Title: Myo-inositol trispyrophosphate (ITPP) did not alter oxygen dissociation from mouse or equine hemoglobin in fresh whole blood.

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Myo-inositol trispyrophosphate (ITPP) enhances performance in mice, and is rumored to be used in the horse racing industry. Improved dissociation of oxygen from hemoglobin following the binding of ITPP to the 2,3 diphospho-D-glycerate (2,3 DPG) binding site is the proposed mechanism of action. ITPP incubated with human, mouse, and porcine blood shifts the oxy-hemoglobin dissociation curve ($P_{50}D$) to the right. The primary goal of the present study was to investigate the hypothesis that ITPP would shift the equine $P_{50}D$ to the right. ITPP was synthesized (Frontier Biopharm), and confirmed to be ITPP by LC-MS-MS analysis. Equine and mouse blood samples were incubated with a saline control or ITPP (60, 120, or 240 mM), and the $P_{50}D$ was measured using the same protocol and instrumentation described by previous investigators (Hemox-Analyzer, TCS, New Hope, PA). ITPP did not elicit a rightward shift in the equine or mouse $P_{50}D$. Surprisingly, the $P_{50}D$ appeared to be shifted to the left in both equids and mice at high concentrations of ITPP. Positive controls demonstrated the ability of the methodology and instrumentation utilized in this study to detect expected $P_{50}D$ shifts in both directions (pH challenge, fetal vs adult blood comparison). The difference between the present study and previous reports may be due to the use of fresh blood by the present study. Previous studies were performed with "stored" blood, and the storage of blood shifts the $P_{50}D$ to the left, presumably due to the release of 2,3 DPG. Subsequent binding of ITPP in the absence of 2,3 DPG would then be expected to shift the $P_{50}D$ to the right. The results of the present study suggest that another mechanism is needed to explain the performance enhancing effects observed in mice administered ITPP.

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