



Effects of fumarate on ruminal ammonia accumulation and fiber digestion in vitro and nutrient utilization in dairy does¹

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ABSTRACT

The objective of this study was to evaluate effects of fumarate on ruminal ammonia accumulation and fiber digestion in vitro and on feed intake and nutrient utilization in dairy does. Batch cultures of mixed rumen microorganisms were used to study effects of different concentrations of fumarate on fermentation with various N sources (ammonia as ammonium bicarbonate, casein amino acids, casein peptides, gelatin peptides) and feeds (bermudagrass hay, mixed diet of 60% bermudagrass hay plus 40% concentrate) for 6 and 24 h, respectively. Substrates were grouped into pairs for separate incubations. Monosodium fumarate was added to incubation tubes to achieve final concentrations of 0, 5, and 10 mM fumarate. More ammonia accumulated at the end of incubation with added ammonium bicarbonate. Ammonia concentration was higher for peptide compared with amino acid incubation, and for casein peptide compared with gelatin peptide. Addition of fumarate linearly decreased ammonia for all N sources and for feed substrates. For all substrate types, fumarate treatment increased acetate, propionate, and total volatile fatty acids (VFA), decreased acetate to propionate ratio, and tended to reduce branched-chain VFA. Digestion of feed neutral detergent fiber (NDF) by rumen microorganisms was improved by fumarate along with elevated endoglucanase and xylanase activities. In an animal metabolism experiment, 8 dairy does (4 per treatment) were used in a completely randomized design for 21 d. Does were fed a hay plus concentrate diet without (control) or with fumarate (6 g/head per day) supplementation to determine feed intake, whole-tract nutrient digestibility, and N utilization. Fumarate treatment did not affect weight change or feed intake but increased whole-tract digestion of gross

energy, crude protein, and cellulose. Digested N was increased by fumarate supplementation; however, N retention was unaffected. Plasma glucose concentration was elevated with fumarate but urea N concentration remained unchanged. Fumarate addition had significant effects on rumen microbial fermentation by decreasing ammonia and branched-chain VFA, and by increasing acetate and propionate, and NDF digestion. These effects were reflected in the improvement in whole-tract gross energy, crude protein, and cellulose digestion and elevated plasma glucose concentration when dairy does were supplemented with fumarate.

Key words: fumarate, rumen, ammonia, fiber digestion

INTRODUCTION

Fumarate is a key intermediate in rumen microbial metabolism. Fumarate is reduced to succinate, which is then decarboxylated to propionate (Asanuma et al., 1999; López et al., 1999). During this process, hydrogen is consumed with the production of carbon dioxide. When hydrogen is used to reduce fumarate, less is available for methanogenesis. By an alternative pathway, fumarate is metabolized to acetate, but to a much smaller extent than it is converted to propionate (Demeyer and Henderickx, 1967). Thus, ruminal fermentation of fumarate could increase both acetate and propionate, although a decrease in acetate to propionate ratio often results (Asanuma et al., 1999; Carro and Ranilla, 2003).

Methane is an energy-rich compound but is unavailable to the microorganisms and the host animal before dissipation to the atmosphere, contributing to greenhouse gas. The reduction in methane by fumarate addition has been shown to increase available energy per unit of fermentable substrate in the rumen (Carro and Ranilla, 2003). Propionate is converted to glucose after absorption and is thus an important glucogenic precursor for ruminants (Wiltrout and Satter, 1972). Glucogenic amino acids are also precursors for glucose. Therefore, increasing propionate by fumarate could potentially spare glucogenic amino acids.

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Asanuma et al. (1999) reported that ATP produced from fumarate metabolism could be used by rumen microorganisms for growth. In addition, Linehan et al. (1978) indicated that fumarate could serve as a carbon precursor for amino acid synthesis by incorporation of ammonia. It appears that fumarate could provide energy and carbon skeletons for ammonia utilization by ruminal microorganisms. Cellulolytic rumen bacteria have a requirement for ammonia (Bryant, 1973) and thus may benefit from fumarate supplementation. Previous studies showed that fiber-degrading rumen bacteria are enhanced by additional fumarate (Isobe and Shibata, 1993; Asanuma et al., 1999; López et al., 1999) and therefore may lead to improved fiber digestion in animals.

Fumarate has been reported to decrease ruminal ammonia (Stallcup and Barr, 1983; Isobe and Shibata, 1993; Bayaru et al., 2001). Whether this reduction is a result of an increase in ammonia utilization or a decrease in peptides and amino acids deamination is not clear. Peptides and amino acids are intermediates in ruminal protein degradation (Chen et al., 1987a). When the supply of ruminally degradable carbohydrate is limited, much of the peptides and amino acids can be deaminated and ammonia will accumulate. Reducing ruminal ammonia could minimize energy cost for converting excess ammonia absorbed into urea.

Diets containing large amounts of forage generally lead to a high-methane, low-propionate type of ruminal fermentation, which incurs an inefficiency of energy utilization by ruminants (Van Soest, 1982). In addition, when animals are fed large amounts of low quality forage and concentrate with a high RDP content, there is often an imbalance between ruminal protein (high CP degradability) and carbohydrate fermentation (low carbohydrate availability), and excess ammonia can accumulate in the rumen (Nocek and Russell, 1988).

Many studies have presented favorable effects of fumarate on ruminal fermentation. However, little research has been conducted to associate these effects with animal performance. Based on potential actions by fumarate to reduce methane and ammonia and to increase propionate and fiber digestion in the rumen, ruminant animals should respond to fumarate supplementation when diets contain large amounts of low quality forage plus small amounts of CP as true protein with high solubility. Replacement dairy does in Taiwan are generally fed low quality forage with restricted concentrate. Such a dietary condition may limit nutrient availability for animals, because of low fiber digestibility and poor N utilization. Our objective was to evaluate effects of fumarate on ruminal ammonia accumulation and fiber digestion *in vitro* and intake and whole-tract nutrient digestibility in dairy goats.

Table 1. Chemical composition of bermudagrass hay and concentrate fed to dairy goats

Item	Hay	Concentrate ¹
DM, %	91.7	89.5
Gross energy, Mcal/kg of DM	4.32	4.38
OM, % of DM	92.1	93.3
CP, % of DM	4.62	19.1
Soluble CP, % of CP	72.7	75.1
Ether extract, % of DM	1.02	3.09
NDF, % of DM	70.1	29.0
ADF, % of DM	32.5	8.22
Cellulose, % of DM	24.4	6.34
Acid detergent lignin, % of DM	6.47	2.48
Hemicellulose, % of DM	37.5	20.7
NFC, ² % of DM	16.5	42.1

¹Contained 52.0% ground corn, 17.3% soybean meal, 10.4% wheat bran, 17.3% alfalfa meal, 1.73% limestone, 0.87% dicalcium phosphate, 0.35% trace mineral salt, on a fresh basis.

²OM – CP – ether extract – NDF.

MATERIALS AND METHODS

In Vitro Experiment

Two ruminally fistulated crossbred (Alpine × Nubian × Saanen) wether dairy goats (approximately 55 kg each) were used as rumen fluid donors. The wethers were housed in individual box pens and given a high forage and low concentrate diet consisting of bermudagrass hay *ad libitum* and 0.115 kg (DM) of a commercial farm concentrate (Table 1) fed twice daily (0800 and 1800 h). Mineral salt block and water were provided for free access. The composition of the mineral salt block was as follows (% of DM): Na (38.4), Mg (1.5), Zn (0.25), Mn (0.075), Se (0.0015), Fe (0.15), Cu (0.035), I (0.0038), and Co (0.0015). Animal procedures and protocols were approved by the National I-Lan University Institutional Animal Care and Use Committee (I-Lan, Taiwan, Republic of China).

Immediately before the morning feeding, rumen contents were collected and combined from the wethers via rumen cannula and squeezed through 4 layers of cheesecloth into a glass flask. This rumen fluid was strained again through 8 layers of cheesecloth to further remove small feed particles. Presumably, microorganisms obtained in the rumen fluid represented mostly fluid-associated populations.

The one-stage *in vitro* rumen fermentation conditions were similar to those described by Goering and Van Soest (1970). Triplicate polyethylene centrifuge tubes (50 mL) were used for incubation (anaerobic, 39°C) containing 5 mL of artificial saliva (McDougall, 1948), 5 mL of strained rumen fluid, and specific substrates, which were ammonium bicarbonate (NH₄HCO₃, 2.3 g/L), casein amino acid (5 g/L, Casein, Acid Hydrolysate, Sigma, St. Louis, MO), casein peptide (5 g/L,

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