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Influence of monensin and lauric acid distillate or palm oil on *in vitro* fermentation kinetics and metabolites produced using forage and high concentrate substrates

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ABSTRACT

Two *in vitro* experiments were conducted to evaluate the interactions of monensin with lauric acid distillate (LAD) or palm oil (PO) in forage and concentrate diets on 24 h *in vitro* rumen fermentation characteristics. Two Holstein steers, each surgically fitted with a ruminal cannula, consuming 0.50 alfalfa cubes and 0.50 cracked corn-based concentrate at 1.75 × NE_m requirements were used as rumen fluid donors. For both experiments *in vitro* gas production was measured in a completely random design with a 3 × 2 × 3 factorial treatment structure. Factors were diet [control (no substrate), forage (alfalfa), and high concentrate (corn)], 3 μg/ml of monensin (±), and LAD (0, .05 and 0.10 of the substrate, Exp. 1) or PO (0, .05 and 0.10 of the substrate, Exp. 2). Gas production was affected by diet ($P < 0.001$), and decreased ($P < 0.001$) for monensin addition in both experiments. Gas production had a diet × monensin interaction ($P < 0.01$). Lauric acid distillate and PO addition did not influence gas production. Degradation rate also responded with a diet × monensin interaction ($P < 0.01$) and lag time had only a diet effect ($P = 0.001$) in both *in vitro* experiments. Both the degradation rate and lag time were not affected by a monensin × fat (LAD or PO) interaction. Monensin addition reduced total volatile fatty acid concentration across all 3 substrates (control, alfalfa and high concentrate). Monensin addition decreased acetate:propionate ($P < 0.001$) and increased the propionate proportion ($P < 0.001$). Butyrate proportion had a diet × monensin interaction ($P < 0.01$) as butyrate proportion was increased with monensin for the alfalfa but decreased for the concentrate substrate. Ammonia concentration was reduced ($P < 0.01$) by monensin on all substrates and was slightly higher ($P < 0.01$) for the alfalfa substrate compared with control and concentrate. Lauric acid distillate or PO addition did not influence fermentation metabolites in the present study. In addition, a monensin by LAD or PO interaction was not observed for the metabolites. Methane production and the methane proportion to total gas production had a diet × monensin interaction ($P < 0.01$). The methane proportion was decreased quadratically ($P = 0.028$) with LAD addition, whereas PO addition linearly increased ($P = 0.014$) the methane proportion; both of these effects were small. Meanwhile, monensin by LAD or PO interactions were not observed for methane concentration and proportion. In conclusion, the combinations of monensin and LAD or PO in forage and high concentrate diets had no interactions on *in vitro* fermentation kinetics and end product production.

Abbreviations: ADF, acid detergent fiber; A:P, acetate:propionate ratio; CP, crude protein; DM, dry matter; LAD, lauric acid distillate; NDF, neutral detergent fiber; NE_m, net energy for maintenance; PO, palm oil; VFA, volatile fatty acid.

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1. Introduction

Monensin sodium is the predominant ionophore fed to ruminants for the purpose of modifying ruminal fermentation. Monensin is highly lipophilic and soluble in a range of organic solvents but is only slightly soluble in water. Monensin functions in the aqueous rumen environment by locating within the lipid membranes of microbes. The effects of monensin include a decrease in methane production (Narvaez et al., 2013), a decrease in protein degradation to ammonia (Dinius et al., 1976; Narvaez et al., 2013) and an increase the production of propionic acid as well as prevention of acidosis, coccidiosis, and bloat (Matsuoka et al., 1996). Previous investigations have not fully resolved whether an interaction exists with monensin and the source of fat. The experiments have found no interaction (Zinn, 1988), or the monensin response was reduced in the presence of fat (Clary et al., 1993). It stands to reason that based on the lipophilic nature of monensin, this interaction may be affected by source of fat.

Palm oil (PO) is a principal vegetable fat with a rich content of vitamins and antioxidants. Palm oil and its by-product, lauric acid distillate (LAD), are major contributors to the world oil and fat production, accounting for .30 of the total world oil output (MPOC, 2006). Palm oil industries provide by-products such as palm kernel cake which are suitable for use in animal feeds. Lauric acid distillate is the by-product which results from the distillation process to obtain purified plant oils from palm kernel and coconut. Although the use of these fat sources may benefit the entire animal feed industry by reducing the cost of feeding, they have rarely been applied to ruminant animals as a feed additive with monensin. Lauric acid distillate is composed of medium-chain saturated fatty acids, whereas PO is composed of long-chain saturated and unsaturated fatty acids. Both fats are solids at room temperature and our hypothesis was that the fatty acid composition of the fat will differentially affect how monensin alters the fermentation. Therefore, the objective of this study was to compare fermentations in the presence of monensin with PO and LAD in diets with differing forage:concentrate to determine how monensin interacts in these altered environments.

2. Materials and methods

2.1. Experimental design

Two *in vitro* experiments were conducted to determine the interactions of monensin with LAD or PO. Both experiments incorporated a control (no substrate), forage (alfalfa) and high concentrate (corn) substrate with and without monensin (monensin sodium, Elanco Animal Health, Greenfield, IN, USA).

The experimental design for each experiment was completely random with a $3 \times 2 \times 3$ factorial treatment structure. Factors were diet (control, forage, and high concentrate), 3 μg/ml of monensin (±), and level of LAD or PO (0, 50 and 100 g/kg of the substrate). Each fat source was evaluated in a separate experiment which included 18 treatments. Each fermentation (LAD or PO) was conducted in duplicate and replicated on 5 separate days ($n = 5$ for statistical analysis).

2.2. Fermentation preparation

Ruminal contents were collected from two ruminally-fistulated steers consuming 500 g/kg alfalfa cubes and 500 g/kg cracked corn-based concentrate. The diet was fed to supply $1.75 \times NE_m$ requirements of the steers in two equal portions at 0700 and 1700 daily (Table 1).

Table 1
Composition of diet used for the ruminal fluid donor steers, and used for *in vitro* fermentation.

Items	g/kg
Ingredient, as-fed basis	
Alfalfa hay cubes	500.0
Cracked corn grain	440.9
Soybean meal	36.4
Fat	11.0
Limestone	6.3
Trace mineral premix[a]	4.9
Vitamin premix[b]	0.5

Composition, DM basis	Alfalfa cubes	Concentrate
Crude protein	165	114
Neutral detergent fiber	519	137
Acid detergent fiber	372	64
Calcium	16.7	5.1
Phosphorus	1.7	3.2
NE_m, Mcal/kg	12.4	20.1

[a] Composition (g/kg): NaCl, 940; Zn, 5.5; Fe, 9.3; Mn, 4.8; Cu, 1.8; I, 0.1; Se, 0.1; Co, 0.1.
[b] Composition (IU/kg): Vitamin A, 1,818,182; D, 363,000; E, 227.