

Title-Subacute Ruminal Acidosis in Zebu Cattle: Clinical and Behavioral Aspects

Supplementary Material S1 – Analytical Controls and Calibration

Table S1. Analytical controls and calibration used in this study.

| Variable | Calibration/Validation |
|------------|---|
| L-Lactate | Biochemical kit (Randox®, Crumlin, UK). Calibrator C2 REF: CAL2350, Randox (1.776 mmol/L); Quality control normal N2 REF: HN1530, Randox (1.443–2.054 mmol/L), recovery >98%; Quality control pathological N3 REF: HE1532, Randox (1.776–4.218 mmol/L), recovery >97%. |
| D-Lactate | D-Lactate Colorimetric Assay Kit REF: K667 (BioVision Inc., Milpitas, CA, USA). D-Lactate calibration standard (100 mmol). $r^2 = 0.98$. Calibration curve from 0.01 to 10 mmol. |
| Glucose | Randox Glucose GOD-PAP assay kit (Randox®). Calibrator C2 REF: CAL2350, Randox: 5.42 mmol/L. Quality control normal N2 REF: HN1530, Randox: 4.78–6.45 mmol/L. Quality control pathological N3 REF: HE1532, Randox: 10.89–14.55 mmol/L, recovery >98%. |
| Osmolarity | Internal precheck calibration. Reference solution with 290 mOsm (Ref.: MA029). Acceptable variation: ± 2 mOsm. Quality control low: 50 mOsm Ref: 3MA005. Quality control high: 850 mOsm, Ref: 3MA085. Advanced®, Norwood, MA, USA. |
| pH | Standard calibration in each day using 4.01 pH, REF: DM-S1B and 7.00 pH, REF: DM-S1D. Digimed, São Paulo, Brazil. |
| SCFA | Calibration with reference standards. Acetic acid analytical standard, GC assay $\geq 99.8\%$, Sigma-Aldrich, San Luis, Mi, USA, REF: 71251. Propionic acid analytical standard, GC assay $\geq 99.5\%$, Sigma-Aldrich, REF: 94425. Butyric acid analytical standard, GC assay $\geq 99.5\%$, Sigma-Aldrich, REF: 19215. |

SCFA: Short-chain Fatty Acid.