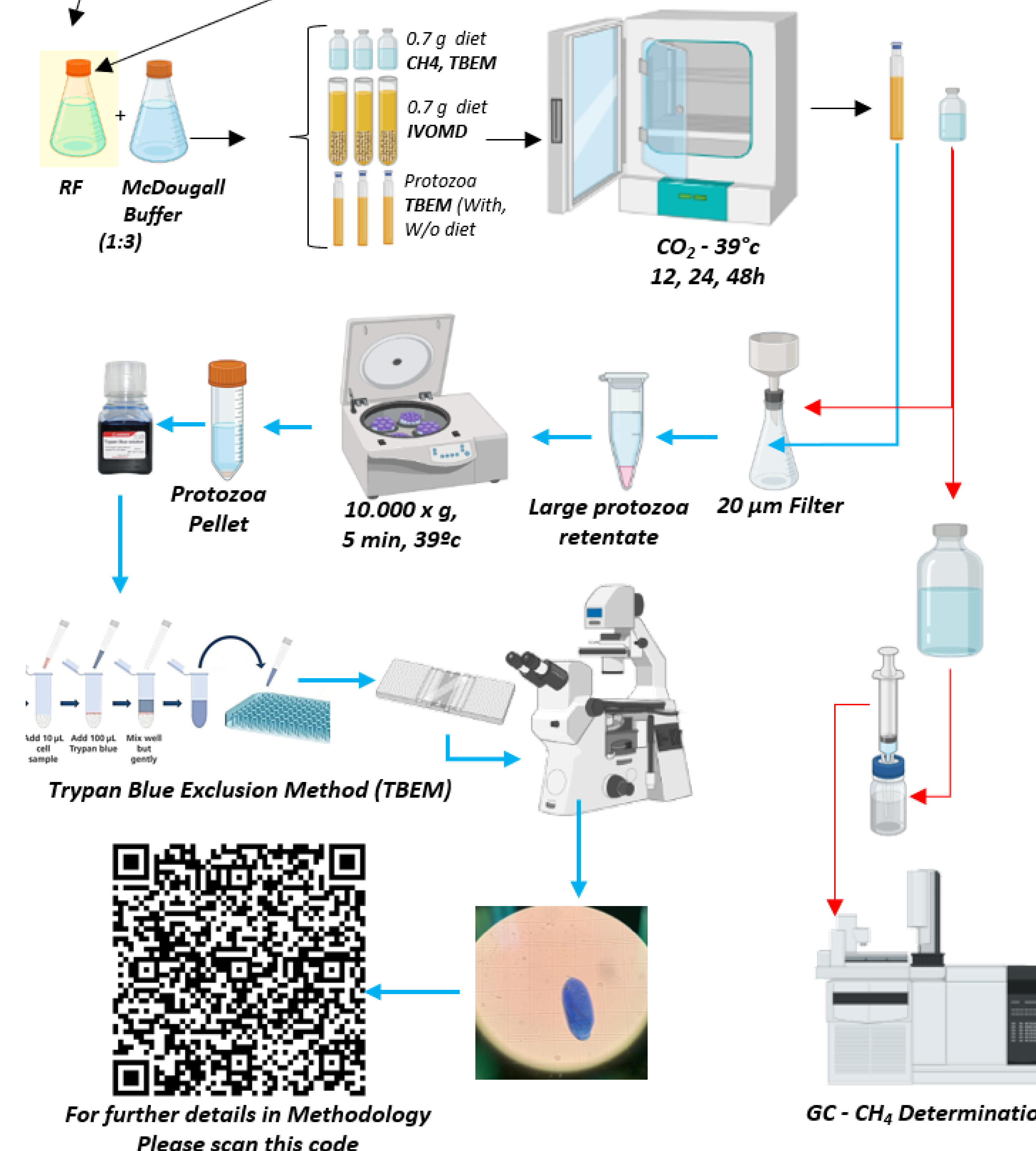


INTRODUCTION

- ✓ Yeast supplementation enhances cattle productivity via increasing ruminal bacteria count.
- ✓ A reliable counting method of ruminal microorganisms is essential to detect differences in growth dynamics, refining analysis of ruminal fermentation parameters.
- ✓ **Objective** = Validate trypan blue exclusion method (TBEM) for ruminal protozoa counting under different culture conditions in fluid from yeast-supplemented steers consuming a grain-based diet.

MATERIALS AND METHODS

Ingredient	(%)
Cracked corn	65
DDGS	10
Cottonseed hulls	5
Bermuda hay	15
Vit&Min Premix	5



RESULTS & DISCUSSION

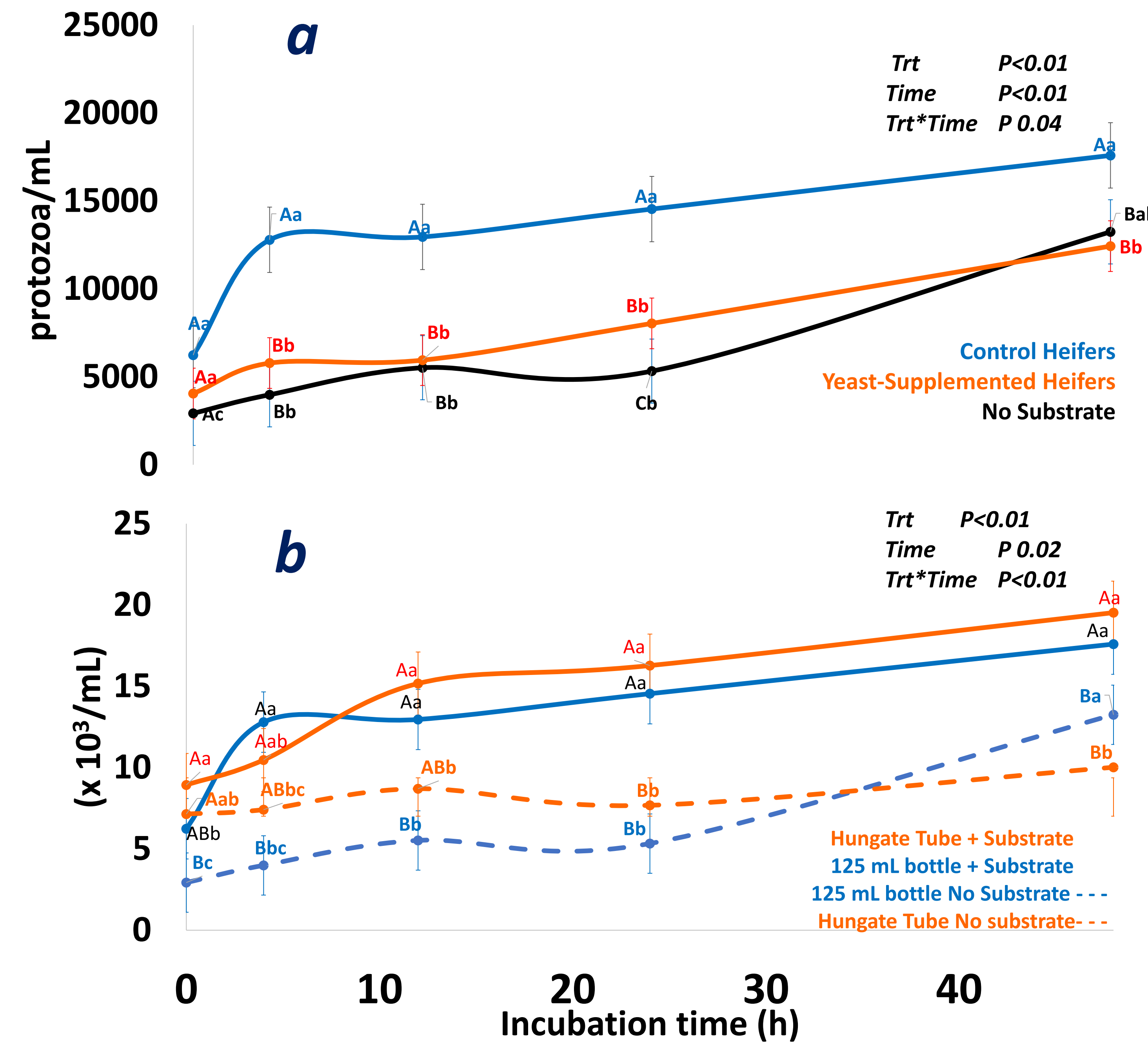


Fig 1a. Effect of yeast supplementation on ruminal live protozoa
Fig 1b. Effect of incubation conditions on live protozoa

Different capital letters = difference between treatments at each time point. Different Lower letters = difference between time points within each treatment

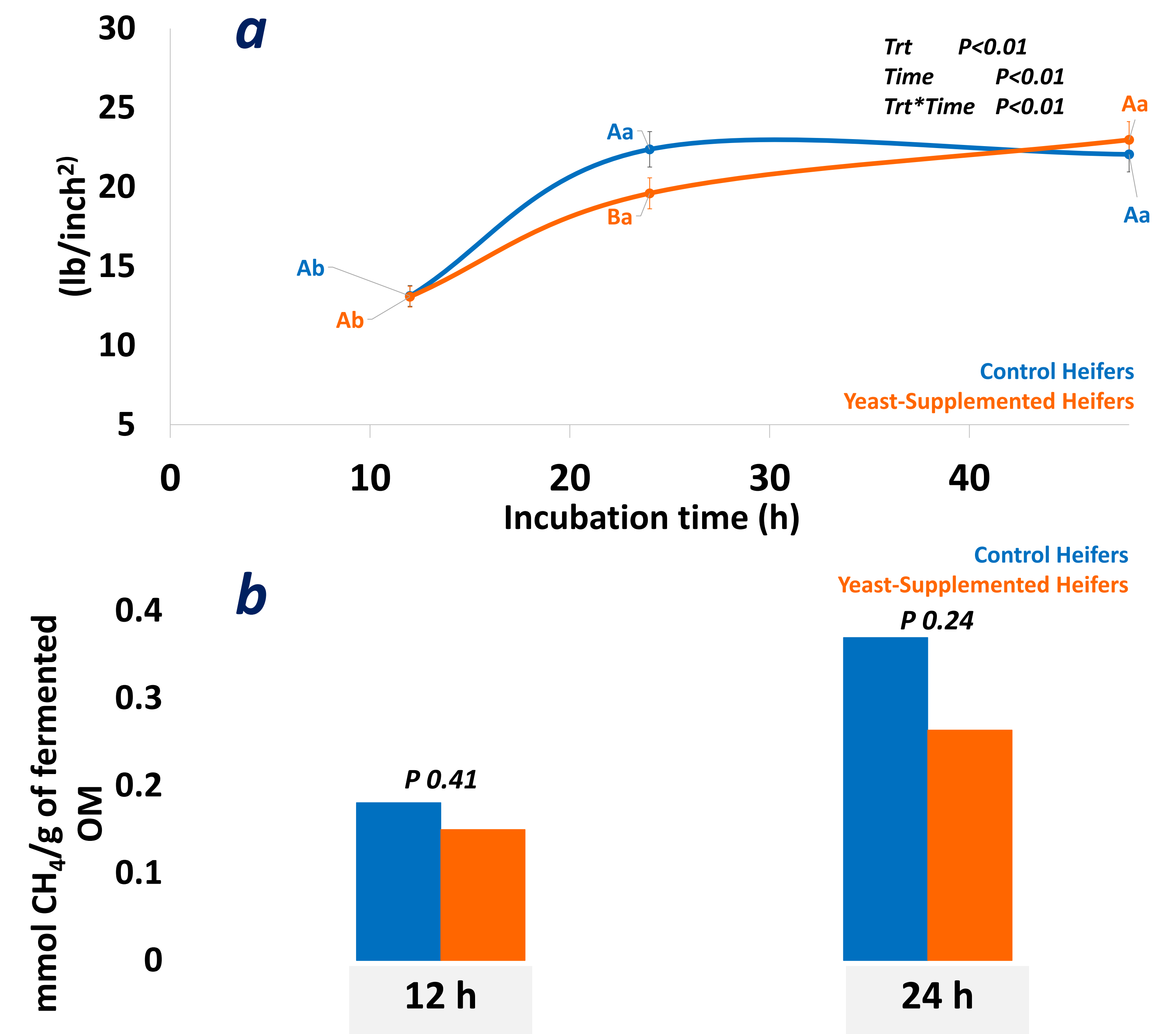


Fig 2a. Effect of yeast supplementation on gas production
Fig 2b. *In vitro* methane production

As with eukaryotic cells, Trypan Blue function as an intracellular probe to evaluate viability of ruminal protozoa (Fig 3), with similar counts described in cows (1.5×10^4) and Steers (3.7×10^3)^{1,2,3}. Ruminal fluid from yeast-supplemented steers exhibited fewer alive protozoa in 125mL bottle and Hungate tubes. Protozoa cultured with substrate displayed higher viability, something essential for further studies on protozoa dynamics. Agreeing with⁴, the observed reduction of protozoa in yeast-supplemented steers coincide with a lower gas production (Fig 2a) and 28% less CH_4 *In vitro* production (Fig 2b).

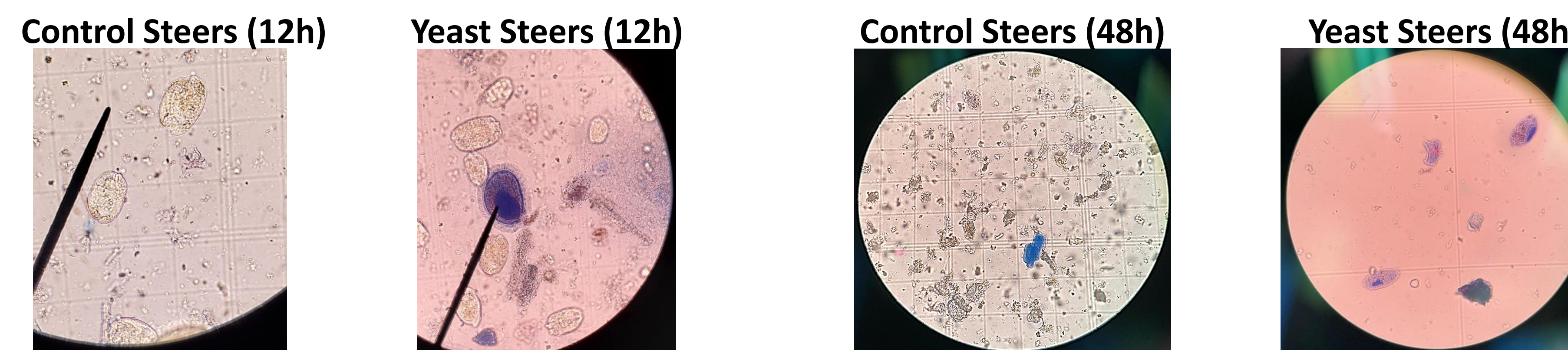


Fig 3 Intracellular probe internalized in ruminal protozoa

CONCLUSIONS

TBEM is a suitable method to determine viability of protozoa under similar experimental conditions of a batch culture using solid substrate.

28 g/d of Yeast supplemented to steers under a High-grain diets reduced ruminal protozoa concentration and viability as detected through TBEM in bottles or Hungate tubes incubated with substrate