***In vitro* fermentation characteristics of four agro-industrial protein by-products compared to soyabean meal**

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**Application**

The alternative to soyabean meal agro-industrial by-products can be used in animal diets due to the reduced embodied carbon footprint but a further reduction in methane production was not confirmed in the present study.

***Introduction***

Soyabean meal (SBM) is one of the most common protein feeds in animal rations due to its high nutritional value and commercial availability (Sasu-Boakye et al., 2014). However, there is concern over the environmental impact of growing soya (land use and degradation, water consumption, and transportation costs (Song et al., 2021). Agro-industrial co-products made from locally grown crops likely carry a lower embodied carbon footprint than SBM and, if high in unsaturated fatty acids, may directly reduce enteric methane emissions. Given the recent concerns around methane emissions from livestock production, most being from rumen fermentation, and the strong effect of animal diet on such emissions, the potential of new feeds for reducing methane emissions is also essential to assess their overall sustainability. The present study aims to assess *in vitro* the effect of four agro-industrial by-products (maize residue (MR), wheat distillers’ grains (WDG), brewers’ spent grains (BG), and corn steep liquor (CSL)) on gas and methane production, compared to SBM.

***Material and methods***

Four food industry by-products either dried and ground (MR; WDG; BG; and SBM as control treatment) or liquid (CSL), were incubated with dried and ground grass silage and wheat in Wheaton flasks, giving a total of 1.0 g of total mixed ration (TMR), with a protein content of 40 g/kg DM. Negative control flasks with no TMR substrate were also included. In each flask, 90 mL buffer and 10 mL rumen fluid were mixed, followed by sealing and incubation at 39 °C. Total gas volume was measured using headspace gas pressure at 2, 4, 6, 8, 10, 12, 24, 32, 48, and 72 h. The experiment was run three times. Gas pressure readings were used to calculate gas volume and a sample (10 mL) of gas was collected for methane via the port valve for analysis using gas chromatography (Bruker 450-GC). At 72 h, the flask content was filtered, and the residue was oven-dried at 100 °C for 4 h. In vitro Dry Matter Digestibility (IVDMD; g/kg) was computed based on residual DM. Curves were fitted to the cumulative production data. The filtrate underwent VFA analysis using GC. Data were analysed (RStudio) using a general linear mixed model analysis of variance that included treatment as a fixed effect and the run as a random effect. Tukey’s Honestly Significant Difference test was used for pairwise comparisons where treatment effects were significant (*P*<0.05).

***Results***

Cumulative gas and methane productions expressed as ml/DM or /digested DM after 72h, and the methane production as % total gas production did not differ (*P*>0.05). IVDMD was lower (*P*<0.001) in the BG compared to all other treatments, and in the MR compared to CSL. The fractional rate of degradation (/h), estimated at 50% of asymptote, was lower (*P*<0.001) in the BG, WDG, and MR compared to SBM, and CSL. The extent of degradation (%) estimated at 0.04/h and 0.025/h passage rates, was lower (*P*<0.01) in the BG than in the other treatments. Rumen fluid VFA profile parameters were not significantly affected by the treatment (*P*>0.05).

***Conclusions***

Despite the lower embodied carbon footprint of the tested agro-industrial by-products, compared with soyabean meal, the overall cumulative gas and methane production between them, was not significantly different. Brewers’ spent grains had lower *in vitro* dry matter digestibility, but this may not raise concerns because previous animal studies using brewers’ grains in beef and lactating dairy cow diets, did not show any adverse effect on animal productivity and feed digestibility (Moate et al., 2011; Duthie et al., 2015).

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