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***Dose-response effects of diets partially replaced by food processing industrial by-products on in vitro ruminal fermentation and methane production***

**Application:** By-products derived from the food processing industry have the potential to replace the conventional ingredients in ruminant feed, as well as improve production performance, energy and nitrogen utilization efficiency, reducing methane emissions and thereby making farms more effective and environmentally friendly.

**Introduction:** There are 1.3 billion tons of food lost or wasted every year around the world, approximately one-third weight of global food production (Calicioglu et al., 2019). In the UK, the quantity of food waste without recycling reaches 15 million tonnes annually (234 kg/person/year or 50% of food), nearly half of which is disposed of in landfilling (Wrap, 2015). It is globally acknowledged that addressing the environmental impact of their disposal is imperative. Simultaneously, there is a pressing challenge in the resource competition between food for human consumption and feed for animal. The resolution to transform the waste into feedstuff for livestock is becoming an increasingly prevailing trend. Prior to feeding ruminants with by-products, more accurate information is needed on their nutritive value and potential to reduce emissions. Therefore, the aim of this study was to explore *in vitro*, the effects of various by-products from the food processing industry on rumen fermentation.

**Material and Methods:** Materials of different by-products, including red apple pomace(AP), green apple pomace(AP2), hempseed cake(HC), coffee hulls(CH), coffee grounds(CG), spent mushroom compost (SMC) and distillers Dried Grains with Solubles (DDGs), were collected from various food processing industry in N. Ireland, freeze-dried and ground at 1-mm screen 2. Tested by-products were incubated *in vitro* in doses of 100, 200, 300 g/kg. These amounts replaced equal amounts of the mixed ration (500 mg), which was composed of grass silage and concentrate (70:30). AP, AP2, CH, CG and SMC were designed to replace the silage portion, while HC and DDG were designed to replace the concentrate portion. Incubations were done anaerobically at 39 °C for 24h in jars containing buffered rumen fluid from 3 dairy cattle from the abattoir. Gas production, pH, methane(CH4) concentration in headspace gas samples, ammonia(NH3-N) and volatile fatty acids (VFA) in the fermentation medium, were measured at 24h. Each of the 21 treatments (7 by-products × 3 doses) was repeated in two independent *in vitro* runs. For statistical analysis, all data were analyzed by the SPSS (Version 20.0, IBM). A linear mixed model (LMM) was used to evaluate the effects of by-products on rumen fermentation, with by-products, dose and interaction as fixed effects, run as random effect. General linear model (GLM) was used to compare the by-products effect within the dose considering run as random factor. Linear and non-linear effects of dose within test by-products were evaluated by orthogonal polynomial contrasts.

**Results:** After 24 h fermentation, based on LMM, gas production, pH and NH3-N were significantly affected by different by-products and doses (*P< 0.001*), while tendency were found in CH4 production affected by doses. There were no significant effects on VFA caused by dose(*P> 0.05*) but by-products (*P< 0.001*), and the CG and SMC have the lowest concentration among all sorts of VFA. For GLM, CH4 production decreased linearly with the increases in doses of all by-products, *(P < 0.05)*, while for CH4 production per unit of degradable dry matter, the AP2, HC, CH, CG and SMC didn’t show linear trends *(P > 0.05)*. The CH4 production decreased by 29%, 33.1%, 33.9%, 31.0%, 30.4%, 27.6% and 28.9% at the dose in 100-300 g/kg, when diets were replaced with AP, AP2, HC, CH, CG, SMC and DDGS, respectively. Gas production and *in vitro* dry matter degradability respond similarly to CH4 production (*P< 0.001*), except the AP and AP2 *(P > 0.05)*. Doses had significant impact on NH3-N concentration in nearly all by-products, often leading to a positive linear dose response (*P< 0.001*), while AP2 had a quadratic response (*P< 0.001*).

**Conclusions:** Based on our *in vitro* study, the by-products tested were proved to be a potential option for replacing conventional feed ingredients but not exceeding the dose at 200 g/kg, especially apple pomace and hempseed cake, making it promising to apply these by-products on ruminant feed. Further *in vivo* studies are needed to be done to confirm these results on CH4 production, but also explore the effect on the quality of animal products (milk. etc.).

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**References**

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