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IN VITRO EVALUATION OF HYDROPONIC BARLEY FODDER AS A DIETARY STRATEGY TO MITIGATE ENTERIC METHANE EMISSIONS AND MAINTAIN RUMEN DIGESTIBILITY

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Livestock agriculture faces the challenge of reducing enteric methane (CH4) emissions without compromising animal performance and feed efficiency. Hydroponic barley fodder (HBF) is a biomass characterized by high enzymatic activity and readily fermentable carbohydrates. This study evaluated the *in vitro* CH4 mitigation potential and apparent organic matter digestibility (IVOMD) of HBF when replacing standard dietary components at two physiological maturities: Day 4 (HBF1) and Day 6 (HBF2). The Hohenheim Gas Test was utilized to simulate rumen fermentation during a 24-hour incubation. Treatments included a standard control diet (CTR; 60% hay, 40% concentrate), pure HBF substrates, and mixed diets where HBF replaced either the hay fraction at graded levels (LOW, MED, HIGH, REPL) or the concentrate fraction (10% and 20% inclusion). A quadrant analysis comparing treatments to the CTR baseline demonstrated that eight dietary formulations achieved the targeted criteria of simultaneously lowering CH4 emissions and improving overall digestibility. Specifically, pure HBF substrates exhibited significantly higher digestibility compared to the CTR (60.08%). HBF1 recorded an IVOMD of 73.56% due to its high content of readily available plant starch. As the plant matured to Day 6, HBF2 IVOMD naturally decreased to 67.99% due to the development of structural fiber. Correspondingly, both maturities successfully lowered the CH4/-CO2 ratio, achieving reductions of 5.8% and 9.0% respectively, when compared to CTR. In the hay-substitution diets, higher inclusion levels of both HBF1 (MED, HIGH, REPL) and HBF2 (HIGH, REPL) consistently shifted the fermentation into the targeted quadrant compared to the control. To statistically validate this relationship, a correlation analysis (n = 130) was performed, revealing a significant negative overall correlation between the CH4/CO2 ratio and IVOMD (Pearson r = -0.557, p < 0.001). This confirms that treatments improving digestibility quantitatively associate with reduced methane output. Furthermore, significant negative correlations were confirmed within the pure HBF1 (r = -0.791, p = 0.011) and HBF2 (r = -0.793, p = 0.019) substrates, as well as in specific HBF1 inclusion levels (LOW, REPL, and LC20). The decrease in CH_4 emissions seen in high-HBF diets is likely due to the rapid fermentation of plant starches. *In vitro* findings suggest that substituting fibrous forage with hydroponic forages at certain maturity stages may help reduce CH_4 emissions; however, further *in vivo* studies are needed to confirm these effects and determine optimal inclusion levels.