

Final Technical Report
FCEB Project #16

Final Report

August 2024

1. Project Title and principal investigator contact information:

Title: Sexual dimorphism of supplementation with rumen-protected Methionine in early gestation in beef cattle systems: impact on fetal development, and postnatal growth and performance (Year 1).

Project number: FCEB No: 16.

Award number: AWD15831

Proponent:

Angela Gonella D; a.gonelladiaza@ufl.edu; (850) 526-1612 (Work) or (850) 693-6465 (cell)

Affiliation: University of Florida – North Florida Research and Education Center

Research team: Daniella Heredia¹ (Co-PD/PI), Nicolas Di Lorenzo¹ (Co-PD/PI), Peter J. Hansen² (Co-PD/PI), Dean Pringle (Co-PD/PI)¹.

2. Significance

Methionine is one of the most limiting amino acids for beef cattle. The rumen rapidly degrades it; therefore, it has a low absorbance in the small intestine and decreased availability in the blood. Previous studies have shown that RPM supplementation increased Methionine ruminal bypass, small intestine degradation, and plasma methionine concentrations (Berthiaume et al., 2000; Zhou et al., 2016). Additionally, supplementation of RPM leads to greater plasma concentrations of sulfur-containing compounds (cystathionine, cysteine, homocysteine, and taurine) and availability of other amino acids (lysine, arginine, tryptophan, threonine, proline, asparagine, alanine, and citrulline; Zhou et al. 2016). There is growing evidence of the potential effects of methionine and other methyl-donor compounds on fetal development and postnatal growth performance in mammals. Peñagaricano et al. (2013) reported that supplementing RPM to lactating dairy cows fed methionine-deficient diets during the periconceptual period altered the expression of genes related to embryonic development in 6 days old embryos when compared with cows receiving no RPM. Additionally, studies with rodents have reported that diets adequate in energy and total protein but deficient in methyl donors (methionine, choline, and folate) during early gestation changed the expression of proteins involved in methionine metabolism, lipid metabolism, and carbohydrate metabolism in the liver of the offspring (McNeil et al., 2008). The economics of beef cattle production systems are driven mainly by pregnancy success. Thus, any interventions that may lead to increased embryonic competence and pregnancy establishment should be attractive to cattle producers. This proposal addresses FCA research priorities #2 "Calf Weaning Rate" and #5 "Herd Nutrition", as our final aim is to improve the pregnancy attainment of multiparous cows by supplementing methyl donors in the diet.

Inside this research line, during the 2021 breeding season, we conducted an experiment that aimed to evaluate the effect of peri-conceptual feeding of RPM in beef cows. In this sense, we fed 114 multiparous cows (Angus and Brangus at the NFREC, Marianna) with 1 pound of the carrier containing the 15 gr of RPM or not. The diet was administrated for 14 days, starting 7 days before and 7 days after the AI (periconceptual period). Our preliminary results show that the resulting offspring of cows fed with the RPM grows more than the control group. Significant differences in the embryo vesicle diameter (day 30 of gestation), birth weight (male calves from the RPM group were 10 kg heavier at birth), and wither height (days 60 and 120) and Body length (Day 180; Figure 1) were found when comparing RPM and control.

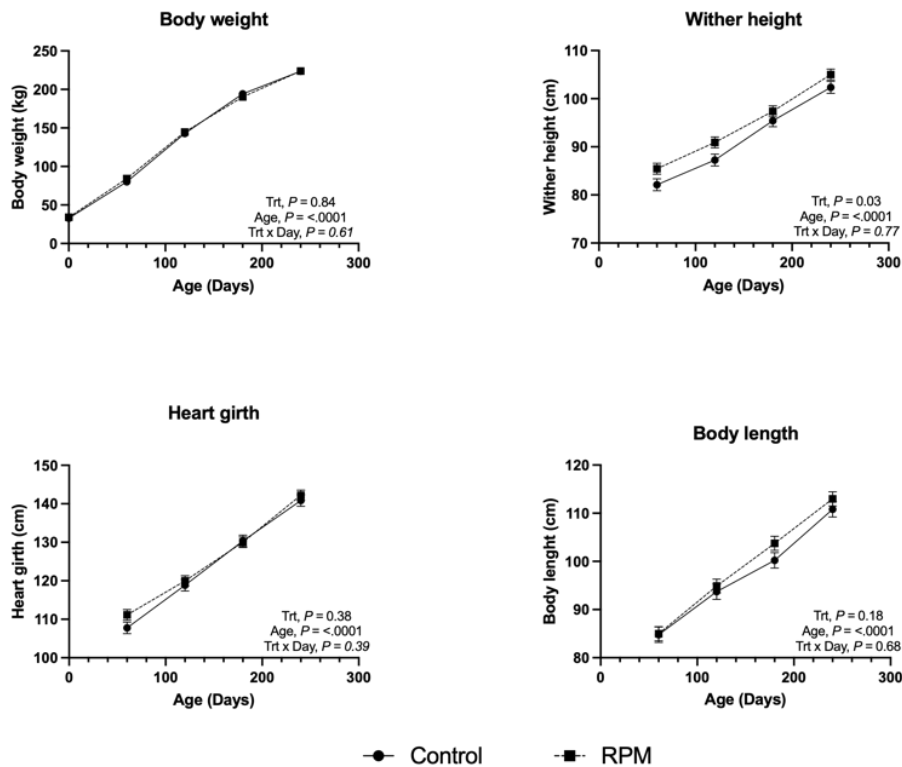


Figure 1: Evaluation of post-natal (pre-weaning) development of heifers from RPM and Control groups. Dams from the RPM group receive 15 gr of RPM 7 days before and 7 days after the artificial insemination.

Those differences remain significant after weaning, and when heifers were 1 year old, the RPM group had a higher wither height, heart girth, and body length (Figure 2). Additionally, RNA sequencing analysis in liver and adipose tissue resulted in having 129 genes downregulated and 24 upregulated in the liver in the RPM group, where the top canonical pathways are associated with the immune system function, and in the adipose tissue, 22 genes were downregulated and 6 genes upregulated in the RPM group, where the top canonical pathways are associated with the enrichment of extracellular matrix and cellular response to extracellular stimulus and enrichment of fibroblast growth factor. In summary, our preliminary data show that female calves derived from cows fed with RPM during the periconceptual period are bigger than control. Also, an increase in downregulated genes was identified in female calves derived from cows fed with RPM.

However, in that experiment, we used sex-sorted semen to produce females, so we only have birth weight data from the male calves. The present proposal aims to evaluate the effect of RPM in both female and male calves. For this RFA (2023), we only request funds to conduct the experiment, feed the experimental diets, generate the pregnancies, and follow those gestations until term.

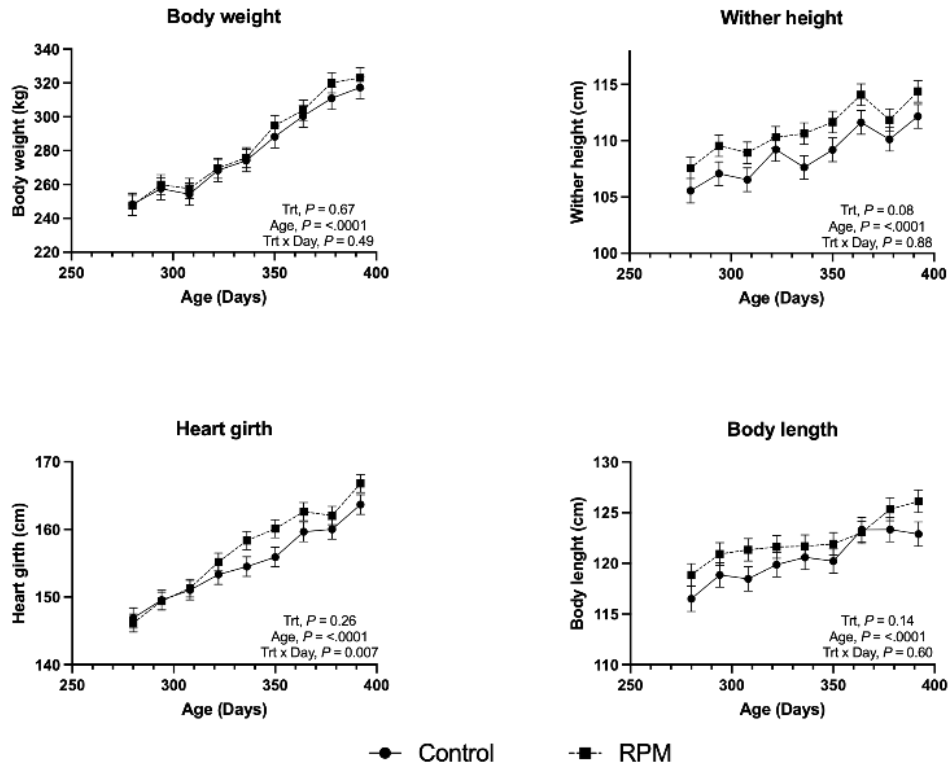


Figure 2: Evaluation of post-weaning development of heifers from RPM and Control groups. Dams from the RPM group receive 15 gr of RPM 7 days before and 7 days after the artificial insemination.

3. Specific aims:

Evaluate the differential responses of male and female fetuses and calves after supplementing rumen-protected Methionine (RPM) to their dams during the periconceptional period.

- Feed RPM 7 days before and 7 days after the AI to multiparous beef cows.
- Evaluate fetal development and placental function.
- On Year 2, Evaluate postnatal development characteristics such as puberty attainment, carcass quality, sperm morphology (males), and follicular count (females).

4. Approach:

We request funds to feed the diets, synchronize and inseminate the animals (generate the pregnancies) and follow gestation until term (Year 1). After calving, resources will be requested from different sources for postnatal evaluations of the progeny.

Animal management and experimental diet supplementation: The experiment will be conducted at the NFREC during the 2024 breeding season. Briefly, 250 cows will be synchronized with the 7 days protocol and timely-artificially inseminated with conventional semen (Figure 2). Twenty-one days before the insemination, each cow will be fed individually 1 lb. of corn gluten feed per day using the Super SmartFeed, to adapt cows to receive the supplement and to eat from the Super SmartFeed feeder. This feeder is a portable and self-contained system designed to measure and control individual daily feed intake in cattle housed as a single group via RFID technology. On Day -7 (a week before the expected day of ovulation and TAI), cows in the MET group (n = 125) will receive the experimental supplementation of 15 g of RPM (Smartamine, Adisseo, Alpharetta, GA) once daily. The RPM will be mixed with the corn gluten feed to continue the delivery of 1 lb/day of the diet-methionine mix. The control group will continue to receive 1 lb/d of the diet. All cows will be artificially inseminated, and the experimental diets will be fed until day 7 (7 days after TAI). All cows will remain together in the same pasture until weaning the calves on the subsequent year's calf crop.

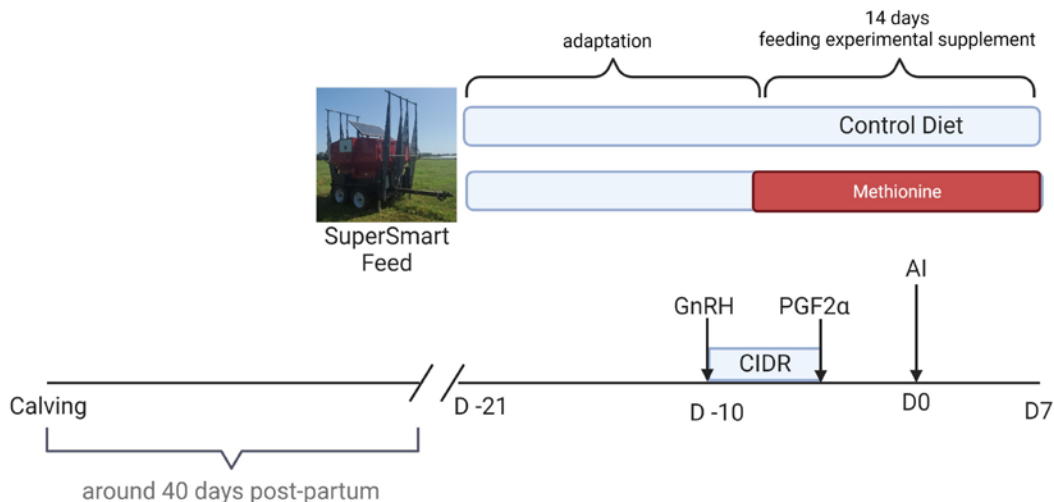


Figure 3: Experimental design and hormonal manipulation protocol.

Pregnancy Diagnoses and embryo/fetus morphology: Pregnancy diagnoses will be conducted 30, 60, and 120 days after the artificial insemination. Also, a blood sample (from pregnant cows only) and videos of the ultrasonographic evaluations will be collected from each cow. All calves will be weighed at birth and will remain together until weaning. Ultrasonographic videos and images will be used for embryonic and fetal morphology. Video and image analysis will be conducted using the My Delta lab software and Image J. Videos will be used to evaluate the following variables:

embryo vesicle diameter, embryo vesicle circumference, embryo length, abdominal cavity width at day 30 of gestation, and head length, eye cavity, head transversal, and wither-tail length at day 60.

Pregnancy-associated glycoproteins (PAGs) assay: Blood samples collected on days 30, 60, and 120 will be used for PAG's determination using commercial kits. PAGs are used commercially for pregnancy diagnoses but are also makers of placental function.

Data Analysis: Continuous data will be analyzed by least-squares analysis of variance using the GLIMMIX procedure of SAS. The main effects will include sex, group, and animal nested within the group. For repeated measures, the animal will be considered as a random effect.

5. Problems encountered and achievements:

So far, we have no problems to report. We successfully completed the treatment supplementation, estrus synchronization, and artificial insemination of the first group of cows, and we are currently doing the experiment with the second group. We have already programmed all the activities for the third group in our calendar. The table below summarizes the dates of the three groups expected to be enrolled in the study:

	Group 1	Group 2	Group 3
Number of cows enrolled	91	98	61
Start of adaptation period	01/25/2024	02/23/2024	03/29/2024
Start of treatment supplementation	02/08/2024	03/07/2024	04/12/2024
Date of the artificial insemination	02/15/2024	03/14/2024	04/19/2024
Finish of supplementation period	02/22/2024	03/21/2024	04/26/2024
Cows with acceptable intake of supplements	50	65	29
Pregnant cows retained for Year 2	25	22	9

A new short-term scholar, Mr. Jose Infante oversaw the execution of this experiment. He successfully complete the first year of this study and was admitted as a master's student at the Animal Sciences Department. Therefore, he will continue working with this study during year 2.

6. References

- Berthiaume R, Lapierre H, Stevenson M, Coté N, McBride BW. 2000. Comparison of the in situ and in vivo intestinal disappearance of ruminally protected methionine. *J Dairy Sci.* 83(9):2049-56.
- McNeil CJ, Hay SM, Rucklidge G, Reid M, Duncan G, Maloney CA, Rees WD. 2008. Disruption of lipid metabolism in the liver of the pregnant rat fed folate deficient and methyl donor deficient diets. *Br J Nutr.* 99:262–271.

- Peñagaricano F, Souza AH, Carvalho PD, Driver AM, Gambra R, Kropp J, Hackbart KS, Luchini D, Shaver RD, Wiltbank MC, Khatib H. 2013. Effect of maternal methionine supplementation on the transcriptome of bovine preimplantation embryos. *PLoS One*. 8(8):e72302.
- Zhou Z, Bulgari O, Vailati-Riboni M, Trevisi E, Ballou MA, Cardoso FC, Luchini DN, Looor JJ. 2016. Rumens-protected methionine compared with rumens-protected choline improves immunometabolic status in dairy cows during the peripartal period. *J Dairy Sci*. 99(11):8956-8969.

PLEASE REMIT TO:

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 Contracts & Grants
 PO Box 931297
 Atlanta, GA 31193-1297

Invoice Date: 08/13/2024
 Invoice Period: 03/01/2024 - 07/31/2024
 Principal Investigator: Gonella Diaza, Angela Maria
 Award Begin Date: 10/30/2023
 Award End Date: 07/31/2024
 UF FEIN: 59-6002052

SPONSOR:

FL CATTLE ENHANCEMENT BOARD
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 Kissimmee FL 34742-1929
 United States

Sponsor Award ID: 16
 Award Title: Sexual dimorphism of supplementation with rumen-protected Methionine in early gestation in beef cattle systems: impact on fetal development, and postnatal growth and performance (Year 1)
 Award Amount: \$58,437.00

Invoice #	I000130454
UF Award #	AWD15831
Primary Project #	P0325271
Primary Department:	60770000
Current Invoice Amount:	\$31,800.94

Description	Current	Cumulative
Personnel - Salary	\$2,702.40	\$2,702.40
Personnel - Fringe Benefits	\$113.47	\$113.47
Materials and Supplies	\$8,383.48	\$9,153.94
Contractual Services	\$15,763.69	\$20,763.69
Animal	\$0.00	\$17,148.48
Other Expenses	\$769.73	\$1,183.73
Domestic Travel	\$660.92	\$876.10
Foreign Travel	\$0.00	\$0.00
Direct Cost	\$28,393.69	\$51,941.81
Facilities and Administrative Costs	\$3,407.25	\$6,233.05
Total	\$31,800.94	\$58,174.86

For billing questions, please call 352.392.1235
 Peterson, Nathan Kyle npeterson82@ufl.edu
 Please reference the UF Award Number and Invoice Number in all correspondence

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).

Nathan Peterson

 Certifying Official

Payment History	
Cumulative Invoices:	\$58,174.86
Payments Received:	\$26,373.92
Outstanding Balance:	\$31,800.94
Note: Outstanding balance includes current invoice amount	