Role of Lactulose as a Modifier in Rumen Fermentation

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Abstract: This *in vitro* experiment was conducted to examine the effects of lactulose (0, 2 or 4%) on ruminal fermentation of mixtures differing in the forage:concentrate ratio (F:C; 20:80, 40:60 or 60:40). Ruminal fluids collected from two Holstein steers were incubated with the mixtures for 48 h. Data were subjected to 2 way ANOVA. Lactulose did not alter ruminal fluid pH, ammonia and lactate concentrations, individual and total Volatile Fatty Acid (VFA) concentrations and gas production. There was a linear increase in ruminal fluid pH from 6.71-6.76 (p<0.0001), a linear decrease in ammonia concentration from 15.84-11.77 mmol L^{-1} (p<0.0001), a quadratic increase in lactate concentration from 25.51-26.91 mmol L^{-1} (p<0.04) as the F:C ratio increased from 10:80-60:40. Moreover, increasing the forage level caused linear increases in the acetate (from 53.89-55.69%; p<0.0002) and valerate (from 3.05-3.25%; p<0.003) proportions and a linear decrease in the butyrate proportion (from 19.74-17.32%; p<0.0001) and a linear decrease in cumulative gas production from 41.14-33.54 mL (p<0.0001). The effects of the F:C ratio and lactulose level on gas kinetics parameters were variable. Lactulose addition failed to modify alterations in rumen fermentation characteristics of the mixtures varying in the F:C ratio. In conclusion, lactulose fermentation in ruminal fluids had minimal effects on fermentability of the mixture consisting of different F:C ratios suggesting that lactulose does not modify alterations in rumen fermentation in cattle during their growth and lactation phases.

Key words: Lactulose, rumen fermentation, gas production, cumulative, proportions, quadrate

INTRODUCTION

Lactulose is a keto analog of lactose [β-D-galactopyranosyl-(1-4)-D-fructofuranose]. It is produced from lactose either via transgalactosylation by means of β-galactosidase obtained from *Aspergillus oryzae* and β-glycosidase obtained from *Pyrococcus furiosus* (Mayer *et al.*, 2004) or isomerisation in alkaline media (Aider and de Halleux, 2007). Lactulose is neither digested nor hydrolyzed in stomach and small intestine due to its resistance to gastric acidity and an absence of disaccharides on the microvillus membrane of enterocytes in humans and monogastric species. That is it is not absorbed and metabolized in the glycolytic pathway or directly stored as glycogen like sugars or starches. It reaches the colon intact and is fermented selectively and extensively by the colonic microflora (e.g., bifidobacteria and lactobacilli) to Volatile Fatty Acids (VFA), lactic acid and gases (Sahota *et al.*, 1982). Pre-caecal and faecal digestibility of lactulose in pigs was reported to be 79 and 100% (Branner *et al.*, 2004). Beaugerie *et al.* (1991) reported that lactitol was slightly absorbable (16%) and contributed 2.3 kcal g^{-1}.

Lactulose has been the subject of numerous research and review articles coping with its effects as a pharmaceutical agent (Conn, 1997), a nutraceutical substance (Bezkorovainy, 2001) and a food additive (Ozer *et al.*, 2005). Because of being a core of the colon-targeted drug delivery system (Yang *et al.*, 2003), it is employed as an indirect estimate of carbohydrate malabsorption in humans (Brighenti *et al.*, 1995), cats (Muir *et al.*, 1991) and calves (Holland *et al.*, 1986). Lactulose also contributes to intestinal immunity through stimulating mRNA expression of anti-inflammatory cytokines and suppressing apoptosis markers (Pie *et al.*, 2007), increasing plasma acute phase proteins (Krueger *et al.*, 2002) and inhibiting the adherence and colonization of pathogens to the host epithelial cell surface (Gibson, 2004).

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Mechanisms by which these benefits occur resulting from prebiotic properties and chemical nature in the hindguts of humans and laboratory animals suggest that lactulose supplementation may also be pertinent to well-being and productive capacity in farm animals. However, its effects on rumen fermentation are largely unknown. It is hypothesized that lactulose fermentation in the rumen could alter fermentation pattern differently depending upon dietary Forage:Concentrate (F:C) ratio. The objective of this *in vitro* experiment was therefore to examine if there is a rumen modifier effect of lactulose using media consisted of different the F:C ratios.

MATERIALS AND METHODS

Animal, diet and management: Two years old Holstein steers weighing an average of 350 kg were cannulated 3 months prior to collection of rumen fluid for the *in vitro* experiment. The basal diet formulated to meet nutrient requirements for maintenance and 0.5 kg daily weight gain (NRC, 2001). The steers were fed twice daily at 0:600 and 1:600 h had free access to water and were kept in individual pens during the experimental period. The animals were offered 4.0 kg concentrate mixture (57.2% barley, 20% wheat bran, 19.0% cottonseed meal, 2.3% limestone, 0.5% salt and 1.0% vitamin-mineral premix each kg provided 12 500 IU vitamin A, 2 500 IU vitamin D_3, 30 mg vitamin E, 10 mg niacin, 0.4 mg Mg, 3.2 mg Zn, 3.0 mg Mn, 8 mg Cu, 0.4 mg I, 0.1 mg Co, 0.1 mg Se and 0.3 g $NaHCO_3$) as well as free choice of the mixture of alfalfa hay and wheat straw.

Media preparation and treatment: For *in vitro* study, approximately 1 L of rumen fluid samples from each steer were obtained 1 h prior to morning feeding through the rumen cannula and then mixed. Rumen liquors was pumped with an automatic air pump from the rumen into pre-warmed thermo flasks (39°C), filtered through four layers of cheesecloth and flushed with CO_2.

Treatments were 3×3 factorial arrangement of three F:C ratios (20:80, 40:60 and 60:40) and lactulose levels (667 mg lactulose mL^{-1}, Osmolak® Solusyon, Biofarma Ilac Sanayi ve Ticaret A.S., Istanbul, Turkey) (0, 2 and 4%) in six replicates. Table 1 shows ingredient and chemical composition of the mixtures. These mixtures were reconstituted with lactulose after grinding them to pass 1 mm sieve for *in vitro* incubation.

For *in vitro* gas production, the media were prepared using Hohenheim Gas Test as outlined by Menke and Steingass (1988). Briefly, after weighing 230±5 mg in duplicates, each of the experimental mixtures was filtered to pass 1.0 mm screen and put into calibrated glass

Table 1: Ingredients of the mixtures for *in vitro* incubation (% of dry matter)

Items	Forage:Concentrate ratio		
	20:80	40:60	60:40
Alfalfa hay	20.0	40.0	60.0
Barley	33.0	24.0	15.0
Wheat	33.0	24.0	15.0
Sunflower meal	9.0	7.0	5.0
Soybean meal	3.0	3.0	3.0
Limestone	1.0	1.0	1.0
Salt	0.5	0.5	0.5
Vitamin-mineral premix[1]	0.5	0.5	0.5
Nutrients	Dry matter (%)		
	91.55	91.75	91.75
Crude protein	18.30	18.00	17.70
Neutral detergent fibre	31.20	35.60	40.10
Ether extract	2.05	2.04	2.07
Crude ash	5.51	7.34	9.74

[1]Per kilogram contained: 12,500 IU vitamin A, 2,500 IU vitamin D_3, 30 g vitamin E, 10 mg niacin, 1.6 g Ca, 1.3 g P, 0.4 mg Mg, 3.2 mg Zn, 3.0 mg Mn, 8 mg Cu, 0.4 mg I, 0.1 mg Co, 0.1 mg Se, 0.5 g NaCl and 0.3 mg $NaHCO_3$

syringes (100 mL). The pistons were previously lubricated with a little vaseline to ease the sliding of pistons and prevent gas escape. The syringes were pre-warmed (39°C) prior to the injection of 30±1.0 mL of the mixture of buffer and rumen liquor (2:1 vol/vol) into each syringe. Samples were collected at 6, 12, 24 and 48 h relative to incubation. Duplicates of each sample were used in five runs by correcting the volume of gas production according to the standard.

Sample collection, analytical procedure and calculation: After determination of pH using a digital pH meter (HI 8314, Hanna Instruments, Amorim-Povoa Varzim, Portugal), fluids were analyzed for NH_3-N concentration using modified Kjeldahl method (AOAC, 1990) and lactate concentration using gas chromatography (GC-15A, Shimadzu, Kyoto, Japan) (Beauchemin *et al.*, 2003). Additional 5 mL of samples collected at 24 h post incubation were centrifuged at 4000 g for 10 min, acidified with 1 mL meta-phosphoric acid (vol/vol, 25%) and allowed to stand for 30 min. After recentrifugation, the supernatants were subjected to gas chromatographic evaluation of VFA (Sigma-Aldrich Corp., Bulletin 856, Bellefonte, PA). Namely, pH and NH_3-N concentrations were measured at 6, 12, 24 and 48 h relative to incubation, whereas lactate concentration and VFA were measured only at 24 h post-incubation. Gas kinetics parameters were generated from an exponential model as described by Orskov and McDonald (1979) using Neway software (Version 5.0, Rowett Research Institute, Aberdeen, UK) which was as follows:

$$P = a+b*(1-e^{-ct})$$

Where:

P = The amount of gas produced at time t

a = The amount of gas produced from soluble portion of the mixture (mL)

b = The amount of gas produced from insoluble but slowly fermentable portion of the mixture (mL)

c = The rate of gas production, % h^{-1}

t = Time relative to incubation, h

The experimental mixtures were analyzed for dry matter, crude protein, ether extract and ash (AOAC, 1990) using wet chemistry as well as neutral detergent fibre (Goering and van Soest, 1970) using fibre analyzer (Ankom[220] Fiber Analyzer, Ankom Co., Macedon, NY).

Statistics: Data were subjected to 2-way ANOVA as repeated measures with time being subplot using the MIXED Procedure (SAS, 1999). The linear model to test the effects of the treatments on response variables was as follows:

$$Y_{ijk} = \mu + FC_i + LL_j + (FC*LL)_{ij} + Error\,A + T_k + (FC*T)_{ik}$$
$$+ (LL*T)_{jk} + (FC*LL*T)_{ijk} + Error\,B$$

Where:

Y_{ijk} = Response variable

μ = Population mean

Fc_i = ith forage:concentrate ratio

L_j = Jth lactulose level

Error A = Whole plot error

T_k = Kth time relative to incubation and

Error B = Subplot error

Because measured only at 24 h relative to incubation, time variable and its corresponding interaction terms were omitted from the linear model for statistical analysis of lactate concentration and VFA pattern. The polynomial contrast was also constructed for attaining changes in variables in response to increasing the F:C ratio and lactulose level. The effects were considered significant at p<0.05.

RESULTS AND DISCUSSION

Less lactate (16.12 vs. 24.88 mmol L^{-1}) and higher VFA (132.1 vs. 108.1 mmol L^{-1}) were produced by fermentation of pure lactulose than by fermentation of the basal mixtures. The proportion (%) of acetate (54.16 vs. 53.99) and butyrate (18.60 vs. 18.86) were similar, the proportion of propionate (23.11 vs. 19.47) was higher and the proportion of valerate (2.34 vs. 3.24) was lower after fermentation of pure lactulose comparing to that of the basal mixtures. The ratio of acetate to propionate was also lower following fermentation of pure lactulose than that of the basal mixture (2.34 vs. 2.80) (Table 2).

Table 2 shows the effects of F:C ratio and lactulose level on rumen variables. Increasing the forage level from 20-60% was associated with a linear increase in ruminal fluid pH from 6.71-6.76 (p<0.0001). Although, ruminal fluid pH never decreased <6.6 during incubation, pH decreased linearly at low forage and decreased quadratically as at high forage level (p<0.04; Fig. 1). Increasing the concentrate level in the mixture linearly increased ruminal fluid NH_3-N concentration from 11.77-15.84 mmol L^{-1} (p<0.0001) whereas it quadratically decreased ruminal fluid 1 actate concentration from

Table 2: The effect of forage:concentrate ratio and lactulose level on rumen variables[*]

| Variables | Forage:Concentrate ratio and lactulose level (%) | | | | | | | | | | Significance, p<F[1] | | |
| | 20:80 | | | 40:60 | | | 60:40 | | | | | | |
	0	2	4	0	2	4	0	2	4	SEM	FC	LL	FC×LL
pH[2]	6.71	6.72	6.71	6.73	6.73	6.73	6.76	6.76	6.75	0.01	0.0001	0.76	0.98
NH_3-N (mmol L^{-1}(3,4))	11.42	12.30	11.60	10.61	11.01	10.76	15.93	15.92	15.67	0.50	0.0001	0.50	0.92
Lactate (mmol L^{-1})	26.99	27.35	26.40	22.54	22.93	23.78	25.10	25.24	26.18	1.78	0.0400	0.92	0.98
Total VFA (mmol L^{-1})	100.10	112.10	111.80	110.50	104.20	102.90	113.80	109.30	106.40	3.90	0.4700	0.89	0.05
Acetate (%)	53.85	53.94	53.89	53.01	54.67	53.87	55.12	55.86	56.09	0.53	0.0001	0.15	0.60
Propionate (%)	18.56	18.39	18.78	20.07	18.66	18.72	19.78	19.55	18.66	0.86	0.5400	0.53	0.83
Isobutyrate (%)	2.30	2.15	2.13	2.06	2.06	2.10	2.10	2.14	2.19	0.08	0.2100	0.87	0.60
Butyrate (%)	19.41	20.07	19.74	19.73	19.36	20.03	17.43	17.02	17.50	0.68	0.0001	0.86	0.91
Isovalerate (%)	2.76	2.48	2.40	1.91	2.22	2.26	2.21	2.24	2.33	0.14	0.0040	0.96	0.15
Valerate (%)	3.13	2.97	3.06	3.23	3.03	3.02	3.35	3.18	3.22	0.08	0.0080	0.02	0.92
Acetate:Propionate	2.90	2.95	2.89	2.69	2.96	2.93	2.81	2.87	3.01	0.14	0.8800	0.39	0.80

[*]Concentrations of lactate and total VFA and percentages of acetate, propionate, isobutyrate, butyrate, isovalerate and valerate and the A:P ratio were 16.12 (2.18) and 132.1 mmol L^{-1} (6.4) and 54.16 (2.53), 23.11 (2.80), 1.17 (1.21), 18.60 (2.97), 0.73 (9.73) and 2.13% (3.32) and 2.34 (3.15), respectively when pure lactulose was incubated. Values in parentheses are percent CV. [1]FC = Effect of the ratio of forage to concentrate; LL = effect of lactulose level; FC×LL = forage:concentrate ratio by lactulose level interaction. [2]Forage:concentrate ratio by time interaction effect, p<0.04. [3]Time effect, p<0.0001. [4]Lactulose level by time interaction effect, p<0.002

26.91-25.51 mmol L^{-1} (p<0.04). The lactulose addition had no effect on ruminal fluid pH and NH$_3$-N and lactate concentrations.

However, increasing the lactulose level started to depress a continuous increase in ruminal fluid NH$_3$-N concentration over time (p<0.0001) at 24 h post-incubation (p<0.0002; Fig. 2). There was no effect of the F:C ratio by lactulose level interaction on ruminal pH and rumen metabolites. Neither the F:C ratio nor lactulose level affected total VFA concentration. There were linear
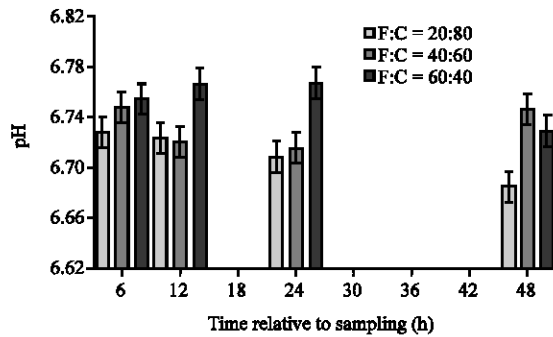
Fig. 1: Changes in ruminal fluid pH during incubation in response to increasing the forage level (p<0.04)
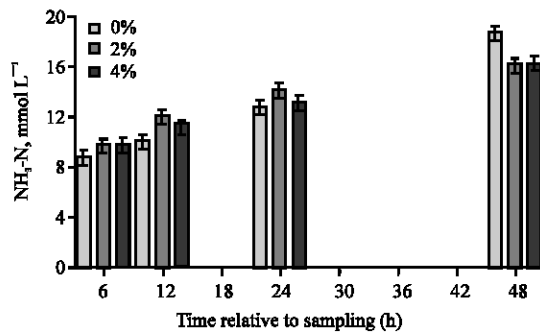

Fig. 2: Changes in ruminal fluid NH$_3$-N concentration during incubation in response to increasing the lactulose level (p<0.002)

increases in percentages of acetate from 53.89-55.69 (p<0.0002) and of valerate from 3.05-3.25 (p<0.003) and linear decreases in percentages of butyrate from 19.74-17.32 (p<0.0001) and of isovalerate from 2.55-2.26 (p<0.02) as the forage level increased from 20-60%. The lactulose addition had minimal effects on the proportions of individual VFA's.

The media added with lactulose tended to have lower acetate (54.72 vs. 53.99%, p<0.06) and had lower valerate (3.08 vs. 3.23%, p<0.007) than those not added with lactulose. Moreover, valerate percentage linearly decreased from 3.23-3.08% with increasing lactulose level from 0-4% (p<0.04).

The acetate:propionate ratio was not affected by the treatments. There was no effect of F:C ratio by lactulose level interaction on any of rumen variables except for total VFA concentration (p<0.05; Table 2) increasing lactulose level increased total VFA concentration at high concentrate whereas it decreased total VFA concentration at low concentrate.

Cumulative gas production and gas production kinetics parameters in response to the F:C ratio and lactulose level are shown in Table 3. Increasing the concentrate level linearly increased cumulative gas production from 33.54-41.14 mL (p<0.0001). Cumulative gas production tended to be greater in the media added with lactulose than those not added with lactulose (37.64 vs. 36.77 m, p<0.10) and tended to increase linearly from 36.77-37.24 mL (p<0.06) as the lactulose level increased. However, there was no F:C ratio by lactulose level interaction effect. Gas production also almost doubled over the incubation period (p<0.0001) with a greater magnitude as the concentrate level decreased from 80-40% (p<0.0005; Fig. 3).

Increasing the forage level did not affect the amount of gas produced from the fraction a of the mixture. However, the amount of gas produced from the fraction b of the mixture linearly decreased from 45.69-35.95 mL as the forage level increased from 20-60% (p<0.0001).

Table 3: The effect of forage:concentrate ratio and lactulose level on rumen gas production and gas kinetics

| | Forage:Concentrate ratio and lactulose level (%) | | | | | | | | | | | | |
| | 20:80 | | | 40:60 | | | 60:40 | | | | Significance, p<F[1] | | |
Variable[2]	0	2	4	0	2	4	0	2	4	SEM	FC	LL	FC×LL
Gas (mL[3,4])	40.890	42.500	40.500	36.700	37.560	37.400	32.710	34.090	33.810	0.740	0.0001	0.120	0.73
a	8.710	11.240	12.320	5.670	8.380	10.540	7.860	6.880	12.040	2.530	0.4800	0.130	0.92
b	47.360	47.290	42.420	43.640	41.580	38.800	35.760	35.810	31.470	1.830	0.0001	0.007	0.97
c	0.072	0.070	0.070	0.087	0.082	0.078	0.083	0.102	0.078	0.010	0.1400	0.570	0.72

[1]FC = Effect of the ratio of forage to concentrate; LL = Effect of lactulose level; FC×LL = Forage:concentrate ratio by lactulose level interaction. [2]a = The amount of gas produced from soluble portion of the mixture (mL); b = The amount of gas produced from insoluble but slowly fermentable portion of the mixture (mL) c = The rate of gas production (%/h). [3]Time effect, p<0.0001. [4]Forage:concentrate ratio by time interaction effect, p<0.0005
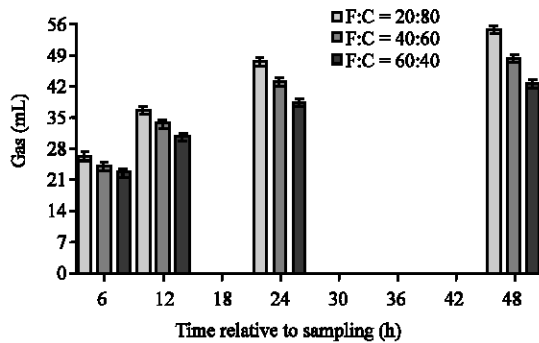
Fig. 3: Changes in gas production during incubation in response to increasing the forage level (p<0.0005)

Moreover, increasing the forage level increased the gas production rate linearly from 0.071-0.088 % h^{-1} (p<0.05). In response to increasing the lactulose level from 0-4% in the media there was a linear increase in the fraction a from 7.41-11.63 mL (p<0.05). The fraction b was lower in the media added with lactulose than those not added with lactulose (39.56 vs. 42.25 mL, p<0.05) and decreased linearly from 42.25-37.56 mL with increasing the lactulose level (p<0.04). The lactulose addition did not alter the gas production rate. There was no effect of the F:C ratio by lactulose level interaction on any of the gas production kinetics parameters.

A great deal of consumer concerns over antibiotic residues have led researcher to seek for safe alternatives in order to improve animal health and performance as well as public health (Schumann, 2002). Lactulose meets prebiotic criteria that include being non-digestible fibre and beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria (e.g., Bifidobacteria and Lactobacilli) and suppressing the growth of potential pathogens in the colon upon fermentation (Bielecka *et al.*, 2002; Gibson, 2004; Gibson *et al.*, 2004). In piglets fed lactulose (66.7 g L^{-1}), Kien *et al.* (2007) however reported no in increase in caecal densities of total anaerobes, total aerobes, Bifidobacteria or Lactobacilli. Although, an intensive fermentation takes place in the rumen similar to caecum and colon of humans and monogastric species, probiotic effects of lactulose on rumen fermentation are limited. Rumen environment structurally and functionally adapt to dietary changes (Liebich *et al.*, 1987). Rumen bacteria (10^9-10^{10} mL^{-1}) are sensitive to changes in diet composition (Therion *et al.*, 1982). Bacteria using fructans as substrates (Bifidobacteria and Lactobacilli) are also present in the rumen (Kamra, 2005). Improving fibre digestion, decreasing protein degradation, reducing methanogenesis, detoxifying feed components and increasing tolerance of rumen environment to high grain diet are some of the major objectives in manipulating rumen ecosystem to support high production (Weimer, 1998). In this experiment, we investigated if there was modifier role of lactulose up to 4% in the rumen at different F:C ratios from 20:80-60:40 which is typical dietary change throughout lactation in dairy cattle and growth and fattening period of beef cattle (Allen, 2000). *In vitro* model is an excellent tool with which to study bioconversion of functional food components such as lactulose and other oligosaccharides (Venema *et al.*, 2003). Lactulose can be used in human at maximum level of 0.26 g kg^{-1} (Oku and Nakamura, 2002) and added to rat diets at 2.5-20% (Scholz-Ahrens and Schrezenmeir, 2002).

Using a fresh human faecal slurry, Palframan *et al.* (2002) demonstrated that lactulose (2%) exerted bifidogenic effect at pH 6. Pierce *et al.* (2006) reported that feeding lactulose (150 g kg^{-1}) to the weanling pig resulted in increased villus height and was associated with increased total VFA in caecum, the population of lactobacilli in the caecum and colon and reduced intestinal pH. The colonic pH reduction effect of lactulose is inconsistent among studies monogastric species. Awati *et al.* (2006) utilized pig faeces and examined pH reducing effect of some carbohydrate sources. It was shown that beet pulp and wheat starch decreased pH but lactulose did not.

Intestinal ammonia arises from amino acids after bacterial deamination (Bongaerts *et al.*, 2005). Lactulose supplementation attenuates colonic ammonia metabolism which covers reduction of urea production rate and increases in ammonia absorption and faecal nitrogen excretion (Remesy and Demigne, 1989) through shifting nitrogen excretion from urine to faeces as shown in humans (Schumann, 2002; De Preter *et al.*, 2006) and dogs (Beynen *et al.*, 2001) in association with the acidification of the contents of the large intestine.

Moreover, the effect of low pH in reducing generation of ammonia appears to be part of a general reduction in bacterial metabolism. Upon lactulose supplementation, turkeys and rats responded to decrease caecal ammonia concentration (Juskiewicz *et al.*, 2005) and horses responded to decrease blood ammonia concentration (Scarratt and Warnick, 1998). Dietary manipulation of gut microbiota by lactulose supplementation to reduce ammonia emission can be beneficial. Resulting from bacterial nitrogen partitioning, lactulose can make nitrogen become available and be utilized more for bacterial mass production (Nocek and Tamminga, 1991; Vanhoof and DeSchrijver, 1996; Foley *et al.*, 2006). However, there was no lactulose effect on ammonia concentration in the present experiment

which could be related to no alteration in ruminal pH (Table 2). Increased ammonia concentration in response to decreasing the F:C ratio is in agreement with the fact that decreased cellulolytic bacteria that utilizes NH_3-N (Hoover, 1986).

It appears that lactulose affects VFA production through altering intestinal morphology and microbiology. Holtug *et al.* (1992) showed that VFA production was high at pH from 5-9 whereas changing pH to <5 or >11 abolished VFA formation in the faecal incubates. Sunvold *et al.* (1995) reported that comparing with 13 fibrous substrates (Solka Floc, oat fiber, fructooligosaccharides, gum karaya, xanthan gum, citrus pectin, guar gum, beet pulp), lactulose yielded highest VFA in *in vitro* using dog faeces as the source of inoculum. Similarly, Demigne *et al.* (1980) evaluated the effects of poorly digested carbohydrates in the small intestine on caecal digestion and absorption in the rat. Except for cellulose, the carbohydrates (bran, pectin, guar-gum, crude potato-starch, lactose, lactulose) favoured considerable development of the caecum and enhanced the quantities of VFA. Reduction in caecal pH in rats fed 8% lactulose (Zdunczyk *et al.*, 2004) and piglets and sows fed lactulose (27-29 and 55-140 g kg^{-1}) (Kamphues *et al.*, 2003) was accompanied by increased weights of caecal wall and digesta, increased concentration of total VFA and lactic acid in caecum, decreased concentration of ammonia in caecum and increased ammonia excretion. In ruminants lactate forms when total VFA concentration exceeds 130 mM because lactate has a lower pK (3.86) than VFA (pKa 4.7-4.9) (Russell and Chow, 1993). Moreover, the F:C ratio affects ruminal pH and the ratio of acetate to propionate these, in turn regulate rumen VFA concentration and methane production (Russell, 1998). In this experiment, total VFA and lactate concentrations were however independent from pH and lactulose addition (Table 2). Changes in rumen fermentation in response to increasing the F:C ratio are in agreement with other reports that showed reductions in ruminal pH, acetate and butyrate percentages and increases in total VFA concentration and propionate percentage in dairy (Krause and Otzel, 2006) and beef (Brown *et al.*, 2000) cattle fed >60% concentrate.

Colonic fermentation contributes >30% of dietary calories in humans. Volatile fatty acids may nourish mucosal cells, spare glutamine utilization and enhance hepatic gluconeogenesis (Elsen and Bistrian, 1991). The degree of polymerization and the solubility may affect VFA formation; lactulose fermentation yields high proportions of acetic acid and low proportions of butyric acid (Nilsson and Nyman, 2005). Peters *et al.* (1992) measured the mean peak concentrations of acetate,

propionate and butyrate in peripheral blood as 240.9, 39.0 and 26.9 μmol L^{-1} after 10 g lactulose supplementation in humans. Mortensen *et al.* (1990) also showed that lactulose was converted to acetate only and increased faecal acidity and the degradation of amino acids to VFA was inhibited at 10-25 mM lactulose. In agreement with the literature, fermentation of pure lactulose in ruminal fluid yielded acetate highest (54%), followed by propionate (23%) and butyrate (19%) (Table 2).

Gas production technique is based on the principle that anaerobic microbial digestion of carbohydrates releases VFA's and gases (hydrogen, carbon dioxide and methane) (Eberhard *et al.*, 2007; Lanzas *et al.*, 2007). Using faeces of unweaned piglets as inoculum, Awati *et al.* (2006) examined gas production on lactulose, molasses-free sugar beet pulp and wheat starch and reported no differences in gas production and kinetics parameters. Fernandes *et al.* (2000) evaluated fermentation profile in response to several substrates using methane producing and non-producing media prepared from human faeces. Acetate production was highest on lactulose comparing to on rhamnose, cornstarch and guar. There was also decrease in methane production due to only lactulose. Comparing with other prebiotic oligosaccharides, VFA and gas productions were shown to be highest on lactulose (Rycroft *et al.*, 2001). In this study, lactulose tended to increase gas production (Table 3).

CONCLUSION

In order to investigate modifier role in rumen fermentation, lactulose was added up to 4% into mixtures differing F:C ratios that mimic changes in dietary composition of the ruminant animals depending on their physiological states. Expectedly, the F:C ratio altered rumen variables including ruminal fluid pH, concentrations of lactate, NH_3-N and total VFA, VFA pattern and gas production. There were no alterations in these variables due to the lactulose addition. Future studies should consider a higher lactulose level, enumeration and specification of microflora in ruminal fluids.

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