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# THE RELATIONSHIP BETWEEN DIETARY INCLUSION OF WHEAT AND PALM KERNEL AND METHANE EMISSIONS DETERMINED USING AN IN VITRO METHODOLOGY

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## Abstract

There has been a lot of interest in determining greenhouse gas (GHG) emissions from ruminant feedstuffs, whether individual raw materials or complete rations that include both pasture and supplements.

Previous work by Meads *et al* (2019) showed a significant change in methane emissions with increasing levels of concentrates in the ration. The current work looked to see if this pattern could be repeated with different diets, and specifically introducing wheat and Palm Kernel Expeller (PKE) to the diet, using an *in vitro* fermentation model.

Unlike the previous study (Meads *et al*, 2019) the total volatile fatty acid (VFA) production was unaffected by the level of concentrate in the ration. However, acetate, propionate and butyrate as a molar proportion of total VFA production changed significantly in a linear fashion with increasing concentrate inclusion. When using a VFA stoichiometric model (Wolin, 1960) as a proxy for methane measurement, significant changes in methane production were associated with the inclusion of higher levels of concentrates ($p<0.05$).

Propionate acts as an alternative $H^+$ ion sink in the rumen, therefore reducing the production of methane. These results would indicate that ruminal digestion of diets with a component of concentrates added in the form of wheat and PKE changed propionate production to significantly impact methanogenesis in an *in vitro* fermentation situation.

## Introduction

There is currently a very large focus on GHG from agriculture in New Zealand and across the world. 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). Recent 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. It has been observed that inclusion of cereal grains into a diet can result in lower methane yield from the diets fed ruminants, with wheat producing the most pronounced effect when compared to corn or either single rolled or double rolled barley (Moate *et al*, 2017). Increasing cereal components in a ration will increase the starch content of such rations, and increasing starch content of diets has been postulated as a legitimate strategy for lowering enteric methane yields, the effect being attributed to an alteration of bacterial populations resulting in increased propionate production (Darabighane *et al*, 2021).

The present *in vitro* work tested the addition of starch to pasture in a similar fashion to Meads *et al* (2019), this time relying on wheat rather than maize silage as a source of starch.

## Methodology

Three mixed rations (100%, 70% and 55% pasture - plus corresponding amounts of a mix of wheat and PKE, 0%, 30% and 45%) were fermented in a closed *in vitro* system for 48 hours. VFA production and total gas production were determined as indicators of ruminal digestion.

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

The mixed rations were dried and samples of treatment rations (0.5 g) ground to a 2 mm size were incubated at 39ºC using a rumen-buffered inoculum for 48h (Mould *et al*, 2005). Rumen fluid to buffer ratio was 20:80. 6 replicates per treatment were used.

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

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 (Figure 1).

$$CH_4 = a + 2b - CO_2$$

$$CO_2 = a/2 + p/4 + 1.5b$$

Where a = acetate
b = butyrate
p = propionate

**Figure 1. The stoichiometry of Wolin (1960).**

## Results

The molar amounts of the various VFAs were determined and are presented in Table 1.

| Pasture in diet (%) | Combined wheat and PKE in diet (%) | Starch (%) | Propionate (p) | Acetate (a) | Butyrate (b) | Methane (mL/gDM) |
|---|---|---|---|---|---|---|
| 100 | 0 | 0.62 | 29.92b | 52.91a | 13.72c | 26.61[a] |
| 70 | 30 | 3.06 | 32.48a | 49.21b | 14.91b | 23.81[b] |
| 55 | 45 | 4.41 | 33.20a | 47.74c | 15.73a | 22.41[b] |

**Table 1. Production of propionate, butyrate and acetate, and calculated methane emission. Differing superscripts within a column denote p<0.01.**

## Discussion

Propionate acts as an alternative hydrogen ion sink in the rumen, meaning the production of propionate competes directly with methane production in the rumen (Ellis *et al*, 2012; Baldwin, 1983). It is expected that any change in the proportion of propionate (p) to the combination of acetate (a) and butyrate (b) will result in altered methane production.

Table 1 shows that in the present work the VFA production profile changed significantly with the inclusion of starch, via the inclusion of a wheat/PKE concentrate mix. These results support the idea that concentrate feeding to supplement pasture can produce a lower enteric methane yield per kg of dry matter consumed.

Because imported feedstuffs have an embedded carbon cost in them from their own production, a life cycle analysis style of assessment may help find the optimum use of supplements in a pasture-based environment from a carbon accounting view.

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