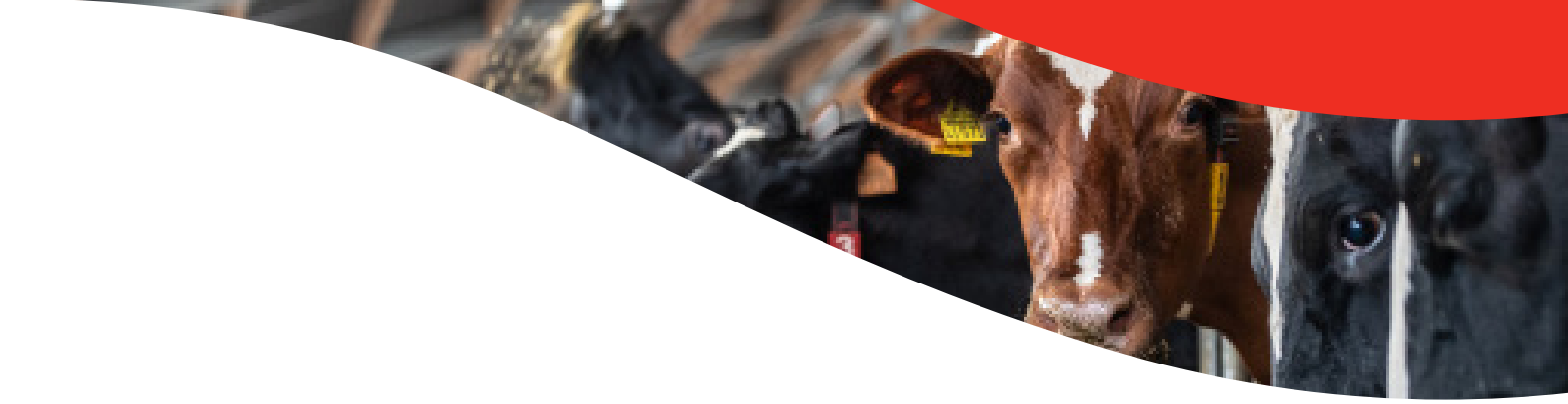

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Comparative analysis of the impact of 2-hydroxy-4-(methylthio)butanoic Acid (HMTBa) and its salt variant on *in vitro* ruminal fermentation dynamics

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The study examines the effects of dry HMTBa acid on in vitro rumen fermentation, comparing it to its calcium salt counterpart using batch culture methodology. The HMTBa sources included KEMSSER H (KEM, 61.6% HMTBa, Kemin Animal Nutrition and Health, Belgium) and a commercial HMB-Ca salt (RS, ≥84.0% HMTBa and its calcium salt). In vitro batch culture of 6 and 24 hours were conducted with rumen fluid from cannulated sheep. The RS and KEM were incubated at 24 mg (providing 20.2 mg and 14.8 mg HMTBa, respectively) in 50 mL diluted rumen fluid, reflecting their respective concentrations. Doses were formulated to align with typical dairy cow supplementation (24 g/day/head). The control group received an unsupplemented basal diet. Measured parameters included gas pressure kinetics, volatile fatty acids (VFA), lactic acid, NH3-N, and pH. Data were analyzed using a randomized block ANOVA, with individual animals as blocking factors, and post-hoc comparisons performed via Fischer's LSD test. Supplementation of KEM did not significantly change functional VFA and lactate productions, pH, N-NH3, and acetic:propionic acid ratio, and total gas production at both timepoints, while total gas pressure at 24 h was significantly higher ($P < 0.001$) for KEM compared with RS (10.1 vs 9.64 ± 0.15). Isovaleric acid production was significantly lower in RS groups than in the control and KEM ($P = 0.017$), while no treatment effect was observed for the rest of VFA. At 6h, both lactate and N-NH3 were lower in treatment groups compared to control (3.91 vs 6.73 ± 0.55; 4.48 vs 4.88 ± 0.14, respectively) as a result of alteration in nitrogen and energy metabolism ($P < 0.033$), while no difference was observed among the groups after 24h incubation. In conclusion, the acid form of HMTBa supplementation shows similar effects to the Ca-salt form in the rumen even in case of lower delivery of equivalent HTMBa. The higher solubility of pure HTMBA, compared to the ion-bound Ca-salt form, may enhance microbial utilization in the rumen, allowing effective biohydrogenation and milk fat production at a lower delivery level. Further trials are needed to confirm this.

KEYWORDS:
Analog, methionine, rumen fermentation