Effects of Dietary Acidogenicity Values on Rumen Fermentation Characteristics and Nutrients Digestibility

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ABSTRACT : This study was conducted to observe effects of dietary acidogenicity value (AV) on rumen fermentation characteristics and nutrients digestibility. The AV of feedstuffs was based on the dissolution of Ca from $CaCO_3$ powder added at the end of a 24 h *in vitro* fermentation. Three diets were formulated to be iso-energetic and iso-nitrogenous with different AV. Two experiments were involved in this study. In experiment 1, it appears that pH, NH_3-N concentration and A:P ratio tended to decrease, but gas production, VFA production and DM disappearance tended to increase with increasing dietary AV. In experiment 2, the rumen pH tended to decrease in order of high AV>medium AV>low AV treatment, respectively. There were no significant effects of dietary AV on NH_3-N concentration, enzyme activity and nutrient digestibility. In addition, total VFA and individual VFA concentrations tended to increase with increasing dietary AV without significance. In fact, we hypothesized that different dietary AV would affect rumen fermentation and nutrients digestibility because dietary AV was adjusted with fermentable carbohydrate sources. The present results indicate that differences in dietary AV between treatments were too small to affect rumen fermentation and its effects were minimal. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1625-1633)***

Key Words : Dietary AV, Rumen Fermentation, Digestibility, VFA

INTRODUCTION

Ruminants are able to utilize low quality roughages and rumen is the place where fibers are degraded with symbiotic function of ruminal microbes. They should be fed diets contain rich fibrolytic feedstuffs for keeping optimal fermentation in the rumen. A desirable level of pH in the rumen is between 6.0 and 7.0 (Owens et al., 1998). In many farms these days, high energy feeds, such as grains and commercial concentrates, have been used to improve production efficiency in dairy industry. However, the ingestion of excessive amounts of grains decline pH level and disrupt microbial population in the rumen, because grains are composed of easily fermentable carbohydrates such as starch, maltose, sucrose, cellobiose, fructose, and glucose (Dunlup, 1972; Russell and Rychlik, 2001). A pH decline of rumen leads to rumen acidosis. The early studies have reported that low rumen pH has negative effects on appetite (Shinozaki, 1959), microbial yield (Russell and Dombrowski, 1980; Oliveira et al., 1997), methane production (Lee et al., 2003), dry matter intake (Dewhurst et al., 2001), milk yield (Wadhwa et al., 2001) and fiber digestion (Terry et al., 1969; Smith et al., 1973; Steward, 1977; Fondevilla et al., 1994). Occasionally, acute rumen acidosis results in severe health problems, such as laminitis, ruminal ulceration, liver abscess, and even death (Elam, 1976; Slyter, 1976).

In our earlier experiments, we have found that each feedstuff has distinctive various acidogenicity value (AV), and shows different characteristics of an *in vitro* rumen fermentation (Choi et al., personal communication). Rumen fermentation can be affected by various environmental factors, of which diet is the main contributor to the nature of fermentation in the rumen. Thus, dietary AV should be taken into account when rations are formulated.

The objective of this study was to evaluate effects of diets having different AV on ruminal fermentation characteristics and nutrients digestibility.

MATERIALS AND METHODS

Feeds

There were three diets with different AV values; low AV (LAV), medium AV (MAV) and high AV (HAV) as concentrate dietary sources. Ingredients and chemical composition of the experimental diets are shown in Table 1. The method of dietary AV determination was based on work of Choi et al. (In preparations). Three diets were formulated to be iso-energetic and iso-nitrogenous. The concentrate ingredients were ground in Wiley mill with 3 mm screen. The formulated AV values (mg of Ca/g of feed DM) were 24.5, 21.0 and 16.8 for HAV, MAV, and LAV, respectively. The AV of corn silage was 13.98.

Experimental procedures

Experiment 1 : The present study was conducted to investigate *in vitro* fermentation characteristics. There were four treatments: HAV, MAV, LAV and corn silage. The incubation times were 0, 3, 6, 12 and 24 h, with five-replications. A fistulated Holstein cow of 500 kg body

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Table 1. Ingredient composition and chemical composition of experimental diets

	HAV	MAV	LAV
Ingredient composition		% as fed basis	
Wheat	34	26	24
Wheat bran	26	10	30
Corn	30	26	-
Rapeseed meal	10	-	-
Coconut meal	-	20	14
Palm meal	-	8	20
Beet pulp	-	-	12
Limestone	1	1	1
Mineral and vitamin premix	0.2	0.2	0.2
Chemical compostion			
DM (as fed; %)	89	89	90
AV (mg Ca/g DM)	24.5	21.0	16.8
TDN (kg)	0.71	0.71	0.69
Crude protein (g)	0.15	0.15	0.15
Crude fiber (kg)	0.05	0.07	0.11
Calcium (g)	4.61	4.32	3.37
Phosphorous (g)	6.15	5.75	6.59
Vitamin A (IU)	7,000	7,000	7,000
Vitamin E (IU)	41	33	29

HAV: high-AV, MAV: medium-AV, LAV: low-AV.

weight was used as a donor of rumen fluid. Tall fescue and commercial concentrate (crude-protein content, 12%) in the ratio of 60 to 40 were fed at 2% of body weight twice daily (9:00 h and 17:00 h). Water and mineral-vitamin block were adapted to self-feeding. The rumen content, 0.25 g the squeezed solids per milliliter of rumen fluid, was collected 3 h after morning feeding. Rumen fluid was filtered through two layers of cheesecloth before mixing with buffer, which was maintained at 39°C.

Feeds were freeze-dried and ground through a 1 mm screen on a laboratory mill before being returned to the freeze-drier. The buffer based on McDougall (1948) buffer for *in vitro* icubation. Cysteine hydrochloride monohydrate (0.025% wt/vol) was added just prior to incubations. One-gram (DM) samples were weighed and incubated with 30 ml of buffered rumen liquor comprising 60% buffer and 40% rumen liquor. The incubations were carried out in 60 ml serum tubes held in incubator at 39°C. To minimize the risk of contamination, all glassware and crucibles were washed overnight in a 6 N HNO_3 acid solution. Distilled deionized water was used to rinse all glass wares.

The total gas production in serum bottles was measured by the method of Theodorou (1994) on every incubation time. Samples (2 ml) were withdrawn from each tube and transferred to 2 ml tubes containing $CaCO_3$ powder for acidogenicity value. The pH values were determined with Mettler®Delta 340 pH meter. The cultures were centrifuged at 16,000 rpm for 15 min, and the supernatant were prepared for determination of NH_3-N, volatile fatty acid, and enzyme activity.

Experiment 2 : Three fistulated sheep with 50 kg body weight received the three experimental concentrates in a 3×3 Latin square design. There were three treatments with different AV that were high acidogenicity value (HAV), medium acidogenicity value (MAV), and low acidogenicity value (LAV). Experimental periods were 17 d. The first 14 d of each period were for adjustment to the new diet and the last 3 d were for sample collection.

On the last day of adaptation period, fermentation characteristics were determined at 0, 2, 4, 6 and 8 h after morning feeding. Rumen contents were sampled in the area of the ventral blind sac, with the composite being strained

Table 2. Effects of different dietary AV on in vitro pH, gas production and NH_3-N concentration

Incubation time	Corn silage	HAV	MAV	LAV	SEM	Significance
pH						
0 h	6.44	6.54	6.53	6.52	0.01	NS
3 h	6.42	6.45	6.42	6.41	0.01	NS
6 h	6.22	6.12	6.11	6.14	0.01	NS
12 h	5.80	5.38	5.47	5.49	0.02	NS
24 h	5.51	5.14	5.25	5.23	0.02	**
Total gas production (ml)						
0 h	3.79	3.08	2.75	2.55	0.12	NS
3 h	13.91	15.93	16.72	15.96	0.41	NS
6 h	21.93	40.46	40.02	37.57	0.64	NS
12 h	52.83	68.00	65.83	67.61	0.51	NS
24 h	64.04	86.42	78.11	78.22	1.48	NS
NH_3-N concentration (mg/100 ml)						
0 h	6.26	6.07	5.64	5.55	0.14	NS
3 h	6.99	5.96	5.85	5.34	0.17	NS
6 h	5.29	4.23	4.25	5.04	0.20	NS
12 h	5.21	3.08	2.88	3.67	0.18	NS
24 h	6.73	3.93	4.06	4.74	0.15	NS

NS: not significant, * $p<0.05$, and ** $p<0.01$. HAV: high-AV, MAV: medium-AV, LAV: low-AV.

Table 3. Effect of different dietary AV on *in vitro* VFA concentration (unit: mM) and A:P ratio

Incubation time	Corn silage	HAV	MAV	LAV	SEM	Significance
Acetate						
0 h	61.94	62.03	37.49	37.49	4.37	NS
3 h	50.40	57.22	49.28	45.61	2.71	NS
6 h	59.32	78.15	98.93	57.19	7.46	*
12 h	90.29	94.02	88.09	103.26	8.96	NS
24 h	107.21	101.77	97.95	95.78	5.30	NS
Propionate						
0 h	18.61	18.24	10.86	13.48	1.33	NS
3 h	16.57	20.70	17.94	16.83	1.12	NS
6 h	27.00	39.12	51.41	24.29	4.79	*
12 h	48.36	55.17	49.30	58.16	5.03	NS
24 h	53.76	67.52	52.83	52.98	3.69	NS
Butyrate						
0 h	8.17	8.36	4.70	6.52	0.67	NS
3 h	6.58	8.75	7.05	6.81	0.52	NS
6 h	9.56	11.51	17.25	7.14	1.83	NS
12 h	17.05	17.67	14.94	20.09	2.10	NS
24 h	19.21	31.77	19.50	16.93	2.48	NS
Valerate						
0 h	0.72	0.61	0.44	0.49	0.04	NS
3 h	0.51	0.65	0.50	0.50	0.04	NS
6 h	0.77	0.94	1.46	0.56	0.16	NS
12 h	1.87	1.52	1.23	1.65	0.20	NS
24 h	2.29	4.99	2.18	0.08	0.72	**
Total VFA						
0 h	89.86	89.65	53.74	64.69	6.32	NS
3 h	74.39	87.73	75.09	69.63	4.38	NS
6 h	97.07	130.21	169.85	89.49	14.21	NS
12 h	158.32	169.00	154.11	183.91	15.86	NS
24 h	183.36	206.79	173.05	166.31	10.67	NS
A:P ratio						
0 h	3.33	3.39	3.46	3.30	0.09	NS
3 h	3.04	2.77	2.75	2.82	0.04	NS
6 h	2.20	2.02	1.94	2.37	0.08	*
12 h	1.87	1.71	1.79	1.77	0.06	NS
24 h	1.99	1.51	1.84	1.82	0.06	NS

NS : not significant, * $p<0.05$, and ** $p<0.01$.

through a double layer of cheesecloth. The pH of ruminal fluid was immediately determined using a Mettler® Delta 340 pH meter.

Apparent total tract digestibilities of DM, crude protein, crude fiber, ash, NDF and ADF were determined. Fecal samples were obtained at 08:00 h during the last 3 d of each period, were composited on an equal wet weight basis, dried in a forced-air oven at 60°C for 72 h and ground in a Wiley mill. Approximate analysis was carried out by the method of AOAC (1990) and ADF and NDF were analyzed by the method of Gooring and Van Soest (1970).

Analytical methods

Acidogenicity value (AV) : In *in vitro* study, samples (2 ml) were taken from each tube after 24 h and transferred to 2ml tubes containing 50 mg of $CaCO_3$ powder (Sigma Chemical Co., St. Louis, MO). The mixture was shaken manually for 5s and then centrifuged at 13,000 rpm for 10 min before analysis of Ca content in the supernatant with a test kit (Sigma-Aldrich Company Ltd., Poole, UK). Blanks (with no feed sample) and standards (silage) were included in each run, though these were not used to adjust the AV. The AV was calculated as the product of Ca concentration (from the analysis) and fluid volume (30 ml) divided by the sample weight (1 g). In *in vivo* study, the AV of rumen fluid was collected and directly measured at every sampling time.

Total gas production : A detachable pressure transducer and digital readout voltmeter (Laurel Electronics, Inc., CA USA) were used to measure the headspace gas pressure of fermenting cultures. For gas production studies, the transducer was modified such a way that it could be connected to the inlet of a disposable Luer-lock three-way stopcock (Theodorou, 1994).

NH_3-N concentration : NH_3-N concentration was analyzed by the method of Chaney and Marbach (1962). The supernatant in an amount of 0.1 ml, prepared as above procedure, was mixed with phenol color reagent and alkali-hypochlorite reagent. NH_3-N concentration was determined by using Shimadzu® UV 1601 spectrophotometer at 630 nm.

VFA concentration : VFA concentration was analyzed by the method of Erwin et al. (1961). The supernatant in an amount of 1.0 ml, prepared as above procedure, was added to the Eppendorf® tube containing 0.2 ml of 25% HPO_3 solution, and then, fixed for 30 min at the room temperature. After centrifuging at 16,000 rpm for 15 min, 0.2 ml of the supernatant were collected and used for the assay of VFA concentration by Hewlett Packard® 6890 GC System.

DM disappearance rate : The residual pellets were filtered through Whatman No. 541. The filtered pellets were dried to a constant weight at 60°C. Dry matter disappearance rates were calculated by converting the differences between filter paper weight before and after incubation to the percentage of the former weight.

Enzyme activity : Activity of CMCase and xylanase was measured by using 2% (w/v) carboxyl methylcellulose (CMC) and 1% (w/v) oat spelt xylan in 0.05 M citrate buffer (pH 5.0) as a substrate, respectively. The medium or rumen sample and respective substrate in 0.5 ml was incubated at 39°C for 1 h for enzyme reaction, put into ice bath for 30 min to stop the reaction and centrifuged at 6,000 rpm for 15 min. To measure the concentration of reducing sugars, 0.2 ml supernatant was mixed with DNS (dinitrosalicyclic acid) reagent, placed in a boiling water bath for 5 min, cooled to room temperature, and OD was measured at A_{540} with standard calibration using glucose or xylose (Miller, 1959). Amylase activity was determined by measuring the increase of reducing sugars formed by the enzymatic hydrolysis of 1% soluble potato starch. The reducing sugar were quantified by DNS method same as above. One unit of enzyme activity was defined as the amount of enzyme that released 1 μmol reducing sugars per min under the above condition (μmol reducing sugar/ml/min).

Statistical analysis

Data were analyzed using the general linear model (GLM) procedure of the Statistical Analysis System Institute, Inc. (SAS; 1995). Differences among means were tested for significance using the least significant difference (LSD) procedure of SAS (1995).

RESULTS AND DISCUSSION

Experiment 1: *in vitro* experiment

The *in vitro* fermentation characteristics are shown in Table 2 and 3. The pH was not significantly different across the treatments on 0, 3, 6 and 12 h. While the pH was highest in corn silage among the treatments on 24 h ($p<0.01$) reflecting dietary AV. Despite no significant differences, there was a tendency of decreased pH in lower AV treatments. Corn silage had higher pH compared with other three treatments. As shown in Table 1, AV was adjusted with dietary grain sources when diets are formulated. Therefore, different dietary AV that has different amounts of the readily available carbohydrate could affect ruminal pH

Table 4. Effect of different dietary AV on *in vitro* enzymes activity (unit: umol/reducing sugar/ml/min)

Incubation time	Corn silage	HAV	MAV	LAV	SEM	Significance
Xylanase						
0 h	0.10	0.16	0.20	0.25	0.03	NS
3 h	0.07	0.18	0.17	0.26	0.02	NS
6 h	0.12	0.30	0.21	0.19	0.02	NS
12 h	0.05	0.16	0.10	0.10	0.02	NS
24 h	0.25	0.21	0.30	0.25	0.02	NS
CMCase						
0 h	0.08	0.17	0.11	0.23	0.04	NS
3 h	0.06	0.18	0.17	0.16	0.02	NS
6 h	0.10	0.21	0.20	0.17	0.01	NS
12 h	0.06	0.10	0.04	0.08	0.01	NS
24 h	0.31	0.29	0.34	0.32	0.02	NS
Amylase						
0 h	0.09	0.10	0.19	0.30	0.03	NS
3 h	0.12	0.28	0.30	0.38	0.02	NS
6 h	0.22	0.41	0.35	0.32	0.02	NS
12 h	0.07	0.18	0.13	0.13	0.02	NS
24 h	0.32	0.29	0.38	0.33	0.02	NS

NS: not significant.

despite lack of significance. Total gas production was not significantly different across the treatments during the incubation. While corn silage had relatively lower gas production compared with other three treatments despite lack of significance. As explained, this is due to difference in readily available carbohydrate in the diets. In addition, corn silage had relatively higher NH_3-N concentration compared with other treatments in spite of no significant differences across the treatments. It has been reported that the lower NH_3-N concentration on the higher energy diets with increasing carbohydrate sources (Henning et al., 1991; Lee et al., 2003). Henning et al. (1993) suggested that the lower NH_3-N concentration on the higher energy diet was a result of a greater microbial utilization of dietary nitrogen from an improved energy and nitrogen synchronization. Therefore, the present NH_3-N results are consistent with the previous results.

Volatile fatty acid (VFA) concentration is shown in Table 3. During the incubation, total VFA and butyrate concentration were not significantly different across the treatments. Acetate and propionate concentrations were highest in MAV treatment and lowest in LAV on 6 h ($p<0.05$), but there were no significant differences across the treatments on 0, 3, 12 and 24 h. Valerate concentration was similar between the treatments until 12 h, but it was highest in HVA and lowest in LAV on 24 h ($p<0.01$). During the incubation, A:P ratio was not significantly different across the treatment, except at 6 h. Apparently, there were no major effects of AV on VFA production during the incubation.

Individual enzyme activities are shown in Table 4. The activities of xylanase, CMCase and amylase were not significantly different across the treatments during the incubation. This is related to the fact that fermentation parameters such as pH, NH_3-N and VFA were not affected to the great extent by treatments. Many studies reported decreased cellulolytic enzyme activity on low pH (Barry and Johnstone, 1976; Stewart et al., 1977; Oliveira et al., 1997). In addition, Oliveira et al. (1997) reported that cellulolytic bacteria were especially sensitive to low pH compared with amylolytic species.

Dry matter disappearance was not affected by the treatments during the incubation (Table 5). As explained, this is also related to the fact that fermentation parameters such as pH, NH_3-N and VFA were not significantly affected by treatments. In addition, similar enzyme activity across

Table 5. Effects of different dietary AV on *in vitro* DM disappearance rate and incubated medium AV

Incubation time	Corn silage	HAV	MAV	LAV	SEM	Significance
DM disappearance rate (%)						
0 h	7.69	11.39	9.06	13.50	0.89	NS
3 h	9.44	12.80	12.53	13.70	0.25	NS
6 h	12.94	18.69	15.27	16.99	0.69	NS
12 h	13.71	20.07	18.52	16.74	0.70	NS
24 h	14.40	21.12	19.09	18.54	0.52	NS
Acidogenicity value (mg Ca/g DM)						
0 h	0.16	ND	ND	ND	0.11	NS
3 h	ND	ND	ND	ND	-	NS
6 h	ND	0.64	0.30	0.07	0.10	NS
12 h	1.54	14.43	8.34	7.21	1.17	**
24 h	7.91	27.97	21.36	19.73	1.51	**

NS: not significant, ND: not detected, and ** $p<0.01$.

Table 6. Effects of different dietary AV on ruminal pH and NH_3-N concentration in sheep

Sampling time	HAV	MAV	LAV	SEM	Significance
pH					
0 h	6.81	6.80	6.95	0.05	NS
2 h	6.30	6.38	6.42	0.06	NS
4 h	6.22	6.32	6.35	0.05	NS
6 h	6.28	6.42	6.46	0.08	*
8 h	6.55	6.57	6.72	0.05	NS
NH_3-N concentration (mg/100 ml)					
0 h	5.39	9.27	8.38	1.10	NS
2 h	9.08	9.52	9.10	0.62	NS
4 h	5.67	3.89	5.55	0.30	***
6 h	3.49	1.45	3.03	0.55	NS
8 h	3.52	3.09	3.27	0.35	NS

NS: not significant, * $p<0.05$, and *** $p<0.001$.

Table 7. Effects of different dietary AV on ruminal VFA concentration (unit: Mm) and A:P ratio in sheep

Sampling time	HAV	MAV	LAV	SEM	Significance
Acetate					
0 h	26.18	25.98	19.71	2.37	NS
2 h	43.69	28.19	30.46	3.31	NS
4 h	47.25	33.95	33.75	3.51	NS
6 h	39.02	29.55	32.73	3.20	NS
8 h	39.54	31.34	32.04	2.54	NS
Propionate					
0 h	6.12	6.09	3.72	1.16	NS
2 h	13.34	8.96	9.48	1.53	NS
4 h	13.74	9.45	8.89	1.82	NS
6 h	11.24	8.16	8.05	1.44	NS
8 h	10.37	7.93	6.78	1.29	*
Butyrate					
0 h	4.06	6.47	3.40	1.02	NS
2 h	6.47	5.42	5.15	0.64	NS
4 h	7.31	6.44	6.09	0.62	*
6 h	6.64	6.05	6.15	0.74	NS
8 h	6.50	6.60	5.44	0.64	NS
Valerate					
0 h	0.29	0.65	0.24	0.15	NS
2 h	0.84	0.69	0.70	0.10	NS
4 h	0.81	0.52	0.51	0.10	NS
6 h	0.74	0.40	0.44	0.09	NS
8 h	0.49	0.37	0.28	0.06	NS
Total VFA					
0 h	37.47	40.60	27.89	4.68	NS
2 h	65.29	44.04	46.64	5.39	NS
4 h	70.02	50.98	49.98	5.93	NS
6 h	58.40	44.67	47.98	5.33	NS
8 h	57.70	46.94	45.16	4.28	NS
A:P ratio					
0 h	5.86	5.02	5.30	0.67	NS
2 h	3.61	3.41	3.21	0.23	*
4 h	4.03	4.03	3.88	0.34	*
6 h	3.92	3.99	4.02	0.27	*
8 h	4.38	4.31	4.69	0.34	NS

NS: not significant, and * $p<0.05$.

treatments may have resulted in similar DM disappearance. While Barry and Johnstone (1976) found that the supplementation of small amounts of starch, far from having a depressive effect, rather enhanced fiber digestion, probably due to a stimulation of the rate of glycocalix formation and therefore, the attachment of microbes to fiber particles (Demeyer, 1981). It was difficult to measure the incubated medium AV until 6 h (Table 5). As expected, the medium AV was higher in order of HAV>MAV>LAV>corn silage, respectively, on 12 and 24 h ($p<0.01$).

Experiment 2: *in vivo* experiment

The ruminal pH and NH_3-N concentration are shown in Table 6. Different dietary AV did not affect the pH on every sampling time, except at 6 h. HAV had lowest ruminal pH at 6 h ($p<0.05$). Apparently, there was a tendency of decreased pH with increasing dietary AV with no significant differences. Davis et al. (1964) observed that saliva output is much reduced under high grain feeding, which lowers the buffering capacity of the rumen, resulting in a lower pH. In addition, Allen (1997) indicated that fermentable carbohydrate was the primary influencing factor governing ruminal pH. In the present study, different dietary AV was adjusted with different amount of fermentable carbohydrate. However, the ruminal pH did not significantly influence actual rumen pH and that differences of dietary AV in the present study did not influence for rumen fermentation. The NH_3-N concentration was highest in HAV and lowest in MAV on 4 h ($p<0.001$). While, the NH_3-N concentration was similar across the treatments on 0, 2, 6 and 8 h. The present NH_3-N concentration results are similar to previous results (Oliveira et al., 1997; Wadhwa et al., 2001). Oliveira et al. (1997) reported that acidogenic condition had no effect on the NH_3-N concentrations, NH_3-N remained in the

Table 8. Effect of different dietary AV on ruminal enzymes activity in the rumen fluid in sheep (unit : umol/reducing sugar/ml/min)

Sampling time	HAV	MAV	LAV	SEM	Significance
Xylanase					
0 h	0.22	0.31	0.26	0.07	NS
2 h	1.08	0.64	0.78	0.11	NS
4 h	0.83	0.64	0.46	0.15	NS
6 h	0.50	0.50	0.45	0.12	NS
8 h	0.32	0.44	0.40	0.12	NS
CMCase					
0 h	0.05	0.05	0.05	0.01	NS
2 h	0.18	0.11	0.13	0.02	NS
4 h	0.12	0.08	0.10	0.02	***
6 h	0.10	0.07	0.09	0.02	*
8 h	0.08	0.07	0.08	0.02	NS
Amylase					
0 h	0.07	0.06	0.05	0.01	NS
2 h	0.16	0.13	0.11	0.02	NS
4 h	0.11	0.10	0.07	0.02	NS
6 h	0.17	0.09	0.08	0.03	NS
8 h	0.07	0.09	0.07	0.02	NS

NS: not significant, * $p<0.05$, and *** $p<0.001$.

Table 9. Effects of different dietary AV on nutrients digestibility in sheep (%)

Item	HAV	MAV	LAV	SEM	Significance
DM	64.59	62.05	58.01	1.63	NS
OM	33.88	34.86	23.83	4.94	NS
Crude protein	63.58	55.48	57.57	2.47	NS
Ether extract	66.43	73.30	73.02	3.07	NS
Crude fiber	45.51	45.13	39.79	2.64	NS
NDF	57.96	58.73	58.21	1.21	NS
ADF	53.75	54.39	51.11	1.24	NS

NS: not significant.

range 150-210 mg/l, and assumed to be adequate to satisfy microbial requirement. Wadhwa et al. (2001) also reported that different dietary AV had no significant effect on the concentrations of NH_3-N in mixed effluent after 24 h of incubation.

Total VFA, acetate and valerate were not significantly different across the treatments on every sampling time (Table 7). However, propionate was higher in order of HAV>MAV>LAV, respectively, on 8 h ($p<0.05$). Butyrate was also higher in order of HAV>MAV>LAV, respectively, on 4 h ($p<0.05$). A:P ratio was not affected by dietary AV on 0 and 8 h. While, A:P ratio was highest in HAV and lowest in LAV on 2 and 4 h ($p<0.05$), but it was highest in LAV and lowest in HAV on 6 h ($p<0.05$). Yang et al. (2002) reported that total VFA increased linearly with increasing fermenter pH (5.5, 6.0 and 6.5).

On every sampling time, amylase and xylanase activities were not significantly different across the treatments (Table 8). CMCase was highest in HAV treatment on 4 and 6 h among the treatments ($p<0.001$ and $p<0.05$, respectively).

There were no significant effects on dietary AV on nutrients digestibility in the total tract (Table 9). Interestingly, although not significant, digestibilities of DM, OM, crude protein and crude fiber were numerically higher for HAV than for MAV or LAV. Possibly, this could indicate that higher amounts of grain source in HAV had no negative effects on a metabolism of ruminants when the amounts of fermentable carbohydrate is in a optimal range. Digestibility of ether extract tended to lower in HAV and higher in MAV or LAV. Hoover (1986) explained that if the fermentation of readily fermentable carbohydrates causes a decrease in rumen pH to 6.0 or below, fiber digestion will be decreased. Therefore, the present results suggested that nutrients digestibility is associated with ruminal pH and enzymes activity data.

The hypothesis of present study was that different dietary AV would affect rumen fermentation and nutrients digestibility when dietary AV was adjusted with different amounts of grain. However, the present results showed that effects of dietary AV on rumen fermentation and nutrients digestibility were minimal. Wider differences in dietary AV may result in altered rumen fermentation, which may warrant further studies.

IMPLICATION

On the contrary to the hypothesis, effects of different levels of dietary AV on rumen fermentation and nutrients digestibility were not observed in the present study. Although the *in vitro* study showed that pH and NH_3-N concentration tended to decrease, but total gas production, VFA and DM disappearance tended to increase with increasing AV. Also, the *in vivo* data are consistent with the *in vitro* data. Therefore, the present study indicates that rumen metabolism will not be inhibited if dietary AV is in an optimal range.

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