UNIVERSIDADE ESTADUAL PAULISTA-UNESP
CÂMPUS DE JABOTICABAL

FEED DIGESTION OF GROWING SAANEN GOATS

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TÍTULO: FEED DIGESTION OF GROWING SAANEN GOATS

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DADOS CURRICULARES DO AUTOR

Rafael Fernandes Leite filho de Marilda Gaspar Fernandes e Joel Leite nasceu no dia 07 de fevereiro de 1983 na cidade de Passa Quatro em Minas Gerais. Iniciou sua graduação em Zootecnia em abril de 2002 na Universidade Federal de Lavras (UFLA), colando grau no dia 27 de agosto de 2007. Em março de 2008 deu início a pós graduação em Zootecnia na Universidade Federal de Lavras na área de nutrição e produção de ruminantes, com a obtenção do título de Mestre no dia 05 de julho de 2010 sob orientação do Prof. Juan Ramon Olalquiaga Perez. Em março de 2011 iniciou o doutorado na Universidade Estadual Paulista (UNESP) em Jaboticabal na mesma área sob orientação da Profa. Izabelle Auxiliadora Molina de Almeida Teixeira. No período de outubro de 2013 à julho de 2014 realizou estágio no exterior sob orientação do prof. Pekka Huhtanen na “Swedish University of Agricultural”, na Suécia.

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SUMMARY

UNIVERSIDADE ESTADUAL PAULISTA
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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 007156/10 do trabalho de pesquisa intitulado **"Taxa de passagem de partículas e fluidos em caprinos submetidos a diferentes níveis nutricionais"**, sob a responsabilidade da Profª Drª Izabelle Auxiliadora Molina de Almeida Teixeira, está de acordo com os Princípios Éticos na Experimentação Animal, adotado pelo Colégio Brasileiro de Experimentação (COBEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 15 de abril de 2010.

Jaboticabal, 19 de abril de 2010.

Prof. Dr. Jeffrey Frederico Lui
Presidente - CEBEA

Med. Vet. Maria Alice de Campos
Secretária - CEBEA

02/05/11

Faculdade de Ciências Agrárias e Veterinárias
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FEED DIGESTION OF GROWING SAANEN GOATS

ABSTRACT - The main objective was to assess the contribution of different segments of the gastrointestinal tract (GIT) to the fiber digestion. Two experiments were conducted, which mean retention time (MRT) of particulate and liquid matter, pool size, and tissues weight in different segments were determined by slaughter technique. The study one evaluated MRT of particulate and liquid matter in growing Saanen goats of different sexes and subjected to different levels of feed restriction. Fifty-four Saanen goats (18 each of females, castrated males and intact males) were used in a 3×3 factorial arrangement comprising the three sexes and three levels of feed restriction (unrestricted/ad libitum, moderate and severe restriction). 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. 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. The second study examined physiological aspects in the digestion of fiber, particulate and liquid matter residence, pool size, and tissues weight in the whole tract of growing Saanen goats. A total of 58 Saanen goats were disposed in a 3×3 factorial arrangement consisting of three sexes (female, castrated males, intact males) and three slaughter weights (target BW 16, 23, and 30 kg at slaughter). Treatment effects were evaluated in a split-plot design, with sex as the main plot and slaughter weight as the subplot. Polynomial contrasts were used to determine linear and quadratic effects of slaughter weight. Thus increased BW at slaughter resulted in greater MRT of particulate and liquid matter in digestive segments characterized by mixing digesta flow. This suggests greater capacity for fiber digestion with increased BW, as also indicated by the greater pool size and iNDF:NDF ratio of digesta in these segments. The results also indicated that on average, more than 95% of fiber digestion occurred in the fore-stomachs of growing Sannen goats.

Key words: gastrointestinal tract, fiber digestibility, mean retention time, pool size

DIGESTÃO DE ALIMENTOS DE CABRITOS EM CRESCIMENTO

RESUMO - O objetivo principal foi determinar a contribuição dos diferentes segmentos do trato gastrointestinal (TGI) para a digestão da fibra. Dois experimentos foram conduzidos, os quais foram determinados o tempo médio de retenção (TMR) de partículas e líquido, conteúdo e pesos dos tecidos nos diferentes segmentos pelo método do abate. O primeiro estudo avaliou TMR de partículas e líquido de cabritos Saanen em crescimento de diferentes sexos e submetidos a diferentes níveis de restrição alimentar. Foram utilizados 54 cabritos da raça Saanen (18 fêmeas, 18 machos castrados e 18 machos inteiros) em um arranjo fatorial 3 × 3 que compreendeu os três sexos e três níveis de restrição alimentar (*ad libitum* / sem restrição, restrição moderada e severa). Contrastes polinomiais foram utilizados para determinar o efeito linear e quadrático da restrição alimentar, enquanto o efeito do sexo foi comparado pelo teste de Tukey. Os efeitos do sexo e interação entre sexo e restrição alimentar não foram significativos na maioria das variáveis avaliadas. A restrição alimentar aumentou o TMR de partículas e fluído. O TMR foi um recurso importante para aumentar a oferta de nutrientes, quando os animais foram submetidos à restrição alimentar, como indicado pelo aumento da digestibilidade total. O segundo estudo avaliou aspectos fisiológicos da digestão da fibra, retenção de partículas e líquido, conteúdo e peso dos tecidos em todo o TGI de cabritos da raça Saanen. O total de 58 cabritos da raça Saanen foram aleatoriamente dispostos em um arranjo fatorial 3 × 3, composto por três sexos (fêmeas, machos castrados, machos inteiros) e três pesos de abate (aproximadamente 16, 23, e 30 kg de peso no abate). Os efeitos do tratamento foram avaliados em um esquema de parcelas subdivididas, com o sexo como efeito principal e peso de abate como sub parcela. Contrastes polinomiais foram utilizados para determinar os efeitos linear e quadráticos de peso de abate. Assim, o aumento do peso de abate resultou em geral em maiores TMR de partículas e líquido nos segmentos do TGI caracterizados por misturar a digesta. Isso sugere maior capacidade de digestão da fibra com o aumento do peso, como também indicado pelo maior tamanho dos conteúdos e pela relação FDNi:FDN da digesta nestes segmentos. Os resultados também indicaram que, em média, mais de 95% da digestão da fibra ocorreu nos pré-estômagos de caprinos Sannen em crescimento.

Palavras-chave: conteúdo da digesta, digestibilidade da fibra, tempo médio de retenção, trato gastrointestinal

THESIS STRUCTURE

Chapter 1 is a literature review, about mean retention time and fiber digestion covering the factors that influence and methods. It was written following the rules of the Graduate Program in Animal Science at UNESP Jaboticabal Campus.

Chapter 2 describes contribution of different segments of the gastrointestinal tract to digestion in growing Saanen goats subjected to different feeding levels. This chapter was written following the guidelines of Journal of Animal Science (JAS), except by letter style and size, and position of tables. The paper authors are R. F. Leite, S. J. Krizsan, F. O. M. Figueiredo, V. B. Carvalho, I. A. M. A. Teixeira and P. Huhtanen.

Chapter 3 describes retention of feed through the gastrointestinal tract in growing Saanen goats using animals with different body weight to study primarily effects of growing phase on passage and digestion kinetics. It was written following the rules of guidelines of Journal of Animal Science (JAS), except by letter style and size, and position of tables. The paper authors are R. F. Leite, S. J. Krizsan, F. O. M. Figueiredo, V. B. Carvalho, I. A. M. A. Teixeira and P. Huhtanen.

Chapter 4 describes the main implications of the research.

CHAPTER1. GENERAL CONSIDERATIONS

1. INTRODUCTION

Ruminants are unique because they can convert fibrous plant material into nutritious feed based on microbial degradation in the fore-stomachs (VAN SOEST, 1994). In most of situations, fiber is the major energy source in ruminant feedstuffs. Variation in forage digestibility in ruminants results almost entirely from the concentration of cell wall carbohydrates, often analyzed as neutral detergent fiber (HUHTANEN et al., 2006). The ruminant digestive system has developed to selectively retain undigested fibrous material in order to maximize ruminal fiber digestion (ALLEN; MERTENS, 1988). Feed must disappear from the rumen by either digestion or passage for a further intake to occur (MERTENS, 1994). On the other hand, longer retention time of feed particles in the rumen improves the utilization of the fiber fraction, however, it may also restrict feed intake, because intake of forage is limited mostly by rumen capacity (VAN SOEST, 1994). Reticulum and omasum also contribute to digesta cell wall, in a complex ecosystem that is influenced by interactions between feeds, microbial population and the host animal (HUHTANEN et al., 2006). Thus, knowledge of the factors influencing the mean retention time (**MRT**) of fiber is essential for predicting forage utilization by ruminants (KRIZSAN et al., 2010). Additionally, understanding the changes in nutrients digestion that occur in the rumen and also in the other segments of the digestive tract may help to explain difference occurring in animal performance (TITGEMEYER, 1997).

This review will discuss the main factors related to passage kinetics. Furthermore, it will discuss the main markers and methods used to determine passage rate (**kp**) or MRT in ruminants.

2. DEFINITION AND FACTORS INFLUENCING PASSAGE KINETICS

Passage, or transit, refers to the flow of undigested residues through the digestive tract (VAN SOEST, 1994). Mean retention time is the inverse of kp for an

indigestible entity and is directly related to feed intake (ELLIS et al., 1994). Furthermore, MRT is a measure of time the digesta is exposed to the processes of mixing, digestion and absorption in the gastrointestinal tract (**GIT**) or a given segment (FAICHNEY, 2005), and it can be calculated also as the contents of segment divided by the inflow per unit time (VAN SOEST et al., 1992).

Retention time in the rumen is the sum of two processes, resulting in a selective retention of particles. The first is an ageing process based on mastication and fermentation (alteration of particle size, functional specific gravity, etc.) and it is required for entering the second process, involving simple passive escape from reticulorumen (ELLIS et al., 1999). Additionally, neutral detergent fiber (**NDF**) is not a homogenous entity, because indigestible NDF (**iNDF**) and potentially digestible NDF (**pdNDF**) have different digestion and passage rates (MERTENS, 1993; HUHTANEN ET AL., 2007). The faster kp of iNDF compared to pdNDF for the small particle size fraction indicates that pdNDF is selectively retained in the rumen (TAMMINGA et al., 1989; RINNE et al., 2002; HUHTANEN et al., 2007; BAYAT et al., 2010). In this regard, differential MRT of NDF, iNDF and pdNDF need attention, for example MRT of NDF and pdNDF are highly biased due to the contemporary digestion (LUND, 2002). Therefore, MRT and kp can only be estimated for entities like iNDF, which are indigestible and disappear only via passage (ELLIS et al., 1999).

Passage rate or MRT can be affected by several factors. In general, it can be described as animal and feed characteristics, which affect passage kinetics. Animal characteristics are related to species, body weight (**BW**), sex, stage of gestation, and lactation. On the other hand, feed characteristics are related to physical characteristics e.g., particle size, rate of particle size reduction, and functional specific gravity and there are also factors related to diet composition e.g. carbohydrate content, protein content, fat supplementation (ELLIS et al., 1994; HUHTANEN et al., 2006).

Animals Characteristics

A central issue to differential passage rate of feeds is the level of intake by the animal. Therefore, all aspects that can influence feed intake may affect passage rate or MRT, and consequently digestibility. Level of intake has been the main mechanism studied to evaluate the MRT, and its consequences in diet digestibility. A negative relationship between feed intake and MRT of particles in the rumen of sheep and cattle was previously reported (COLUCCI et al., 1990; HUHTANEN; KUKKONEN, 1995; ATTI et al., 2002; DIAS et al., 2011). As result of this interaction influences diet digestibility. For instance, a decreased feed intake in general resulted in increased diet digestibility (DOREAU et al., 2003, 2004; GALVANI et al., 2010; DIAS et al., 2011), due to the increased MRT.

Rumen passage rate has often been considered species specific (EVANS, 1981; OWENS; GOETSCH, 1986; CSIRO, 1990; LESCOAT; SAUVANT, 1995) owing to morphological differences (i.e., salivary glands, lips, tongue, etc.), body size and digestive capacity between species (HOFMANN, 1989; VAN SOEST, 1994). Therefore, differences in the passage kinetics and digestibility between species may be possible. However, no differences between sheep and cattle in the ruminal passage rate were found (CANNAS; VAN SOEST, 2000; CANNAS; VAN SOEST; PELL, 2003).

Body weight has a positive influence on MRT and a correlation between BW and MRT has been reported in studies comparing different herbivorous species (DEMMENT; VAN SOEST, 1983; ILLIUS; GORDON, 1992; GORDON; ILLIUS, 1994). Additionally, forage intake can be limited by the capacity of the GIT and, in particular, of the reticulorumen (MERTENS, 1994; VAN SOEST, 1994). Consequently, large animals should have greater capacity to retain feed for longer time and digest it more extensively than smaller animals (HACKMANN 2008; HACKMANN; SPAIN, 2010; STEUER et al., 2011).

Passage rate can also be influenced by physiological state as gestation and lactation (FAICHNEY; WHITE, 1988; WESTON, 1988). There is a high nutrient demand in the end of pregnancy, due to fetus and placenta development (CRONJE, 2000). Simultaneously, the increase of fetal size promotes compression in the rumen

and other segments by uterus, decreasing feed intake. However, feed intake decreases lesser than gastrointestinal tract volume because passage rate increases (MACEDO JUNIOR et al., 2012). In this case, the mechanisms that control passage rate are different compared to growing or lactating animals. Apparently, the uterus compression in the gastrointestinal tract increases intra-ruminal pressure and stimulates the motility, increasing passage rate, irrespective of decreased intake. Whereas, lactating ewes increases particle passage rate compared to nonlactating ewes (COFFEY et al., 1989), it is related to greater DMI (OKINE; MATHISON, 1991), due to high demand of nutrient to produce milk.

Sex is inherent animal characteristic that influences nutritional requirement (NRC, 2007). Previous studies to date have reported that intact males have higher dry matter intake (DMI) compared to castrated males and females, due to the different nutrient requirements related to higher weight gain and maintenance (Bailey and Duff, 2005; NRC, 2007). Therefore, since DMI may change according to sex, and intake level is the factor with the greatest influence on MRT, sex could have a significant impact on MRT.

Feed Characteristics

It has been widely accepted that the probability of feed particles to leave the reticulorumen depends on their physical characteristics, i.e. the time required to reduce particle size (WELCH, 1982) and increase particle density (EHLE, et al., 1984). Consequently, the rumen passage is based on particle size (POPPI et al., 1980), specific gravity (HOOPER; WELCH, 1985), and particle density (SUTHERLAND, 1988). Further, fermentation gases from microbial feed degradation can influence the specific gravity of feed particles, e.g. by increasing their buoyancy due to gas entrapment in feed particles (SUTHERLAND, 1988) and, thereby reducing their probability of rumen escape. In contrast, small particles show greater density due to low gas entrapment, which increases their probability of rumen escape. Additionally, particle shape (TROELSEN; CAMPBELL, 1968) and physical location of feed particles within the rumen (WELCH, 1982; POPPI et al., 2001) might be relevant to escape of rumen (WARNER, 2013).

Passage kinetics is also affected by diet composition. It can be attributed to different dietary components, which can affect passage and digestion kinetics through unbalance of nutrients (HUHTAHEN et al., 2006). Dietary components have different effects on rumen microbes, and interactions between them may occur. For low quality forages, limitations in the rate and extent of digestion can be attributed to a deficiency in the supply of essential nutrients (HOOVER, 1986). In contrast, in high producing ruminants fed mixed diets, the rate of cell wall digestion can be strongly retarded by substrates that inhibit the growth of rumen cellulolytic bacteria.

Forage to concentrate ratio and physical form of diet may also affect passage rate (GOETSCH; GALYEAN, 1982) so that animals fed diet with small particle size and low level of fiber presents a greater rate of passage.

Regarding to these factors (animals and feed characteristics), Krämer et al. (2013) highlighted that modern feed evaluation systems are taking into account animal characteristics to estimate passage rate, whereas differences between forage type are often not taken into account (NRC, 2001; FOX et al., 2004; VOLDEN, 2011). For instance, Krizsan et al. (2010) showed in a meta-analysis that inclusion of forage type affect particulate matter passage. However, the authors also pointed out that more research is needed to confirm the importance of relative forage differences in a rumen model and to separate animal effects from feed factors in predictions of ruminal particulate matter.

3.MEASUREMENT OF PASSAGE KINETICS

Procedures used to estimate passage kinetics in ruminants have differed with regard to the marker and the methods. The markers are related to the type (external and internal), number of markers (simple, double and triple markers), marker dosing (pulse dose or continuous infusion) and sampling site. Furthermore, passage kinetics can be estimated by direct (slaughter method or rumen evacuation) or by indirect method (compartmental model method).

Markers

In order to obtain an accurate and precise measurement on the passage kinetics through the GIT, particles or liquid have to be labelled (UDÉN et al., 1980). Several markers have been investigated (ELLIS et al., 1982; FAHEY; JUNG, 1983; SATTER et al., 1986; OWENS; HANSON, 1992). According to Owens and Hanson (1992), an ideal marker must not be separated from the labelled fractions, must be physically similar to or intimately associated with the labelled material; must not be affected or affect the digestive tract or its microbes, must not be absorbed in the GIT and must have a specific and sensitive method of estimation. In general, markers can be described in external or internal markers.

The external marker should remain associated with the specific undigested nutrient in question. Rare earth and chromium are the most frequently used external markers to study passage kinetics of particulate matter (CANNAS; VAN SOEST; PELL, 2003; HUHTANEN et al., 2006). However, chromium has been criticized for altering physical characteristics and the digestibility of labelled feedstuffs (EHLE et al., 1984), and rare earths have been observed to migrate to rumen liquid (COMBS; SHAVER; SATTER, 1992) and to mainly bind to small particles (SIDDONS et al., 1985). On the other hand, internal markers are intrinsic to the feed and, hence, circumvent the inherent limitation of external markers. There are many indigestible inert components, which can be present in feed in low concentration (e.g. lignin, acid-insoluble ash) or in more abundant (e.g. indigestible fiber) to determine particulate matter (WARNER, 2013). Internal markers are advantageous because they are intrinsic of feeds, and thus no preparation of markers is needed. Lignin has been extensively studied as an internal marker (FAHEY; JUNG, 1983), but there are problems with incomplete and variable recovery.

Several indigestible cell wall components, e.g. cellulose (PENNING; JOHNSON, 1983), neutral detergent fiber and acid detergent fiber (ADF) (COCHRAN; ADAMS; WALLACE, 1986; JUDKINS; KRYSL; BARTON, 1990) have been used. Huhtanen et al. (1994) concluded that iNDF is probably more uniformly distributed in the solid phase than external markers. In this regard, the use of iNDF as a particulate marker may decrease errors, which originate from unrepresentative

sampling from a duodenal cannula. Also in this study, the authors found that faecal recoveries of iNDF and iADF were more acceptable when determined by 288 h ruminal incubation than by in vitro incubation.

Liquid in the rumen acts as a lubricant and provides a medium for microbes to access feed particles and buffer (SEO et al., 2007), therefore it is important to determine liquid MRT. Polyethylene Glycol (**PEG**) can be used is a fluid marker in ruminants. However, it has been shown that PEG concentration is influenced by high tannin levels and there is adsorption to particulate matter, which decreases recovery in rumen fluid for feed with higher digestibility (SUTHERLAND, 1962; TEETER et al., 1979, VAN SOEST, 1994).

Downes and McDonald (1964) also reported the lack of a specific, sensitive and accurate method of analysis for PEG. Thus, these authors proposed the Chromium-EDTA as an alternative to PEG fluid marker. Another possibility was proposed by Udén et al. (1980), which suggested to use the Co-EDTA as fluid marker in combination with Cr-mordanted fiber as particulate marker, whereas Cr-EDTA and Cr-mordanted fiber could not be used together.

Liquid markers generally have fewer problems, and tend to have a much lower marker migration than particulate marker (Udén et al., 1980). In this context, the main liquid markers used currently are Co-EDTA and Cr-EDTA, due to the good affinity to the liquid phase, precision and simplicity to determine the concentration (BERCHIELLI; OLIVEIRA; DE VEGA, 2005).

Single Marker, Double or Triple Marker

Digesta consist of a heterogeneous mixture of liquid and particulate matter (FAICHNEY, 2005). If only one marker is used, it is impossible to know whether samples are representative of total flow (TITGEMEYER, 1997). Faichney (1975) suggested that digesta could be considered as two phases such that unrepresentative sampling occurs due to a tendency for digesta to separate during collection. Then, the relative proportion of each phase in collected samples can become biased compared to true digesta. If the assumption of two homogeneous phases holds true, unrepresentative samples can be reconstituted to represent true

digesta using markers that exhibit different affinities for individual digesta phases (FAICHNEY, 1975). Accordingly, assumption of a single homogeneous particulate phase is too simplistic (SIDDONS et al., 1985; SATTER et al., 1987; HUHTANEN et al., 1994), and no single marker gives reliable values for digesta flow (FAICHNEY, 2005). The use of the double marker system has been extended to accommodate three or more phase (FAICHNEY, 1975; FRANCE; SIDDONS, 1986; AHVENJÄRVI et al., 2003). Therefore, in passage kinetics evaluations is required at least dual-phase marker system to access the true digesta, due problems associated with unrepresentative samples.

Marker Dosing and Sampling Method

All markers procedures use one of two types of dosing and one of two types of sampling. For dosing, the marker can be administered either as a pulse-dose, or it can be provided constantly (or frequently) for a period of days in an attempt to reach steady state conditions. Pulse dosing typically is used to estimate digesta volume and retention time in specific parts of GIT. From knowledge of volume and retention time, passage rate can be calculated. While, continuous dosing is used primarily to measure instantaneous flow at a specific point in the digestive tract (OWENS; HANSON, 1992).

Digesta samples can be obtained from a specific site at successive times, obtaining the excretion curve of marker concentration. Alternatively, it is possible to determine the marker concentration in the rumen and also in various sections of digestive tract by rumen evacuation or slaughter the animals (OWENS; HANSON, 1992).

4. METHOD TO ESTIMATE PASSAGE KINETICS

Passage kinetics in the gastrointestinal tract can be estimated by direct evaluation method (rumen evacuation or also slaughter method) or by indirect method (compartmental model method).

The slaughter technique estimates the residence time in different segments of the digestive tract (PALOHEIMO; MÄKELÄ, 1959). Robinson et al. (1987) had applied the rumen evacuation (adaptation of slaughter technique) for estimation of digestive and passage kinetics of cell wall digestion. After this, the rumen evacuation method has been widely used (HUHTANEN et al., 2007). Additionally, this methodology has also been applied for starch kinetics (OBA; ALLEN, 2003; TOTHI et al., 2003). The slaughter technique and rumen evacuation estimate MRT as the time required for influx rate of indigestible entity (IE) to replace IE within a physically definable pool of digesta. These techniques are considered the standard method to measure MRT (HUHTANEN et al., 2007). Further, the slaughter method provides the unique advantage of information of digesta MRT separate in several segments of the GIT, and are independent of mathematical descriptions and use of external markers. Unfortunately, the slaughter technique demands terminating the life of the experimental animals, and is also time-consuming, expensive and laborious. This clearly precludes its use for routine analysis. In order to obtain reliable estimates of MRT using rumen evacuation or slaughter technique, it is crucial that the GIT of the animals is in steady state, which the evacuation is carried out to allow accurate and precise estimation of the average rumen pool size, to determine the flux and compartmental mass of an indigestible entity (HUHTANEN et al., 2007). In summary, rumen evacuation and slaughter technique are based on following assumptions: instantaneous mix of influx with all resident particles in the compartment (an age-independent mixing compartment); steady state condition, i.e., constant influx equaling constant efflux; and equal opportunity for escape particles of all resident ages (ELLIS, et al., 1994). Additionally, to apply this method using external markers is required to use continuous infusion to reach the steady state condition.

The compartmental model method (CMM) assumes a distribution of residence times, based on external marker profiles, resulting from specific mechanisms regulating indigestible entity flux within mixing pools or non-mixing segments (ELLIS et al., 1994; 2002). It has been determined using associated markers, administrated as a pulse dose, followed by study of duodenal or feacal excretion curves of the used marker (LUND, 2002).

Several models are available to estimate the passage of digesta particles through ruminant gut. Grovum and Williams (1973) described a model with two age-independent compartments and a time delay. The parameters are estimated by using natural log transformed marker concentrations. Digesta kinetics can also be estimated by two compartmental models with gamma age dependency in the first compartment using non-linear procedures as described by Pond et al. (1988). These models suggest that the probability of a particle escaping from the rumen increases with its residence time in the rumen (MATIS, 1972).

In the two compartment models, the ascending part of excretion curve is related to residence of particles in the initial age-dependent compartment, whereas the descending part is related to the residence in the terminal age-independent compartment. The age-dependent compartment has been assigned as the lag-rumination pool in order to describe its content of retained particles subjected to rumination, and the age independent compartment has been assigned as the mass action turnover escapable pool in order to illustrate the mass action turnover of small particles, all available for passage (ELLIS et al., 1999; 2000). The different models mostly deviates at the ascending part of the curve, representing the age-dependent compartment, whereas differences in the descending part, representing the age-independent compartment are minor. (LUND, 2002).

In conclusion, the MRT determined by slaughter technique allows to determined the instantaneous flow in different points in the digestive tract, and is independent of mathematical descriptions. It is based on one compartment model with steady state conditions, which mean, that all particles, irrespective of age in the rumen, size, specific gravity etc., have the same probability to escape (WALDO; SMITH; COX, 1972). Nevertheless, MRT can only be estimated for entities like iNDF, which are indigestible and disappear only via passage (ELLIS et al., 1999). Additionally, it is necessary to use at least dual-phase marker system to access the true digesta, due problems associated with unrepresentative samples.

Objective

In this context, the main objective of the research described in this thesis is to assess digestion and passage kinetics of feed in the different segments of the gastrointestinal tract in growing goats, in order to determine the contribution of different segments of the gastrointestinal tract to digestion of fiber using the slaughter technique.

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CHAPTER 2. Contribution of different segments of the gastrointestinal tract to digestion in growing Saanen goats

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 behaviour 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 three sexes and three levels of feed restriction (unrestricted/ad libitum, moderate and severe restriction). Six blocks per sex group, each consisting of three goats, were randomly formed and the goats within each block were randomly allocated to one of three 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-labelled 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 ($P < 0.03$). Diet digestibility increased quadratically for DM and OM, and linearly for NDF as feed intake decreased ($P < 0.03$). 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 caecum 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, caecum 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

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

Cannas (2000) compared kp predictions from the Cornell Net Carbohydrate and Protein System developed for cattle against observed kp in small ruminants and found that the predictions underestimated the ruminal passage of feed particles. In the Small Ruminant Nutrition System, the kp 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.

Sex is inherent animal characteristic that influences nutritional requirement (NRC, 2007). Previous studies to date have reported that intact males have higher dry matter intake (DMI) compared to castrated males and females, due to the different nutrient requirements related to higher weight gain and maintenance (Bailey and Duff, 2005; NRC, 2007). Therefore, since DMI may change according to sex, and intake level is the factor with the greatest influence on MRT, sex could have a significant impact on MRT. 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-labelled 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 predictions 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 behaviour and total tract digestibility.

2. MATERIALS AND METHODS

The experiment was carried out at the Goat Facility of FCAV, Animal Science Department, Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil (21°14'S; 48º17'W, 595 m altitude). 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 carried out 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 and 35.7ºC, 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 sub-plot.

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 animals were weighed weekly. Duration of experimental period was determined by the ad libitum animals, when they reached 30 kg BW, then the block of each ad libitum animal were slaughtered.

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 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℃ until further processing and chemical analysis. Chemical composition of the diet was calculated from the individual ingredients.

Feeding Behaviour

Feeding behaviour 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.

Table 1. Ingredient and chemical composition of the experimental diet

Item	Value
Dietary ingredient[3], % 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.
[3]Chemical composition of the diet was calculated from the individual ingredients.

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 faeces. The feed supplied, orts and faeces were weighed daily and subsamples of 10% weight were collected and stored at -10^{o}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-labelled diet (external marker) or by in situ determination of indigestible NDF (**iNDF**; internal marker). The Yb-labelled diet was prepared according de Vega et al. (1998). The particles were labelled with Yb by soaking the whole diet in acetate buffer (0.1 M acetic acid adjusted to pH 6.0 with NH4OH) for 3 h and then overnight in the same solution with an exposure of 17 g Yb-acetate per kg DM. The Yb-labelled 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^{o}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 prior to 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 labelled pellet per dose.

In order to determine MRT, different segments of the digestive tract were evacuated after the animals were slaughtered (2.5 ± 0.5 h after morning feeding), each block on the same day. The GIT was removed and separated into reticulorumen, omasum, abomasum, small intestine, caecum 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 caecum was measured by digital potentiometer (TEC-5; Tecnal, Piracicaba, Sao Paulo, Brazil). The digesta in each GIT segment was sampled and stored at -10^{o}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, caecum and colon.

Chemical Analysis

The dietary ingredients, orts and faeces were dried in an oven at 60^{o}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, faeces and the digesta 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℃ for 24 h (AOAC, 1995; method 930.15) and ash content by complete combustion in a muffle furnace at 600℃ for 3 h (AOAC, 1990; method 942.05). The concentration of NDF was measured in an Ankom 220 Fiber Analyser (Ankom Technology Corp., Fairport, NY) using heat-stable α-amylase without sodium sulphite (Van Soest et al., 1991). 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., 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 solubilisation of cellulose in 12 M sulphuric 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, Colorado) 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 (Valente et al., 2011). 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 Analyser (Ankom Technology Corp., Fairport, NY) as described by Van Soest et al. (1991).

Statistical Analyses

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

$$Yijk = \mu + Si + Bj(i) + SBij(i) + Fk + SFik + eijk \quad [2]$$

where Yijk = dependent variable, μ = overall mean, Si = effect of sex i (main plot), Bj(i) = effect of block j nested in sex i, SBij(i) = interaction between sex i and block j nested within sex i (main plot error); Fk = effect of feed restriction k, SFik = interaction between sex i and feed restriction k, and eijk ~ N (0, σe^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 effect 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.

3. RESULTS

Feed Intake and BW

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 behaviour, digestibility and slaughter; Tables 2, 3 and 4). The intake of iNDF and NDF (% of BW) in the last 5 d prior to slaughter also decreased linearly with increased level of feed restriction ($P < 0.01$). During observations of feeding behaviour, 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 behaviour and digestibility. Therefore, these interactions are not presented in Tables 2 and 3. During the last 5 d prior to 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 prior to 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 Behaviour

Animals fed ad libitum linearly increased the time spent eating and ruminating ($P \leq 0.03$) and linearly 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 ($P \leq 0.03$). Faecal metabolic OM, calculated as faecal OM/kg DMI – faecal 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.

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

Item	Sex			SEM	Feed restriction			SEM	*P*-value[1]	
	Females	Castrated males	Intact males		None	Moderate	Severe		Sex	FL[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]FL = linear effect of feed restriction; FQ = 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 behaviour.
[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

Item	Sex			SEM	Feed restriction			SEM	*P*-value[1]		
	Females	Castrated males	Intact males		None	Moderate	Severe		Sex	FL[2]	FQ[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]FL = linear effect of feed restriction; FQ = quadratic effect of feed restriction.
[3]BW and feed intake presented were measured during the evaluation period of digestibility.
[4]Faecal metabolic OM =100 x [faecal OM – faecal NDF) / (DMI)].

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, but neither group was significantly different from intact males. 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 to 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, caecum and colon was 1.8% of BW across all treatments.

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

Item	Sex			SEM	Feed restriction			SEM	*P*-value[1]			
	Females	Castrated males	Intact males		None	Moderate	Severe		Sex	FL[2]	FQ[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
Caecum	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]FL = linear effect of feed restriction; FQ = 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.

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

Item	Sex			SEM	Feed restriction			SEM	*P*-value[1]		
	Females	Castrated males	Intact males		None	Moderate	Severe		Sex	FL[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
Caecum	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]FL = linear effect of feed restriction; FQ = quadratic effect of feed restriction was not significant ($P > 0.11$; 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, caecum and colon.

Digesta Pool Size

The pool size of fresh matter in the reticulorumen and fresh matter, DM and NDF in the caecum changed quadratically ($P \leq 0.02$), displayed 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 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 castrated males and 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 analysed 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 caecum (across all treatments).

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

Item	Sex			SEM	Feed restriction			SEM	*P*-value[1]		
	Females	Castrated males	Intact males		None	Moderate	Severe		Sex	FL[2]	FQ[2]
Reticulorumen, g											
Fresh	3720	3664	4019	203.0	4064	4193	3146	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
Caecum, 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	5074	5136	5371	290	5674	5605	4302	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 ($P > 0.09$; data not shown).
[2]FL = linear effect of feed restriction; FQ = quadratic effect of feed restriction.
[3]Colon = colon and rectum.

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

Item	Sex			SEM	Feed restriction			SEM	*P*-value[1]			
	Females	Castrated males	Intact males		None	Moderate	Severe		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
Caecum	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]FL = linear effect of feed restriction; FQ = quadratic effect of feed restriction.
[3]SxF = interaction between sex and feed restriction.
[4]Colon = colon and rectum.

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 caecum 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 caecum 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 ($P \geq 0.12$).

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.

Item	Sex			SEM	Feed restriction			SEM	*P*-Value[1]		
	Females	Castrated Males	Intact Males		None	Moderate	Severe		Sex	FL[2]	FQ[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	9.2	8.8	7.8	0.88	7.2	9.0	9.5	0.65	0.53	<0.01	0.25
Total GIT	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	8.2	9.1	7.5	0.80	8.0	8.4	8.4	0.80	0.36	0.76	0.87
Total GIT	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	6.3	5.1	5.2	0.39	4.6	5.5	6.5	0.38	0.10	<0.01	0.91
Total GIT	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 ($P > 0.06$; data not shown).
[2]FL = linear effect of feed restriction; FQ = quadratic effect of feed restriction.
[3]Colon = colon and rectum.
[4]Total GIT = sum MRT of reticulorumen, omasum, abomasum, small intestine, caecum and colon.

Chromium. The MRT of liquid was quadratically ($P \leq 0.04$) affected by level of feed restriction in the reticulorumen, caecum 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.

4. DISCUSSION

A TMR was used in this study in order 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 prior to 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 in further below.

In order 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 carried out 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 behaviour data and considering 3 hours 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 previously to the slaughter was 1.5, 1.4 and 1.4 g/kg BW for ad libitum, moderate and severe restriction, respectively. Taking these aspects into account, we are certain of animals at slaughter were close of daily rumen pool size.

Feeding Behaviour 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, 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 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 can have caused the difference between studies. For instance, most sheep diets (74%) reviewed by Cannas et al. (2003) were exclusively forage, which can 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 pre-duodenal 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 labelled 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 labelled 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 post-duodenal (small intestine + caecum + colon) were compared. This resulted from a greater MRT estimated by Yb in the small intestine and caecum and a greater MRT using iNDF in the colon. Huhtanen and Kukkonen (1995) dosed the markers

into the duodenum and found similar post-duodenal MRT of Yb-labelled 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, caecum 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

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. Based on iNDF:NDF ratio, more than 90% of fibre 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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CHAPTER 3. Retention time of digesta in the gastrointestinal tract of growing Saanen goats[1]

ABSTRACT: This study examined physiological aspects of fiber digestion and particulate and liquid matter residence in the whole tract of growing Saanen goats using the slaughter technique. A total of 58 Saanen goats with initial BW 15.7 ± 0.9 kg were disposed in a 3 × 3 factorial arrangement consisting of three sexes (female, castrated males, intact males) and three slaughter weights (initial, intermediate, and final; target BW 16, 23, and 30 kg at slaughter). They were fed ad libitum, with the same diet offered twice daily (0700 and 1600 h). Mean retention time (MRT) of particulate matter was estimated by in situ determination of iNDF (internal marker) and MRT of the liquid phase using Cr-EDTA as the marker. Treatment effects were evaluated in a split-plot design, with sex as the main plot and slaughter weight as the subplot. Orthogonal polynomial contrasts were used to determine linear and quadratic effect of slaughter weight. The results showed that DMI increased linearly with increasing slaughter weight ($P < 0.01$). Gastrointestinal tissue fresh weight and pool size generally increased linearly with increasing BW of the slaughtered animals ($P \leq 0.01$). The iNDF:NDF ratio in digesta in the reticulorumen, small intestine, and cecum increased linearly with increasing slaughter weight ($P \leq 0.03$). Mean retention time of particulate matter increased linearly in the omasum, but decreased linearly in the abomasum with increasing slaughter weight ($P < 0.01$). Mean retention time of liquid in the fore-stomachs increased linearly with increasing slaughter weight ($P \leq 0.01$). Thus increased BW at slaughter resulted in generally greater MRT of particulate and liquid matter in digestive compartments characterized by mixing digesta flow. This suggests greater capacity for fiber digestion with increased BW, as

also indicated by the greater pool size and iNDF:NDF ratio of digesta in these compartments. The results also indicated that on average, more than 95% of fiber digestion occurred in the fore-stomachs of growing Sannen goats.

Key words: fiber digestibility, markers, mean retention time, pool size, slaughter weight

1. INTRODUCTION

The gastrointestinal tract (**GIT**) of ruminants consists of sequential segments characterized by mixing or non-mixing tubular digesta flows. Therefore particulate matter has been suggested to have an age-dependent or age-independent distribution of residence times in different segments of the GIT (Ellis et al., 1994). Mean retention time (**MRT**) of digesta, especially in the reticulorumen, is one of the most important factors affecting the extent of digestion (Ellis, 1978). Additionally, when animals are fed ad libitum, physical constraints can limit intake, since feed must disappear from the rumen either by digestion or passage (Van Soest, 1994). Furthermore, a positive relationship between digesta MRT and BW has been reported (Demment and Van Soest, 1983; Illius and Gordon, 1992; Gordon and Illius, 1994).

Estimates of MRT in the reticulorumen are generally based on the descending phase of marker excretion curves (Cannas and Van Soest, 2000; NRC, 2001). However, the compartmental MRT estimated from the descending phase of marker excretion curves has been found to be markedly shorter than the MRT determined by lignin and indigestible NDF (**iNDF**) recovery in slaughter or rumen evacuation studies

(Paloheimo and Mäkelä, 1959; Huhtanen and Kukkonen, 1995; Walz et al., 2004). The flux/compartmental pool method also gives estimates of an age-independent rate constant, and the inverse of the mass action dilution turnover for an indigestible entity (**IE**) is equal to whole diet ruminal MRT (Ellis et al., 1994). The slaughter technique makes it possible to determine the contribution of different segments of the GIT to the digestion of fiber (Walz et al., 2004; Ahvenjärvi et al., 2010).

The aim of this experiment was to study the physiological aspects of fiber digestion and MRT of particulate and liquid matter in the whole tract of growing Saanen goats, using the slaughter technique.

2. MATERIALS AND METHODS

The experiment was carried out at the Goat Facility of FCAV, Universidade Estadual Paulista in Jaboticabal, Sao Paulo, Brazil (21°14'S; 48º17'W, 595 m above sea level). All animals were registered and cared for according to guidelines approved by the Human Animal Care and Handling Committee of the Faculty of Agricultural and Veterinarian Sciences, and the experiment was carried out in accordance with the laws and regulations controlling experiments performed with live animals in Brazil. During the experiment, the daily average minimum and maximum temperature was 20.7 and 35.7°C, respectively, and the minimum and maximum relative humidity of the air was 36.1 and 83.4%, respectively.

Animals, Experimental Design, and Diets

A total of 58 Saanen goats (20 intact males, 20 castrated males, and 18 females) with initial BW 15.7 ± 0.9 kg was used in the experiment. The animals were

housed in individual 0.5 m × 1.0 m pens with free access to water and were fed twice daily at 0700 and 1600 h. Treatments were administered in a 3 × 3 factorial arrangement consisting of three sexes (females, castrated males, and intact males) and three slaughter weights (initial, intermediate, and final, with a target BW of 16, 23, and 30 kg, respectively). The effects of treatments were evaluated in a split-plot design, with sex as the main plot and slaughter weight as the subplot. The goats within each sex were randomly allocated to one of three slaughter weights. All animals were fed the experimental diet before the start of the experiment, since they were weaned at 12 kg BW.

The experimental diet consisted of dehydrated corn plant (*Zea mays*), cracked corn grain, soybean (*Glycine max*) meal, soybean oil, limestone, mineral supplement, and ammonium chloride, and was fed as a total mixed ration (**TMR**). Feeding rate was adjusted to yield orts of approximately 10% of intake. Orts were weighed and representative samples were taken on a daily basis. Dehydrated corn plant was made from whole corn plants harvested and chopped when the kernel milk line was approximately two-thirds of the distance down the kernel, air-dried for approximately 72 h or to a DM content of approximately 90% and milled to pass a 10 mm screen (Mexon charger 15.0 hay mill, G3 Mexon Maquinas Agricolas, Cajuru, Sao Paulo, Brazil). The ingredients of the diet were sampled individually before the diet was mixed. All samples of feed ingredients and orts were stored at -10℃ before further processing and chemical analysis. All animals were fed ad libitum during the whole experiment (139 d), but only the feed intake in the last 5 d prior to slaughter (administration of marker) was used to calculate the MRT, and therefore only those

data are presented. Chemical composition of the diet was calculated from the individual ingredients (Table 1).

Table 1. Ingredients and chemical composition of the experimental diet

Item	Value
Dietary ingredient[3], % of DM	
Dehydrated corn plant[1]	45.4
Cracked corn 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	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 corn plant consisted of whole corn plants harvested and chopped when the kernel milk line was approximately two-thirds of the distance down the kernel.
[2]Mean and standard deviation of 10 composite samples.
[3]Chemical composition of the diet was calculated from the individual ingredients.

Markers, Administration of Marker, and Slaughter

The retention time of particulate matter was estimated by in situ determination of iNDF (internal marker). The retention time of the liquid phase was determined using Cr-EDTA prepared according to Downes and McDonald (1964). The Cr-EDTA was administered orally over 5 d to all animals when they had reached the target slaughter weight, at 0100, 0700, 1300 and 1900 h on each day prior to slaughter. Due to inconsistency of results obtained using Yb labelling of particulate marker in a preliminary study (Leite et al., 2015), the MRT of particulate matter obtained by Yb labelling are not presented.

To obtain measurements of MRT for different GIT compartments, different segments of the digestive tract were evacuated after the animals had been slaughtered (2.2 ± 0.8 h after feeding). The GIT was removed and separated into reticulorumen, omasum, abomasum, small intestine, cecum, and colon including rectum. The segments were weighed before and after emptying to determine the amount of digesta and the weight of tissue in each segment. The total GIT tissues and pool size were calculated from the sum of each tissue or pool size in the GIT. Measurements of digesta pH were made in the reticulorumen and cecum using a digital potentiometer (TEC-5; Tecnal, Piracicaba, Sao Paulo, Brazil). The digesta in each GIT segment was sampled and stored at -10°C for later processing and chemical analysis.

Mean Retention Time

Mean retention time of particulate and liquid matter in different segments of the GIT was determined by the flux/compartmental pool method using Eqs. 1 and 2, as described by Ellis et al. (1994):

$$\text{Ke of IE} = \text{Intake rate of IE} / \text{Compartmental mass of IE} \quad [1]$$

where Ke is the fractional rate of escape in h^{-1}, IE is the intake rate of indigestible entity in g/h and compartmental mass of indigestible entity in the segment in g.

$$\text{MRT} = 1 / \text{Ke} \quad [2]$$

where MRT is measured in h.

The feed intake during the last 5 d before slaughter was used to determine the IE of iNDF. The amount of Cr administered in the same period was also used to determine IE. Total MRT in the GIT was calculated as the sum of MRT in the reticulorumen, omasum, abomasum, small intestine, cecum, and colon.

Fiber Digestibility Determination by iNDF:NDF Ratio

Fiber digestibility was calculated for each segment using the iNDF:NDF ratio in the diet and in each segment (reticulorumen, omasum, abomasum, small intestine, cecum, and colon) and expressed as a percentage (Eq. 3 and 4):

$$\text{iNDF:NDF}_{segment} = \{1 - [(\text{iNDF: NDF}_{diet})/(\text{iNDF: NDF}_{segment})]\} \quad [3]$$

$$\text{Fore-stomachs NDF Dig (\%)} = \{100 \text{ x } [(\text{iNDF: NDF}_{cecum})/(\text{iNDF}_{colon})]\} \quad [4]$$

where iNDF:NDFdiet is the ratio between indigestible NDF and NDF in the diet and iNDF:NDFsegment is the ratio between indigestible NDF and NDF in each segment. To calculate the fore-stomachs NDF digestibility (Dig of NDF) was considered the ratio between cecum and colon. Since, it was assumed that no fiber digestion occurred between omasum and cecum.

Chemical Analysis

The ingredients of diet and orts were oven-dried at 60℃ for 72 h. The digesta from different GIT segments were freeze-dried for 96 h. Thereafter, all samples were milled to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). Concentrations of DM, ash, NDF, and Cr were determined in diet ingredients,

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

The concentration of DM was quantified by drying the material in an oven at 105℃ for 24 h (AOAC, 1995; method 930.15) and ash content by complete combustion in a muffle furnace at 600℃ for 3 h (AOAC, 1990; method 942.05). The concentration of NDF was analyzed according to the technique described by Van Soest et al. (1991), using heat-stable α-amylase but without sodium sulfite, in an Ankom 220 Fiber Analyzer (Ankom Technology Corp., Fairport, NY). Furthermore, the concentration of NDF was expressed free of residual ash. The concentration of CP was estimated using the Dumas combustion method (LECO FP-528, LECO Corp., Michigan, AOAC, 1990; method 990.03). The crude fat concentration was determined by extraction with petroleum ether in a Soxhlet apparatus for 6 h (AOAC, 1990; method 930.15). The lignin was analyzed by solubilization of cellulose in 12 M sulfuric acid after extraction with acid detergent (AOAC, 1990; method 973.18). The concentration of Cr 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 concentration was analyzed with an atomic absorption spectrometer (Varian, model Spectra AA 220 FS, Alchem Technology, Denver, Colorado) with an acetylene and nitrous oxide flame (de Vega et al., 1998).

The iNDF content in the diet, orts, and digesta from all GIT segments was quantified 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 (Valente et al., 2011). After this in situ incubation, the bags were washed manually for 30 min and the content of

iNDF was determined using an Ankom 220 Fiber Analyzer (Ankom Technology Corp., Fairport, NY), as described by Van Soest et al. (1991).

Statistical Analyses

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

$$Yij = \mu + Si + Wj + Si \times Wj + eij,$$

where Yij is dependent variable, μ is overall mean, Si is the effect of sex *i* (main plot), Wj is the effect of slaughter weight *j*, Si × Wj is the interaction between sex *i* and slaughter weight *j* (main plot error), and eij $\underset{\sim}{}$ N (0, σe^2) is the random residual error. The effect of sex, slaughter weight, and their interactions were considered fixed effects. Orthogonal polynomial contrasts were used to determine linear and quadratic effect of slaughter weight. The effect of sex was compared by Tukey test. Statistical significance was set at $P \leq 0.05$.

Residuals were plotted against the predicted values to check the model assumptions regarding 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.

3. RESULTS

Feed Intake and BW

There was no consistent effect of sex or interaction between sex × slaughter weight, so only the results for slaughter weight are presented in Tables. Males had a greater BW ($P < 0.01$) than castrated males or females. There was a significant effect of sex × slaughter weight on BW ($P < 0.01$), with intact males slaughtered at

intermediate and final weight having a greater ($P \leq 0.03$) BW than castrated males and females. Intake of DM, OM, NDF, and iNDF increased linearly with increasing slaughter weight ($P < 0.01$; Table 2). Intake of NDF as a percentage of BW changed quadratically, with the greatest value observed for the animals slaughtered at intermediate weight ($P = 0.03$).

Measurements of pH and Fresh Weight of Gastrointestinal Tissues

The pH of ruminal digesta changed quadratically as slaughter weight increased ($P = 0.01$), with the highest value observed for animals slaughtered at the intermediate weight (Table 2). The fresh weight of reticulorumen, omasum, abomasum, cecum, and colon increased linearly with increasing slaughter weight ($P < 0.01$). The fresh weight of small intestine and total GIT tissues changed quadratically as the slaughter weight increased ($P = 0.01$). The percentage of total GIT tissue fresh weight (% of BW) was 7.9, 7.5, and 6.2 % for initial, intermediate, and final slaughter weight, respectively. However, when fresh weight of each tissue was expressed as a percentage of total GIT tissue, there was an increase of 4.9% in the combined weights of the reticulorumen, omasum, and colon with increasing slaughter weight. In contrast, there was a decrease of 3.0% in the combined weight of the abomasum, small intestine, and cecum with increasing slaughter weight.

Table 2. Body weight, feed intake prior to slaughter, pH of digesta in the reticulorumen and cecum, and fresh weight of gastrointestinal tissues (without digesta) in goats of different sexes and slaughtered at three different target weights (initial, intermediate, final)

	Sex			SEM	Slaughter Weight			SEM	*P*-Value[1]			
Item	Females	Castrated Males	Intact Males		Initial	Intermediate	Final		Sex	SWL[2]	SWQ[2]	SxSW[3]
BW	23.1[b]	23.5[b]	24.5[a]	0.25	16.6	23.1	31.3	0.25	<0.01	<0.01	<0.01	0.01
Intake, g / day												
DM	775	837	843	29.1	632	838	985	29.7	0.21	<0.01	0.41	0.65
OM	722	780	784	27.4	590	781	915	27.9	0.23	<0.01	0.40	0.62
NDF	269	273	280	12.1	202	285	334	12.3	0.80	<0.01	0.27	0.61
NDFi	78	79	83	4.6	55	78	107	4.7	0.76	<0.01	0.60	0.26
NDF, % of BW	1.19	1.17	1.16	0.05	1.22	1.23	1.06	0.049	0.91	0.78	0.03	0.13
pH												
Reticulorumen	5.72	5.74	5.62	0.038	5.62	5.78	5.68	0.037	0.07	0.22	0.01	0.52
Cecum	6.36	6.47	6.41	0.060	6.31	6.47	6.46	0.060	0.41	0.07	0.26	0.19
Weight, g												
Reticulorumen	514	523	513	13.9	397	529	624	13.6	0.86	<0.01	0.29	0.51
Omasum	56[b]	63[a]	66[a]	2.1	41	60	84	2.1	0.01	<0.01	0.29	0.02
Abomasum	105[b]	118[a]	116[ab]	3.3	95	115	129	3.4	0.02	<0.01	0.40	0.09
Small Intestine	642[b]	712[a]	624[b]	19.6	535	700	743	19.3	0.01	<0.01	0.01	0.98
Cecum	33	30	33	1.2	26	33	37	1.2	0.08	<0.01	0.20	0.78
Colon	293	303	278	7.3	218	301	353	7.3	0.06	<0.01	0.10	<0.01
GIT	1629	1730	1616	36.0	1312	1730	1933	34.6	0.06	<0.01	0.02	0.65

[1]Main effect of slaughter weight.
[2]WL = Linear effect of slaughter weight; WQ = quadratic effect of slaughter weight.
[3]iNDF= Indigestible NDF.

Digesta Pool Size and iNDF:NDF Ratio

Pool size of fresh matter, DM, and NDF in the reticulorumen, omasum, cecum, colon, and total GIT increased linearly as slaughter weight increased ($P \leq 0.01$; Table 3). Pool size of fresh matter, DM and NDF in the small intestine changed quadratically as slaughter weight increased, with the greatest value observed for animals slaughtered at the final weight ($P \leq 0.05$).

The digesta ratio of iNDF:NDF in the reticulorumen, small intestine, and cecum increased linearly as slaughter weight increased ($P \leq 0.03$; Table 4). The iNDF:NDF ratio in abomasum digesta changed quadratically as slaughter weight increased, with the smallest ratio observed for the animals slaughtered at intermediate weight ($P = 0.01$).

Mean Retention Time

Mean retention time of digesta in the omasum increased linearly and MRT in the abomasum decreased linearly as slaughter weight increased ($P < 0.01$; Table 5). There was a significant ($P \leq 0.04$) effect of sex × slaughter weight on MRT in the omasum, colon, and total GIT (results not shown). Additionally, MRT of liquid in the reticulorumen, omasum, and GIT increased linearly with increasing slaughter weight ($P \leq 0.01$; Table 5).

Table 3. Pool size of different gastrointestinal tract (GIT) segments in goats of different sexes and slaughtered at three different target weights (initial, intermediate, final)

	Sex				Slaughter Weight				*P*-Value[1]			
Item	Females	Castrated	Intact	SEM	Initial	Intermediate	Final	SEM	Sex	WL[2]	WQ[2]	SxW[3]
Reticulorumen,g												
Fresh	3201	3356	3339	127.6	2407	3428	4061	130.0	0.66	<0.01	0.23	0.45
DM	432[b]	501[a]	479[ab]	19.2	354	478	580	19.5	0.05	<0.01	0.64	0.20
NDF	217	239	230	10.7	172	231	283	11.0	0.37	<0.01	0.82	0.10
Omasum,g												
Fresh	75[b]	101[a]	104[a]	7.8	49	87	145	7.9	0.03	<0.01	0.34	0.62
DM	18[b]	25[a]	26[a]	2.0	12	21	35	2.0	0.01	<0.01	0.34	0.62
NDF	7[b]	9[a]	10[a]	0.8	5	8	13	0.8	0.04	<0.01	0.44	0.57
Abomasum,g												
Fresh	354	417	350	26.3	356	401	364	26.8	0.14	0.83	0.22	0.86
DM	46	57	43	4.8	54	47	45	4.9	0.10	0.24	0.74	0.89
NDF	17	19	16	1.3	16	19	18	1.3	0.33	0.44	0.19	0.74
Small Intestine,g												
Fresh	385	378	416	22.2	323	354	502	21.5	0.42	<0.01	0.03	0.90
DM	35	36	41	2.4	32	33	46	2.4	0.18	<0.01	0.05	0.79
NDF	7[b]	8[b]	10[a]	0.6	8	7	10	0.6	0.03	<0.01	0.04	0.43
Cecum,g												
Fresh	149	134	127	9.4	113	134	163	9.9	0.36	<0.01	0.75	0.02
DM	19	18	17	1.3	16	17	21	1.4	0.49	0.01	0.44	0.06
NDF	8	7	7	0.6	6	7	9	0.6	0.82	0.01	0.47	0.12
Colon,g												
Fresh	387	379	379	20.1	271	408	513	20.5	0.22	<0.01	0.54	0.09
DM	78	73	81	5.1	55	72	105	5.2	0.55	<0.01	0.23	0.20
NDF	33	31	35	2.1	23	31	45	2.1	0.35	<0.01	0.22	0.15
GIT,g												
Fresh	4520	4766	4759	166.8	3524	4811	5710	166.8	0.51	<0.01	0.35	0.57
DM	613[b]	710[a]	686[ab]	26.8	525	664	820	27.7	0.05	<0.01	0.81	0.63
NDF	284	315	306	15.5	234	300	371	15.5	0.37	<0.01	0.91	0.33

[1]Main effect of slaughter weight.
[2]WL = Linear effect of slaughter weight; WQ = quadratic effect of slaughter weight.
[3]Colon = Colon and rectum.
[4]Total GIT = Sum pool size of reticulorumen, omasum, abomasum, small intestine, cecum, and colon.

Table 4. Ratio of indigestible NDF to NDF (iNDF:NDF) in different gastrointestinal tract (GIT) segments of goats of different sexes and slaughtered at three different target weights (initial, intermediate, final)

Item	Sex			SEM	Slaughter Weight			SEM	*P*-Value[1]			
	Females	Castrated	Intact		Initial	Intermediate	Final		Sex	WL[2]	WQ[2]	SxW[3]
iNDF:NDF												
Reticulorumen	0.52	0.51	0.49	0.015	0.47	0.52	0.53	0.015	0.47	<0.01	0.45	0.09
Omasum	0.54	0.56	0.50	0.023	0.51	0.51	0.57	0.023	0.21	0.09	0.25	0.15
Abomasum	0.74	0.74	0.65	0.032	0.78	0.65	0.71	0.031	0.07	0.19	0.01	0.68
Small Intestine	0.49	0.46	0.42	0.021	0.41	0.48	0.48	0.021	0.11	0.03	0.16	0.99
Cecum	0.69	0.68	0.64	0.024	0.62	0.68	0.70	0.025	0.37	0.03	0.64	0.23
Colon[4]	0.71	0.72	0.70	0.016	0.65	0.75	0.74	0.016	0.45	<0.01	0.01	0.01

[1]Main effect of slaughter weight.
[2]WL = Linear effect of slaughter weight; WQ = quadratic effect of slaughter weight.
[3]Colon = colon and rectum.

Table 5. Mean retention time (MRT) of particulate and liquid matter in different gastrointestinal tract (GIT) segments of goats of different sexes and slaughtered at three different target weights (initial, intermediate, final), estimated by indigestible NDF (iNDF) for particulate matter and by Cr-EDTA for liquid matter

	Sex				Slaughter Weight				*P*-Value[1]			
Item	Females	Castrated Males	Intact Males	SEM	Initial	Intermediate	Final	SEM	Sex	SWL[2]	SWQ[2]	SxSW[3]
MRT by iNDF, h												
Reticulorumen	38.3	38.3	34.1	2.30	35.6	39.3	35.8	2.30	0.33	0.94	0.21	0.36
Omasum	1.3	1.6	1.4	0.14	1.1	1.4	1.8	0.12	0.26	<0.01	0.85	0.01
Abomasum	5.1	4.8	3.8	0.43	6.4	4.3	3.0	0.42	0.08	<0.01	0.50	0.09
Small Instestine	1.2	1.0	1.3	0.08	1.3	1.1	1.1	0.08	0.23	0.17	0.71	0.90
Cecum	1.7	1.7	1.5	0.19	1.8	1.7	1.7	0.19	0.62	0.15	0.61	0.07
Colon	7.7	7.0	7.9	0.60	6.6	8.0	8.0	0.56	0.58	0.09	0.35	<0.01
GIT	55.1	52.7	49.1	3.37	53.3	54.7	49.0	3.37	0.47	0.37	0.39	0.04
MRT by Cr-EDTA, h												
Reticulorumen	4.1	4.4	4.0	0.35	3.1	4.1	5.2	0.36	0.65	<0.01	0.90	0.28
Omasum	0.1	0.1	0.1	0.01	0.1	0.1	0.2	0.01	0.26	<0.01	0.87	0.14
Abomasum	0.5	0.6	0.5	0.05	0.5	0.5	0.5	0.05	0.30	0.36	0.55	0.32
Small Instestine	0.9	1.1	1.1	0.08	1.1	0.9	1.1	0.08	0.40	0.67	0.16	0.43
Cecum	1.2	1.1	0.9	0.10	1.1	1.0	1.0	0.10	0.08	0.27	0.74	0.02
Colon	4.4	4.2	4.0	0.26	4.0	3.9	4.7	0.26	0.61	0.06	0.18	0.02
GIT	10.8	11.0	10.3	0.42	9.8	10.1	12.3	0.42	0.44	<0.01	0.08	<0.01

[1]Main effect of slaughter weight.
[2]WL = Linear effect of slaughter weight; WQ = quadratic effect of slaughter weight.
[3]Colon = Colon and rectum.
[4]Total GIT = Sum pool size of reticulorumen, omasum, abomasum, small intestine, cecum, and colon.

4. DISCUSSION

A TMR was used in this study to avoid feed selection by the animals that could have resulted in different proportions of ingredients being eaten. The greater BW of intact males than castrated males and females at intermediate and final slaughter weight may explain most of the differences between sexes and the sex × slaughter weight interactions as in the previous study (Leite et al., 2015). Therefore, the effects of sex and sex × slaughter weight interactions are not discussed further below.

Methodological Aspects

Procedures used previously to estimate feed retention in ruminants differ with regard to marker type, compartmental model, sampling site, amount applied, and particle size of labelled feed (Ellis et al., 1994; Huhtanen et al., 2006). Mean retention time of undigested residues in the GIT can be estimated by a compartmental model method (**CMM**) or by the IE pool dilution (flux/compartmental pool) method. The flux/compartmental pool method estimates MRT as the time required for influx rate of IE to replace IE within a physically definable pool of digesta. The CMM assumes a distribution of residence times, based on external marker profiles, resulting from specific mechanisms regulating IE flux within mixing pools or non-mixing segments (Ellis et al., 1994; Ellis et al, 2002). However, both the rumen evacuation and slaughter technique can be used to obtain information on digesta MRT based on the assumptions of the flux/compartmental pool method. The slaughter technique has the unique added advantage of providing data on digesta MRT in separate segments of the GIT that are independent of mathematical descriptions and use of external markers. Differences between experiments in these last factors have confounded

biological interpretations, i.e. relative differences due to animal and diets have not been clearly separated from methodological effects. Unfortunately, the slaughter technique involves terminating the life of the experimental animals, and is also time-consuming, expensive, and laborious. This clearly precludes its use for routine analysis. Furthermore, techniques based on the assumptions of the flux/compartmental pool method are limited as regards providing information on MRT of the whole diet. It is a well-known fact that forage particles are retained longer in the rumen than concentrate particles (Colucci et al., 1990; NRC, 2001; Seo et al., 2009), but the difference is also likely to be dependent on the consistency of the rumen raft as a consequence of the basal dietary forage type (and thereby length of entrapment of concentrate particles in the reticulorumen; Ellis et al., 1994). Duodenal marker profiles of labelled concentrate feeds (Mambrini and Peyraud, 1997) or fecal particles (Wylie et al., 2002) clearly show an ascending phase in the marker excretion curve, indicating selective retention and showing that the passage kinetics cannot be described by a first-order single pool model even for small particles. Accurate estimation of rumen pool size requires steady state conditions or evacuation are carried out to allow having a representative estimation of the average rumen pool size (Ellis et al., 2002; Huhtanen et al., 2007). To avoid maximum and minimum rumen fill, the animals in the present study were slaughtered 2.2 ± 0.8 h after post-feeding, which could represent the average pool size. Therefore, it can be assumed that the GIT was close to steady state in the present study.

Estimates of Mean Retention Time of Particulate and Liquid Matter

Feed passage rate is often measured using rare earths or Cr-mordanted fiber as markers and estimated from the descending phase of the marker excretion curve (Cannas et al., 2002; Huhtanen et al., 2006). Studies using the flux/compartmental pool method with iNDF as the internal marker can give more biologically relevant predictions (Krizsan et al., 2010a). However, in the present study there was no alteration in MRT in the reticulorumen with increased slaughter weight, as determined by iNDF measurements. This was an unexpected finding, as reduced NDF intake as a proportion of BW could be expected to increase MRT in the rumen (Krizsan et al., 2010b).

The reticulorumen, omasum, and abomasum together were responsible for 90% of the difference in retention time between particulate and liquid matter. The segment mainly responsible for this difference in total MRT was the reticulorumen (77%), as it comprises a large mixing compartment in the GIT of ruminants (Grovum and Williams, 1973). The omasum was responsible for 3% and the abomasum for 10%. Apart from the mixing segments of the GIT, there are other segments characterized by non-mixing types of motility, or containing relatively dry digesta which is resistant to mixing (Ellis et al., 1994). In the present study, the particulate MRT in the reticulorumen represented 70% of total GIT retention time (on average across all treatments). Similarly, Ahvenjärvi et al. (2010) found that 72% and 71% of total MIT occurred in the reticulorumen of dairy cows and goats, respectively.

There was a negative relationship between intake and rumen MRT of liquid, as reported in earlier studies (Grovum and Williams, 1977; Colucci et al., 1990). In

addition, it has been reported that particulate and liquid matter have comparable flow rates after the fore-stomachs (Ellis et al., 1994; Huhtanen and Kukkonen, 1995).

Physiological Aspects Related to Digesta Retention and Fiber Digestion

Body weight has a positive influence on MRT and a correlation between BW and MRT has been reported in studies comparing different herbivorous species (Demment and Van Soest, 1983; Illius and Gordon, 1992; Gordon and Illius, 1994). According to those studies, MRT is proportional to $BW^{0.25}$, the volume of the GIT in herbivorous animals increases in proportion to $BW^{1.0}$ (Parra, 1978; Demment and Van Soest 1985, Hackmann and Spain, 2010), and the energy requirement most commonly increases in proportion to $BW^{0.75}$ (Kleiber, 1932). Additionally, forage intake can be limited by the capacity of the GIT and, in particular, of the reticulorumen (Mertens, 1994; Van Soest, 1994). Consequently, large animals should have greater capacity to retain feed for a longer time and digest it more extensively than smaller animals. Owing to this, larger animals may be able to utilize poor quality material better, because they can consume relatively large amount to meet their metabolic requirement, whereas smaller species are constrained because they can eat relatively little (Hackmann 2008; Hackmann and Spain, 2010; Steuer et al., 2011).

In the present study there was a decrease in intake when DMI was expressed as a proportion of BW (38.1; 36.3, and 31.5 g/kg BW for initial, intermediate, and final slaughter weight, respectively). Additionally, total GIT tissue fresh weight (% of BW) decreased with increased slaughter weight. On the other hand, the proportion of each segment to the total GIT tissue fresh weight (%) increased for the reticulorumen

(2.0%), omasum (1.2%) and colon (1.7%) as slaughter weight increased. Increased relative GIT size indicates a relatively larger area available for absorption of nutrients, allowing greater nutrient absorption and thus increased digestibility (Van Soest, 1994).

The iNDF:NDF ratio in digesta can be used to determine the contribution of different segments of the GIT to fiber digestion (Walz et al., 2004; Ahvenjärvi et al., 2010). A negative relationship between DMI and MRT has been reported (Colucci, 1990; Huhtanen and Kukkonen, 1995; Dias et al., 2011), and results in increased diet digestibility with reduced feed intake. In this study the DMI expressed as g/d increased with increasing slaughter weight. However, this study used growing animals and when DMI was related to BW, it actually decreased with increased slaughter weight. This may explain the increased reticuloruminal iNDF:NDF ratio with increasing slaughter weight.

Walz et al. (2004) found a progressive decrease in potential digestible NDF relative to iNDF through different segments of the ruminant GIT with the exception of the small intestine. In addition, it has been shown in previous studies that additional cell wall digestion occurs in the omasum (Ahvenjärvi et al., 2000; Walz et al., 2004; Huhtanen et al., 2010), an effect also observed in the present study. However, the observed iNDF:NDF ratio in the abomasal digesta for the initial slaughter weight animals in the present study may be unrepresentative, because observed values in the cecum and colon were lower. Therefore, to calculate the proportion of NDF digested, it was assumed that no fiber digestion occurred between omasum and cecum. That assumption resulted in values for ruminal NDF digestibility as a proportion of total NDF digestibility of 96, 93, and 96 % for the initial, intermediate,

and final slaughter weight animals, respectively. These values confirm observations in a meta-analysis by Huhtanen et al. (2010) quantifying NDF digestion in cattle.

Conclusions

In the growing Saanen goats studied here, the results suggested greater capacity for fiber digestion with increasing BW, as supported by greater pool sizes and iNDF:NDF ratio. The results also indicated that on average, 95% of fiber digestion occurred in the fore-stomachs of the goats.

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CHAPTER 4. IMPLICATIONS

Mean retention time (**MRT**) is a key point in feed evaluation. The results of the present research confirm the importance of fore-stomachs for fiber digestibility, which their contribution can be more than 90% of total fiber digestion. The main responsible GIT segment is the reticulorumen as expected, which correspond to 85 - 90% of total fiber digestion. Furthermore, the omasum can contribute to 3 – 5% of total fiber digestion. The omasum contribution to digest fiber has not been completely accepted, but the microorganism adhered in the particles that escaped from reticulorumen associated to the favourable environment could be one possible reason to keep fiber digestion in this segment. The contribution of the hindgut to total fiber digestion may be similar or somewhat lower compared to the omasum. In special situations, the hindgut can increase the contribution when passage rate in the reticulorumen increases, then more fermentable product will reach these segments, consequently the digesta can be fermented and the animals utilise the volatile fatty acids to compensate the lower digestibility in the fore-stomachs. Nevertheless, the fiber digestion efficiency in the hindgut is lower compared to reticulorumen, because there is a limited mean retention time of digesta and the animal cannot use the microbial protein in these compartments.

It is expected lower mean retention time in goats compared to cattle and sheep. For instance, passage and digestion kinetics has often been considered species specific, owing to morphological differences (salivary glands, lips, tongue, etc.), body size and digestive capacity between them. However, the results in the present study compared to literature reports (cattle and sheep) indicated that MRT

were similar among cattle, sheep and goat. Additionally, it is expected difference between sex, owing to different feed intake. However, our results suggest that sex does not influence feed intake and MRT, thus sex may not be an important factor to be considered in models for growing Saanen goats.

Due to the importance of feed factors influencing the digestion and passage kinetics, there is an extensive evaluation of these factors in the literature compared to the animal factors. Nevertheless, to study and determine the relationships between animal factors and passage kinetics is also important and it may contribute to adjust feeding systems applied to ruminants, as well as the development of models to improve field routine.

Regarding the method to determine mean retention time, it is important to emphasize that slaughter technique measures the instantaneous flow in different segments of digestive tract, and it is not dependent on mathematical descriptions. However, it is based on one compartment model with steady state conditions, which all particles have the same probability to escape. The steady state condition is a crucial aspect in this method, however it is a theoretical assumption. In this context, to obtain an accurate and precise estimative is necessary to determine the average rumen pool size. Thereby, when using slaughter technique one alternative should be aware of the best moment to slaughtering the animals, avoiding the minimum and maximum rumen fill. Another one is the slaughter of several animals at different times to estimate the pool size changes over a day.

Digesta consists of a heterogeneous mixture of liquid and particulate matter, consequently, the use of at least dual-phase marker system is necessary to assess the true digesta, due to problems associated with unrepresentative samples. For

instance, most of recent studies have used triple markers system to determine the retention or flow of larger particles, small particles and liquid fraction. The reconstitution of true digesta should be based on system that contains markers associated with large particle phases (Cr or iNDF), small particle (Yb) and liquid (Co or Cr, when Cr is not used to large particles).

The present research indicates that the use of Yb as external marker of whole diet should be avoided. Ytterbium is associated with small particles and also Yb can migrate from the labelled particles to fine particles and microbial residues. The Yb migration from large to small particles leads to underestimation of rumen retention time due to faster passage rate of small particles and liquid. Furthermore, rare earths can be displaced from their feedstuff binding sites by protons at pH values comparable to more acidic abomasal digesta.

In order to avoid some of problems mention above is recommended to label only the fiber forage with Yb by boiling the forage in NDF solution and afterwards perform the label process. In addition, during the label process is necessary to wash the material to 0.1 M acetic acid to remove any loosely bound rare earth that would be displaced by a proton concentration equivalent to 0.1 M acetic acid.

The iNDF marker was a good marker to estimate MRT in different gastrointestinal tract segments. Although, some adjustments in the method used in this study can increase the precision and consequently decrease the number of replicates. For instance, the ankom bag may not be the most appropriate clothes, which imply in the overestimation of indigestible material, due to the small pore in the bags. Additionally, iNDF should be expressed ash free, especially for samples with high ash concentration.

The main liquid markers currently used are Co-EDTA and Cr-EDTA, due to the good affinity to the liquid phase, precision and simplicity to determine the concentration. It acts as a lubricant and provides a medium for microbes to access feed particles and buffer.

All in all, in spite of the contribution of the present study in determine the importance of segments to digest fiber, studies evaluating mean retention time in ruminants are still necessary, especially assessing the contribution of fore-stomach to digest fiber and synthesis of microbial protein. The next gap in the literature in this field of study is to determine the interrelationship between feed and animal factors, especially in pregnant and lactating Saanen goats. Furthermore, the similar mean retention time in goats compared to cattle and sheep, associated with no difference among sex may create the opportunity to use a bigger database with cattle, sheep and goats, irrespective of sex, to development a model more robust and accurate models.