IJAS Iranian Journal of Applied Animal Science

Effects of Specific Gravity and Particle Size of Passage Marker on Particulate Rumen Turnover in Holestine Dairy Cattle

Research Article

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Received on: 18 Nov 2016
Revised on: 16 Mar 2017
Accepted on: 31 Mar 2017
Online Published on: Sep 2017

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Online version is available on: www.ijas.ir

ABSTRACT

This experiment was carried out to evaluate the effect of specific gravity (SG) and particle size (PS) of Chromium-mordanated alfalfa neutral detergent fiber (NDF) markers on estimation of particulate rumen turnover. Thirty-two multiparous, mid lactation Holstein dairy cows (body weight=654±24 kg) were allotted to a completely randomized design experiment with two replications in a 4×4 factorial method. The experiment was accomplished over 21 d (adaptation, 14 d; sample collection, 7 d). Cows were *ad libitum* fed twice daily at 09:00 and 21:00 with similar total mixed rations. To prepare the marker, alfalfa in the individual bales was chopped for theoretical cut length 19, 10 and 5 mm. The fine alfalfa was prepared by grinding the 5 mm cut alfalfa through a 2 mm screen. The marker preparation followed a modified procedure of Uden *et al*. (1980) by different PS (8.45, 4.38, 3.00 and 1.10 mm) and $K_2Cr_2O_7$ concentration in mordanting solution (50, 300, 500 and 900 g/kg $K_2Cr_2O_7$ instead of 300 g/kg). Fecal grab samples were taken at 0, 6, 12, 16, 20, 24, 30, 36, 48, 60, 72, 96, 120 and 144 h after dosing. Reduction of PS and enhancement of $K_2Cr_2O_7$ concentration increased SG of marker, ruminal passage rate, but decreased ruminal mean retention time, time delay and total mean retention time. The coefficient of correlation between SG and PS of marker, ruminal passage rate, time delay and ruminal mean retention time were -0.62, -0.70 and 0.65; 0.45, 0.75 and 0.12, respectively. The SG of themarker was the most influential factor affecting ruminal passage rate and mean retention time.

KEY WORDS chromium-mordanated marker, particle size, ruminal particulate turn over, specific gravity.

INTRODUCTION

According to Van Soest's "Hotel Theory" feed particles in rumen compete on retention for digestion or passage (Van Soest, 1994). Ruminants consuming forage diets need to balance the advantages of rapid transit of digesta through the gut to maintain high feed intake, against allowing sufficient time for fermentation by rumen microbes of fiber to maximize the digestion and yield of energy. In order to predict the quantitative importance of rumen digestion on fiber, information is needed on the flow rate of feed particles from rumen. The K_p can be measured by tagging the feed with various indigestible markers. An ideal marker should: 1) be inert without toxic physiological effects on the animal or microflora, 2) not be absorbed or metabolized within the gastrointestinal tract, 3) not influence gastrointestinal secretion, digestion, absorption, or motility, 4) have physiochemical properties that allow for precise, quantitative analysis, and it must not interfere with other analyses and finally and 5) flow parallel with or be physically similar to or intimately associated with the material it is to mark. Rare earth-labeled particles and chromium (Cr)-

mordanated fiber often are used to estimate the K_p of particle-associated components, whereas polyethylene glycol and EDTA chelates of Cr and cobalt often are used to measure fluid K_p (Galyean, 1987). The Cr mordanating fulfils most of desirable criteria for particulate marking. It yields a stable marker of solids forming hexacoordinate ligands with hydroxyl groups that are very difficult to hydrolyze (Udén *et al*. 1980). The Cr mordanated fiber is the most tenaciously bound of the particulate markers, which have been examined. Concentrations of Cr in excess of 80 mg/g dry matter (DM) render NDF essentially indigestible (Colucci *et al*. 1982); as the content of Cr on NDF decreases, the digestibility of NDF increased. However, the single dose procedure provides estimates of passage rate, mean retention time and gastrointestinal trace fill. The Cr mordanating of forages increase their density, reduce their digestibility and alter their chemical composition (Ehle, 1984; Lindberg, 1985; Ramanzin *et al.* 1991; Udén *et al.* 1980). Therefore, all of those alterations should be considered on estimation of turnover rate parameters.

Many experiments showed that physicochemical properties of mordanated markers change with marking. Passage rates measured with Cr mordanated particles can give a fairly accurate estimation of indigestible cell wall components (Bosch and Bruining, 1995). The method can seriously overestimate the passage rates of digestible cell wall components as measured by evacuation techniques (Aitchison *et al*. 1986). These differences indicate that mordanated particles do not readily simulate the passage of natural feed components. This may be because the markers have a different PS distribution and functional specific gravity (FSG) compared to the digesta component and the rate of PS reduction for the marker particles and feeds may be different. Mordanated hay particles are rendered indigestible by the preparation method, and are broken down only by physical mechanisms (Offer and Dixon, 2000) and it seems their rate of breakdown is vary greater than the feeds. Without an accurate description of the PS range and FSG of the markers, it is difficult to interpret differences in outflow rates measured in different experiments.

Bruining and Bosch (1992) found that PS of the Cr-NDF has a great influence on the calculated fractional passage rate from the rumen. Bruining and Bosch (1992) reported that passage rate of mordanated hay particles is dependent on their size and particles lower than 0.3 mm have twice passage rate (4.1±1.0 %/h) compared to that for those of size 0.6 - 1.0 mm (2.1±0.5 %/h). Chopped particles were found to have a slower rate than ground particles, and these in turn, had a slower rate than ground particles in which the NDF had been removed prior to mordanating (Ramanzin *et al*. 1991).

Ehle and Stern (1986) reported that labeling of feed particles with high Cr concentrations alters density of markers, which changes the availability of particles to rumination and passage. Therefore, for making prediction of quantitative importance of rumen digestion the use of suitable marker is critical.

The current experiment was carried out to test effects of PS distributions and density of a Cr-mordanated marker on the passage rate, ruminal mean retention time (RMRT), total mean retention time (TMRT) and time delay (TD) of markers.

MATERIALS AND METHODS

Particle size measurements

Alfalfa harvested at early flowering on one day from a single field without rain was cut and dried. Individual bales were chopped with a forage field harvester (Jaguar#62, Class Company, Germany) to 19, 10 and 5 mm theoretical cut length (TCL) for preparation of three different PS distributions. The fine alfalfa was prepared by grinding the 5 mm cut alfalfa using a farm grinder (2 mm screen size; Behsaz company#11.02 Jahadeh-e-Daneshgahie Mashhad, Iran).

The GM and the standard deviation of GM in each type of alfalfa were determined as ASAE (2002) (Table 1). Also, the GM and the standard deviation of GM of corn silage and alfalfa used total mixed ration (TMR) and four sizes of alfalfa used to marking and four sizes of markers were determined as ASAE Standard (2002) S424.1 (Table 1).

The four types of alfalfa analyzed for dry matter (DM), organic matter (OM), Kjeldahl N, ether extracts (EE) (Feldsine *et al*. 2002), NDF, acid detergent fiber (ADF) (Van Soest *et al*. 1991) and ash at 605 ˚C. Nonfiber carbohydrates (NFC) was calculated by 1000 - (CP(g/kg of DM) + NDF (g/kgof DM) + Ash (g/kgof DM) + EE (g/kgof DM)) (Table 1).

Marker Preparation

Cr-mordanated alfalfa NDF was prepared using a modified procedure of Udén *et al*. (1980). Basic modifications were to vary $K_2Cr_2O_7$ concentration in mordanating solution (50, 300, 500 and 900 g/kg) and feeds PS. Dichromate potassium ($K_2Cr_2O_7$; 231-906-6, solid crystallize, Merck Co.) was used for preparation of markers. The concentration of H_2O in $K_2Cr_2O_7$ was determined by titration with acidic 0.1 *N* cerium sulphate using ferroin as an indicator. Markers were analyzed for DM, OM, Kjeldahl N, ether extract NDF, ADF and ash as described above. NFC was calculated as described in equation 1 (Table 3). Also, the GM of markers determined according to ASAE (2002); Table 3.

Table 1 Chemical composition, functional specific gravity and particle size distribution of alfalfa, total mixed ration and four sizes of alfalfa used for marker preparation according to chop length

Screen size	TMR	Theoretical length cut of alfalfa			
		19 mm	10 mm	5 mm	Fine
Dry matter (g/kg)	625	938.3	938.3	938.3	938.3
Neutral detergent fiber (g/kg)	334	450.0	450.6	450.9	451.5
Acid detergent fiber (g/kg)	178	313.3	313.5	313.6	313.9
Nonfiber carbohydrates (g/kg)	432	326.7	324.7	324.1	323.0
Crude protein (g/kg)	170	166.2	167.5	167.9	168.1
Ether extracts (g/kg)	25	15.3	15.1	15.2	15.2
Ash (g/kg)	49	41.8	42.1	41.9	42.2
FSG	-	0.890^{d}	1.003^{c}	1.121^{b}	1.208^{a}
19 mm	12.51	27.19	12.40	11.19	0.00
12.7 mm	8.52	23.51	8.51	9.26	0.00
6.3 mm	16.50	14.20	16.30	6.68	0.00
3.96 mm	3.82	14.00	23.70	21.02	5.90
1.18 mm	15.12	15.30	23.00	22.93	37.00
Pan	16.14	5.30	16.0	28.92	57.10
Geometric mean	4.75	9.13^{a}	4.51^{b}	3.34^{c}	1.20^{d}
Standard deviation of GM	3.02	3.01^{de}	3.84^{c}	2.95^{b}	1.66^{f}

TMR: total mixed ration and FSG: functional specific gravity.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 2 Chemical composition of feeds used in the total mixed ration

Feeds	Chemical composition									
	DM (g/kg)	OM (g/kg)	CP (g/kg)	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	EE (g/kg)	ASH (g/kg)	NFC (g/kg)	Chromium (ng/g DM)
Alfalfa	938.3^{a}	938.7^{ef}	164.3^{d}	442.1^{g}	314.9^{bc}	106.0^{b}	14.7^{h}	61.3^{ef}	326^{e}	204^{b}
Corn silage	316.7^{e}	929.3^{hg}	89^{g}	463.3^{f}	298.4^{c}	37.3^{e}	32.5^{c}	70.7^{cd}	345.6^{d}	318^{a}
Barely	925.3^{bc}	975.9^{a}	142.5^{e}	210.8^{i}	80.8^{f}	22.7^{g}	23.4^{e}	24.1^{j}	599.1^{b}	104^{d}
Beet pulp	943.6^{a}	926.9^{hi}	96.1^{f}	396.7^{h}	238.8^{d}	16.6^{h}	7.8^{i}	73.1^{bc}	426.3^{c}	116^{d}
Soybean meal	912.3^{c}	923.3^{hi}	444.7^{a}	183.8^{j}	110.9^{f}	3.7^{i}	18.7^{g}	76.7^{bc}	266.2^{g}	16^{c}
Wheat bran	916.1^{c}	942.9^{e}	164.9^{d}	498.9^{e}	168.5^{e}	29.6^{f}	46.4^{b}	57.1^{f}	232.6^{h}	81^{e}

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
DM: dry matter; OM: organic matter; CP: crud protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; EE: ether extract and NFC : non-fiber carbohydrate.

Table 3 Density and geometric mean of types of alfalfa used for marker preparation and markers and mordanated chromium % of alfalfa NDF, according to chop length

Item	Theoretical length cut of alfalfa															
	19 mm				10 mm				5 mm				Fine			
$K_2Cr_2O_7$ Concentration (g/kg of DM)	50	300	500	900	50	300	500	900	50	300	500	900	50	300	500	900
Alfalfa FSG GM (mm)	0.89^{d} 9.13 mm^{a}				1.003^{c} 4.51 mm^{b}				1.121^{b} 3.34 mm^{c}				1.208^{a} 1.20 mm^{d}			
Markers																
Composition of marker (g/kg of DM)																
Mordanated Cr	22.4^{j}	137^{h}	202^{f}	375^{c}	225^{j}	133^{i}	204^{f}	376^{c}	23.7^{j}	136^{hi}	208^{e}	3802^{b}	2.43^{j}	14.1^{g}	22.0^{d}	40.3^{a}
NDF	955^{a}	841^{b}	775^{d}	603^{g}	955^{a}	844^{b}	773^{d}	601^{g}	954^{a}	866^{c}	756^{f}	596^{h}	953^{a}	837^{c}	758^{f}	574^{i}
ADF	691^{a}	609^{b}	562^{d}	437^{g}	691^{a}	611^{b}	560^{d}	436^{g}	690^{a}	610^{b}	557^{e}	432^{h}	689^{a}	606^{c}	548^{f}	417^{i}
CP	16.2	16.4	16.8	16.6	16.5	16.9	16.4	16.5	16.6	16.2	16.4	16.8	16.8	16.6	16.4	16.5
EE	0.14	1.5	1.5	1.4	1.5	1.5	1.5	1.5	1.5	1.4	1.5	1.5	1.5	1.5	1.4	1.5
Ash	27.4^{j}	141^{h}	207^{f}	379^{c}	270^{j}	138^{i}	209^{f}	381^{c}	27.9^{j}	116^{hi}	226^{e}	386^{b}	28.7^{j}	145^{g}	224^{d}	408^{a}
Physical characteristics																
Initial FSG	11^{k}	12.6^{i}	15.8^{f}	19^{c}	11.6^{j}	13.1^{h}	16^{ef}	19.2^{c}	12.5^{i}	13.2^{h}	16.2^{e}	19.6^{b}	12.9^{h}	14.2^{g}	16.7^{d}	20.1^{a}
FSG12	12.1^{j}	13.1^{h}	15.6^{f}	19^{c}	12.1^{j}	13.3^{h}	16^{ef}	19.1^{c}	12.6^{i}	13.3^{h}	16.2^{e}	19.5^{b}	13.1^{h}	14.3^{g}	16.5^{d}	19.9^{a}
FSG24	12.5^{i}	13.2^{h}	15.6^{f}	18.9^{c}	12.4^{i}	13.4^{h}	15.9^{ef}	19.1^{c}	12.6^{i}	13.4^{h}	16.3^{e}	19.6^{b}	13.1^{h}	14.2^{g}	16.5^{d}	20^{a}
GM (mm)	84.2^{a}	84.6^{a}	84.5^{a}	84.6^{a}	43.6^{b}	43.5^{b}	43.9^{b}	44.2^{b}	30.2^{c}	30^{c}	30^{c}	30.1^{c}	11.1^{d}	11^{d}	10.6^{d}	11.4^{d}

FSG: functional specific gravity; FSG12: functional specific gravity of materials after 12 h incubation and FSG24: functional specific gravity of materials after 24 h incubation.
DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crud protein and EE: ether extract.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Functional specific gravity measurements

The functional specific gravity (FSG) of four alfalfa types, markers and TMRs were measured using 100 mL pycnometer (Wattiaux, 1990).

The dry samples (1.5 g) were incubated for 24 h in a 50 mL pycnometers with thermometer (Ambala Cantt, Ambala- 133001, Haryana) pycnometer and their FSG were measured at 12 and 24 h after incubation (Table 3). All the measurements for the kinetics of hydration were made at 39.0 ± 0.5 ˚C. The mixed rumen fluid from two steers fed only alfalfa were collected prior to feeding via a cannula and rinsed through 8 layers of cheese cloth, centrifuged at 30000 × g, for 10 min and the supernatant with density 1.0039 ± 0.0024 g/mL was used as the hydration solution. Sodium azide (0.50 g/L) and penicillin G (25000 units/L) were added to the hydration solution to prevent microbial growth.

Animal and diets

All procedures used in this study were approved based on Proposing a National Ethical Framework for Animal Research in Iran (16). Thirty-two multiparous, mid lactation Holstein dairy cows (body weight=654±24 kg) were allotted to a completely randomized design with two replications in a 4 × 4 factorial for evaluation of four size of markers with 8.45, 4.38, 3.00 and 1.10 mm of the GM and four $K_2Cr_2O_7$ concentration (50, 300, 500 and 900 g/kg of $K_2Cr_2O_7$ per DM were used for preparation of Cr-mordanated alfalfa NDF markers in different specific gravity). The experiment was undertaken over 21 d (adaptation, 14 d; sample collection, 7 d). During the experiment, cows were housed in tie-stalls and fed twice daily at 09:00 and 21:00.

The TMRs were offered ad libitum, allowing for at least 100 g resiual (as-fed basis). All diets had a 40 forage:60 concentrate ratio and contained 200, 200, 350, 70, 75, 100, 3, 1 and 1 g/kg of DM alfalfa, corn silage, barely grain, soybean meal, beet pulp, wheat bran, DCP, vitamin premix and salt, respectively. Water and mineralized salt were available for all cows over the experiment. The TMRs were formulated using the NRC (2001). The TMRs had similar chemical component and contained 334, 178, 432, 170, 5 and 5 (g/kg of DM) NDF, ADF, NFC, CP, EE, calcium and phosphors, respectively.

Markers were mixed with 400 g concentrate and 200 g molasses, fed to all cows at d 15, at the time of the morning feeding. Fecal grab samples were taken at 0, 6, 10, 12, 14, 18, 22, 26, 30, 36, 42, 48, 54, 60, 72, 84, 96, 120 and 144 h after dosing to determine the rate of passage, RMRT, TMRT and TD. Feeds, fecal samples and markers were dry-ashed and concentrations of Cr were determined by direct current plasma emission spectroscopy (Feldsine *et al.* 2002).

Sample collection

Body weights were recorded weekly. Dry matter intake was measured daily for all cows. Samples of forage, concentrates, and residual samples were collected each day.

Samples were dried at 55 ˚C, ground through a Wiley mill (1-mm screen), and composited by animal within period. Total collection of feces was carried out for all cows over d 14 to 20. The feces were dried at 55 ˚C and ground through a Wiley mill (1-mm screen). Feeds and TMRs were analyzed as descried above and digestibility was calculated for DM, OM and all nutrients (Table 4).

Statistical analysis

The data were analyzed as a completely randomized design in a 4 × 4 factorial with two replicates using the following the model:

$$Y_{ijk} = \mu + PS_i + SG_j + PS \times SG_{ij} + e_{ijk}$$

Where:

Y_{ijk}: depended variable.

μ: overall mean.

PS_i: random effect of PS (i=1, 2, 3 and 4).

SG_j: random effect of specific gravity (j=1, 2, 3 and 4).

e_{ijk}: experimental error.

PROC GLM in SAS (2002) was used for analyses. In the first analysis, dry matter intake (DMI) was significantly different between the treatments; therefore DMI was considered as a covariate in future analysis. The data of PS were analyzed as a completely randomized design by using the REML variance component and PROC MIXEDof SAS (2002). Mean separation was determined using the PDIFF procedure and significance was declared at $P < 0.05$.

Fecal Cr excretion curves were fitted to the double compartment model represented by two exponential constants and a time delay (Grovum and Williams, 1973):

$$Y_t = Ae^{-k_1(t-TD)} - Ae^{-k_2(t-TD)},\ k_1 = k_2 \text{ for } t \geq T,$$
$$Y = 0 \text{ for } t = TD,$$

Where:

Y_t: marker concentration (ppm).

A: scale parameter.

k_1: ruminal rate of passage (%/h).

k_2: lower digestive tract rate of passage (%/h).

t: sampling time post dosing (h).

TD: time delay.

The TMRT in the digestive tract was calculated as the sum of RMRT ($1/k_1$) and in the lower digestive tract ($1/k_2$) plus the TD (Table 4). Data were analyzed by nonlinear regression using the SAS (2002) (PROC NLIN®, iterative Marquardt method). The model in SAS® programming language (G4G1) that was used for fitting the excretion curves of markers are listed in appendix 1. Values used in the PARAMS statement were as follows: C-zero, 100, 500, 1200, 2400; Lambda, 0.1, 0.4, 0.8, 2; K, 0.03, 0.05, 0.07 and TD, 1, 10 (Moore *et al*. 1992). All of estimated parameters were analyzed as a completely randomized design in 4×4 factorial with two replicates. The orthogonal methods were used to compare means of two factors and all treatments (Table 4).

Analysis of correlation between PS and FSG of markers and ruminal turnover parameters was done using the PROC CORR of SAS (2002); (Table 5 and 6). The concentration of $K_2Cr_2O_7$ in mordanating solutions and the GM of alfalfa were used in analysis of regression for describing the relationship with CR concentrations in CR mordanated alfalfa NDF.

RESULTS AND DISCUSSION

The Cr concentrations of feeds rarely are measured, but all feeds used in the TMR had Cr concentrations in the normal range. Chemical composition and FSG of four sizes of alfalfa used for marker preparation are shown in Table 2. There were no significant differences between the chemical compositions of four sizes of alfalfa. But FSG of alfalfa types were significantly different and increased as PS of alfalfa was reduced because reduction of PS decreases the volume of feeds. Also, as theoretical cut length (TLC) of alfalfa reduced, the GM of alfalfa particles reduced. The GM of particle was different for four sizes of alfalfa. The GM of alfalfa and corn silage particles that used in TMRs were 4.43, 4.42 and 3.94 mm, respectively (Table 3). All of TMRs had the same composition and the same distribution of PS.

Particle size of alfalfa ($P<0.0001$) and $K_2Cr_2O_7$ concentration as a percentage of DM of materials ($P<0.0001$) and their interaction ($P=0.0045$) had significantly effect on the density of markers. Density of marker increased as $K_2Cr_2O_7$ concentration as a percentage of DM of materials was increased and PS was reduced (Table 4). Regardless the size, when $K_2Cr_2O_7$ concentration as 900 g/kg of DM of alfalfa was used, all markers had density more than 1.90 g/ml. However, alfalfa with TLC 19 mm produced the lowest dense markers, but fine alfalfa produced the highest dense markers. As ratios of concentration of $K_2Cr_2O_7$ in mordanating solutions to DM were increased, there was a concomitant increase of bounded Cr to alfalfa NDF.

The concentration of $K_2Cr_2O_7$ in mordanating solutions and the GM of alfalfa were used in analysis of regression for describing the relationship with Cr concentrations in Cr mordanated alfalfa NDF.
The equations describing this relationship are:
$Y= 0.38631 + 0.42099X_1$, $R^2= 99.6$ (Equation 1)
$Y= 19.21436 – 0.05762X_2$, $R^2= 4.0$ (Equation 2)
$Y= 0.33958 + 0.42119X_1 + 0.00537X_2$, $R^2= 99.6$ (Equation 3)

Where:
Y: Cr concentrations in Cr mordanated alfalfa NDF (mg/g cell wall).
X_1: concentration of $K_2Cr_2O_7$ in mordanating solutions.
X_2: GM of alfalfa that used for marker preparation (mm).

Ehle (1984) used 20, 40, 80, 160 and 320 g/kg Cr per cell wall and found that as ratios of Cr to fiber DM were increased, Cr bound to alfalfa fiber was increased (24.23, 28.17, 31.95, 38.99 and 41.13 mg/g cell wall in each Cr concentration; respectively) and the equation described this relationship was:

$Y= 0.533X + 26.28$ with $R^2= 91$

Where:
Y: Cr concentration (mg/g cell wall).
X: Cr concentration in mordanating solutions.

However, the PS of mordanated alfalfa in Ehle's study was 2.07 mm, but in the current study, PS of alfalfa that used for marking was significantly different.

As ratios of concentration of $K_2Cr_2O_7$ in mordanating solutions to DM were increased, the density of Cr mordanated alfalfa NDF was increased. The results were similar to Ehle (1984), Ramanzin *et al*. (1991). We used the concentration of Cr mandated alfalfa NDF and GM in the regression analyses to describe the relationship between density and Cr concentration and marker PS in the current study. The equations describing this relationship are:

$Y= 1.1124 + 0.00217X_1$, $R^2= 94.0$ (Equation 4)
$Y= 1.597 – 0.0179X_2$, $R^2= 3.3$ (Equation 5)
$Y= 1.1772 + 0.0216X_1 – 0.0149X_2$, $R^2= 96.0$ (Equation 6)

Where:
Y: SG of markers.
X_1: Cr concentration (mg/g cell wall).
X_2: GM of Cr mordanated alfalfa NDF.

The recommended $K_2Cr_2O_7$ or $Na_2Cr_2O_7$ application by Udén *et al*. (1980) is between 300 to 330 g/kg of fiber

weight to get a Cr concentration equivalent to 12-14 g/kg (average 13 g/kg) in the cell wall. Based on our current study using the different concentrations of $K_2Cr_2O_7$ resulted in Cr concentration between 22.4 to 403.1 g/kg of Cr mordanated NDF.

The data of FSG of markers after 12 and 24 h incubation in pycnometer are shown in Table 4. Particle size (P=0.0241) had a significant effect on FSG changes over the incubation time, but, concentrations of $K_2Cr_2O_7$ had no significant effect (P=0.4563). Over the incubation time, only FSG of markers that originated with alfalfa in 19 and 10 mm TCL and mordanated by 50 g/kg concentrations of $K_2Cr_2O_7$ were increased.

The GM of alfalfa types and markers were significantly different. Preparation of markers reduced the GM of alfalfa types and amount of reduction were 8, 3, 11 and 9% for 19, 10 and 5 mm TLC and fine alfalfa, respectively. Markers that prepared with alfalfa with TLC 19 mm had the largest GM but markers that prepared with fine alfalfa had the lowest GM (Table 4). There were no significant differences between the GM of prepared markers in each TLC size of alfalfa. The geometric means of markers were 8.45, 4.38, 3.00 and 1.10 mm that originated from 19, 10, 10 mm TLC and fine alfalfa, respectively. Therefore, it can be expected that different digestion parameters result in different density of markers.

Chemical compositions of markers are shown in Table 2. There were no significant differences for CP and EE between the markers. There were significant different for NDF, ADF, ash and mordanated Cr between the markers. As ratios of concentration of $K_2Cr_2O_7$ in mordanating solutions to DM were increased, ash and mordanated Cr were increased and NDF and ADF were decreased. It seems that the concentration of $K_2Cr_2O_7$ in mordanating solutions was the most influence factor affected on chemical compositions and physical properties of markers.

BW, DMI and digestibility of nutrients used in the ration are shown in Table 4. There were no significantly difference in BW between the cows. DMI was significantly different between cows in different treatments. Therefore, DMI was considered as a covariate in the final model of ANOVA. However, digestibility of DM, NDF, ADF and CP were not significantly different between the treatments (Table 4). The passage rate and digestibility are correlated and digestibility can be easily related to the digestive mechanism and it is a function of the kinetic of digestion and passage (Colucci *et al*. 1982; Grovum and Williams, 1973; Allen and Mertens, 1988; Van Soest, 1994). Also, DMI is related to fiber digestion because it is limited by the rate of disappearance of material from the digestive tract (Allen and Mertens, 1988). There is clear evidence that the major dietary factors affecting rumen outflow of liquid and particle components are BW (body size), level of feeding, ration composition and form, forage:concentrate ratio and digestibility of nutrient.

In current experiment, BW, level of feeding, forage: concentrate ratio and digestibility of nutrient, especially fiber components (NDF and ADF) were not significantly different between treatments and all cows used in experiment were multiparous, therefore, it seems significant difference between the cows in DMI may be result of different ruminal capacity.

Digestion kinetic parameters are shown in Table 4. Marker PS (P=0.0003) and concentration of $K_2Cr_2O_7$ in mordanating solutions (P<0.0001) and interaction of PS and concentration of $K_2Cr_2O_7$ (P<0.0001) had significant effects on Kp. Regardless the size, as concentration of $K_2Cr_2O_7$ was increased or density of markers were increased, Kp significantly were decreased. The highest Kp was observed when marker was prepared with 300 g/kg of DM concentration of $K_2Cr_2O_7$ by using alfalfa in 19 mm TLC. In all size markers, markers that were prepared with 5% of DM concentration of $K_2Cr_2O_7$ had higher Kp than the others.

Ehle (1984) used 20, 40, 80,160 and 320 g/kg Cr per cell wall (with density 1.126, 1.165, 1.242, 1.396 and 1.703 in each Cr concentration; respectively) and found that rumen turnover rates increased linearly with increased Cr concentration (0.0107, 0.0072, 0.0191, 0.0228 and 0.0194 per h in each Cr concentration; respectively). Lirette and Milligan (1989) used 0.1 and 5 g/kg DM Cr to NDF of stem in 1-2 or 10 mm in length and found that Cr concentration had no significant effect on turnover rate parameters, but PS had a significant effect. However, in current study, we increased Cr concentration to 400 g/kg of cell wall and obtained greater density than previous studies (we obtained markers with density greater than 1.90 with 900 g/kg of DM concentration of $K_2Cr_2O_7$ in all sizes). The Kp was increased when concentration of $K_2Cr_2O_7$ increased from 50 to 300 g/kg but decreased when concentration of $K_2Cr_2O_7$ more than 300 g/kg were used for marking.

Marker PS (P=0.0381) and concentration of $K_2Cr_2O_7$ in mordanating solutions (P=0.0034) had a significant effect on RMRT, but interaction of PS and concentration of $K_2Cr_2O_7$ (P=0.3148) did not have a significant effect on RMRT.

Lirette and Milligan (1989) used 0.1 and 5 g/kg DM Cr to NDF of stem in 1-2 or 10 mm lengths and found that Cr concentration had not significantly effect on RMRT.

In normal rations the coarsest material floats in an upper layer and forms aruminal mat. The mat is one of the main sorting mechanisms and elimination allows the escape of larger particles (Van Soest, 1994). Rumen contents are stratified into several layers. PS and FSG of the mat contents are important in the consistency of ruminal mat.

Table 4 Dry matter intake, digestible nutrients of rations and kinetic of digestion

$K_2Cr_2O_7$ Concentration (g/kg of DM)	Theoretical length cut of alfalfa															
	19 mm				10 mm				5 mm				Fine			
	50	300	500	900	50	300	500	900	50	300	500	900	50	300	500	900
BW (kg)	650.5	657.5	656.5	656	660	655	655	657	661.5	656.5	661	657.5	655	655	655	653.5
DMI (Kg/d)	24.9^{abcd}	23.8^{abcd}	24.9^{d}	24.8^{abcd}	24.6^{abcd}	24.4^{cd}	25.7^{abc}	25.0^{abcd}	24.5^{bcd}	24.7^{abcd}	25.3^{abc}	24.9^{abcd}	26.0^{ab}	23.7^{d}	23.8^{d}	26.0^{a}
Digestible nutrients (g/kg)																
DM	763	773	774	765	770	753	762	764	760	771	770	764	763	764	763	767
NDF	566	568	572	559	568	572	563	557	559	560	563	571	568	558	556	562
ADF	515	521	522	513	501	516	498	503	496	490	521	506	512	496	505	514
CP	753	760	764	753	751	750	761	753	746	753	754	748	762	754	752	750
Kinetic of digestion																
Kp (%/h)	4.5^{bc}	5.45^{a}	5.0^{ab}	3.1^{e}	4.2^{cd}	5.0^{ab}	3.4^{e}	3.6^{de}	5.0^{ab}	3.6^{de}	3.1^{e}	3.1^{e}	5.1^{ab}	3.7^{de}	3.4^{e}	3.1^{e}
K_2 (%/h)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RMRT (h)	22.2^{de}	18.4^{e}	19.9^{e}	32.2^{ab}	25.2^{cd}	19.8^{e}	29.4^{abc}	27.9^{bc}	19.9^{e}	28.0^{bc}	32.8a	32.8^{a}	19.7^{e}	27.6^{bc}	32.2^{ab}	32.2^{ab}
FMRT (h)	11.9^{ab}	10.9^{bc}	10.9^{bc}	10.2^{bcd}	10.3^{bcd}	10.9^{bc}	11.6^{abc}	9.88^{bcd}	10.2^{bcd}	9.2^{cd}	13.6^{a}	10.9^{bc}	9.38^{bcd}	10.2^{bcd}	10.5^{bc}	7.79^{d}
TMRT (h)	53.2^{a}	46.3^{b}	45.5^{b}	51.6^{a}	52.2^{a}	45.8^{b}	51^{a}	45.5^{b}	39.4^{dc}	42.3^{bc}	53^{a}	52^{a}	37.8^{d}	44.7^{b}	44.6^{b}	43.8^{b}
TD (h)	20.0^{a}	17.0^{b}	14.7^{c}	9.3^{de}	16.7^{b}	15.1^{c}	10.0^{d}	7.7^{fg}	9.3^{de}	5.1^{i}	6.6^{h}	8.3^{ef}	8.7^{e}	6.8^{hg}	4.7^{ij}	3.8^{j}
Recovery rate (%)	97.55	97.6	97.0	96.8	97.3	97.4	97.2	96.75	97.4	96.8	97.0	97.1	97.3	97.4	97.5	96.9

BW: body weight and DMI: dry matter intake.
Kp: ruminal passage rate; K_2: lower compartments passage rate; RMRT: ruminal mean retention time; FMRT: mean retention time in lower compartments; TMRT: total mean retention time and TD: tTime delay.
DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber and CP: crud protein.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 5 The effects of particle size and $K_2Cr_2O_7$ concentration on ruminal turnover

Item	P-value		
	Particle size	$K_2Cr_2O_7$ concentration	Interaction
Kp (%/h)	0.0003	< 0.0001	< 0.0001
K_2 (%/h)	0.0012	< 0.0001	0.0704
RMRT (h)	0.0004	< 0.0001	< 0.0001
FMRT (h)	0.0381	0.0034	0.3148
TMRT (h)	< 0.0001	0.0297	< 0.0001
Time delay (h)	< 0.0001	< 0.0001	< 0.0001

Kp: ruminal passage rate; K_2: lower compartments passage rate; RMRT: ruminal mean retention time; FMRT: mean retention time in lower compartments and TMRT: total mean retention time.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 6 Correlation between particle size and density of markers and ruminal turn over parameters (%, above diagonal) and their P-value (below diagonal)

Item	DMI (Kg/d)	Kp (%/h)	K_2 (%/h)	RMRT (h)	FMRT (h)	TMRT (h)	Time delay (h)	Particle size	Density of markers
DMI (kg/d)		-0.13	0.18	-0.03	0.16	0.06	-0.11	-0.09	0.16
Kp (%/h)	0.4825	-	-0.16	0.07	-0.96	-0.43	0.63	0.37	-0.55
K_2 (%/h)	0.3267	0.3814	-	-0.95	0.14	-0.52	-0.41	-0.40	0.40
RMRT (h)	0.8923	0.6956	< 0.0001	-	-0.04	0.54	0.30	0.29	-0.33
FMRT (h)	0.3866	< 0.0001	0.4510	0.8186	-	0.47	-0.64	-0.33	0.59
TMRT (h)	0.7604	0.0124	0.0024	0.0016	0.0064	-	0.35	0.43	-0.12
Time delay (h)	0.5312	0.0001	0.0196	0.0918	< 0.0001	0.0518	-	0.74	-0.70
Particle size	0.3789	0.0364	0.0248	0.1057	0.0695	0.0133	< 0.0001	-	-0.16
Density of markers	0.6124	0.0010	0.0241	0.0636	0.0004	0.5224	< 0.0001	0.3692	-

DMI: dry matter intake; Kp: ruminal passage rate; K_2: lower compartments passage rate; RMRT: ruminal mean retention time; FMRT: mean retention time in lower compartments and TMRT: total mean retention time.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Evans *et al.* (1973) reported that FSG is a major factor in the separation of ruminal mat contents. Fine and heavy materials cause cessation of rumination and the relative elimination of the floating mat (Van Soest, 1994).

Heavy material sinks through the rumen pack whereas light material floats and forms the upper part of the fibrous mass. Material with SG less than 1 was heavily ruminated and slowly passed (DesBordes and Welch, 1984).

Intermediate SG (1.17 and 1.42) passed most rapidly. The densest particles (1.77 and 2.14) passed more slowly with little evidence of remastication. Apparently, few of the heavy particles were incorporated in the active moving materials in the region of the cardiac, where rumination boluses are formed. Particle lengths from 1 to 10 mm showed the same trends: light SG passed very slowly and the 1.17 to 1.42 range passed most rapidly.

Marker PS ($P<0.0001$) and concentration of $K_2Cr_2O_7$ in mordanating solutions ($P=0.0297$) and interaction of PS and interaction of PS and concentration of $K_2Cr_2O_7$ ($P<0.0001$) had significant effects on TMRT (Table 4). Marker PS ($P<0.0001$) and concentration of $K_2Cr_2O_7$ in mordanating solutions ($P<0.0001$) and interaction of PS and interaction of PS and concentration of $K_2Cr_2O_7$ ($P<0.0001$) had significantly effect on transit time of markers. As TCL was decreased and concentration of $K_2Cr_2O_7$ in mordanating solutions was increased, time delay was decreased. Regardless the density, the markers that originated with TCL 19 mm and fine alfalfa had highest and lowest time delay, respectively. Marker PS ($P=0.3452$) and concentration of $K_2Cr_2O_7$ in mordanating solutions ($P=0.6542$) and interaction of PS and interaction of PS and concentration of $K_2Cr_2O_7$ ($P=0.5874$) had not significantly effect on recovery rate of markers (Table 4).

CONCLUSION

The results obtained in this experiment showed that the Cr mordanting procedure can greatly affect the physicochemical properties of feed that use for marking, thus altering their passage rate estimation. Calculated outflow rates are highly dependent on marker properties such as density and PS distribution, which should be properly, defined in future experiments. The results of the current experiment indicate that outflow from the rumen is greatly affected by PS and FSG and FSG is the most important factor that influenced the ruminal turn over rate parameters. The Cr mordanating of forages increase their density, reduce their digestibility and alter their chemical composition. Therefore, all of those alterations should be considered on estimation of turnover rate parameters. Without an accurate description of the PS range and FSG of the markers, it is difficult to interpret differences in outflow rates measured in different experiments. Thus the passage rate of mordanated alfalfa NDF is dependent on their FSG and PS distributions (geometric mean).

ACKNOWLEDGEMENT

This work was supported by the research grant of Sari Agricultural and Natural Resources University (SANRU), Mazandaran, Iran. The author thank from Mrs. Maassoumeh Vahmian and Rohangiz Vahmian for preparation of Cr-Mordanated marker of forage particles. Also, the authors thank Mr. Mousa and Esmaiel Teimouri, Ali Mahdavi and Abolfazl Hydarian, the staff of the dairy unit for care of the cows and sample collection, for their assistance.

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