53.8)	173.5 (63.9)	0.170	**0.033**	0.885
GLOB(g/dL)	3.0 (0.5)	3.0 (0.5)	3.04 (0.5)	0.690	**<0.001**	0.889
ALB/GLOB ratio	1.3 (0.2)	1.3 (1.0)	1.2 (0.2)	0.387	**0.023**	0.649
ALP (U/L)	181.9 (75.5)	169.0 (53.8)	173.5 (63.9)	0.258	**<0.001**	0.208
AST (U/L)	72.3 (12.0) [a]	69.1 (12.7) [b]	74.4 (13.7) [a]	**0.005**	**<0.001**	0.490
GGT (U/L)	16.8 (4.4)	15.4 (3.5)	16.1 (4.3)	0.376	0.295	**0.053**
BUN (mg/dL)	13.2 (7.2)	12.8 (6.5)	13.1 (6.6)	0.630	**<0.001**	0.186
Ca (mg/dL)	10.6 (0.7)	10.5 (0.5)	10.4 (0.5)	0.202	**<0.001**	0.245
P (mg/dL)	8.9 (1.0)	9.0 (1.0)	9.1 (1.1)	0.643	**0.012**	0.815
Ca/P ratio	1.2 (0.1)	1.2 (0.1)	1.2 (0.2)	0.221	**<0.001**	0.644
Mg (mg/dL)	2.5 (0.2)	2.5 (0.3)	2.5 (0.2)	0.8265	**<0.001**	0.878
CK (UL)	267.3 (145) [a]	247.9 (139) [b]	290.3 (205) [a]	**0.096**	0.509	0.266

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Results here showed that calves treated with ITM seven days before weaning and shipping (ITM pre-weaning) had lower concentrations of the enzymes that indicate damage of liver cells or muscle damage (AST, Aspartate aminotransferase; and CK, creatine kinase; tendency; P= 0.09) compared to the Control group (CONT). The calves that received ITM on the day of weaning had similar AST and CK (tendency; P= 0.09) concentrations than the control group (Table 1).
There were variations within each group in the concentration of the serum biochemicals analyzed depending on the day of the collection (Table 1).

Table 2. Mean ± SD of Hematology parameters in control-treated calves (CONT), calves treated with injectable trace minerals (ITM) 7 days before weaning (ITM pre-weaning), and calves treated with ITM at weaning (ITM weaning).

	CONT	ITM pre-weaning	ITM weaning	*P-value*		
				Group	*Day*	*Group*Day*
WBC (10^9/l)	12.1 (3.2)	11.7 (3.0)	12.2 (3.3)	0.366	<0.001	0.710
LYM (10^9/l)	7.9 (2.9)	7.9 (8.7)	7.5 (2.6)	0.708	0.136	0.549
MON (10^9/l)	0.8 (0.6)	0.8 (0.5)	0.9 (0.5)	0.184	0.090	0.935
NEU (10^9/l)	3.4 (1.5)	3.3 (1.7)	3.6 (1.8)	0.369	<0.001	0.969
EOS (10^9/l)	0.17 (0.2) [a]	0.16 (0.1) [b]	0.20 (0.2) [a]	**0.048**	<0.001	0.405
BAS (10^9/l)	0.1 (0.1) [a]	0.07 (0.0) [b]	0.1 (0.1) [a]	**0.056**	<0.001	0.653
RBC (10^12/l)	13.3 (11.1)	11.4 (1.3)	11.7 (1.3)	0.011	0.001	**0.029**
HGB (g/dl)	11.4 (0.9)	10.9 (0.9)	11.1 (0.8)	**<0.001**	<0.001	0.994
HCT (%)	38.4 (2.7)	37.4 (4.3)	38.1 (3.8)	0.088	0.380	0.432
MCV	31.8 (3.0)	33.1 (2.8)	32.6 (2.7)	**0.002**	<0.001	0.999
MCH	9.4 (0.6)	9.6 (1.2)	9.4 (1.0)	0.250	0.027	0.995
MCHC	29.8 (1.8)	29.0 (2.9)	28.9 (2.6)	**0.014**	<0.001	0.977
RDWc	31.7 (3.1)	30.1 (2.9)	30.5 (2.9)	**<0.001**	<0.001	0.867
RSWs	36.5 (3.4)	36.4 (3.8)	36.2 (2.7)	0.749	<0.001	0.250
PLT (10^9/l)	444.9 (256) [a]	671.7 (178) [b]	436.6 (166) [a]	**0.105**	0.670	0.963
MPV	5.8 (0.6)	6.2 (1.3)	6.0 (0.5)	**0.005**	0.392	0.999
PCT	0.3 (0.2)	0.6 (2.8)	0.3 (0.1)	0.098	0.871	0.942

Results here showed similarity in white blood cells (WBC), Lymphocytes (LYM), Monocytes (MON), and Neutrophils (NEU) between all three groups (Table 2).
The ITM pre-weaning group had higher Eosinophils (EOS) and Basophils (BAS) concentrations in the blood than the Control (CONT) and ITM weaning groups (Table 2).

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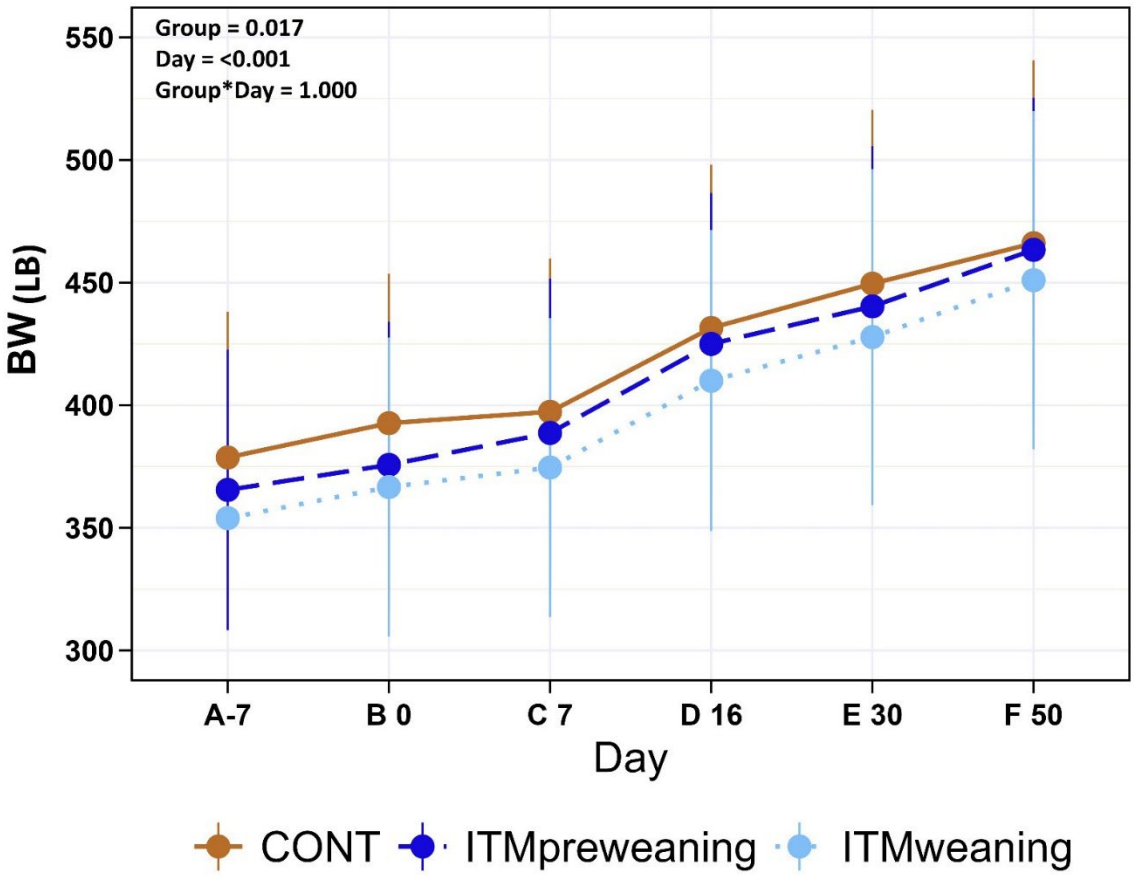
There is a tendency (P= 0.105) of the ITM pre-weaning group to have higher Platelets concentration (PLT) than the Control (CONT) and ITM weaning groups (Table 2).

Figure 1. Mean ± SD of Live Body Weight in control-treated calves (CONT; tan), ITM-treated calves 7 days before weaning (ITM pre-weaning; blue violin), and ITM-treated calves at weaning (ITM-weaning; light blue).



Calves from the control group (CONT) had higher Live Body Weight at the start of the study and on the day of weaning compared to both ITM-treated groups. However, there were no differences in Live Body Weights between all three groups after weaning (Figure 1).

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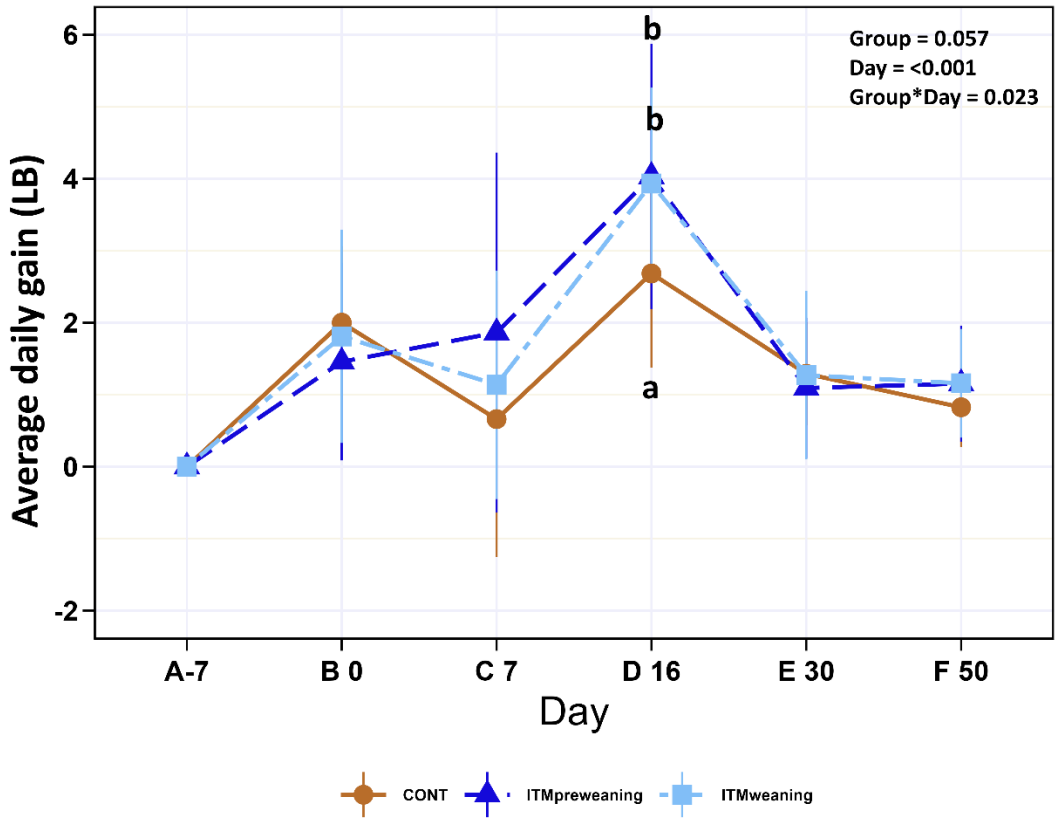
Figure 2. Mean ± SD of Average Daily Gain (ADG) in control-treated calves (CONT; tan), ITM-treated calves 7 days before weaning (ITM pre-weaning; blue), and ITM-treated calves at weaning (ITM weaning; light blue)



Calves treated with ITM had higher ADG from the first 23 days of the study (from Day -7 to Day 16) compared to the control group (CONT) (Figure 2).
Both ITM-treated calves had similar ADGs throughout the study (Figure 2).

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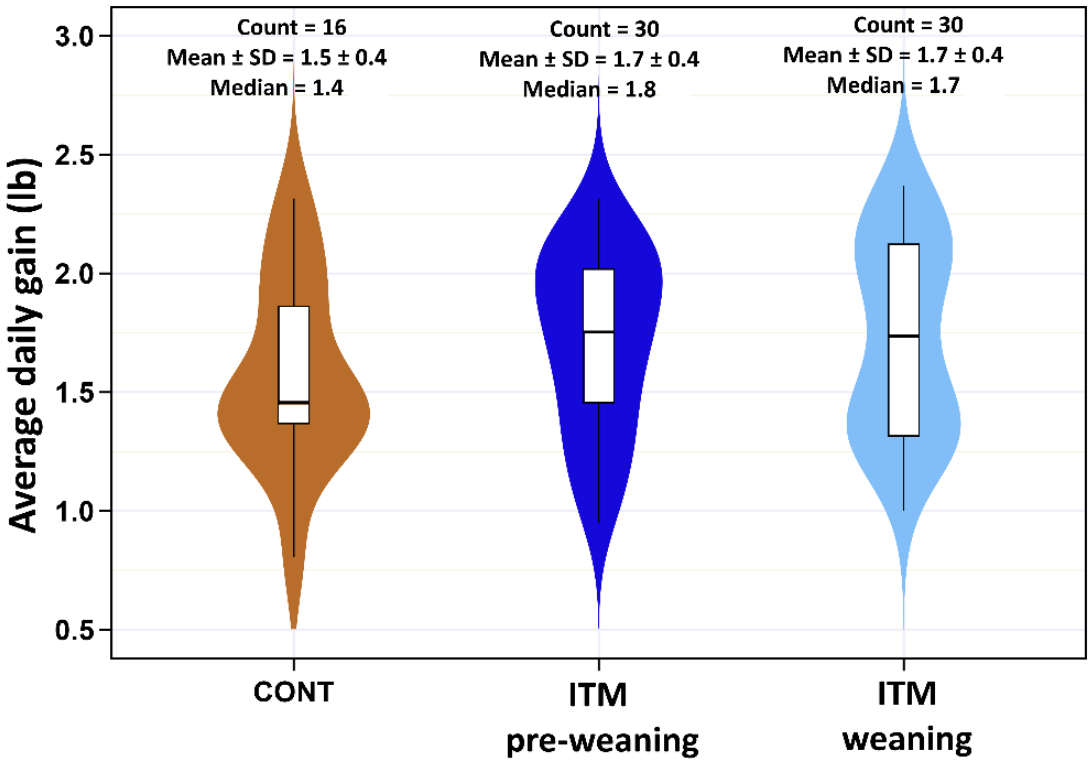
Figure 3. Box plot of the Overall Average Daily Gain (ADG) in control-treated calves (CONT; tan violin plot), ITM-treated calves 7 days before weaning (ITM pre-weaning; blue violin plot), and ITM-treated calves at weaning (ITM-weaning; light blue violin plot).



Calves from the control group (CONT) had lower Overall Average Daily Gain (ADG) in the study compared to both ITM-treated groups (Figure 3).
The ITM pre-weaning group had 0.4 lb. higher ADG (when evaluating the Median) compared to the control group (CONT), and 0.2 lb. higher ADG (when evaluating the Mean compared to the control group (CONT) in the overall study. The ITM weaning group had similar differences to CONT (Figure 3).
However, the calves treated with ITM seven days before weaning (ITM pre-weaning) had more calves with ADG in the upper quartile compared to both other groups (CONT and ITM weaning; Figure 3).

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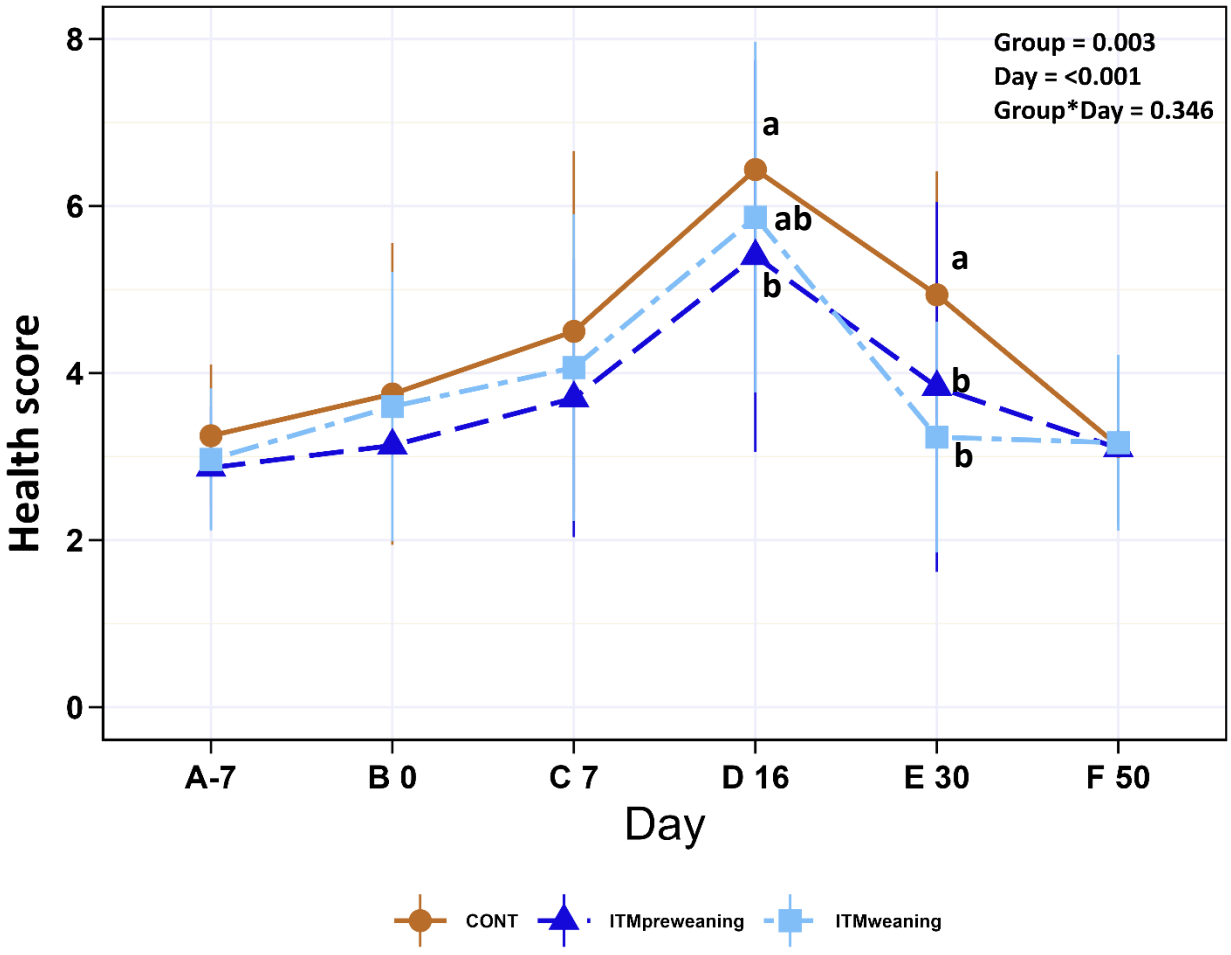
Figure 4. Mean ± SD of Sum Health Scores in control-treated calves (CONT; tan), ITM-treated calves 7 days before weaning (ITM pre-weaning; blue), and ITM-treated calves at weaning (ITM weaning; light blue). A lower value indicates better health.



Calves from the control group (CONT) had a higher Sum Health Score in the study on Day 16 and on Day 30 compared to the ITM pre-weaning group (ITM preweaning) and similar scores to the ITM weaning group (ITM weaning) on Day 16 (Figure 4).
Both ITM-treated groups had similar Sum Health Score throughout the study (Figure 4).

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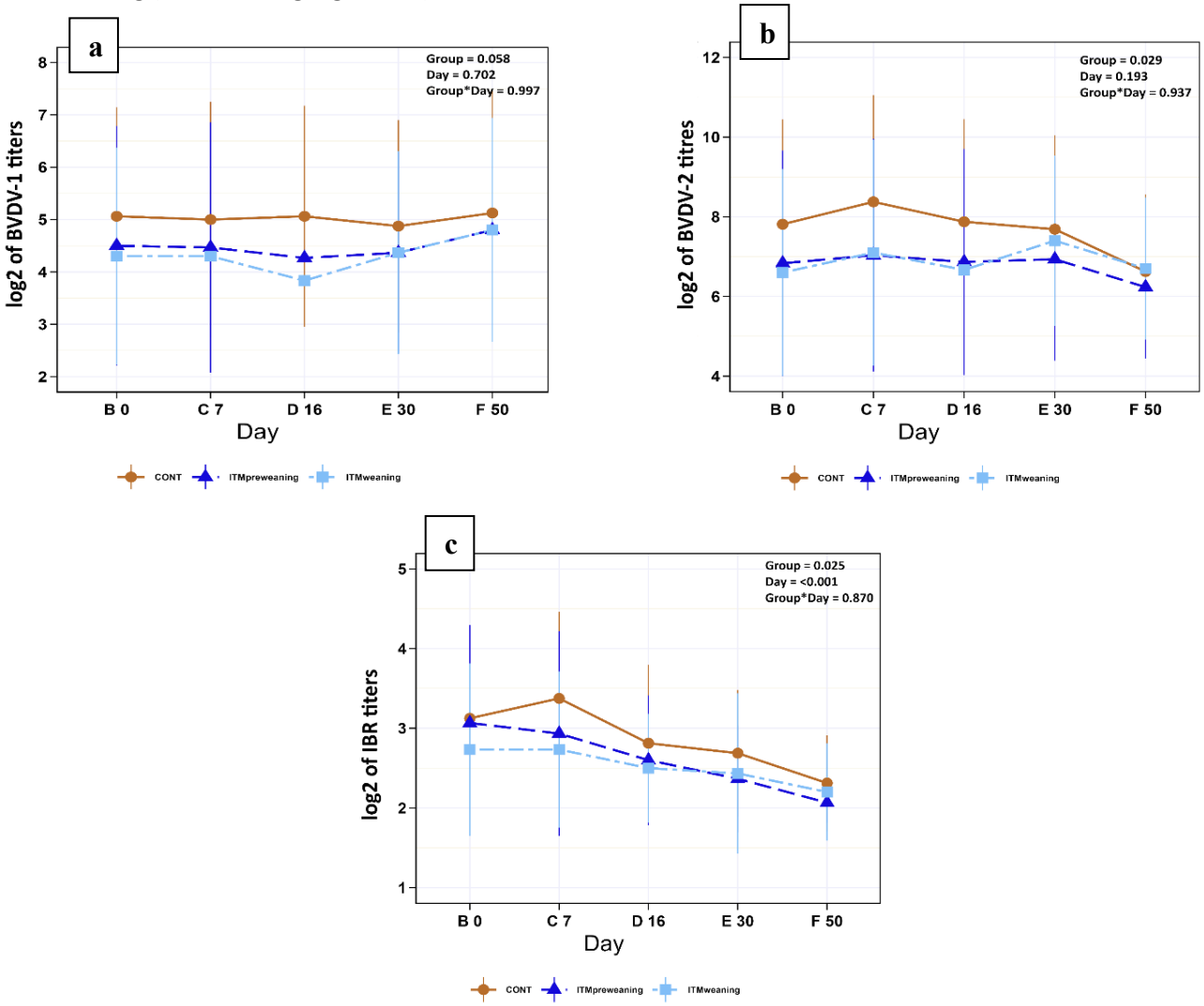
Figure 5. Mean ± SD of Serum Neutralizing Antibody titers (SNAb) for BVDV-1 (a), BVDV-2 (b), and IBR (c) in control-treated calves (CONT; tan), ITM-treated calves 7 days before weaning (ITM pre-weaning; blue), and ITM-treated calves at weaning (ITM weaning; light blue).



The titers for Serum-Neutralizing Antibody for BVDV-1, BVDV-2, and IBR in all the calves were high on the weaning day (Day 0; B 0), indicating the presence of maternal antibodies in the calves' blood (Figure 5).

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Calves from the control group (CONT) had higher BVDV-1 SNAb titers than ITM-treated groups on the first four collections (B 0, C 7, D 16, and E 30), and ITM-treated groups had similar titers on those days (Figure 5a).
Calves from the control group (CONT) had higher BVDV-2 SNAb titers than ITM-treated groups on the first three collections (B 0, C 7, and D 16), and ITM-treated groups had similar titers on those days (Figure 5b).
Calves from the control group (CONT) had higher IBR SNAb titers than ITM-treated groups only one week after weaning (Day 7; C 7), and ITM-treated groups had similar titers on those days (Figure 5b).

There was no mortality in the study, and no calves were treated for common diseases during the study.

Remarks and Conclusion:

Once we analyze the samples for cortisol, as we proposed but are only waiting for our machine to become available for use, we can evaluate the data and draw conclusions about the effects of the treatment used on stress in this model. The evaluation of the common parameters in blood and serum (Tables 1 and 2) did not show many differences between the groups. Also, there is a difference between calves treated and those not treated with ITM regarding Serum-Neutralizing Antibodies (SNAb; of the three viruses evaluated), and the high starting point of SNAb in the calves in the study, supports the adequate passive transfer of these calves had in which there is the presence of Maternal SNAb in blood that can interfere in the response to the vaccines used. Further analysis of seroconversion will be done to better understand the effects of the used treatment on SNAb. The Sum Health Score was higher in the control groups for several days after weaning compared to ITM-treated calves and appeared to be lower in the calves treated with ITM seven days before weaning. These findings can add additional support to the hypothesis that the ITM treatment before weaning and shipping is beneficial in reducing stress and translating into better health. However, we need to be cautious because there was no mortality or morbidity (and this could be associated with deficiencies in looking for sick calves).
More importantly, and in relation to our expected potential benefits, we saw a difference in the average daily gain between the groups, in which the use of ITM seven days before weaning and shipping was associated with an additional 0.4 lb. ADG per calf compared to the control group. Also, the ADG distribution in the ITM pre-weaned calves concentrated in the higher quartiles (Fig. 3) demonstrated trends in more uniform ADG to the higher numbers than the other ITM-treated group, which also had similar ADG but with a larger range.
We conclude at this point, with the results available, that ITM treatment seven days before weaning-shipping increased calf performance and, considering even current beef cattle prices that are higher than when we planned, at the submission of this project, that the return on the investment for the use of this

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strategic treatment can be even higher than what we proposed, therefore giving sound data that support for its implementation.

As a final consideration, the authors of this study are very grateful for the important support that the Florida Cattlemen's Association gave to our research group. The authors will also carry out and finalize all the analyses needed and work in writing a scientific manuscript to be submitted to the peer-reviewed journal of beef cattle production-health.

Sincerely

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Principal Investigator:	Jabur Bittar,Joao Henrique
Award Begin Date:	10/30/2023
Award End Date:	07/31/2024
UF FEIN:	59-6002052

Sponsor Award ID:	60
Award Title:	Effect of injectable trace minerals use at two different
Award Amount:	$55,553.00

Invoice #	M000414413
UF Award #	AWD15782
Primary Project #	P0324549
Primary Department	28040000
Current Invoice Amount:	$52,155.07

Description	Current	Cumulative
Final	$52,155.07	$52,155.07
Total	$52,155.07	$52,155.07

For billing questions, please call 352.392.1235
Canziani,Nicole Knapp nknapp@ufl.edu
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By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).

Nicole Knapp Canziani

Certifying Official

Payment History	
Cumulative Invoices:	$52,155.07
Payments Received:	$0.00
Outstanding Balance:	$52,155.07
Note: Outstanding balance includes current invoice amount	

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Project ID	Deptid	Department Name	Current	Cumulative
P0324549	28040000	VM-LACS	$52,155.07	$52,155.07