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Effects of isopropyl ester of hydroxy analog of methionine (HMBi) on *in vitro* rumen fermentation, enzymatic activity and nutrient digestibility

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The study aimed to explore *in vitro* ruminal effects of isopropyl ester of hydroxy analog of methionine (HMBi) to enhance understanding of rumen degradable portion of it. In Exp 1, HMBi (KMFD, KESSENT® MF Dry, Kemin Animal Nutrition and Health, Belgium) was added to a basal feed (10% as fed) and incubated in Hohenheimer Futterwerttest (HFT) and Daisy incubator (3 runs x 6 replicates). Basal feed was incubated as control (CON). In Exp 2, *in vitro* 24h rumen incubation was performed followed by Radial enzyme diffusion (RED) test of KMFD to observe enzymatic activities (EA) of amylase (Amy), cellulase (Cel), and xylanase (Xyl) over time (n = 60, expressed as area of hydrolysis). A mixed model was used with REML (JMP 16.1, SAS) with fixed effects of treatment and random effects of each run of incubators. In RED, time was assigned as a fixed effect with random effect of each petri. After 24h incubation in HFT, KMFD did not affect pH, neither the molar level of acetic, isobutyric, isovaleric, valeric, caproic, and lactic acids as well as total gas and methane production (ml/g DM). Molar concentrations of ammonia-N (20.3 vs 17.4 ± 1.2), butyric acid (8.2 vs. 7.2 ± 1.0), and Ace:Pro ratio (5.2 vs 4.8 ± 0.1) decreased with KMFD supplementation compared to CON, while propionic acid increased (P < 0.05). KMFD improved *in vitro* (%) total true digestibility and nutrient digestibility compared to CON (P < 0.05). Percentages of IVTD, IVTDDM, IVTDNDF, and IVTDOM were 71.6 vs. 74.2 ± 1.5; 68.7 vs. 71.6 ± 1.6; 19.7 vs 27.1 ± 4.2 and 59.6 vs. 63.4 ± 2.3 for KMFD vs. CON, respectively. Compared to time-zero, Xyl EA linearly decreased (1.9 vs 1.4 ± 0.1) while Cel EA increased (3.1 vs 4.1 ± 0.1) after 2h (P < 0.05) over time. Initial Amy EA was maintained by 12h (P > 0.05) and increased at 24h (3.3 vs 4.0 ± 0.2, P < 0.05). In conclusion, observed reduction in the rumen-free ammonia-N and alterations in some rumen fermentation patterns and EAs with KMFD supplementation suggest an effect of degradable portion of its HMBi concentration on nutrient utilization under *in vitro* rumen conditions.

KEYWORDS:

HMBi, rumen, enzyme