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Effects of Maize Silage Particle Size and Feeding Method on Ruminal Fermentation, Chewing Activity and Passage Rate of Lactation Cows

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Abstract: This study was to evaluate the effects of maize silage particle size and feeding method on dry matter intake, ruminal fermentation, chewing activity and passage rate of lactating cows. Four multiparous Holstein cows fitted with ruminal cannulas, averaging 625 kg of body weight and 195 days in milk, were assigned to a 4×4 Latin square design. The geometric mean of particle length was calculated according to Penn State Particle Separator. Treatments consisted of Total Mixed Ration (TMR) with 100% short maize silage (SS, 6.3 mm), TMR with 50% short maize silage+50% long maize silage (SL, 10.7 mm), TMR with 100% long maize silage (LL, 16.4 mm) and Separate Ingredients (SI) feeding compared with TMR. With the increasing of maize silage particle size, ruminating ($p = 0.05$) and chewing time ($p = 0.03$) responded quadratically. There was a significant linear increment ($p = 0.01$) of ruminal pH from 6.20-6.39 with increased particle size. Significant linear effects of diets were detected on the total Volatile Fatty Acids (VFA) ($p = 0.01$) and propionate ($p = 0.02$) concentrations of which the greatest value appeared in the diet-LL. However, concentrations of acetate ($p = 0.01$) and butyate ($p = 0.01$) were linearly decreased with increasing particle size. Cows fed diet-SI spent more time ruminating ($p = 0.05$) and consumed less acid detergent fibre ($p = 0.04$) than cows fed diet-SS. Feeding method did not affect ruminal pH and total VFA concentration.

Key words: Maize silage particle size, feeding method, ruminal fermentation, chewing activity, cows, China

INTRODUCTION

Maize silage is viewed as one of ideal forages for cows due to its high energy, low fibre content and high yield ha^{-1}. It is commonly believed that hybrid, maturity and dry matter are indicators of nutritional value of maize silage (Johnson *et al*., 2002). Besides these factors, particle size distribution of maize silage also has great effect on cows' ruminal fermentation and chewing activity. Physically effective fibre, which is defined as the proportion of diet that stimulates chewing activity and saliva secretion, is a primary factor to affect rumen health and function (Yang *et al*., 2001). The physical characteristics become critical when attempting to define the lower limit for acceptable ratio of concentrate to forage (C:F) in dairy ration (Mertens, 1997). Cows often suffer a variety of metabolic disorders when minimum fibre levels are insufficient (Sudweeks *et al*., 1981). However, even if cows consume sufficient NDF without adequate amount of long chopped forage, they may still experience the same metabolic problems due to fibre deficiency (Fahey and Berger, 1988). Some researchers have shed light on how chopping length of maize silage affects cows in early and mid lactation (Beauchemin and Yang, 2005; Yang and Beauchemin, 2006; Krause *et al*., 2002; Kononoff *et al*., 2003; Kononoff and Heinrichs, 2003a; Onetti *et al*., 2003; Schwab *et al*., 2002). In this experiment, we evaluated the DMI, particle size and fibre content of feed offered and leftovers, which make it different from previous studies. Also, this study was based on the realistic situation of Chinese dairy cows. In China, the average milk production was 4800 kg/305 day (Dairy Association of China, 2009). Such a low milk production was mainly because of the poor quality forage situation, especially the poor quality maize silage. The hypothesis was that there were some different regularities between China and Europe or North America. So the results were useful and helpful for the farmers whose cows had low milk production and low quality forages.

It is well known that Total Mixed Ration (TMR) avoids feeding large meals of grains, which reduces the risk of acidosis and this feeding method also has other advantages, such as saving time and labor, accurate administration of mineral and nonprotein nitrogen

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supplements etc. However, there is limited information (Maekawa *et al*., 2002; Li, 2006) concerning the comparison of ruminal fermentation and chewing activity of cows between TMR feeding and SI feeding.

This study was to evaluate the effects of maize particle size and feeding method on DMI, ruminal fermentation, chewing activity and passage rate of lactating cows.

MATERIALS AND METHODS

Cows and diets: Four multiparous Holstein cows with ruminal cannulas (10 cm in diameter, Bar Diamond, Parma, ID, USA), averaged 625 kg (SD = 63) of body weight, 18.2 kg day^{-1} (SD = 2.2) of milk production and 195 days in milk (SD = 21) were assigned to a 4×4 Latin square design. For 21 days periods were started with 14th day of treatment adaptation, followed by 7 days of sample collection. The long maize silage was chopped with a forage chopper (Model No. 93ZP-08, Fengcheng Co., Liaoning, China) and provided as short maize silage. Maize silage and TMR particle size were measured by Penn State Particle Separator (PSPS) as described by Kononoff *et al.* (2002). The Geometric Mean Particle Length (GMPL) was calculated according to the particle size distribution of feeds.

Treatments consisted of TMR with 100% short maize silage (SS, GMPL = 6.3 mm), TMR with 50% short maize silage + 50% long maize silage (SL, GMPL = 10.7 mm), TMR with 100% long maize silage (LL, GMPL = 16.4 mm) and Separate Ingredient (SI) feeding compared with TMR. Short maize silage was used in SI feeding. All diets were formulated to meet or exceed the requirements of a 600 kg multiparous cow producing 20 kg day^{-1} of milk with 4.0% fat (China NY/t34, 2004). Diet ingredients and chemical composition are given in Table 1.

The cows were fed in individual tie stalls bedded with rubber mattresses. Diets were fed as TMR (CAU-mixer wagon model JZC-200, Beijing, China) or SI with a ratio of 48:52 (C:F; DM basis). The TMR was offered for ad libitum intake (10% refusals) at 0730 and 1930 h each day. For cow fed SI, concentrate was fed at the same time as the TMR and the forage was offered 30 min later (0800 and 2000 h). Cows were milked twice daily using a DeLaval mobile milking unit (VP32B2, DeLaval (China), Shanghai, China). Water was available for *ad libitum* consumption. Orts were gathered at 0730 h before the morning feeding.

Ruminal pH and VFA concentration: Ruminal fluid (50 mL) was collected with a rumen filter probe tube via the ruminal cannulas at 2, 4, 6, 8, 10 and 12 h after the morning feeding on day 15 of each period. Samples were filtered through four layers of gauze.

Table 1: Ingredients and chemical composition of the diets

		TMR[2]		
		SS	SL	LL
Ingredients	SI[1]	DM (%)		
Maize silage	30.7	30.7	30.7	30.7
Lucerne hay	21.7	21.7	21.7	21.7
Cracked maize	24.8	24.8	24.8	24.8
Wheat bran	6.00	6.00	6.00	6.00
Soybean meal	8.56	8.56	8.56	8.56
Cotton seed meal	5.73	5.73	5.73	5.73
Limestone	0.54	0.54	0.54	0.54
Dicalcium phosphate	0.79	0.79	0.79	0.79
Sodium chloride	0.55	0.55	0.55	0.55
Minerals+vitamins[3]	0.61	0.61	0.61	0.61
Chemical composition				
NE_L[4] (MJ kg^{-1} DM)	6.34	6.34	6.34	6.34
DM (%)	47.5	47.3	48.4	47.5
OM (%)	92.4	92.7	92.3	92.5
CP (%)	12.2	12.6	12.9	12.5
NDF (%)	32.8	32.5	33.2	32.2
ADF (%)	21.6	21.9	22.7	19.7
Starch (%)	24.3	24.2	24.7	23.9

[1]Separate Ingredient with 100% short maize silage. [2]SS = 100% short maize silage-TMR, SL = (50% short maize silage+50% long maize silage)-TMR, LL = 100% long maize silage-TMR; [3]Minerals+Vitamins = 3305 IU of vitamin A g^{-1}, 1125 IU of vitamin D g^{-1} and 10.98 IU of 526 vitamin E g^{-1} and 0.52% Mn, 0.53% Zn, 0.36% Fe, 0.15% Cu, 0.008% I, 0.006% Se and 0.002% 527 Co. [4]NE_L was calculated based on China NY/t 34 (2004) values for individual feedstuffs

Ruminal pH was immediately determined using a hand held pH electrode (Model pHB-4, Shanghai Chemical Co., Shanghai, China). Approximately 15 mL filtered sample was put into a plastic bottle containing 3 mL of 25% metaphosphoric acid and 3 mL of 0.6% 2-ethyl butyric acid (internal standard) and stored at -20°C until analysis of VFA using gas chromatography. Gas chromatography (6890 N, Agilent technologies, Avondale, PA, USA) was equipped with a 30 m HP-INNOWax 19091N-213 (Agilent) capillary column (0.32 mm i.d. and 0.50 mm film thickness).

The chromatograph oven was programmed as follows: 120°C for 3 min, 10°C min^{-1} increment to 180°C and then was held for 1 min. The injector and detector were maintained at 220 and 250°C, respectively. Nitrogen was used as carrier gas (flow rate 2.0 mL min^{-1}). Additionally, 10 mL filtered ruminal fluid was centrifuged at 4000x g for 30 min at 4°C to obtain a clear supernatant, which was analyzed for ammonia using a phenol-hypochlorite assay (Broderick and Kang, 1980).

Chewing activity: Eating and ruminating activities were monitored 24 h a day by computer-camera system (Infrared Monitor Apparatus, China Agricultural University, Beijing, China) for three 24 h periods from day 18-21. Data were expressed as daily eating, ruminating, or total chewing activity. Total chewing time was calculated as the sum of eating and ruminating time. This criterion was derived from the description of meal by Wangsness *et al.* (1976). A period of rumination was defined as at least 5 min of ruminating occurring after at least 5 min without ruminating activity. Dry matter intake for that day was used to calculate the time spent eating per kg of DM and NDF. The results of DM and NDF intake per unit time were calculated by dividing total minutes of the mean of the measured variables (eating, ruminating and chewing time).

Rumen pool size and rate of passage: Ruminal digesta kinetics was determined using Sodium-Co-EDTA and Cr-mordanted fibre prepared as described by Udén *et al.* (1980). Sodium-Co-EDTA and Cr-mordanted fibre were used as markers for liquid and solid passage rates, respectively. Cr-mordanted fibre (250 g) and 500 mL solution containing 15 g of Co-EDTA were inserted into the rumen via the rumen cannula before the morning feeding.

Fecal grab samples were taken at 0, 6, 10, 14, 18, 22, 26, 30, 36, 42, 48, 54, 60, 72, 84, 96 and 120 h after dosing (starting from day 15) to determine the rate of passage (Yang *et al.*, 2002). Samples were dry-ashed and fecal marker concentrations of Cr and Co were determined by direct current plasma emission spectroscopy (Combs and Satter, 1992).

Before the morning feeding on the last day of each experimental period, rumen fill was determined by a complete rumen evacuation. After weighing and thorough mixing, two representative samples (1.0 kg) were taken. One sample was used to determine DM of ruminal contents and the other sample was immediately squeezed through four layers of gauze to determine the proportions of liquid and solid sample. Digesta were immediately placed back into rumen after sample collection.

Experimental measures and sample analysis: Feed samples were collected once a week and ort samples were taken twice weekly for DM determination. Dry matter (65°C) of feed components was determined weekly and diets were adjusted to account for changes in DM content. In sampling periods (from day 15-21), orts from each cow were gathered daily for calculation of DM and NDF intake. The particle size distribution of the orts was also measured by PSPS.

All samples were oven-dried at 65°C and ground (Model 2000, Experimental Mill Co., Beijing, China). The DM of feeds and faeces were determined at 105°C in oven for 16 h, OM was calculated after ashing at 550°C for 8 h and the micro-Kjeldahl method was utilized to analyze the CP (AOAC, 1990). Neutral detergent fibre was determined based on the method of Van Soest *et al.* (1991) and was not corrected for ash. Heat-stable α-amylase (order code 2325609, Sigma Co., St. Louis, MO, USA) was used in the NDF procedure. Acid detergent fibre was determined according to AOAC (1990).

Statistical analysis: Data on intake, chewing activity and rumen pool size were analyzed as a 4×4 Latin square with model effects of cow, period and treatment. The first autoregressive covariance structure and the GLM procedure of SAS (2001) were used to analyze all of the data. Repeated measurements of ration and orts and ruminal pH, ruminal ammonia and ruminal VFA were analyzed a REPEATED model statement, as well as terms of time and interaction of treatment by time. Contrasts were used to test for linear and quadratic effects of the increasing of maize silage particle size in the TMR and to determine differences between SS-TMR and SI. Significance was declared at $p = 0.05$. A trend was considered to exist if $0.05<p\leq0.10$. All reported values were least square means unless otherwise stated.

RESULTS

Feed particle size and intake: Particle size distribution of maize silage, lucerne hay and TMR is presented in Table 2. The proportion of particles ≥19 mm screen of PSPS increased from 14.1-46.6% (DM basis) for Short Maize Silage (SMS) and Long Maize Silage (LMS), respectively. The amount of particles retained on 1.18 mm screen of SMS increased compared with LMS. As a result, the GMPL was 6.3 and 16.4 mm for SMS and LMS, respectively.

Chemical composition of the diets was not altered by treatments, but physical characteristics were affected by particle size. Therefore, the comparisons among diets reflected differences in particle size rather than differences in ingredients and nutrient composition. As expected, diet-LL had more material retained on 19 and 8 mm screens than diet-SS and diet-SL (Table 2). Diet-SL was a mixture of equal parts of SMS and LMS, consequently it had more material retained on 19 and 8 mm screens but less on 1.18 mm screen than diet-SS. The three TMRs had different particle size distribution, but similar GMPL (4.03, 4.35 and 5.02 mm for diet-SS, diet-SL and diet-LL, respectively).

Particle size of orts at 4, 8 and 12 h after feeding is presented in Table 3. Some sorting behavior was clearly observed among all diets. Proportion of particles >19 mm increased from 9.5% in the original diet-SS to 39.7% in orts at 12 h after feeding. Highest sorting was found on diet-LL that proportion of particles >19 mm increased from 15.3% in the original TMR to 51.5% in orts at 12 h after feeding. The diet-SS and diet-LL contained less orts on the 19 mm screen at 12 h post-feeding than that at 8 h. In comparison with the orts of diet-SS during the

Table 2: Geometric mean particle size of maize silage, lucerne hay and TMR (DM basis)

	Individual feed[5]			TMR[6]		
Values	SMS	LMS	lucerne hay	SS	SL	LL
>19.0 mm (%)	14.10	46.60	2.40	9.50	11.70	15.30
19.0-8.0 mm (%)	36.90	43.70	25.20	27.40	28.40	30.60
8.0-1.18 mm (%)	35.00	9.10	42.40	37.40	31.70	28.70
<1.18 mm (%)	14.00	0.60	30.00	25.70	28.20	25.40
Xgm[1] mm	6.27	16.43	2.58	4.03	4.35	5.02
Sgm[2] mm	3.23	1.98	2.25	3.21	3.44	3.53
Pef[3] (%)	0.51	0.90	0.31	0.37	0.40	0.45
peNDF[4] (%)	21.00	29.70	12.20	12.70	13.10	14.90

[1]Xgm was the geometric mean particle size of sample. [2]Sgm was the standard deviation of sample. [3]Physically effectively factor determined as the sum of the proportions of particles retained on 19 and 8 mm screens. [4]NDF content of the silage (DM basis) multiplies by the pef. [5]SMS = Short Maize Silage, LMS = Long Maize Silage. [6]SS-TMR = 100% short maize silage-TMR, SL-TMR = (50% short maize silage+50% long maize silage)-TMR, LL-TMR = 100% long maize silage-TMR

Table 3: Particle size distribution of orts at each time

		TMR [2](DM %)				p-value[3]		
Time (h)	SI[1]	SS	SL	LL	SEM	L	Q	SS-TMR vs. SI
>19.0 mm								
0	14.1	9.5	11.7	15.3	0.5	0.46	0.88	0.77
4	43.9	31.4	28.1	51.3	8.4	0.56	0.16	0.10
8	39.6	50.1	41.9	55.2	7.8	0.21	0.79	0.27
12	42.0	39.7	43.4	51.5	9.4	0.50	0.56	0.56
<19.0-8.0 mm								
0	36.9	27.4	28.4	30.6	0.7	0.76	0.94	0.88
4	31.1	30.0	20.7	25.9	2.3	0.16	0.63	0.15
8	33.4	25.1	23.3	24.6	2.2	0.03	0.19	0.67
12	28.0	22.5	22.8	22.3	3.8	0.33	0.60	0.93
<8.0-1.18 mm								
0	35.0	37.4	31.7	28.7	0.2	0.59	0.57	0.58
4	19.3	22.8	25.1	13.3	3.4	0.27	0.17	0.05
8	19.5	14.4	21.3	11.6	4.0	0.21	0.83	0.14
12	21.1	20.5	19.4	14.4	4.6	0.35	0.64	0.47
<1.18 mm								
0	14.0	25.7	28.2	25.4	0.1	0.47	0.11	0.57
4	5.7	15.8	26.1	9.5	3.9	0.52	0.14	0.02
8	7.5	10.4	13.5	8.6	2.2	0.74	0.42	0.17
12	8.9	17.3	14.4	11.8	3.0	0.53	0.11	0.56

[1]Separate Ingredient with 100% short maize silage. [2]SS = 100% short maize silage-TMR, SL = (50% short maize silage+50% long maize silage)-TMR, LL = 100% long maize silage-TMR. [3]L = Linear contrast calculated for increasing the maize particle size; Q = Quadratic contrast calculated for the maize silage particle size. SS-TMR vs. SI = Effects of 100% short maize silage-TMR vs. Separate ingredient with 100% short maize silage

Table 4: Effects of maize silage particle size and feeding method on DMI

		TMR[2]				p-value[3]		
Intake (kg day^{-1})	SI[1]	SS	SL	LL	SEM	L	Q	SS-TMR vs. SI
DM	16.2	16.8	18.1	17.1	0.9	0.57	0.86	0.16
OM	15.0	15.5	16.7	15.8	0.6	0.51	0.50	0.38
NDF	5.09	5.48	5.59	5.20	0.36	0.86	0.50	0.48
ADF	3.35	3.51	3.81	3.13	0.17	0.08	0.78	0.04

[1]Separate Ingredient with 100% short maize silage. [2]SS = 100% short maize silage-TMR, SL = (50% short maize silage+50% long maize silage)-TMR, LL = 100% long maize silage-TMR. [3]L = Linear contrast calculated for increasing the maize particle size; Q = Quadratic contrast calculated for the maize silage particle size. SS-TMR vs. SI = Effects of 100% short maize silage-TMR vs. Separate ingredient with 100% short maize silage

first 4 h, diet-SI tended to increase the orts retained on the 19 mm screen (43.9% vs. 31.4%, p = 0.10) and decrease the proportion of particles on 1.18 mm screen (p = 0.05) and pan (5.7% vs. 15.8%, p = 0.02).

Maize silage particle size did not affect intakes of DM, OM and NDF in this study (Table 4). But ADF intake tended to linearly decrease (p = 0.08) with the increasing particle size. In this study, cows with diet-SI ate less forage than allocated due to the difficulty in maintaining a desired C:F ratio during eating when ingredients were offered separately. Cows fed diet-SS consumed more ADF (3.51 kg day^{-1} vs. 3.35 kg day^{-1}, p = 0.04) compared with cows fed diet-SI.

Ruminal fermentation: Effects of maize silage particle size and feeding method on ruminal pH, ruminal ammonia and VFA concentration are shown in Table 5. There was a significant linear pH increase (p = 0.01) from 6.20-6.39 with increased maize silage particle size. Diurnal ruminal pH reached peak before feeding and fell to the lowest value

Table 5: Effects of maize silage particle size and feeding method on ruminal pH, NH_3-N and VFA concentrations

		TMR[2]				p-value[3]		
Components	SI[1]	SS	SL	LL	SEM	L	Q	SS-TMR vs. SI
pH	6.55	6.20	6.35	6.39	0.04	0.01	<0.001	0.99
NH_3-N (mg L^{-1})	88.60	97.70	89.50	87.30	5.60	0.88	0.16	0.79
Total (mM)	100.20	120.30	111.30	121.20	5.70	0.01	0.17	0.22
mM								
Acetate	64.10	77.20	68.90	75.60	3.00	0.01	0.05	0.12
Propionate	20.60	23.50	26.00	26.40	1.70	0.02	0.98	0.88
Isobutyrate	0.92	1.05	0.91	1.05	0.05	0.08	0.24	0.05
Butyrate	12.40	15.90	13.20	15.40	0.70	0.01	0.02	0.02
Isovalerate	1.70	2.04	1.61	1.95	0.09	0.05	0.01	0.01
Valerate	1.22	1.45	1.38	1.53	0.07	0.004	0.38	0.17
A:P ratio	3.24	3.86	3.01	2.94	0.27	0.44	0.02	0.85

Table 6: Effects of maize silage particle size and feeding method on eating and ruminating

		TMR[2]				p-value[3]		
Eating and ruminating	SI[1]	SS	SL	LL	SEM	L	Q	SS-TMR vs. SI
min day^{-1}								
Eating	301	271	291	306	16	0.83	0.15	0.52
Ruminating	484	445	446	503	16	0.45	0.05	0.05
Chewing	785	716	737	809	22	0.48	0.03	0.06
min kg^{-1} of DMI								
Eating	19.8	16.6	18.3	16.2	1.5	0.57	0.17	0.24
Ruminating	28.9	26.8	24.7	29.6	1.3	0.67	0.11	0.02
Chewing	48.7	43.4	43.0	46.8	2.3	0.88	0.11	0.05
min kg^{-1} of NDFI								
Eating	61.8	50.7	54.5	61.9	5.7	0.99	0.11	0.27
Ruminating	91.9	82.3	82.1	99.2	4.4	0.46	0.12	0.08
Chewing	154	133	136	161	8.0	0.65	0.10	0.12

[1]Separate Ingredient with 100% short maize silage. [2]SS = 100% short maize silage-TMR, SL = (50% short maize silage+50% long maize silage)-TMR, LL = 100% long maize silage-TMR. [3]L = Linear contrast calculated for increasing the maize particle size; Q = Quadratic contrast calculated for the maize silage particle size. SS-TMR vs. SI = Effects of 100% short maize silage-TMR vs. Separate ingredient with 100% short maize silage

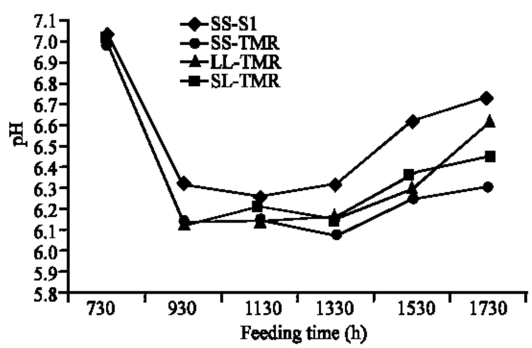

Fig. 1: Effects of maize silage particle size and feeding management on daily fluctuation of pH. X axis signifies time of sampling. SS-SI = Separate Ingredient with 100% short maize silage; SS-TMR = 100% short maize silage; SL-TMR = 50% short maize silage +50% long maize silage; LL-TMR = 100% long maize silage

at approximately 2 h after 0730 meal (Fig. 1). Ruminal pH was similar for cows fed diet-SS and SI. Ruminal ammonia concentration was not affected by maize silage particle size and feeding method and the mean value varied from 87.3-97.7 mg L^{-1}. Total VFA concentration was affected by the hour of sampling and there was no interaction between diet and hour, so only the mean values for dietary treatments are shown. Total VFA concentration increased linearly ($p = 0.01$) with increased particle size. A similar effect was observed for concentration of propionate ($p = 0.02$). However, concentrations of acetate ($p = 0.01$) and butyate ($p = 0.01$) decreased linearly with increased particle size. The ratio of Acetate to Propionate (A: P) ($p = 0.02$) was quadratically affected by the maize silage particle size. Concentrations of isobutyrate ($p = 0.05$), butyrate ($p = 0.02$) and isovalerate ($p = 0.01$) were higher for diet-SS than diet-SI.

Chewing behavior: The effects of maize silage particle size and feeding method on eating and ruminating are presented in Table 6. No difference in eating time was detected with the increase of maize silage particle size. However, ruminating and chewing time exhibited a quadratic effect on cows with longer particle size diet. Because of similar DM and NDF intake among treatments in this study, there was no difference among treatments of total time spent on chewing per kilogram of DM and NDF intake.

Feeding method had little effect on eating time ($p = 0.52$), but cows fed diet-SI spent more time ruminating than fed diet-SS, even when expressed as per kg of

Table 7: Effects of maize silage particle size and feeding method on rumen pool size

		TMR[2]				p-value[3]		
Rumen pool size	SI[1]	SS	SL	LL	SEM	L	Q	SS-TMR vs. SI
Total (kg)	78.6	80.7	80.3	81.2	1.7	0.23	0.81	0.55
DM content (%)	16.2	14.9	14.4	15.6	0.3	0.16	0.02	0.02
DM (kg)	12.7	12.0	11.6	12.8	0.4	0.92	0.16	0.06
Ruminal solid passage rate (% h^{-1})	5.5	6.4	6.1	5.8	0.2	0.13	0.54	0.18
Ruminal liquid passage rate (% h^{-1})	11.3	11.4	12.4	11.9	0.2	0.06	0.92	0.33

[1]Separate Ingredient with 100% short maize silage. [2]SS = 100% short maize silage-TMR, SL = (50% short maize silage+50% long maize silage)-TMR, LL = 100% long maize silage-TMR. [3]L = Linear contrast calculated for increasing the maize particle size; Q = Quadratic contrast calculated for the maize silage particle size. SS-TMR vs. SI = Effects of 100% short maize silage-TMR vs. Separate ingredient with 100% short maize silage

DM (p = 0.02). When ruminating time was expressed on the basis of NDF, results indicated that there was the tendency (p = 0.08) to promote rumination when the silage was allocated separately versus the silage included in a TMR.

Rumen pool size and ruminal passage rate: Total rumen pool size averaged 80.2 kg and DM of rumen contents averaged 12.3 kg were not affected by any treatment (Table 7). Ruminal solids passage rate was not affected by maize silage particle size, which changed from 6.4-5.8% h^{-1} of diet-SS and diet-LL, respectively. There was no difference in ruminal solid passage rate between diet-SI and diet-SS. Results of ruminal liquid passage rate indicated that there was the tendency to be linearly (p = 0.06) higher for diet-SL than for diet-SS and diet-LL. Although, ruminating activity increased when cows fed diet-SI compared with those fed diet-SS, ruminal liquid passage rate was not affected by feeding method.

DISCUSSION

Based on the study of Heinrichs *et al.* (1999), the distribution of TMR used in this study was representative of commercial dairy farms. Some sorting behavior was found among all TMR diets. It indicated that cows preferred smaller feed particles to larger ones on the initiation of feeding, but might intentionally consume long feed particles subsequently to meet their need for physically effective fibre (Beauchemin and Yang, 2005). The sorting activity also occurred in both diet-SI and diet-SS during the whole feeding-to-feeding interval, which resulted in the reduced intake of long particles. However, since diet-SS was offered 30 min earlier than forage administration of the diet-SI, the cows had more time to access to the long particles in each feeding period.

Previous studies have not been in consistent with the effect of chop length of maize silage on DMI. Some (Clark and Armentano, 1999; Bal *et al.*, 2000; Schwab *et al.*, 2002) showed a lack of influence, whereas the effects were observed in others (Kononoff and Heinrichs, 2003a; Krause and Combs, 2003; Onetti *et al.*, 2003). There may be an interaction between diet particle size and the C:F ratio on DMI. When high forage diets were provided to lactating cows, a reduction of particle size that resulted in higher particulate passage rate from the rumen would allow for greater feed intake (Allen, 2000). But, for high concentrate diets (50%), a metabolic, rather than a physical, constraint on feed intake was expected to be rate limiting (Allen, 2000). The lack of effect of maize silage particle size on DMI observed in the present study can also be explained by such theory. In other words, forage particle size has less effect on DMI with balanced TMR, but decreasing chop length of forage might be an effective way to improve the DMI when poor quality, high fibre rations were used.

The reason for cows eating less forage than allocated should be attributed to more sorting activity with diet-SI which resulted in a higher C:F ratio diet consumed. It also demonstrated that TMR could decrease sorting of ingredients (Coppock *et al.*, 1981; Maekawa *et al.*, 2002) and the consumption of nutrient could be more likely the composition of the TMR offered than the diet-SI. The results from DeVries and von Keyserlingk (2009) also suggest that the provision of a TMR to growing dairy heifers promoted a more balanced intake of nutrients compared with the separate provision of concentrate and forage across the day.

The effect of maize silage particle size on ruminal pH is consistent with the widely accepted theory that increasing particle length promotes chewing activity and thus increases buffering capacity within the rumen (Yang and Beauchemin, 2006). A previous study Maekawa *et al.* (2002) reported that cows fed diet-SI had a similar mean ruminal pH as those provided TMR with the C:F ratio (40:60), however in comparison with cows fed a TMR with the same targeted ratio of C:F (50:50), cows fed diet-SI had a lower minimum pH and experienced a greater risk for ruminal acidosis (Maekawa *et al.*, 2002). Feeding a TMR avoided cows eating large meals, which was thought to be beneficial in terms of maintaining a high ruminal pH that was desirable for fibre digestion

(Orskov, 1999). The results were in accordance with the hypothesis that increasing chewing activity would induce higher ruminal pH. All mean ruminal pH values were higher than 5.8, which indicated a positive effect on reducing the risk of acidosis (Beauchemin *et al*., 2003). In this study, the diets consisted of 48% maize-based concentrate and 52% forage (30.7% maize silage), whereas the diets fed by Maekawa *et al*. (2002) contained 57% barley-based concentrate and 43% forage.

So it indicates that ruminal pH is more subjected to the change of C:F ratio and forage varieties and the distribution of particle size can exert great effects on total chewing activity although only small changes are observed in ruminal pH (Cao *et al*., 2008). The ruminal pH was not continuously measured in this study. Thus, the single value might not give enough information regarding the risk of ruminal acidosis because the ruminal pH fluctuated strongly.

Soita *et al*. (2000) and Yang *et al*. (2001) observed that ruminal ammonia increased by an increase of dietary particle size. However, in the studies of Beauchemin *et al*. (2003), Calberry *et al*. (2003) and Kononoff and Henrichs (2003b), the distribution of particle size did not affect ruminal ammonia. Based on the results, it is believed that differences in protein contents of diets, rather than differences in dietary particle size, were responsible for variation of ruminal ammonia.

Yang *et al*. (2001) reported that the diets with short forage particle size increased total VFA concentration and had no effect on the ratio of A:P. Krause *et al*. (2002) found total VFA concentration increased and the ratio of A:P decreased when cows fed short maize silage. However, Beauchemin *et al*. (2003) observed no significant effect on total VFA concentration and the ratio of A:P, when cows fed short maize silage to long maize silage.

The disparities indicated that there were other factors which could affect VFA concentration and the ratio of A:P, such as level of concentrate, forage source, rumen fill, passage rate, individual difference and the interaction between these factors and forage particle size. In fact, VFA production can not be perfectly reflected by VFA concentration, which conjunctly affected by VFA production, rumen cell absorption, liquid rumen fill and passage rate (Van Soest, 1994).

Due to the short periods and small number of cows used, the results of ruminal fermentation variables should be interpreted with caution. Concentrations of acetate and propionate were not affected by feeding method. It indicates that feeding method has less effect on modeling VFA concentration. In this study, Cows spent 271-306 min eating per day, which was within the normal range for cows with daily consumption of 4-6 kg NDF (Beauchemin, 1991). Longer particle size resulted in more chewing time, which derived from re-chewing during increased rumination rather than those during eating. These results were consistent with observations of Kononoff and Heinrichs (2003b) and Kononoff *et al*. (2003), in which they concluded that eating time was not affected even though the Theoretical Cut Length (TCL) of maize silage was reduced from 22.3-4.8 mm. In contrast, both eating and ruminating activities were reduced due to decreasing TCL of forages from 20-4 mm in some studies (Soita *et al*., 2000; Kononoff and Heinrichs, 2003a). The change in chewing time can be the consequence of ruminating activity alone or a combination of rumination and eating.

Total rumen pool size was not affected by maize silage particle size in this study. These results agreed with the findings of Yang *et al*. (2001) and Kononoff and Heinrichs (2003a). Whereas, Yang *et al*. (2002) observed that feeding ground lucerne hay rather than chopped lucerne hay reduced rumen pool size in high silage diets and explained that rumen contents with chopped hay diet had a greater capacity to imbibe water.

In fact, cows usually stopped eating before reaching maximal ruminal capacities, therefore the parameter of rumen fill sometimes didn't seem to be a reliable indication of DMI (Cao *et al*., 2008).

The higher ruminating potential of silage may indicate a greater ruminal fill effect for cows fed diet-SI than for diet-SS (Maekawa *et al*., 2002). While we measured the rumen pool size, there was no difference between diet-SI and diet-SS. But the DM percentage of ruminal digesta increased in diet-SI compared with diet-SS.

In this study, ruminal solids passage rate was not affected by maize silage particle size. Whereas, Yang *et al*. (2002) reported that decreasing forage particle size could decrease mean retention time of solids and it allowed a more rapid turnover of feed from the rumen. The different result could be attributed to the particle size and C:F ratio of the diets consumed.

The result of ruminal liquid passage rate was in agreement with Krause *et al*. (2002). These researchers explained that the increased ruminal liquid passage rate was probably due to a higher saliva production of cows fed coarse silage.

In this study, chewing activity was found to be enhanced, but the saliva production was not affected by the increase of maize silage particle size. However, these conclusions came from a simple 4×4 Latin square design experiment. To be more confirmative, a further evaluation with more experimental units should be conducted.

CONCLUSION

With the increasing of maize silage particle size in the diet of lactating cows, the time spent on ruminating and chewing increased, thus mean ruminal pH and total VFA concentration increased. Cows in mid lactation can be fed diets that contain short maize silage and without leading to negative effects on ruminal pH.

Feeding method did not affect mean ruminal pH and total VFA concentration. Cows fed diet-SI spent more time ruminating than cows fed diet-SS. Cows in mid lactation can be fed diet-SI without induction of negative effects on ruminal fermentation, chewing activity and nutrient intake.

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