

# DE SANIDADE BOVINA Tema: Saúde Gastrointestinal





# Study of thiobarbituric acid reactive substances (TBARS) in suckling Holstein calves

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#### INTRODUCTION

The increase in oxidative stress markers, such as thiobarbituric acid reactive substances (TBARS), reflects the inefficiency of antioxidant mechanisms under physiological or pathological conditions.

## **OBJECTIVES**

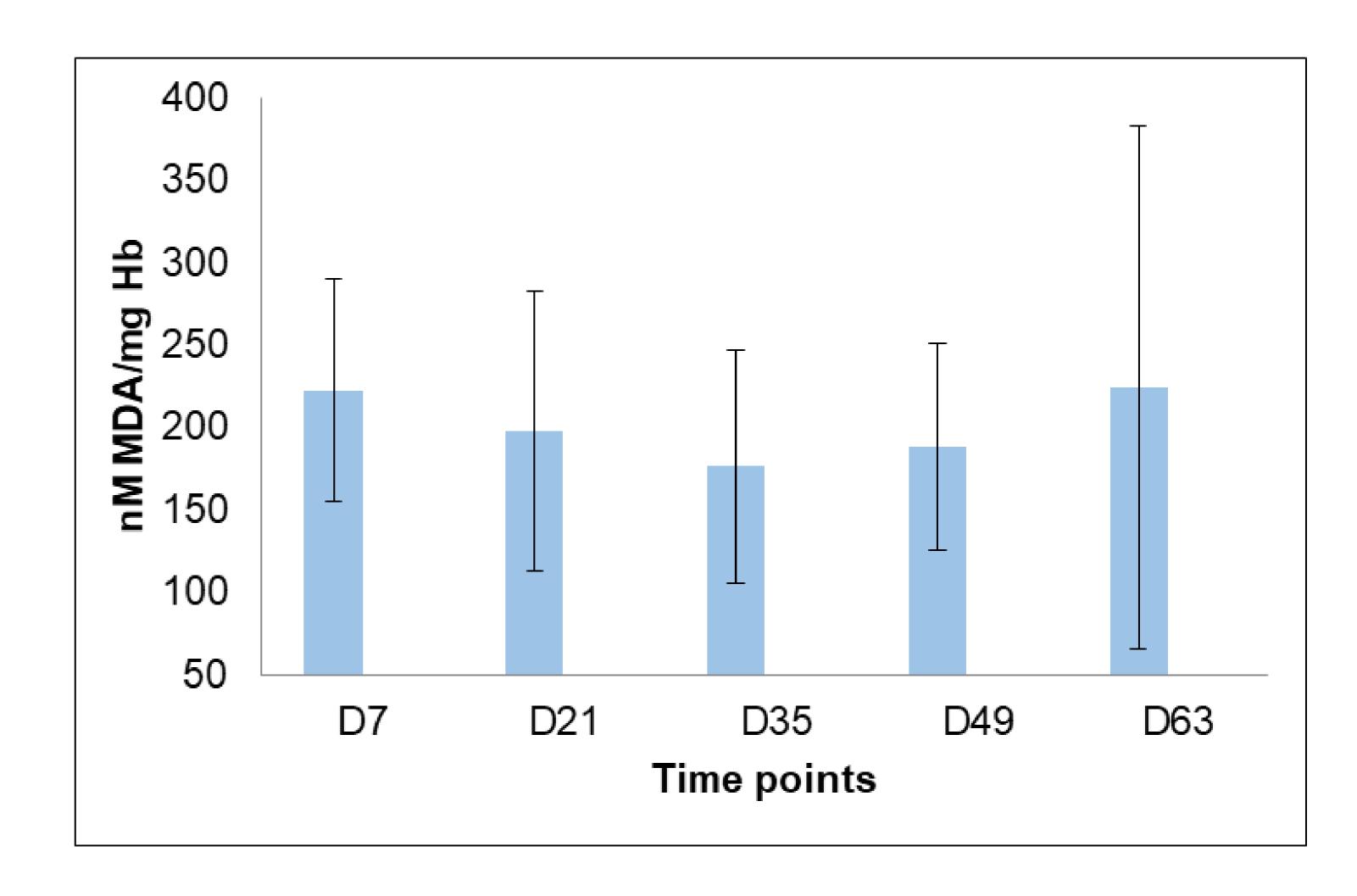
The aim was to evaluate age-related physiological adaptations of the GIT in calves.

# **MATERIAL AND METHODS**

Nine male Holstein animals were used, housed in individual suspended cages of 3m<sup>2</sup>, in a closed environment with controlled ventilation and biosafety measures at the Calf Research Laboratory of FMVZ/USP. The feeding system was intensive, with a daily volume of 6L in two meals, in addition to ad libitum water. At 25 days of age, calves began to eat 300g of starter feed with 22% crude protein (AgMilk, Agroceres®), with 10% excess established. Daily health scores, such as for diarrhea and respiratory disease, were recorded. Whole blood samples were collected by jugular vein puncture into vacuum tubes containing sodium heparin and immediately placed on ice in a cooler at the following time points: D7, D21, D35, D49, and D63. The washed red blood cell technique was then performed, and the samples were stored at -80°C. An assay was conducted to determine TBARS by absorbance in a microplate reader spectrophotometer with a wavelength of 535nm (Figure 1). Results were expressed in malondialdehyde (MDA - nM) per hemoglobin (Hb - mg). Analysis of variance (ANOVA) was employed.

#### RESULTS AND DISCUSSION

A trend (**Figure 2**; P=0.7858) was observed with higher MDA concentration at D7 (222.56  $\pm$  67.02nM/mg Hb), persisting until D21 (197.69  $\pm$  84.90nM/mg Hb). A decrease in values was noted in the second month of life (D35 - 176.55  $\pm$  70.75nM/mg Hb). During this phase, in addition to physiological adaptations of the neonatal period, calves experienced stress due to acclimatization to the new facilities after transportation from the original farm to the experimental calf barn. It should also be noted that the greatest microbial colonization of the gastrointestinal tract occurs in the first 20 days of life, and despite being raised in a controlled environment, the animals showed a higher incidence of diarrhea score 3.



**Figure 2.** TBARS concentration (nM of MDA/mg of hemoglobin) of nine Holstein suckling calves from 7<sup>th</sup> to 63<sup>rd</sup> days of life.

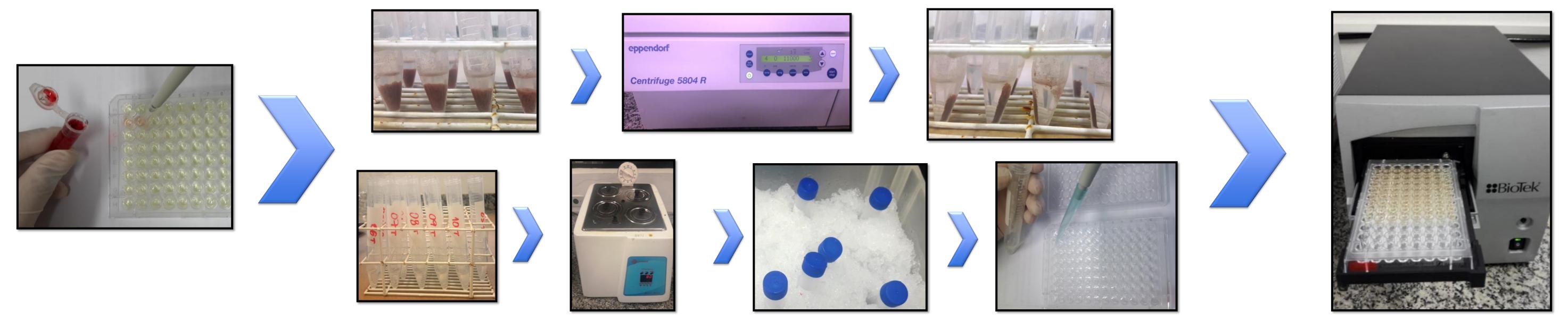


Figure 1. Hemoglobin (Hb) quantification in the washed erythrocytes with purified water, and Drabkin solution. Steps of in-house determination of TBARS by absorbance in a microplate reader spectrophotometer, according to Sinzato et al. (2023).

### CONCLUSION

The combined action of the innate immune system interfacing with the balance of oxidant and antioxidant agents was efficient for the homeostasis and survival of these calves during this challenging period.



















