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THE EFFECT OF EXOGENOUS ENZYME SUPPLEMENTATION ON METHANE EMISSIONS OF SELECTED FEEDSTUFFS DETERMINED USING AN IN VITRO FERMENTATION METHODOLOGY

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Abstract

Using selected raw materials from the New Zealand dairy industry, 14 separate trials were conducted using a closed *in vitro* gas production system to determine differences in digestibility and the amount of methane produced both per kilogram of dry matter (DM), and per kilogram of digestible DM when one of two proprietary exogenous enzyme preparations were added. Methane production was determined by stoichiometric calculation from volatile fatty acid production.

The addition of enzymes resulted in numerically lower methane production on both DM and digestible DM basis in 70% of the samples. On a DM basis, the methane difference was highly significant ($p<0.01$) among four out of ten of these samples, and one sample was significant at $p<0.05$, ranging from 11-35% lower per gram of DM. All five of these were also highly significant differences when considering the methane production on a digestible DM basis ($p<0.01$), ranging from 19-43% less methane per gram of digestible DM.

No significant difference was found in the four treatments that showed numerically greater methane production.

The results reported here support the hypothesis that exogenous enzyme addition to ruminant diets may help lower methane emissions from cattle. The data reported provides a platform for the design of further *in vitro* work as a further pre-screen before engaging *in vivo* work. Investigating different enzyme addition levels will help better understand the optimum level of enzyme addition to the ruminant's diet.

Introduction

There is currently a very large focus on greenhouse gas (GHG) from agriculture in New Zealand. Agriculture is said to contribute 48% of New Zealand's GHG profile, and a large part of this is from enteric methane production from the rumen (Ministry for the Environment, 2020). Research has focussed on identifying and understanding any nutritional influences on methane production.

Enteric production of methane is determined in part from the ruminal fermentation of dietary raw materials. Considerable effort is being expended by industry looking at a range of zootechnical additives in ruminant diets to reduce the amount of dietary energy converted to enteric methane. A first step in discovering nutritional mitigation options is to use an *in vitro* digestibility assay to pre-screen compounds for potential use.

Traditionally, exogenous enzyme supplementation to animal rations has been widespread in monogastric diets and has not had a large focus in ruminant nutrition. However, enzyme addition to ruminant diets has been investigated from as early as 1998 (e.g., Heintzelman, 1998).

Enzymes are thought to be rapidly degraded by microbes in the rumen. However, natural glycosylation (Wu and Wang, 2001, van de Vyver *et al*, 2003) of fungal enzymes such as Fibrozyme® (FZ, Alltech Inc.) mitigates microbial degradation.

Enzyme addition work in ruminants has largely focussed on direct dry matter digestibility measurements. The present work goes further and examines some of the end points of ruminal digestion.

Methodology

Seven samples of canola, four of distillers dried grains with solubles (DDGS), two of palm kernel expeller (PKE) and one of grass seed offal (GSO) were gathered from the New Zealand marketplace.

Test materials were dried and subjected to an *in vitro* fermentation in a closed gas production system. Test materials were fermented with or without one of two proprietary enzymes, either FZ or a multi-activity complex produced by a solid-state fermentation process, Allzyme SSF® (ASSF, Alltech Inc).

To account for weekly variation in donor rumen fluid, a known standard ration with known fermentation characteristics was fermented as part of each weekly fermentation to provide a weekly correction factor that was applied to metrics determined.

Donor rumen fluid was taken from a lactating dairy cow fed a pasture-based ration.

Dried samples of test raw materials (0.5 g) ground to a 2 mm size were incubated at 39^{o}C using a rumen-buffered inoculum for 48h (Mould *et al*, 2005). Rumen fluid to buffer ratio was 20:80.

During the incubation period, gas production was measured continuously using an automated pressure transducer system (IFM, Alltech Laboratory, Auckland, NZ).

True *in vitro* digestible dry matter (TDMD, %) was determined by substrate disappearance after 48 hours incubation (Menke *et al*, 1979) and then correcting this for microbial growth. Microbial growth was determined according to the method of Goering and Van Soest (1970).

VFA concentrations were measured by gas chromatography (Erwin *et al*, 1967) on samples taken at 48h of incubation. The stoichiometry of Wolin (1960) was used to estimate methane production based on VFA production.

Results

A graphical representation of methane production (Figure 1) shows an overall trend towards reductions in methane production with the addition of enzymes. Methane production was numerically lower in 10 out of 14 samples, with statistical significance ($p<0.05$) in one sample and a highly significant difference (<0.01) in four samples.

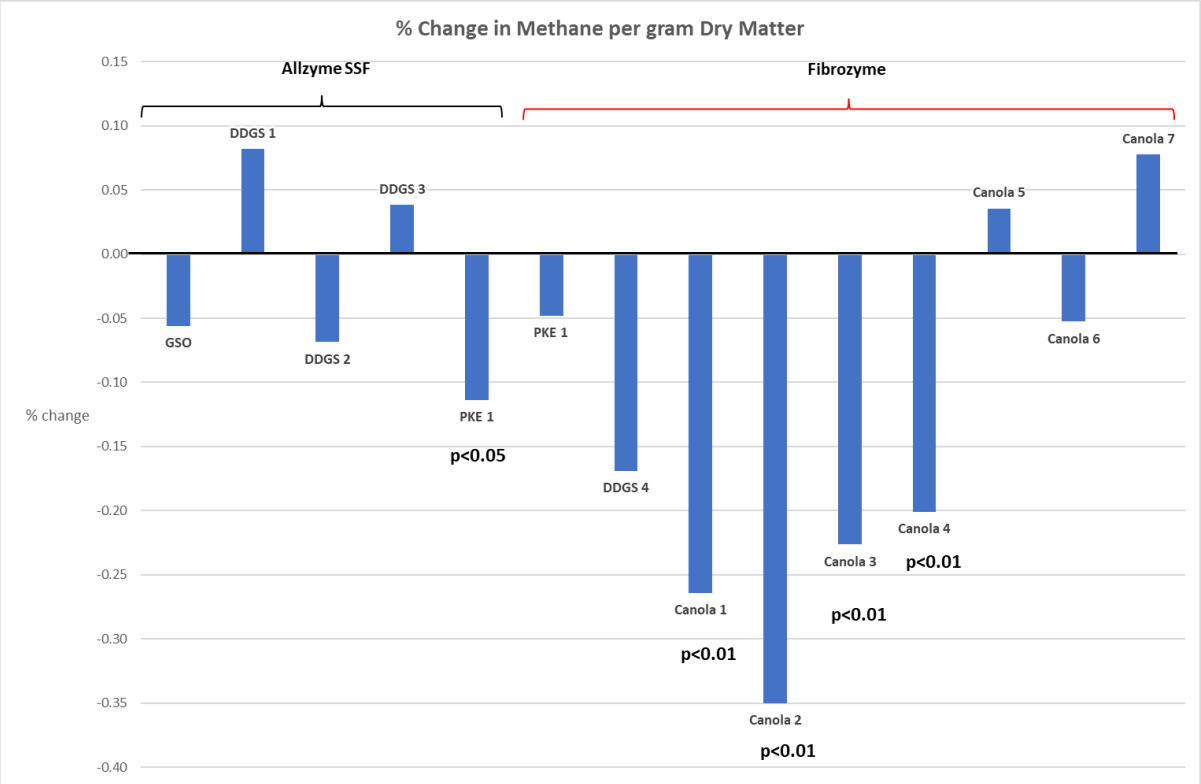

Figure 1. % Change in methane production calculated from volatile fatty acid production in 14 *in vitro* trials of exogenous enzyme addition to selected raw materials.

A key objective in enzyme supplementation is to improve digestibility. Digestibility changes were mostly not significant in the present set of experiments (Table 1) with only two trials reaching a degree of highly significant ($p<0.01$) and one trial reaching significance ($p<0.05$).

When methane production (g CH_4/kg DM) was converted to a true digestible dry matter basis there were no changes to the trials that had significant differences, however the single PKE sample that had significance on a dry matter basis at $p<0.05$ was highly significant ($p<0.01$) when methane production was expressed on a true digestible dry matter basis.

		CH_4 g/kgDM		TDMD (%)		CH_4 g/kgDigDM	
Raw Material	Enzyme	Control	Treatment	Control	Treatment	Control	Treatment
Grass Seed	ASSF	16.23	15.37	64	63	25.30	24.01
DDGS 1	ASSF	13.22	14.40	77	80	17.15	17.95
DDGS 2	ASSF	15.72	14.71	75	76	20.85	19.16
DDGS 3	ASSF	13.27	13.79	80	80	16.50	16.76
PKE 1	ASSF	16.18^a	14.52^b	60	64	27.06^a	22.68^c
PKE 2	FZ	15.32	14.62	75	79	20.34	18.44
DDGS 4	FZ	16.80	14.37	85	87	19.49	16.46
Canola Meal 1	FZ	23.32^a	18.45^c	84^a	93^c	27.55^a	19.71^c
Canola meal 2	FZ	21.06^a	15.6^c	86^a	91^c	24.49^a	17.12^c
Canola meal 3	FZ	26.23^a	21.39^c	87	86	30.05^a	24.78^c
Canola meal 4	FZ	30.08^a	25.05^c	84	84	35.44^a	29.67^c
Canola meal 5	FZ	20.72	21.48	75	75	27.48	28.58
Canola meal 6	FZ	21.76	20.67	76	75	28.51	27.39
Canola meal 7	FZ	17.98	19.50	81^a	84^b	21.99	23.10

Table1. Methane production on a DM basis, True dry matter digestibility, methane production on a true digestible dry matter basis. Superscripts a, b between control and treatment denotes $p<0.05$, superscripts a, c between control and treatment denotes $p<0.01$.

Discussion

In terms of methane production, most of the differences, both by numerical average and the proportion of trials with a negative difference in methane production, came from the FZ treatment.

Enzyme supplementation of ruminant diets is usually targeted at fibre digestibility. The exogenous enzyme FZ is a fibrolytic enzyme which targets the cell wall structure, particularly cellulose and xylan. Increasingly opening the structure of fibres with enzymes will allow increased microbial access and attachment to the substrate in the rumen.

The composition of plant cell walls is specific to each plant species and canola and PKE can both contain more than 50% cellulose (Wickramasuriya *et al*, 2015 and Noorshamsiana *et al*, 2017) with cellulose being a target of FZ. In the present report, FZ addition to the *in vitro* system showed differences from control in terms of methane and digestibility in some trials.

In the current set of trials, only 4/14 samples showed highly significant differences in digestibility with enzyme addition. Other workers in ruminants have also produced a range of results.

Morris and DeBreaux (2002) also worked with the FZ used in this trial and found higher DM digestibility of a TMR diet both *in vitro* ($p<0.01$) and *in vivo* ($p<0.05$).

Although not direct digestibility studies, Ware *et al.* (2002) found greater growth rates ($p<0.05$) in beef cattle supplemented with FZ. Similarly, Wu and Wang (2001) found addition of FZ resulted in higher FCM milk production ($p<0.05$) in Holstein Friesian cows. Del Pino Caicedo

and Hollwg (1999) found greater fat corrected milk production ($p<0.05$) on cows fed tropical grass silage supplemented with FZ, but no liveweight gain or condition score difference for cows fed either tropical pasture silage or corn silage.

Heintzelman (1998) reported field observations that 13 from 15 commercial dairy herds supplying a mid-Atlantic dairy co-operative in the USA showed positive improvements when FZ was added to their mixed rations. Howes (1999) found that FZ tended to result in higher milk yield (7.08%; $p=0.08$) over a 63-day trial.

Dry matter digestibility is associated particularly with neutral detergent fibre (NDF) digestibility in the case of ruminant animals. An increase in NDF digestibility results in increased VFA production which are precursors to milk production and body fat.

Because propionate functions as an alternative hydrogen sink in the rumen, it is expected that any change in the proportion of propionate to the combination of acetate and butyrate will result in altered methane production. Table 2 shows that in the present 14 trials, seven from nine cases of supplementation with FZ, and in three from five cases of supplementation with ASSF, resulted in higher P:(A+B) ratios.

Raw-Material	Enzyme	Propionate: (Acetate+Butyrate)	
		Control	Treatment
Grass Seed	ASSF	0.46	0.46
DDGS 1	ASSF	0.80	0.73
DDGS 2	ASSF	0.74	0.77
DDGS 3	ASSF	0.41a	0.53c
PKE 1	ASSF	0.63a	0.61b
PKE 2	FZ	0.58	0.58
DDGS 4	FZ	0.79	0.82
Canola Meal 1	FZ	0.70a	0.65b
Canola meal 2	FZ	0.72a	0.66c
Canola meal 3	FZ	0.39a	0.45c
Canola meal 4	FZ	0.40a	0.45b
Canola meal 5	FZ	19.22	21.0
Canola meal 6	FZ	20.32	20.79
Canola meal 7	FZ	21.51	23.99

Table 2. ratio of Propionate to combined acetate and butyrate for various feedstuffs when treated with exogenous enzymes. Superscripts a, b between control and treatment denotes $p<0.05$, superscripts a, c between control and treatment denotes $p<0.01$.

The higher ratio of propionate relative to the combined acetate and butyrate productions is supported by Ranilla *et al.* (2008) reporting decreasing acetate:propionate ratios when FZ was used *in vitro*.

Tirado-Estrada *et al.* (2011) found higher molar proportions of propionate and decreased molar proportions of butyrate and acetate when FZ was fed to lambs. Giraldo *et al* (2008) found no change in total VFA but higher molar proportions of propionate and decreased molar proportions of butyrate and acetate when FZ was fed directly into cannulated sheep.

Overall, the current set of 14 trials show encouraging results when examining differences between raw materials with and without the addition of exogenous enzymes, particularly FZ.

Future work will focus on further understanding the partitioning of dietary dry matter to different VFAs and will also focus on addressing graded doses of enzymes, and the application of enzymes in total rations.

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