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Advancing recovery strategies for 2-hydroxy-4-methylthiobutanoic isopropyl esters (HMBi): long-term stability of HMBi in pelleted concentrate feed

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Methionine analogs offer an alternative to encapsulated forms due to their stability during handling or thermo-physical processing. While HPLC UV has been used to assess the stability of isopropyl esters of hydroxy methionine analogues (HMBi) in pelleted feed, the need for a more precise and efficient approach has emerged due to challenges in recovery from complex feed matrices and labor-intensive in/ex vivo methods. Building on our previous studies, this research aimed to develop an optimized extraction and analysis method based on liquid chromatography-mass spectrometry (LC-MS/MS) to evaluate HMBi in pelleted concentrate feed over 110 days. A high concentrated liquid HMBi product (≥97% HMBi, KESSENT® MF (KESS), Kemin Animal Nutrition and Health, Belgium) was incorporated at 3 kg/ton into a grain- and soybean-based feed containing 18% crude protein prior to pelleting. The basal diet (without product) was also pelleted and served as a control. Concentrations of HMBi were analyzed on day 19th (short term, n = 1) and 110th (long-term, n = 3) of storage at 16±4 °C. Liquid extraction was followed by LC-MS/MS analysis. Method capabilities were tested using replicate analyses and a mixed spike approach with final product and hydroxy methionine analogue (2-hydroxy-4-methylthiobutanoic acid, HMBa) and HMBi standards. The method showed high linearity ($R^2 \geq 0.99$) for both neat solvent and pelleted feed extract with minimal matrix effect. Pre-extraction laboratory spike recoveries of the liquid HMBi product on the basal diet confirmed complete HMBi recovery from the pellet feed matrix (104.8% on d19 and 107.6±2.4% on d110). As an innovative part of the LC-MS/MS based approach, concurrent HMBa and HMBi analyses quantified the hydrolysis of HMBi into HMBa under the storage conditions. During storage, the intact HMBi recovery decreased with 4.8%, while hydrolysis into HMBa remained minimal (0.5% increase). The developed LC-MS/MS method enabled full HMBi recovery from the pelleted feed matrix. Furthermore, it showed that hydrolysis of HMBi into HMBa in KESS was marginal during storage of pelleted feed that was treated with liquid HMBi product at 3 kg/ton. These findings confirm the stability of liquid HMBi in KESS in pelleted feed over long-term storage, demonstrating the effectiveness of this methodology.

KEYWORDS:
HMBi, Pelleted feed, Methionine