Contribution of different segments of the gastrointestinal tract to digestion in growing Saanen goats[1]

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ABSTRACT: This study examined mean retention time (MRT) of particulate and liquid matter in different segments of the gastrointestinal tract (GIT) of growing Saanen goats of different sexes and subjected to different levels of feed restriction. In addition, feeding behavior and total tract digestibility were determined for all animals ahead of slaughter. In total, 54 Saanen goats (18 each of females, castrated males, and intact males) with initial BW 15.3 ± 0.4 kg were used in a 3 × 3 factorial arrangement comprising the 3 sexes and 3 levels of feed restriction (unrestricted/ad libitum, moderate, and severe restriction). Six blocks per sex group, each consisting of 3 goats, were randomly formed and the goats within each block were randomly allocated to 1 of 3 different feed restrictions. The daily amounts of feed offered to animals subjected to moderate and severe feed restriction (approximately 75% and 50% of ad libitum rate, respectively) were determined within block based on the DMI by ad libitum fed goats on the previous day. The MRT of particulate matter was determined either using Yb-labeled diet or indigestible NDF (iNDF) determined in situ as markers. Mean retention time of the liquid phase was determined by Cr-EDTA. Orthogonal polynomial contrasts were used to determine linear and quadratic effect of feed restriction, while the effect of sex was compared by Tukey test. The effects of sex and the interaction between sex and feed restriction were not significant on most of variables evaluated. Eating, ruminating, and total chewing time per g DM and NDF intake increased linearly as feed restriction increased ($P \leq 0.03$). Diet digestibility increased quadratically for DM and OM, and linearly for NDF as feed intake decreased ($P \leq 0.03$). The MRT of iNDF in the reticulorumen, omasum, abomasum, colon, and total GIT increased linearly with increased feed restriction ($P \leq 0.01$). Mean retention time in the cecum varied quadratically, being greatest for animals with moderate feed restriction. The MRT of liquid was quadratically ($P \leq 0.04$) affected by feed restriction in the reticulorumen, cecum, and total GIT, with the greatest MRT observed for animals subjected to moderate feed restriction. In conclusion, the level of feed restriction increased the MRT of particulate and liquid matter. The MRT was an important mechanism to increase nutrient supply when animals were subjected to feed restriction, as indicated by increased total tract digestibility.

Key words: gastrointestinal tract, markers, mean retention time, pool size

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INTRODUCTION

Passage and digestion of feed in the reticulorumen are competitive processes that determine feed digestibility in ruminants (Mertens and Ely, 1982). Predicted passage rate ($\mathbf{k_p}$) is used in calculations of the ruminal digestibility of carbohydrate and protein fractions in compartmental models of dairy cattle (NRC, 2001; Fox et al., 2004). The mean retention time (**MRT**) is

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the inverse of k_p for an indigestible entity and is directly related to feed intake (Ellis et al., 1994).

Cannas (2000) compared k_p predictions from the Cornell Net Carbohydrate and Protein System developed for cattle against observed k_p in small ruminants and found that the predictions underestimated the ruminal passage of feed particles. In the Small Ruminant Nutrition System, the k_p equations incorporated were developed from a database with pooled measurements on small and large ruminants (Tedeschi et al., 2010). The effects of species were not significant in these predictions (Cannas and Van Soest, 2000), but it remains unclear whether other factors confounded the results. More research is needed to generate reliable predictions of feed digestion in small ruminants.

Huhtanen and Kukkonen (1995) compared estimates of ruminal MRT using either rumen evacuation or marker techniques with sampling from the duodenum in growing cattle. The results indicated that Yb-labeled feed underestimated MRT compared with the rumen evacuation method, whereas Cr-mordanted particles resulted in comparable estimates. The rumen evacuation and slaughter techniques provide biologically relevant estimates of rumen MRT and also make it possible to determine the contribution of different segments of the gastrointestinal tract (**GIT**) to total digestibility (Ahvenjärvi et al., 2010a).

The aim of this study was to determine the MRT of particulate and liquid matter in the whole tract of growing Saanen goats of different sexes and levels of feed restriction. An additional aim was to determine feeding behavior and total tract digestibility.

MATERIALS AND METHODS

The experiment was performed at the Goat Facility of FCAV, Animal Science Department, Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil (21°14′ S; 48°17′ W, 595 m elevation). All animals were registered and cared for according to guidelines approved by the Human Animal Care and Handling Committee of the Faculty of Agriculture and Veterinary Science, and the experiment was performed in accordance with the laws and regulations controlling experiments performed on live animals in Brazil. During the experiment, mean daily minimum and maximum temperature was 20.7°C and 35.7°C, respectively, and minimum and maximum relative humidity of the air was 36.1% and 83.4%, respectively.

Experimental Design, Animals, and Diets

Treatments were applied in a 3 × 3 factorial arrangement, consisting of 3 different sexes (female, castrated males, and intact males) and 3 levels of feed restriction (unrestricted/ad libitum, moderate restriction, and severe restriction). The effects of sex and level of feed restriction were evaluated in a split-plot design, where sex was the main plot observation and level of feed restriction was the subplot.

A total of 54 Saanen goats (18 animals of each sex) with initial BW 15.3 ± 0.4 kg at 102 ± 15 d of age were used in the trial. The animals were housed in individual pens measuring 0.5 m × 1.0 m, had free access to water, and were fed twice daily at 0700 and 1600 h. Six blocks of 3 goats per sex with equal initial BW were randomly formed. The goats within each block were then randomly allocated to one of 3 different levels of feed restriction. The feed intake of the ad libitum animals was adjusted to give 10% daily orts. The amount of feed offered daily to the animals subjected to moderate and severe feed restriction (approximately 75% and 50% of the ad libitum rate, respectively) in each block was determined based on the DMI of the ad libitum fed goats the previous day. Orts were weighed and representative samples were taken on a daily basis.

The experimental diet (Table 1) consisted of dehydrated maize (*Zea mays*) forage, cracked maize grain, soybean (*Glycine max*) meal, soybean oil, limestone, mineral supplement, and ammonium chloride. It was fed as a total mixed ration (**TMR**). Dehydrated maize forage was made from whole maize plants harvested and chopped when the kernel milk line was approximately two-thirds of the way down the kernel. The

Table 1. Ingredient and chemical composition of the experimental diet

Item	Value
Dietary ingredient,[2] % of DM	
Dehydrated maize forage[1]	45.4
Cracked maize grain	26.6
Soybean meal	22.3
Soybean oil	1.6
Limestone	1.0
Mineral supplement	2.2
Ammonium chloride	0.9
Diet chemical composition[2], g/kg of DM ± SD	
DM, g/kg	854 ± 10.9
OM	935 ± 2.0
Crude protein	204 ± 5.4
Crude fat	80 ± 4.9
NDF	355 ± 25.0
Indigestible NDF (iNDF)	108 ± 10.5
Lignin	57 ± 3.4

[1]Dehydrated maize forage consisted of whole maize plants harvested and chopped when the kernel milk line was approximately two-thirds of the way down the kernel.

[2]Mean and standard deviation for 10 samples of each ingredient. Chemical composition of the diet was calculated from the individual ingredients

whole plants were then air dried for approximately 72 h or to DM content of approximately 90%. Thereafter, the dried, chopped material was ground to pass a 10-mm screen in a hay mill (Mexon charger 15.0; G3 Mexon Maquinas Agricolas, Cajuru, Sao Paulo, Brazil). The ingredients of the diet were sampled before the diet was mixed. All samples (feed ingredients and orts) were stored at −10°C until further processing and chemical analysis. Chemical composition of the diet was calculated from the individual ingredients.

Feeding Behavior

Feeding behavior of all animals was recorded when the ad libitum fed animals in each block had reached BW of 22 kg and approximately 40 d in experiment. The time spent on feeding, drinking, ruminating, resting, and other activities (all activities not previously defined) were recorded during 24 h by 2 trained observers who made visual observations every 5 min. The observers were strategically positioned to avoid disturbing the daily activities of the animals.

Apparent Digestibility

The total apparent digestibility for all animals was determined when the ad libitum fed goat in each block had reached BW of 24 kg and approximately 50 d in experiment. During measurements, the animals were housed in individual metabolic cages for 10 d, of which the first 5 d were used to allow the animals to adapt to the metabolic cages and during the last 5 d digestibility was measured by total collection of feces with separation of urine and feces (Robbins and Bakke, 1967). The orts and feces were weighed daily and subsamples of 10% weight were collected and stored at −10°C. At the end of period, these samples were pooled to provide composite samples for further processing and chemical analysis.

Markers, Administration of Markers, and Slaughter

The retention time of particulate matter was determined either by administration of a Yb-labeled diet (external marker) or by in situ determination of indigestible NDF (**iNDF**; internal marker). The Yb-labeled diet was prepared according to de Vega et al. (1998). The particles were labeled with Yb by soaking the whole diet in acetate buffer (0.1 M acetic acid adjusted to pH 6.0 with NH_4OH) for 3 h and then overnight in the same solution with an exposure of 17 g Yb-acetate per kg DM. The Yb-labeled material was placed in a nylon bag (50 µm pore size), rinsed with tap water until obtain rinsed clean water, and thereafter dried at 60°C for 72 h. The retention time of the liquid phase was determined using Cr-EDTA prepared according to Downes and McDonald (1964).

External markers were administered to all animals when the ad libitum fed animal in the same block had reached 30 kg BW and approximately 100 d in experiment. Both external markers were administered orally during 5 d before slaughtering the animals, at 0100, 0700, 1300, and 1900 h each day. The average daily dose of Yb and Cr was 0.02 and 0.4 g, respectively. Ytterbium was given in 1.5 g labeled pellet per dose.

To determine MRT, different segments of the digestive tract were evacuated after the animals were slaughtered (2.5 ± 0.5 h after morning feeding), in each block on the same day. The GIT was removed and separated into reticulorumen, omasum, abomasum, small intestine, cecum, and colon (colon + rectum). The compartments were weighed before and after emptying to determine the amount of digesta and the weight of tissues in each segment. Total GIT tissue weight and pool size were calculated from the sum of each tissue or pool size in the GIT. The pH in the digesta from the reticulorumen and cecum was measured by digital potentiometer (TEC-5; Tecnal, Piracicaba, Sao Paulo, Brazil). The digesta in each GIT segment was sampled and stored at −10°C until processing and chemical analysis.

Mean Retention Time

The MRT in the digestive compartments was calculated following Eq. [1] as described by Van Soest et al. (1992):

$$MRT = Q/F, \quad [1]$$

where MRT is in h, Q is the marker quantity in g, and F is the marker administration rate in g/h. The feed intake during the last 5 d before slaughtering the animals was used to determine F for iNDF. The amount of Yb and Cr administered in the same period was also used to determine F. The total GIT MRT was calculated as the sum of MRT in the reticulorumen, omasum, abomasum, small intestine, cecum, and colon.

Chemical Analysis

The dietary ingredients, orts, and feces were dried in an oven at 60°C for 72 h, while the digesta from different segments of the GIT were freeze dried for 96 h. After drying, the samples were ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). Concentrations of DM, ash, NDF, Cr, and Yb were determined in the dietary ingredients; orts, feces, and the digesta were collected

from the different segments of the GIT. The CP, crude fat, and lignin concentrations were also determined for the ingredients of the diet.

The DM concentration was determined by drying the material in an oven at 105°C for 24 h (AOAC, 1995; method 930.15) and ash content by complete combustion in a muffle furnace at 600°C for 3 h (AOAC, 1990; method 942.05). The concentration of NDF was measured in an Ankom 220 Fiber Analyzer (Ankom Technology Corp., Fairport, NY) using heat-stable α-amylase without sodium sulphite (Mertens et al., 2002). The concentration of NDF was expressed on an ash-free basis. The CP was established using Dumas's combustion method (LECO FP-528; LECO Corp., St. Joseph, Michigan; AOAC, 1990; method 990.03). The crude fat content was determined by extraction with petroleum ether in a Soxhlet apparatus for 6 h (AOAC, 1990; method 930.15). The lignin concentration was determined by solubilization of cellulose in 12 M sulfuric acid after extraction with acid detergent (AOAC, 1990; method 973.18). The concentration of Cr and Yb was determined by adding 5 mL of a 5:1 mixture of nitric and perchloric acids to 0.2 g DM of sample. Samples were kept in the acidic solution overnight and thereafter gradually heated until completely digested. Marker concentrations were determined with an atomic absorption spectrometer (Varian, model Spectra AA 220 FS; Alchem Technology, Denver, CO) with an acetylene and nitrous oxide flame (de Vega et al., 1998).

The iNDF content of the diet, orts, and digesta of GIT segments was determined by incubating 0.6 g DM of sample in F57 bags (Ankom Technology Corp., Fairport, NY) in the rumen of fistulated cattle for 288 h. After the in situ incubation, the bags were manually washed for 30 min and the content of iNDF was determined using an Ankom 220 Fiber Analyzer (Ankom Technology Corp., Fairport, NY) as described by Mertens et al., (2002).

Statistical Analyses

The data were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC) by the model

$$Y_{ijk} = \mu + S_i + B_{j(i)} + SB_{ij(i)} + F_k + SF_{ik} + e_{ijk}, \qquad [2]$$

where Y_{ijk} = dependent variable, μ = overall mean, S_i = effect of sex i (main plot), $B_{j(i)}$ = effect of block j nested in sex i, $SB_{ij(i)}$ = interaction between sex i and block j nested within sex i (main plot error), F_k = effect of feed restriction k, SF_{ik} = interaction between sex i and feed restriction k, and $e_{ijk} \sim N(0, \sigma_e^2)$ is the random residual error. The effect of sex, level of feed restriction, and their interactions were considered fixed effects, and block nested in sex was considered a random effect. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of feed restriction. The effect of sex was compared by the Tukey test. The significance was declared at $P \leq 0.05$.

Residuals were plotted against the predicted values to check the model assumptions regarding the homoscedasticity, independence, and normality of the errors. A data point was deemed to be an outlier and removed from the database if the studentized residual was outside the ± 3.0 range.

RESULTS

Feed Intake and Body Weight

As expected from the experimental design, feed intake (DM, OM, and NDF) decreased linearly ($P < 0.01$) with increased level of feed restriction during all measurements (feeding behavior, digestibility, and slaughter; Tables 2, 3, and 4). The intake of iNDF and NDF (% of BW) in the last 5 d before slaughter also decreased linearly with increased level of feed restriction ($P < 0.01$). During observations of feeding behavior, castrated males displayed greater DM and OM intake than intact males ($P \leq 0.01$), but neither of these groups was significantly different from the females. The interaction between sex and level of feed restriction was not significant ($P \geq 0.15$) for feeding behavior and digestibility. Therefore, these interactions are not presented in Tables 2 and 3. During the last 5 d before slaughter, DMI in goats under moderate and severe feed restriction was 71% and 49%, respectively, of that in the ad libitum treatment (Table 4). As a consequence of increased level of feed restriction, the BW of all animals also decreased quadratically ($P = 0.03$; Table 4). The interaction between sex and level of feed restriction on BW before slaughter was significant ($P = 0.02$). Intact males fed ad libitum had greater BW than females ($P = 0.02$), but neither group was different to castrated males. Intact males fed at the moderate level of restriction had greater BW than castrated males ($P < 0.01$). However, when intact males were subjected to the severe level of restriction, their BW was not different from that of castrated males and females ($P \geq 0.82$). Castrated males fed ad libitum had similar BW to females ($P = 0.11$), but when fed at moderate restriction their BW was lower than that of females ($P = 0.02$). Females and intact males had similar BW when subjected to moderate feed restriction ($P = 0.40$; data not shown).

Feeding Behavior

Animals fed ad libitum linearly increased the time spent eating and ruminating ($P \leq 0.03$) and linearly

Table 2. Body weight, feed intake and activities measured during feeding behavior in goats of different sexes subjected to feed restriction

	Sex				Feed restriction				*P*-value[1]	
Item	Females	Castrated males	Intact males	SEM	None	Moderate	Severe	SEM	Sex	F_L[2]
BW[3]	20.1	19.8	19.2	0.47	22.5	19.5	17.2	0.33	0.42	< 0.01
Intake[3], g/d										
DM	602[ab]	670[a]	557[b]	26.8	826	599	404	22.5	0.03	< 0.01
OM	562[ab]	626[a]	521[b]	24.9	771	560	378	21.0	0.03	< 0.01
NDF	212	207	184	13.6	259	207	138	10.9	0.33	< 0.01
Activity										
Eating										
min/d	146	155	124	12.9	162	146	116	10.5	0.24	< 0.01
min/g DMI	0.26	0.24	0.25	0.029	0.20	0.24	0.30	0.026	0.94	< 0.01
min/g NDFI[4]	0.72	0.76	0.75	0.099	0.64	0.70	0.89	0.081	0.96	0.03
Ruminating										
min/d	303	317	322	20.8	347	310	285	19.6	0.81	0.03
min/g DMI	0.54	0.50	0.64	0.043	0.43	0.52	0.73	0.042	0.10	< 0.01
min/g NDFI[4]	1.55	1.59	1.96	0.134	1.43	1.53	2.13	0.131	0.09	< 0.01
Total chewing time[5]										
min/d	450	471	447	28.4	508	458	403	23.5	0.81	< 0.01
min/g DMI	0.80	0.74	0.88	0.063	0.62	0.76	1.04	0.059	0.31	< 0.01
min/g NDFI[4]	2.28	2.35	2.70	0.212	2.08	2.23	3.02	0.186	0.35	< 0.01
Resting, min/d	815	816	808	31.6	770	808	861	23.8	0.98	< 0.01
Drinking, min/d	11	12	15	2.3	13	13	12	2.0	0.32	0.52
Other activity, min/d	163	141	168	15.7	149	160	164	14.0	0.46	0.45

[a,b]Means in the same row with different superscripts are different according to Tukey's test ($P \leq 0.01$).

[1]Only main effects are presented; the interaction between sex and feed restriction was not significant ($P \geq 0.25$; data not shown).

[2]F_L = linear effect of feed restriction; F_Q = quadratic effect of feed restriction was not significant ($P \geq 0.13$; data not shown).

[3]BW and feed intake presented were measured during the evaluation period of feeding behavior.

[4] NDFI = NDF intake.

[5]Chewing time = eating + ruminating.

Table 3. Body weight, feed intake, digestibility of DM and NDF in goats of different sexes subjected to feed restriction

	Sex				Feed restriction				*P*-value[1]		
Item	Females	Castrated males	Intact males	SEM	None	Moderate	Severe	SEM	Sex	F_L[2]	F_Q[2]
BW[3]	21.0	20.6	21.1	0.65	24.5	20.9	17.4	0.43	0.88	< 0.01	0.84
Intake[3], g/d											
DM	630	596	559	32.1	790	597	398	20.7	0.32	< 0.01	0.81
OM	589	557	522	30.1	737	558	372	19.3	0.32	< 0.01	0.75
NDF	215	212	201	10.1	267	216	145	6.9	0.62	< 0.01	0.07
Digestibility, %											
DM	74.9	76.1	75.5	0.55	74.6	74.4	77.5	0.57	0.38	< 0.01	0.02
OM	76.3	77.3	76.8	0.53	75.8	75.8	78.8	0.54	0.43	< 0.01	0.03
NDF	64.8	67.9	66.8	1.05	65.0	65.1	69.4	1.04	0.14	0.01	0.09
FOM[4]	10.2	9.8	9.6	0.32	10.8	10.0	8.7	0.29	0.41	< 0.01	0.42

[1]Only main effects are presented, the interaction between sex and feed restriction was not significant ($P \geq 0.15$; data not shown).

[2]F_L = linear effect of feed restriction; F_Q = quadratic effect of feed restriction.

[3]BW and feed intake presented were measured during the evaluation period of digestibility.

[4]Fecal metabolic OM = 100 × [fecal OM – fecal NDF/(DMI)].

Table 4. Body weight, feed intake before slaughter and pH of the ruminal and caecal digesta in goats of different sexes subjected to feed restriction

	Sex				Feed restriction				*P*-value[1]			
Item	Females	Castrated males	Intact males	SEM	None	Moderate	Severe	SEM	Sex	F_L[2]	F_Q[2]	SxF[3]
BW[4], kg	25.7[b]	25.5[b]	27.0[a]	0.36	31.2	26.7	20.2	0.35	0.02	< 0.01	0.03	0.02
Intake[4], g/d												
DM	730	681	779	45.0	995	705	489	30.1	0.33	< 0.01	0.11	0.24
OM	680	634	725	42.3	925	658	457	28.4	0.34	< 0.01	0.13	0.23
NDF	251	245	282	15.8	347	254	177	11.2	0.24	< 0.01	0.40	0.72
iNDF[5]	79	78	90	5.8	108	81	56	4.2	0.29	< 0.01	0.79	0.76
NDF, % of BW	0.93	0.98	1.03	0.055	1.11	0.95	0.89	0.038	0.44	< 0.01	0.13	0.87
pH												
Reticulorumen	5.9	5.9	5.8	0.06	5.7	5.9	6.0	0.05	0.24	< 0.01	0.42	0.93
Cecum	6.7	6.6	6.6	0.06	6.5	6.6	6.7	0.05	0.66	< 0.01	0.56	0.89

[a,b]Means in the same row with different superscripts are different according to Tukey's test ($P \leq 0.02$).

[1]Main effects and interaction between sex and feed restriction.

[2]F_L = linear effect of feed restriction; F_Q = quadratic effect of feed restriction.

[3]SxF = interaction between sex and feed restriction.

[4]BW and feed intake presented were measured during the period of marker infusion.

[5]iNDF = indigestible NDF.

decreased the time spent resting ($P < 0.01$) compared with animals fed restrictively. On the other hand, animals fed restrictively linearly increased the time of eating, ruminating, and total chewing time (eating + ruminating) per g DM and NDF intake ($P \leq 0.03$; Table 2).

Digestibility

The digestibility of NDF increased linearly ($P = 0.01$) when feed intake was restricted (Table 3). The digestibility of DM and OM responded in a quadratic manner, being greater for animals subjected to severe feed restriction than for the other treatment groups ($P \leq 0.03$). Fecal metabolic OM, calculated as fecal OM/kg DMI– fecal NDF/kg DMI, decreased linearly with increased level of feed restriction ($P < 0.01$). The digestibility of DM and OM increased by 4% and NDF by 6% when ad libitum treatment groups were compared with severe restriction.

Measurements of pH and Gastrointestinal Tissue Weight

The pH of ruminal and caecal digesta increased linearly as the level of feed restriction increased ($P < 0.01$; Table 4).

The fresh weight of empty tissues in the GIT linearly decreased ($P < 0.01$) as the level of feed restriction increased (Table 5). Females and castrated males had greater colon tissue weight than intact males ($P \leq 0.01$), but females and castrated males were not significantly different. There was a significant ($P \leq 0.04$) effect between sex and level of feed restriction on the tissue weight of reticulorumen, abomasum, and total GIT. Females had a higher recorded tissue weight of reticulorumen when fed at the moderate feed restriction compared with castrated males ($P < 0.01$), but neither group was significantly different from intact males ($P \geq 0.07$). Intact and castrated males displayed greater weight of abomasal tissue than females in the ad libitum treatment ($P < 0.01$). Castrated males had greater total GIT tissue weight than intact males in the ad libitum treatment ($P < 0.03$), but neither group was significantly different from female animals. Females had greater total GIT tissue weight than intact and castrated males when fed at moderate feed restriction ($P = 0.03$; data not shown). When the fresh weight of each tissue and total GIT were expressed as a percentage of BW, there was no difference between treatments. On average, the fresh tissue weight of the reticulorumen, small intestine and total GIT was 2.0%, 2.4%, and 6.3% of BW, respectively, across all treatments. The sum of omasum, abomasum, cecum, and colon was 1.8% of BW across all treatments.

Digesta Pool Size

The pool size of fresh matter in the reticulorumen and fresh matter, DM, and NDF in the cecum changed quadratically ($P \leq 0.02$), displaying the greatest value for animals subjected to moderate feed restriction (Table 6). The pool size of fresh matter, DM, and NDF in the omasum, small intestine, and colon and the pool size of DM in the abomasum decreased

Table 5. Fresh weight of gastrointestinal tissues (without digesta) in goats of different sexes subjected to feed restriction

	Sex				Feed restriction				*P*-value[1]		
Item	Females	Castrated males	Intact males	SEM	None	Moderate	Severe	SEM	Sex	F_L[2]	SxF[3]
Weight, g											
Reticulorumen	538	513	509	15.1	620	520	420	12.8	0.35	< 0.01	0.01
Omasum	65	67	73	2.5	84	65	55	2.4	0.08	< 0.01	0.11
Abomasum	107	119	109	3.3	129	112	95	3.1	0.06	< 0.01	0.01
Small intestine	640	668	605	23.1	733	621	559	20.6	0.18	< 0.01	0.47
Cecum	34	30	31	1.6	37	32	26	1.5	0.25	< 0.01	0.23
Colon[4]	306[a]	291[a]	253[b]	9.0	347	271	232	9.3	< 0.01	< 0.01	0.59
Total GIT[5]	1692	1665	1564	43.5	1929	1604	1388	39.2	0.13	< 0.01	0.04

[a,b]Means in the same row with different superscripts are different according to Tukey's test ($P \leq 0.01$).

[1]Main effects and interaction between sex and feed restriction.

[2]F_L = linear effect of feed restriction; F_Q = quadratic effect of feed restriction was not significant ($P \geq 0.11$; data not shown).

[3]SxF = interaction between sex and feed restriction.

[4]Colon = colon and rectum.

[5]Total GIT = sum fresh weight of reticulorumen, omasum, abomasum, small intestine, cecum, and colon.

linearly as feed restriction increased ($P \leq 0.03$). The pool size of DM and NDF in the reticulorumen and fresh matter and NDF in the total GIT decreased quadratically, with smaller value for animals subjected to severe feed restriction ($P \leq 0.05$). The pool size of DM in the total GIT decreased linearly with increased feed restriction ($P < 0.01$). The pool size of fresh matter, DM, and NDF in the reticulorumen represented around 74% of total GIT across all treatments. Intact males had a greater DM pool in the reticulorumen than female animals ($P \leq 0.01$), but neither group was significantly different from castrated males. Furthermore, intact males had a greater amount of NDF in the reticulorumen and a greater amount of DM and NDF in the omasum than females ($P \leq 0.04$).

The ratio iNDF:NDF changed quadratically in digesta collected from the omasum and colon, reaching its highest value at moderate feed restriction ($P \leq 0.05$; Table 7). The interaction between sex and level of feed restriction was significant for the iNDF:NDF ratio analyzed in the abomasum ($P = 0.03$). The ratio iNDF:NDF numerically increased in digesta throughout the GIT across all treatments except in the small intestine, where it decreased by approximately 25% compared with iNDF:NDF in the abomasum to cecum (across all treatments).

Mean Retention Time

Indigestible NDF. The MRT of digesta estimated from iNDF in the reticulorumen, omasum, abomasum, colon, and total GIT increased linearly ($P \leq 0.01$) with increasing level of feed restriction (Table 8). The MRT in the cecum varied quadratically, reaching the greatest value for animals fed at the moderate level of feed restriction ($P < 0.01$). The MRT in the reticulorumen represented on average 71% of MRT in the total GIT. Total MRT in the GIT increased by 20% for moderate restriction and 24% for severe restriction compared with ad libitum fed animals. The MRT of iNDF in the abomasum of castrated males was greater than that of intact males ($P < 0.01$), but neither group was significantly different from female animals.

Ytterbium. The MRT of digesta estimated from Yb increased linearly as feed restriction increased for the abomasum ($P = 0.01$; Table 8). The MRT of digesta in the cecum varied quadratically ($P = 0.01$), with the greatest value for the animals fed at moderate feed restriction. The MRT of digesta in the reticulorumen and total GIT decreased linearly as feed restriction increased ($P \leq 0.04$). The MRT of the reticulorumen represented on average 71% of total MRT of total GIT. The MRT of digesta in the omasum of castrated males was greater than that of intact males and females ($P < 0.01$). Castrated males had a greater MRT of digesta in the abomasum than intact males ($P \leq 0.01$), but neither group was significantly different from female animals ($P \geq 0.12$).

Chromium. The MRT of liquid was quadratically ($P \leq 0.04$) affected by level of feed restriction in the reticulorumen, cecum, and total GIT, with the greatest MRT for the animals subjected to the moderate level of feed restriction (Table 8). The MRT of liquid in the abomasum and in the colon increased linearly with increasing feed restriction in the animals ($P \leq 0.03$). The liquid MRT in the reticulorumen represented on average 48% of the liquid MRT in the total GIT, while the liquid MRT in the colon represented on average 33% of the liquid MRT in the total GIT.

Table 6. Pool size of different gastrointestinal tract (GIT) segments in goats of different sexes subjected to feed restriction

	Sex				Feed restriction				*P*-value[1]		
Item	Females	Castrated males	Intact males	SEM	None	Moderate	Severe	SEM	Sex	F_L[2]	F_Q[2]
Reticulorumen, g											
Fresh	3,720	3,664	4,019	203.0	4,064	4,193	3,146	177.4	0.43	< 0.01	0.01
DM	478[b]	525[ab]	612[a]	31.9	641	581	394	26.1	0.03	< 0.01	0.03
NDF	221[b]	234[b]	294[a]	18.3	305	271	173	14.5	0.03	< 0.01	0.03
Omasum, g											
Fresh	100	127	126	8.8	140	121	93	8.2	0.07	< 0.01	0.65
DM	22[b]	30[a]	29[a]	2.2	34	27	21	2.0	0.05	< 0.01	0.78
NDF	9[b]	11[a]	12[a]	0.8	12	11	8	0.8	0.03	< 0.01	0.78
Abomasum, g											
Fresh	307	399	318	33.5	349	364	311	29.2	0.15	0.32	0.33
DM	34	46	35	5.0	42	40	33	3.7	0.23	0.03	0.37
NDF	14	18	15	1.9	16	17	14	1.6	0.34	0.34	0.32
Small intestine, g											
Fresh	359	371	400	31.7	476	378	276	28.0	0.64	< 0.01	0.94
DM	32	34	36	2.9	46	33	23	2.5	0.58	< 0.01	0.65
NDF	7	7	8	0.8	9	7	5	0.8	0.70	< 0.01	0.90
Cecum, g											
Fresh	151	141	155	13.2	155	173	119	11.4	0.75	0.02	0.01
DM	18	18	19	1.7	20	21	15	1.4	0.84	0.01	0.02
NDF	8	8	8	0.7	8	9	6	0.6	0.84	0.02	0.01
Colon[3], g											
Fresh	425	407	433	26.3	503	422	340	22.5	0.77	< 0.01	0.99
DM	86	79	84	6.1	104	81	64	5.4	0.77	< 0.01	0.61
NDF	38	35	37	2.7	45	36	29	2.2	0.70	< 0.01	0.82
Total GIT[4], g											
Fresh	5,074	5,136	5,371	290	5,674	5,605	4,302	233	0.75	< 0.01	0.02
DM	672	734	796	48	882	769	551	36	0.21	< 0.01	0.15
NDF	295	325	375	22	402	356	237	17	0.06	< 0.01	0.05

[a,b]Means in the same row with different superscripts are different according to Tukey's test ($P \leq 0.04$).

[1]Only main effects are presented, the interaction between sex and feed restriction was not significant ($P \geq 0.09$; data not shown).

[2]F_L = linear effect of feed restriction; F_Q = quadratic effect of feed restriction.

[3]Colon = colon and rectum.

[4]Total GIT = sum of pool size of reticulorumen, omasum, abomasum, small intestine, cecum, and colon.

DISCUSSION

A TMR was used in this study to avoid any feed selection bias created by ad libitum fed animals that could result in different proportions of ingredients being eaten compared with animals fed restrictively. The variation in BW before slaughter may have been the main factor determining the differences observed between sexes and the interaction between sex and feed restriction for the variables evaluated. In summary, sex did not influence most of variables evaluated and is therefore not discussed further below.

To obtain reliable estimates of MRT using the rumen evacuation or slaughter technique, it is crucial that the GIT of the animals is in steady state, that evacuation is performed to allow accurate and precise estimation of the average rumen pool size, and that the flux and compartmental mass of an indigestible entity are measured (Huhtanen et al., 2007). However, Huhtanen et al. (2007) found that feeding frequency and rumen evacuation time in growing cattle did not significantly affect the mean pool size of iNDF. To avoid timing rumen evacuation at maximum or minimum rumen fill, the animals in the present study were slaughtered 2.5 ± 0.5 h after feeding. Additionally, using the feeding behavior data in the present study and considering 3 h after offering feed in the morning, the animals spent on average 48, 58, and 41 min on the eating activity (data not shown) for ad libitum, moderate, and severe restriction, respectively. Furthermore, iNDF intake prior to slaughter was 1.5, 1.4, and 1.4 g/kg BW for ad libitum, moderate, and severe restriction, respectively. Taking

Table 7. Ratio of indigestible NDF to NDF (iNDF:NDF) in gastrointestinal tract (GIT) segments of goats of different sexes subjected to feed restriction

	Sex				Feed restriction				*P*-value[1]			
Item	Females	Castrated males	Intact males	SEM	None	Moderate	Severe	SEM	Sex	FL[2]	FQ[2]	SxF[3]
iNDF:NDF												
Reticulorumen	0.57	0.53	0.53	0.030	0.53	0.55	0.57	0.021	0.60	0.07	0.97	0.53
Omasum	0.66	0.62	0.62	0.040	0.58	0.67	0.65	0.028	0.72	0.02	0.03	0.16
Abomasum	0.80[a]	0.75[ab]	0.69[b]	0.025	0.72	0.78	0.76	0.024	0.03	0.25	0.21	0.03
Small intestine	0.59	0.55	0.55	0.039	0.51	0.59	0.59	0.035	0.74	0.08	0.30	0.90
Cecum	0.78	0.71	0.73	0.029	0.71	0.76	0.75	0.023	0.21	0.20	0.13	0.16
Colon[4]	0.80	0.77	0.77	0.031	0.74	0.81	0.80	0.022	0.74	0.01	0.05	0.30

[a,b]Means in the same row with different superscripts are different according to Tukey's test ($P \leq 0.01$).

[1]Main effects and interaction between sex and feed restriction.

[2]F_L = linear effect of feed restriction; F_Q = quadratic effect of feed restriction.

[3]SxF = interaction between sex and feed restriction.

[4]Colon = colon and rectum.

Table 8. Mean retention time (MRT) of particulate and liquid matter in gastrointestinal tract (GIT) segments of goats of different sexes subjected to feed restriction estimated by indigestible NDF (iNDF) and Yb for particulate, and Cr-EDTA for liquid

	Sex				Feed restriction				*P*-value[1]		
Item	Females	Castrated Males	Intact Males	SEM	None	Moderate	Severe	SEM	Sex	F_L[2]	F_Q[2]
MRT by iNDF, h											
Reticulorumen	40.0	37.5	43.4	3.91	36.1	42.1	42.8	2.60	0.57	< 0.01	0.18
Omasum	1.8	2.1	2.0	0.19	1.6	2.2	2.2	0.15	0.60	0.01	0.06
Abomasum	3.6[ab]	4.5[a]	2.8[b]	0.32	2.5	3.6	4.7	0.24	0.01	< 0.01	0.99
Small Instestine	1.1	1.0	1.0	0.08	1.0	1.1	1.1	0.08	0.42	0.64	0.55
Cecum	2.0	1.8	1.8	0.23	1.3	2.2	2.0	0.18	0.68	< 0.01	< 0.01
Colon[3]	9.2	8.8	7.8	0.88	7.2	9.0	9.5	0.65	0.53	< 0.01	0.25
Total GIT[4]	57.5	54.7	58.6	4.86	49.5	59.6	61.6	3.15	0.84	< 0.01	0.08
MRT by Yb, h											
Reticulorumen	31.5	34.3	35.3	2.26	35.6	35.7	29.8	1.89	0.48	0.02	0.15
Omasum	1.5[b]	2.2[a]	1.7[b]	0.11	1.9	1.7	1.8	0.11	< 0.01	0.54	0.31
Abomasum	1.3[ab]	1.6[a]	1.0[b]	0.15	1.0	1.4	1.5	0.14	0.04	0.01	0.35
Small instestine	1.3	1.2	1.2	0.12	1.2	1.4	1.1	0.12	0.82	0.35	0.15
Cecum	2.1	2.1	1.9	0.29	1.6	2.5	2.1	0.22	0.92	0.09	0.01
Colon[3]	8.2	9.1	7.5	0.80	8.0	8.4	8.4	0.80	0.36	0.76	0.87
Total GIT[4]	45.1	47.9	48.1	2.62	48.8	49.6	42.7	2.21	0.68	0.04	0.15
MRT by Cr, h											
Reticulorumen	8.6	7.6	7.5	0.60	6.9	8.8	8.0	0.55	0.43	0.16	0.04
Omasum	0.2	0.2	0.2	0.02	0.2	0.2	0.2	0.02	0.65	0.52	0.80
Abomasum	0.5	0.6	0.5	0.07	0.4	0.6	0.6	0.07	0.27	0.03	0.31
Small instestine	1.1	1.0	1.1	0.13	1.0	1.2	1.0	0.13	0.65	0.87	0.20
Cecum	1.5	1.3	1.3	0.14	0.9	1.7	1.4	0.13	0.70	0.01	< 0.01
Colon[3]	6.3	5.1	5.2	0.39	4.6	5.5	6.5	0.38	0.10	< 0.01	0.91
Total GIT[4]	17.7	16.2	15.7	0.88	14.1	18.5	17.0	0.86	0.24	0.02	0.01

[a,b]Means in the same row with different superscripts are different according to Tukey's test ($P \leq 0.05$).

[1]Only main effects are presented, the interaction between sex and feed restriction was not significant ($P \geq 0.06$; data not shown).

[2]F_L = linear effect of feed restriction; F_Q = quadratic effect of feed restriction.

[3]Colon = colon and rectum.

[4]Total GIT = sum MRT of reticulorumen, omasum, abomasum, small intestine, cecum, and colon.

these aspects into account, we are certain that animals at slaughter were close to daily rumen pool size.

Feeding Behavior and Digestibility

In agreement with previous studies (Doreau et al., 2003, 2004; Galvani et al., 2010; Dias et al., 2011), it was found that decreased feed intake in general resulted in increased diet digestibility. In addition, more efficient mastication was observed, i.e., the animals fed restrictively spent more time eating and ruminating per kg DM or NDF ingested feed. Furthermore, the MRT of ruminal digesta increased when animals were subjected to restricted feeding. The increases in ruminal MRT, ruminal pH, and ruminating activity (min per kg of DMI) can explain the improved digestibility when feed intake decreased. However, in previous experiments on animals fed at intake levels below maintenance requirements, the digestibility decreased, despite increased MRT (Grimaud et al., 1998, 1999; Atti et al., 2002). This has been attributed to limitations imposed on microbial attachment and activity (Doreau et al., 2003).

Gastrointestinal Tissues and Pool Size

A decrease in the fresh weight of GIT tissues with decreasing feed intake has been reported previously (Johnson et al., 1990; Fluharty and McClure, 1997; Nozière et al., 1999). Decreased feed intake can result in decreased mass of splenic and liver tissue within a few weeks of restricted feeding (Johnson et al., 1990). This depends directly on blood flow and energy expenditure, which is related to intake (Atti et al., 2000). Similarly, in the present study, the empty weight of GIT tissues decreased with increasing feed restriction. However, when the fresh weight of GIT tissues was expressed as a percentage of BW, the values were similar between the treatments. Atti et al. (2000) found that tissue weight of the abomasum and small intestine was not influenced by long-term severe undernutrition in adult ewes of 49 kg BW. Those authors concluded that below a given BW, the abomasum and small intestine do not respond to variations in intake. In the present study, the animals were growing and BW changed from approximately 15 to 30 kg during the experiment, with the latter being around 70% of adult weight. This may explain the observed influence of feed intake variation on fresh weight for all GIT tissues. The results indicate that BW is an important factor in determining the fresh weight of GIT tissues, irrespective of level of intake.

It has been shown that a decrease in feed intake results in decreased content of digesta in the reticulorumen (Robinson et al., 1987; Atti et al., 2000, 2002). Cannas et al. (2003) observed a curvilinear relationship between rumen NDF pool size and forage intake. de Vega et al. (1998) concluded that the reticulorumen and hindgut are the main mixing compartments of the GIT of ruminants, which is confirmed by the observed curvilinearity. As reported previously by Atti et al. (2000), in the present study, the contents of the digestive tract varied in the same way as feed intake. This is likely to apply especially when the diet composition is constant.

The iNDF:NDF ratio of digesta from different segments of the GIT can be used to estimate the contribution of these different compartments to NDF digestion (Ahvenjärvi et al., 2010b). Walz et al. (2004) found a progressive increase in the digesta ratio of iNDF to potential digestible NDF through different segments of the ruminant GIT, with the exception of the small intestine. A similar exception for the iNDF:NDF ratio was observed in the present study and suggests that particles with a low iNDF:NDF ratio are selectively retained in the small intestine. The proportion of NDF digested before the abomasum was 93.8% across all treatments in this study, confirming observations in a meta-analysis by Huhtanen et al. (2010) quantifying NDF digestion in cattle. In a slaughter study with goats fed low-quality diets, 85% of NDF digestion occurred before the abomasum when calculated from iNDF:NDF ratio (Walz et al., 2004). Furthermore, the increased iNDF:NDF ratio between the reticulorumen and the abomasum observed in the present study indicated digestion of NDF in the omasum, confirming results presented by Ahvenjärvi et al. (2000).

Mean Retention Time

There is generally a negative relationship between feed intake and MRT of particles in the rumen of sheep and cattle (Colucci et al., 1990; Huhtanen and Kukkonen, 1995; Atti et al., 2002; Dias et al., 2011). However, in the present study, the difference between moderate and severe feed restriction was numerically marginal. The MRT in the reticulorumen represented a proportion of 0.71 of total GIT retention time (on average, across all treatments). Ahvenjärvi et al. (2010b) reported a corresponding proportion of 0.72 in dairy cows and Walz et al. (2004) a proportion of 0.71 in goats.

Cannas et al. (2003) compared rumen turnover in small and large ruminants using lignin and iNDF as an internal marker by compiling published rumen evacuation studies. On average, sheep and cattle had 49.0 and 40.7 h rumen turnover, respectively. In the present study, MRT estimated by iNDF was 40.3 h (across all treatments). Both these MRT averages estimated using iNDF as a marker are realistic, and factors such as quality of forage, physical, and chemical characteristics of diets and level of intake could have caused the

difference between studies. For instance, most sheep diets (74%) reviewed by Cannas et al. (2003) were exclusively forage, which could explain the greater MRT than estimated for cattle as stated by the authors. Furthermore, lignin cannot be regarded as an ideal marker due to the frequently observed incomplete recovery. Analytical variability between samples and between the methods applied could explain the reported apparent digestibility of ADL in dairy cow production trials throughout the literature (Fahey and Jung, 1983; Huhtanen et al., 1994). Moreover, Huhtanen and Kukkonen (1995) found shorter MRT using lignin compared with iNDF or iADF and attributed this to incomplete recovery of lignin in ruminal digesta.

The MRT is shorter with Yb than when Cr or iNDF are used as particulate markers (Huhtanen and Kukkonen, 1995; Ahvenjärvi et al., 2010a; Krizsan et al., 2011). Huhtanen and Kukkonen (1995) compared markers (Yb and Cr) and found that the in situ disappearance of Yb-hay was much greater than that of Cr-hay. Beauchemin and Buchanan-Smith (1989) also compared Yb and Cr and found faster rumen turnover for Yb-silage than Cr-mordanted silage. Krizsan et al. (2011) showed that preduodenal retention time was longer when estimated with Cr-mordanted feeds compared with rare earths. Ytterbium is associated with small particles, which would result in a shorter MRT for Yb than Cr (Erdman and Smith, 1985; Siddons et al., 1985). Furthermore, Yb can migrate from the labeled particles to fine feed and microbial residues (Combs et al., 1992). This migration of Yb from large to small particles could lead to underestimation of rumen retention time due to the faster passage rate of small particles and liquid (Dixon and Milligan, 1985). In addition to possible migration of Yb from labeled particles, it is possible that the contribution of concentrates to Yb was greater than to iNDF. If this were true, shorter MRT of concentrates compared to forages (Colucci et al., 1990) could also have contributed to the difference in MRT between iNDF and Yb.

The rare earths can be displaced from their feedstuff binding sites by protons at pH values comparable to the more acidic abomasal and duodenal digesta. However, such displacement is of little consequence, at least for ruminants, because the ruminal digesta is the primary, if not sole, source of variation in flow of particulate matter and solutes. In the present study, the MRT of reticulorumen determined by Yb was on average, across all treatments, 6.6 h shorter than the MRT determined by iNDF. Furthermore, there was 2.3 h shorter abomasal MRT based on Yb compared with iNDF as marker. These results could indicate displacement of Yb in acidic conditions or could reflect shorter MRT of small particles and fluid compared with large particles in the abomasum. The reticulorumen, omasum, and abomasum were responsible for 93% of the difference between Yb and iNDF in the present study. In addition, there was marginal difference when markers (Yb and iNDF) to estimate MRT postduodenal (small intestine + cecum + colon) were compared. This resulted from a greater MRT estimated by Yb in the small intestine and cecum and a greater MRT using iNDF in the colon. Huhtanen and Kukkonen (1995) dosed the markers into the duodenum and found similar postduodenal MRT of Yb-labeled and Cr-mondanted particles.

The rumen MRT of liquid increased as level of feed restriction increased, in agreement with previous studies (Grovum and Williams, 1977; Colucci et al., 1990; Huhtanen and Kukkonen, 1995). The difference in liquid flow may be mainly due to the reticulorumen acting as a large mixing compartment in the GIT of ruminants (Ellis et al., 1994). Furthermore, particulate and liquid matter should have comparable flow rates after the abomasum because the digesta flow is mainly tubular according to previous studies (Grovum and Williams, 1973; Huhtanen and Kukkonen, 1995). In the present study, 90% of the difference between particulate (Yb and iNDF) and liquid matter (Cr-EDTA) was related to the reticulorumen, omasum, and abomasum and 10% to the small intestine, cecum, and colon. Furthermore, the total MRT of particles estimated by iNDF (across level of feed restriction) represented 3.4-fold total MRT of liquid.

Conclusions

Mean retention time of feed particles in different segments of the digestive tract of growing Saanen goats increased with decreased feed intake when determined by the slaughter technique using internal marker (iNDF). Increased MRT with restrictively fed goats was associated with improved diet digestibility. External marker (Yb) resulted in shorter MRT estimates in the reticulorumen and total tract than slaughter techniques with iNDF. The MRT of Yb was shortest in goats fed the lowest level of intake iNDF, indicating that MRT cannot be reliably estimated with a single marker in a slaughter study. The iNDF:NDF ratio increased progressively with the passage of digesta through the digestive tract except in the small intestine. Based on iNDF:NDF ratio, more than 90% of fiber digestion occurred in the reticulorumen and omasum. Sex was not an important factor affecting MRT in different segments of the GIT in growing Saanen goats.

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