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DIGESTA PASSAGE RATE IN SAANEN GOATS: A META-ANALYTIC APPROACH

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TÍTULO DA TESE: DIGESTA PASSAGE RATE IN SAANEN GOATS: A META-ANALYTIC APPROACH

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BIOGRAPHY

MARCELO GINDRI was born on December 02, 1991, in São Francisco de Assis, Rio Grande do Sul, Brazil. From 2009 to 2013, he did his undergraduate study in Animal Science at Universiade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. During his undergrad, he completed an internship at Instituto Nacional de Investigación Agropecuaria del Uruguay, Tacuarembó, Uruguay, from March to June 2013. He became a graduate student at Universiade Federal de Santa Maria and concluded his Master's degree in Animal Science in February 2016. He focused on developing faecal near-infrared spectroscopy models for estimating the nutritional value of ingested diets by free-ranging sheep in southern Brazil under the supervision of Dr Gilberto Kozloski. In 2016, he began his doctoral degree at Universidade Estadual Paulista, Jaboticabal, São Paulo, Brasil, and he has been working on a meta-analysis of digesta passage rate in growing Saanen goats under supervision of Dr Izabelle Auxiliadora Molina de Almeida Teixeira. He also completed a graduate internship at The Ohio State University, Columbus, Ohio, USA, from July 2018 to June 2019 under supervision of Dr Luis Moraes.

... Mostremos valor, constância
Nesta ímpia e injusta guerra
Sirvam nossas façanhas
De modelo a toda Terra...

Chorus of the Rio-Grandense anthem composed by Francisco Pinto da Fontoura

To my family for giving me love, affection, and support.

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SUMMARY

DIGESTA PASSAGE RATE IN SAANEN GOATS: A META-ANALYTIC APPROACH

ABSTRACT: The digesta passage rate (k_p) in ruminants is a fractional passage of digesta per hour, i.e. /h. The k_p is a very complex and dynamic process and affects feed digestion and the environmental impact of livestock. In this study, we aimed to understand the factors related to k_p in the whole gastrointestinal tract (GIT) of growing goats and goats fed with feeding levels and propose equations for predicting k_p of particles and solutes in reticulorumen. For that, we used a database from two studies with Saanen goats that was split into two different databases: database Growth was composed by individual records of castrated males (n = 36), females (n = 34), and intact males (n = 33) fed ad libitum and slaughtered at 15, 22, 30, 37, and 45 kg body weight (BW); and, database Feeding Levels was composed by individual records of castrated males (n = 38), females (n = 33), and intact males (n = 36) fed ad libitum, 75 or 50% of ad libitum over 109 ± 10 days. Our results demonstrated digesta passage rate had different responses whether evaluated in growing goats or goats fed with feeding levels over long-term. Reticulorumen k_p of particles was not affected by growth or feeding level. However, reticulorumen k_p of particles and large intestines k_p of particles and solutes were positively related among them in growing goats. Reticulorumen k_p of solutes decreased with aging followed by increasing on reticulorumen capacity and reducing on dry matter (DM) intake level and chewing investment (chews/g DM intake). Moreover, goats fed with feeding levels over long-term had k_p of solutes in all GIT segments positively related to feeding levels and capacity. Sex effect on k_p seemed to be dependent on chewing investment, however, it must be further investigated. According to developed models, reticulorumen k_p of solutes demonstrated to be more dependent on feed intake level than reticulorumen k_p of particles, that demonstrated to be more dependent on diet composition and k_p of solutes.

Key words: dairy goat, digesta kinetic, feed intake, sex, equation

TAXA DE PASSAGEN DE DIGESTA EM CAPRINOS SAANEN: UMA ABORDAGEM META-ANALÍTICA

RESUMO: A taxa de passagem da digesta (k_p) em ruminantes é a passagem fracionada da digesta por hora, isto é /h. A k_p é um processo complexo e dinâmico e afeta a digestão dos alimentos e o impacto ambiental da produção animal. Neste estudo, objetivamos compreender os fatores relacionados à k_p em todo o trato gastrointestinal (TGI) de caprinos em crescimento e caprinos alimentados com níveis de alimentação e propor equações para prever k_p de partículas e solutos no reticulo-rúmen. Para isso, utilizamos um banco de dados de dois estudos com caprinos Saanen, que foi dividido em dois bancos de dados diferentes: o banco de dados Crescimento foi composto por registros individuais de machos castrados (n = 36), fêmeas (n = 34) e machos intactos (n = 33) alimentados ad libitum e abatidos aos 15, 22, 30, 37 e 45 kg de peso corporal (PC); e, o banco de dados níveis de alimentação foi composto por registros individuais de machos castrados (n = 38), fêmeas (n = 33) e machos intactos (n = 36) alimentados ad libitum, 75 ou 50% ad libitum durante 109 ± 10 dias . Nossos resultados demonstraram que a taxa de passagem da digesta tem respostas diferentes, quando avaliada em cabras em crescimento ou cabras alimentadas com níveis de alimentação a longo prazo. O k_p de partículas no reticulo-rúmen não foi afetado pelo crescimento animal ou pelo nível de alimentação. No entanto, o k_p de partículas no reticulo-rúmen e o k_p de partículas e solutos no intestino grosso demonstraram ser positivamente relacionados nos animais em crescimento. O k_p de solutos no reticulo-rúmen diminuiu com o crescimento animal, seguido pelo aumento da capacidade do reticulo-rúmen e redução do nível de ingestão de matéria seca (MS) e investimento na mastigação (mastigação/g de ingestão de MS). Já os caprinos alimentados com níveis de alimentação a longo prazo demonstraram k_p de solutos em todos os segmentos do TGI positivamente relacionados aos níveis de alimentação e capacidade do TGI. O efeito do sexo no k_p pareceu depender do investimento em mastigação, no entanto, isso deve ser investigado mais detalhadamente. De acordo com modelos desenvolvidos para reticulo-rúmen, o k_p de solutos demonstrou ser mais dependente do nível de ingestão de alimento do que o k_p de partículas, que demonstrou ser mais dependente da composição da dieta e do

k_p de solutos.

Palavras-chave: cabra leiteira, cinética da digesta, consumo de ração, sexo, equação

LIST OF ABBREVIATIONS

Ab	Abomasum
AICc	Akaike information criterion corrected for small samples
BW	Body weight
BW_L	Linear effect of BW
BW_{Qd}	Quadratic effect of BW
BW_C	Cubic effect of BW
BW_{Qt}	Quartic effect of BW
C	Castrated males
CCC	Lin’s concordance correlation coefficient
Ce	Cecum
ConCP	Crude protein concentration in the ingested diet
ConNDF	NDF concentration in the ingested diet
CP	Crude protein
CR	Colon and rectum
DM	Dry matter
DMfor	Forage DM intake
DMI	DM intake
DMI*	Standardized DMI
F	Females
Fa	Factor
FL	Feeding level
FL_L	Linear effect of FL
FL_{Qd}	Quadratic effect of FL
GIT	Gastrointestinal tract
I	Intact males
IE	Indigestible entity
iNDF	Indigestible NDF
iNDFI	iNDF intake
iNDF:NDF	Ratio between iNDF and NDF
iNDFI:NDFI	iNDF:NDF of ingested diet

iNDFI:NDFI*	Standardized iNDFI:NDFI
KMO	Kaiser–Meyer–Olkin
k_p	Digesta passage rate
k_p Cr	k_p estimated by Cr-EDTA as a marker
k_p iNDF	k_p estimated by iNDF as a marker
L	Metabolizable energy requirements for maintenance intake
ln	Natural logarithm
LS	Least squares
ME	Metabolizable energy
MS	Matéria seca
MFA	Multivariate factor analysis
MRT	mean retention time
MRT_{Cr}	MRT estimated by Cr-EDTA as a marker
MRT_{iNDF}	MRT estimated by iNDF as a marker
MSPE	Mean square prediction error
NDF	Neutral detergent fiber
NDFI	NDF intake
NDFI*	Standardized NDFI
NDFD	Total NDF digestibility
O	Omasum
OM	Organic matter
OMI	OM intake
PC	Peso corporal
PCA	Principal component analysis
pdNDF	Potentially digestible NDF
pdNDFI	pdNDF intake
pdNDFI*	Standardized pdNDFI
PS	Particle size
R^2	Coefficient of determination
RMSPE	Root MSPE
RMSPEp	RMSPE as a proportion of independent variable mean
RR	Reticulorumen

RRwetPS	RR wet pool size
RRwetPS*	Standardized RRwetPS
RRwettissues	RR wet tissues
RRwettissues*	Standardized RRwettissues
S-BW	Slaughter BW
SD	Standard deviation
SI	Small intestine
TGI	Trato gastrointestinal
VIF	Variance inflection factor

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DISSERTATION STRUCTURE

Chapter 1 is a literature review, about digesta passage rate in the whole digestive tract of ruminants, covering factors that influence, and a review of models for predicting digesta passage rate in reticulorumen of goats. This was written following the guidelines of the Graduate Program in Animal Science of Unesp, Jaboticabal Campus.

Chapter 2 describes the effects of growth and sex on digestive variables of growing Saanen goats, including digesta passage rate of particles and solutes. This chapter was also written following the guidelines of the Journal of Translational Animal Science except by the letter style, spaces between lines, and position of tables. The paper authors are M. Gindri, R. F. Leite, C. J. Härter, S. P. Silva, N. St-Pierre, M. H. M. R. Fernandez, T. T. Berchielli, I. A. M. A. Teixeira.

Chapter 3 describes the relationship among the digestive variables in growing goats and goats fed with feeding levels over long-term, emphasizing the variables related to digesta passage rate in the whole digestive tract. This chapter was also written following the guidelines of the journal Animal except by the letter style, spaces between lines, and position of tables. The authors are M. Gindri, L. E. Moraes, I. A. M. A. Teixeira.

Chapter 4 describes developed models for predicting passage rate of particles and solutes in goats' reticulorumen. This chapter was also written following the guidelines of the journal Animal except by the letter style, spaces between lines, and position of tables. The authors are M. Gindri, L. E. Moraes, I. A. M. A. Teixeira.
Chapter 5 describes the main implications of this study, written following the guidelines of the Graduate Program in Animal Science of Unesp, Jaboticabal Campus

CHAPTER 1 – General considerations

INTRODUCTION

Digesta passage rate (k_p) in ruminants is a fractional passage of digesta per hour, i.e. /h. Digesta passage rate is a dynamic process and affects feed digestion and the environmental impact of livestock. Digesta passage rate in ruminants is negatively related to the extent of digestion of feeds because it is negatively related to the length of time that feed components are exposed to digestion and absorption. In reticulorumen, where most of the fibre and protein is digested, k_p is yet positively associated to reticulorumen microbes' growth and feed digestion rate and negatively related to methane emission per unit of digested fibre (Clauss and Hummel, 2017; Grandl et al., 2018). Moreover, in the distal tract, k_p is also positively associated with water losses by faeces (Clauss et al., 2016). Digesta passage rate has been considered dependent on feed intake, body weight (BW), gastrointestinal tract (GIT) capacity, sex, age, physical and chemical composition of the diet, animal's physiological state (i.e. pregnancy and lactation) and behaviour, and weather. However, all these factors are related among them and they synergistically act on k_p. Moreover, k_p can be methodologically split into particles and solutes or also in each of the chemical components of digesta, and each of these k_p has an individual behaviour in the GIT segments, and they can also be related among them within or between GIT segments. All these factors have made the understand and prediction of k_p in ruminants a challenge.

The k_p can be predicted by internal or external markers of feed, considering a steady-state condition, in which the markers are constantly administrated and the concentration of the markers are measured in the GIT segments, or also by an unstable condition over time, in which a pulse dose of markers is administrated and based on their excretion curve the k_p is estimated accounting for dependency between pools of digesta. Moreover, k_p can also be estimated with empirical or mechanistic models, that usually consider the effects of animal and diet as predictor variables. There are few models in the literature for predicting k_p in goats, and most of them are empirical models and have feed intake and diet composition as predictor variables. However, none of them considers the digesta fractions (i.e. solutes and particles) have different

k_p, and that GIT capacity also affects k_p. Moreover, based on own brief literature review of models for predicting k_p in the reticulorumen segment of cattle, mechanistic models have demonstrated over twice better behaviour than empirical equations, based on root mean square prediction error. This demonstrates reticulorumen k_p is a very complex and dynamic process, and for accurately predicting k_p, all the related factors must be taken into consideration.

Thus, the objective of this review is demonstrating, in a mechanistic steady-state condition, how those factors stated above (i.e., feed intake, BW, GIT capacity, sex, age, physical and chemical composition of the diet, and animal’s physiological state and behaviour) are related to k_p in ruminants and a review about models for predicting k_p in reticulorumen of goats.

MECHANISTIC STEADY-STATE REGULATION OF k_p IN GROWING RUMINANTS

Feed intake and GIT capacity are considered the most important factors regulating k_p in ruminants. However, BW has been historically considered the driving force of k_p in ruminants (Müller et al., 2013). Feed intake is driven by animal’s requirements, however, limited by satiety (Forbes, 2007), that depends on GIT capacity and k_p (Clauss et al., 2007b). Animal’s requirements depend on BW because BW implies changes in animal body composition that is related to animals’ requirements (Kleiber, 1932; Blaxter et al., 1982; Souza, 2017). Thus, BW affects feed intake in ruminants (Clauss et al., 2007b; Steuer et al., 2011; Müller et al., 2013; Souza et al., 2019) because both of them are related to animals’ requirements. These relationships have also been demonstrated in models for predicting feed intake in which BW or BW changes over time are used as predictor variables (Almeida et al., 2019). However, BW and feed intake are not linearly related. Studies that evaluated the relationship between BW and feed intake have demonstrated feed intake is allometrically related to BW, with an allometric exponent around 0.75 (Clauss et al., 2007b; Müller et al., 2013). However, this allometric exponent has been demonstrated a bit lower for goats, around 0.67 (Almeida et al., 2019). Gastrointestinal tract capacity, defined as the total of wet contents in GIT (Clauss et al., 2007b), has been linearly related to BW (BW^1; Müller et al., 2013) even GIT wet tissues are allometric related to BW, with an allometric exponent around 0.63 (Andrade et al., 2020). Thus, these differences on the relationship of feed intake and capacity to BW have been used to explain the relationship between BW and k_p in ruminants, that has been scaled to 0.25 ($k_p = BW^{0.25}$; Müller et al. (2013).

Considering GIT k_p is mainly affected by GIT capacity and feed intake, it makes us wonder how other factors (i.e. sex, age, physical and chemical composition of diet, and animal's physiological state and behaviour, and weather) act on k_p in the GIT segments. For this, in the next sections, GIT k_p will be split into particles and solutes and GIT segments (reticulorumen, omasum, abomasum, small intestine, cecum, and colon-rectum). This is necessary because each of these GIT segments has different tissues growth rate over time according to their functions (Andrade et al., 2020), with a possible effect on capacity and consequently on k_p. Moreover, reticulorumen can distend and is considered a mixing segment (Faichney, 2005) that makes its k_p to be different between particles and solutes (Clauss et al., 2016b) and slower than in the other GIT segments, that are not able to distend and have similar k_p between particles and solutes (Ellis et al., 1994; Faichney, 2005).

HOW DOES RETICULORUMEN k_p WORK?

Reticulorumen accounts for 90% of neutral detergent fibre (NDF) digestion and around 72% of total GIT k_p (Ahvenjärvi et al., 2000, 2010; Walz et al., 2004; Leite et al., 2015a), and affects the k_p of far segments, as distal tract (Dijkstra et al., 2005; Huhtanen et al., 2006). Thus, these facts lead us to assume reticulorumen is the most important segment for understanding GIT k_p in ruminants. Around many theories about k_p in reticulorumen, one of the most mechanistic and acceptable theory assumes that: reticulorumen digesta has an escapable pool located in the bottom of reticulorumen and an inescapable pool located in the top of reticulorumen; the passage rate of digesta from the inescapable to the escapable pool is always faster than the passage out of reticulorumen, then the passage of the escapable pool through the reticulo-omasal orifice is the rate-limiting step in reticulorumen k_p; and, the flow of particles out of the reticulorumen follow the dynamics of solutes passage (Seo et al., 2007, 2009).

Pools of digesta in reticulorumen

The escapable pool of digesta from reticulorumen is mostly composed of small particles, however, particles with high size also may be in the escapable pool of digesta (Seo et al., 2009). Digesta particles are taken from the inescapable pool to escapable pool by the density-dependent sorting mechanism that is affected by changes on particles functional specific gravity and reticulorumen mixing movements (Kaske and Engelhardt, 1990; Seo et al., 2009). Once the particles are in the escapable pool, they only depend on reticulo-omasal orifice opening and flowing for escaping from reticulorumen (Seo et al., 2009). According to that, the concept of critical particle size for rumen passage that states reticulo-omasal orifice acts as a screen (Poppi et al., 1980) is not valid. Moreover, the structure of the reticulo-omasal orifice does not seem to act as a screen (Mathison et al., 1995). Therefore, solutes in the reticulorumen are important in this context by providing a lubricated medium for the transit of particles from the inescapable to the escapable pool of digesta and out of reticulorumen (Clauss et al., 2016a).

Digestion and comminution processes are responsible by changes on particle functional specific gravity by hydrating the particles and reducing the amount of gas in the particles (Poppi et al., 1981; Kaske and Engelhardt, 1990; Seo et al., 2009). These two processes are dependent on chewing activity (Wattiaux et al., 1992). Moreover, chewing activity is positively related to saliva secretion (Bailey, 1961; Grandl et al., 2018), that contributes for increasing the solutes content in reticulorumen, and in turn, the dynamics of particles between pools and the digesta transport out of the reticulorumen (Faichney et al., 1981; Poppi et al., 1981; Seo et al., 2009).

Reticulo-omasal orifice opening

A complex and coordinated system of reticulorumen contractions has been correlated to the reticulo-omasal orifice opening (Seo et al., 2007). Two types of reticuloruminal cycle of contractions have been described (Ruckebusch and Thivend, 1980). One commences with a reticular contraction and it is followed by a contraction running caudally across the rumen, that is mainly responsible by mixing the digesta and creating the escapable and inescapable pool of reticulorumen digesta. The other, that does not involve the reticulum, commences with a contraction of the caudal ventral blind sac and is followed by a contraction running cranially across the rumen, that is mainly associated with eructation. The first cycle of reticuloruminal contraction is also composed of two phases with a fixed time interval. In the first phase, a pool of digesta, composed by small particles of digesta and solutes, is separated in the bottom of reticulum from the escapable pool of rumen digesta, and in the second phase, the reticulo-omasal orifice is opened and then that digesta located in the bottom of the reticulum can flow out of the reticulorumen (Reid, 1984; Lechner-Doll et al., 1991). Following, during the second reticuloruminal cycle of contractions, with relaxing of reticulum, digesta located in the escapable pool of rumen may flow out of reticulorumen by reticulo-omasal orifice without selection of particle size. This flow has a variable time interval, because it is controlled by the difference of pressure between omasum and reticulorumen, and it is more intense in situations of high reticulorumen pressure related to high feed and water intake, saliva secretion, and low reticulorumen capacity (Deswysen and Ellis, 1988; Seo et al., 2007).

The primary reticular contractions are characterized by their frequency, duration, and amplitude and are mostly and positively influenced by the chewing behaviour (Mathison et al., 1995). Seo et al. (2007, 2009) developing mechanistic models for predicting k_p in reticulorumen of dairy cattle demonstrated frequency, duration, and amplitude of primary reticular contractions are related to frequency, duration, and amplitude of reticulo-omasal orifice opening and strongly related to solutes outflow rate from reticulorumen. Frequency is characterized by the number of reticulo-omasal orifice opening per minute, duration is characterized by how long the reticulo-omasal orifice stays open per opening, and amplitude is characterized by the pressure of digesta passing through reticulo-omasal orifice per opening. Moreover, Seo et al. (2007, 2009) determined that the reticulo-omasal orifice frequency is higher during eating than ruminating or resting periods, however, the reticulo-omasal orifice stays open longer during ruminating than eating or resting periods. On the other hand, they demonstrated that amplitude of reticulo-omasal orifice opening is higher during resting than during eating or ruminating periods.

Therefore, the scape of particles and solutes from reticulorumen is basically regulated by intake, capacity, particles functional specific gravity, and reticulo-omasal orifice opening. However, there are other factors, relate to them, that may also indirectly affect the reticulorumen k_p, that are related to diet and animal and will be discussed in the next section.

EXTRINSIC FACTORS CONTRIBUTING TO RETICULORUMEN k_p

Feed intake level, age, sex, animal's physiological state (i.e. pregnancy and lactation) and behaviour, weather, and chemical and physical composition of diet play an important role on water intake, chewing activity, digestion process, comminution process, density-dependent sorting mechanism, reticulorumen contractions, and saliva secretion, that are processes regulating k_p in reticulorumen. Feed intake level has been negatively related to water intake (Teixeira et al., 2006) and positively related to changes on digesta dry matter (DM) concentration, in the contents of the ventral rumen and animals fed with feeding levels (Clauss et al., 2016b). Changes on digesta DM concentrations of rumen are considered part of a mechanism to save space and reduce the internal pressure of reticulorumen and keep humid other parts of rumen without full fill of the segment (Clauss et al., 2016b). This happens to guarantee the normal operation of the density-dependent sorting mechanism in reticulorumen, that needs humidity (Clauss et al., 2016b). Moreover, feed intake level has been negatively related to chewing activity per kg of ingested diet and positively related to daily chewing activity (Galvani et al., 2010; Grimaud et al., 2010). Chewing activity has also been related to ageing. Young ruminants are lesser efficient chewers than old ruminants, chewing more per kilogram of ingested diet (Bae et al., 1983; Grandl et al., 2016b, 2018). This is explained most parsimoniously by the addition of additional chewing surface area in the form of the three molars with ageing (Grandl et al., 2018). Occlusal molars' surface area is considered one of the main determinants of herbivore chewing efficiency (Pérez-Barbería and Gordon, 1998). Moreover, sex may also affect chewing investment. Studies have demonstrated females' goats are 50% lesser efficient chewers than males' goats, chewing more per kilogram of ingested diet (Gross et al., 1995). This has been related to their small molar surface area, that is around 15% smaller than males (Fandos and Vigal, 1993; Gross et al., 1995; Loe et al., 2003). Moreover, reticulorumen k_p tends to be faster during gestation and lactation phases. During these phases, the reticulorumen contractions increase as an effect of compression in the reticulorumen by uterus (Faichney and White, 1988). Reticulorumen k_p can also be decreased by exposure to heat and increased by exposure to cold conditions (Dijkstra et al., 2005; Bernabucci et al., 2009). Moreover, animals consuming high saline water may present lower particulate k_p as a response to particles specific gravity (Kattnig et al., 1992). On the contrary, a study has reported an increase in the k_p of solutes of sheep fed with high salt content (Potter et al., 1972).

After intake and capacity, diet chemical and physical composition can be considered the most important factors affecting k_p. Diet affects digestibility, chewing investment, and comminution, that in turn affect the density-dependent sorting mechanism and reticulo-omasal orifice opening, that are factors directly related to k_p. First of all, diet composition is driven by animals age because it is related to experience to sorting (Forbes and Kyriazakis, 1995; Miller-Cushon et al., 2013) and changes on nutrients requirements (Kleiber, 1932; Souza et al., 2017). Young ruminants select feed with high energy and digestibility, less NDF and indigestible NDF (iNDF) contents, and high crude protein content (Clauss et al., 2013; Müller et al., 2013; Leite et al., 2015b). From chemical composition, the cell wall is the most important factor affecting k_p. The NDF and iNDF contents of ingested diet are positively related to chewing activity (Schulze et al., 2014), that positively affects saliva secretion and reticulo-orifice opening and in turn reticulorumen k_p. On the other hand, NDF content is positively related to particles resistance to comminution (Kennedy and Doyle, 1993; Pérez-Barbería and Gordon, 1998). And, comminution is positively related to the density-dependent sorting mechanism (Thomson and Beever, 1980; Faichney, 1983). Thus, the NDF content of the ingested diet has been considered the most limiting fraction to voluntary feed intake in ruminants (Schulze et al., 2014) by limiting the reticulorumen outflow of particles. Moreover, forage particles with low NDF content and high OM content have high DM digestion rate and consequently fast increase on functional specific gravity (Bhatti and Firkins, 1995; Karabulut et al., 2007). Moreover, ingested diets composed by feed particles with high content of iNDF are quickly taken to the escapable pool (Krizsan et al., 2010) because of their reduced fermentation activity, and consequently high functional specific gravity and low flotation capacity (Huhtanen et al., 2006).

Additionally, cell wall characteristics associated with anatomical structure of forage species affect comminution and digestion, and therefore the size and shape of the resultant particles which affect their k_p from the reticulorumen (Wilson, 1994). Comminution during chewing appears difficult in tropical grasses because of their stronger cell junctions than when walls are straight-sided, as in most of the common temperate pasture grasses, or lobed as in legumes (Wilson, 1994). Shedding of the epidermis from the leaf is another breakdown process which is also difficult in most tropical pasture grasses and then chewed tropical grass leaves do not easily split or lose their epidermis, and the resultant particles are often a composite of many vascular bundles in width (Wilson, 1994). In contrast, most higher quality temperate grasses and legumes cells are easily disrupted by chewing to allow rapid shedding of the epidermis (Wilson, 1994). In stems of grasses, the epidermal walls are much thicker than in leaves and the chewing effort needed is greater than for leaves (Mcleod et al., 1990). In legume stems, the cortical and central pith cells are unlignified and digestion quickly results in the loss of these cells, leaving only the lignified xylem ring to be disrupted by rumination (Wilson, 1994). Regarding the shape of the resultant particles, there are no obvious points of weakness for natural breakage within the length of a strand arising from a leaf in grasses or from a stem internode in all forages. They require extensive rumination to break across many walls within the strand to reduce fibre length. Only in legume leaves, with their reticulate venation, are there natural points of breakage at the angular vein junctions (Wilson, 1994). Moreover, legumes have fewer hydrophobic molecules in cell walls when compared to grasses that give then higher functional specific gravity or lower buoyancy of particles than grasses Gates et al. 1987). Therefore, legumes are well recognized to require less rumination than grasses and to break up into short chunky particles (Kelly and Sinclair, 1989) and have faster k_p than grasses (Troelsen and Campbell, 1968).

Moreover, increasing the amount of concentrate in the diet has demonstrated to reduce reticulorumen k_p (Faichney, 1975; Faichney and White, 1977). This has been also observed for ground diets composed of small particles (Faichney, 2005). This is observed because concentrate particles and ground diets require less mastication and then the motility of the reticulorumen decreases (Owens and Goetsch, 1986). Moreover, concentrate particles and ground diets can produce a close-packed array in the reticulorumen because of their small particle size leading to a reduction on reticulorumen fill and internal pressure (Faichney, 2005). On the other hand, findings available in the literature on the effect of concentrate particles and ground diets on k_p are conflicting (Faichney, 1983) because concentrated particles and ground diets have been found to increase reticulorumen k_p because they have fast increased on functional specific gravity (Thomson and Beever, 1980; Faichney, 1983). This controversy illustrates the complexity of the mechanism determining the retention of particles in the reticulorumen (Bernard et al., 2000).

OMASUM, ABOMASUM, AND INTESTINES k_p

Omasum, abomasum and intestines are considered tubular segments (Faichney, 2005), and they are no able to distend like the reticulorumen does (Clauss et al., 2016b). Because of this, their k_p is considered laminar and no differences between k_p of particles and solutes are observed (Ellis et al., 1994; Faichney, 2005). However, based on higher iNDF:NDF relationship in the small intestine than in the other GIT segments, studies have questioned the general assumption of laminar flow in the small intestine (Hristov et al., 2019). The k_p in those segments is a function of feed intake and capacity, and the last acts as a limiting factor and is related to tissues size (Van Soest, 1994; Clauss et al., 2007b; a; Müller et al., 2013). Thus, based on that the k_p on those segments basically dependents on their capacity that is limited by their tissues size and the flow from previous segments. Omasum is the least GIT segment to reach full development because of its slowest growth rate of tissues (Andrade et al., 2020). Because of that, omasum's growth stabilization may occur around the 36th week of ruminant's life (Lyford Jr, 1993). Omasum is largely known for absorbing water, and, in goats, omasum absorbs at least 18% of digesta water (Holtenius and Björnhag, 1989). Moreover, omasum may also contribute to fibre digestion (Leite et al., 2015a). Abomasum and small intestine have early and fast development because they are responsible by enzymatic digestion of non-fibrous feed, and their maximum growth rate occurs around 15th days of goats' life (Lyford Jr, 1993; Andrade et al., 2020). This happens because ruminants are born with non-functional forestomach, and when they are stimulated with fibrous diets, they face a transition period to becoming a functional ruminant (Lyford Jr, 1993). In goat kids, this transition period occurs from 3rd to 4th week of goats' life, according to their feeding management. After this period, around 2 months old in goats, the forestomach, cecum, and colon-rectum reach the maximum growth rate (Lyford Jr, 1993; Andrade et al., 2020). Reticulorumen, cecum, and colon-rectum have late development because of their relationship to fibre digestion. Cecum and colon-rectum may account for 10% of total fibre digestion (Leite et al., 2015a). However, this value may change according to reticulorumen k_p because reticulorumen k_p can be compensated for, at least in part, by changes in the distal tract (Faichney, 2005).

REVIEW ABOUT MODELS FOR PREDICTING k_p IN RETICULORUMEN OF GOATS

Predictions of reticulorumen k_p of particles and solutes are essential to optimize the use of ingested feed by ruminants. Reticulorumen segment accounts for 72% of the total k_p of particulate material on GIT (Ahvenjärvi et al., 2000, 2010; Walz et al., 2004; Leite et al., 2015a) and influences k_p of far GIT segments, as distal tract (Dijkstra et al., 2005; Huhtanen et al., 2006). Additionally, reticulorumen k_p of particles affects the extend of NDF digestibility (Fox et al., 2004). Moreover, knowledge about reticulorumen k_p of solutes is very important because solutes in reticulorumen act a lubricant and provide a medium for the density-dependent sorting mechanism and microbes to access particles (Seo et al., 2007; Clauss et al., 2016a). Moreover, reticulorumen k_p of solutes is positively related to efficient production of microbial protein and methane emission per unit of DM intake (Clauss and Hummel, 2017; Grandl et al., 2018). Reticulorumen k_p of solutes is also related to the outflow of soluble nutrients and particles from reticulorumen (Seo et al., 2009). Thus, this demonstrates how necessary are models for predicting separately the reticulorumen k_p of particles and solutes.

Forestomach physiology has been considered a species-specific characteristic (Müller et al., 2013; Steuer et al., 2013; Dittmann et al., 2015). Despite goats have been considered “cattle-type” ruminant, they are considered intermediate feeders and their rumen particle stratification mechanisms have been demonstrated somewhat different from cattle (Clauss et al., 2016a). The consistency of digesta in the dorsal rumen of the goats, as compared to the cattle, is generally more fluid (Dziuk and McCauley, 1965), that may facilitate the transit of particles between pools. Once reticulorumen k_p has been considered dependent on rumen particle stratification (Poppi et al., 1981; Kaske and Engelhardt, 1990; Seo et al., 2009), the k_p models for goats must be developed considering this. Moreover, studies have demonstrated reticulorumen motility pattern is different between goats and cattle (Dziuk and

McCauley, 1965). They demonstrated goats, during rest, had lesser relaxations between contractions during the biphasic contractions than cattle. Moreover, goats had lesser variable the sequence of contractions than cattle, and the secondary caudal dorsal blind sac contractions of lower magnitude and shorter duration than the primary contractions, when compared to cattle. Once the transit of particles between pools and reticulo-omasal orifice opening depend on reticulorumen contractions, those differences between goats and cattle must be considered. Moreover, studies have demonstrated k_p of particles are strongly different than k_p of solutes and they are affected by such different factors, as previously described (Seo et al., 2007, 2009). Therefore, this justifies the necessity of developing exclusive models for predicting separately reticulorumen k_p of particles and solutes in goats.

Additionally, models for growing ruminants must take into account rumen capacity, feed intake, and chewing activity, that has actually been considered the driving force of reticulorumen k_p in growing ruminants (Seo et al., 2007, 2009; Grandl et al., 2016a, 2018). Moreover, reticulorumen k_p of particles have been related to reticulorumen k_p of solutes and diet composition (Seo et al., 2007, 2009; Grandl et al., 2016a, 2018). Thus, all these characteristics of reticulorumen k_p must be considered when models are being developed. Few models have been found in the literature for predicting k_p in reticulorumen of goats, thus the current scientific landscape in modelling research k_p in goats can be described as incipient and contradictory (Bompadre, 2015).

The following models have been described in the literature as adequate for predicting reticulorumen k_p (Tedeschi et al., 2012). However, none of them separately predicts k_p of solutes and particles and considers capacity, chewing investment, and the relationship between k_p of particles and solutes. The developed empirical equations to predict forage and concentrate k_p for cattle, sheep and goats presented by Cannas and Soest (2000):

$$k_p \text{ of forage (\%/h)} = 1.82*\text{DMFor}^{0.34}+e^{0.036*\text{ConCP}}$$

where, DMFor is forage DM intake expressed as % BW and ConCP is crude protein concentration in ingested diet expressed as % DM. This model was lately revised and improved by Tedeschi et al. (2012), in which the study effect was considered for parameterizing the model:

$$k_p \text{ of forage (/h)} = 0.0182*DMFor^{0.40}+e^{0.0046*ConCP}$$

where, DMFor is forage DM intake expressed as g/kg BW and ConCP is crude protein concentration in ingested diet expressed as g/kg DM intake. The developed empirical equation for the prediction of fractional k_p of goat presented by Tedeschi et al. (2012):

$$k_p \text{ (/h)} = 0.00161*NDFI^{1.503}*e^{(0.022*BW - 0.00375*ConNDF}$$

where, NDFI is NDF intake expressed as g/kg BW, BW is expressed as kg, and ConNDF is NDF concentration in ingested diet expressed as g/kg DM intake. For the development of this model, the authors used a database of four studies with dairy cattle, and an independent database with five studies with goats was used for validating the model.

OBJECTIVE

The main objective of the research described in this dissertation is to understand the factors related to k_p in the whole digestive tract of growing Saanen goats and goats fed with feeding levels and propose equations for predicting k_p of particles and solutes in reticulorumen of Saanen goats.

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CHAPTER 2 – Body weight and sex effects on digesta mean retention time in growing Saanen goats

ABSTRACT: Despite the important role of digesta mean retention time (MRT) on digestive efficiency of ruminants, it is poorly investigated in total gastrointestinal tract (GIT) of growing ruminants, especially in goats. The objective of this study was to evaluate the effect of body weight (BW) and sex on GIT MRT of particles and solutes in growing Saanen goats. A dataset from two studies, comprising 103 individual records of castrated males (n = 36), females (n = 34), and intact males (n = 33) Saanen goats slaughtered at 15, 22, 30, 37, and 45 kg BW, was used. Goats were fed basically with total mixed ration composed by dehydrated corn plant (Zea mays) milled to pass a 10- mm screen, cracked corn grain, and soybean (Glycine max) meal. Variables evaluated were BW, feed intake, feed intake level, composition of ingested diet, wet weight of GIT tissues, wet digesta pool size, digesta composition (dry matter and neutral detergent fiber (NDF)), indigestible NDF:NDF ratio of ingested diet and GIT digesta, MRT of particles (MRT_{iNDF}) and solutes (MRT_{Cr}), and reticulorumen selectivity factors (large particles/solutes). Reticulorumen, omasum, abomasum, small intestine, cecum, and colon-rectum segments were evaluated. The dataset was analyzed as mixed models considering sex, BW, and sex × BW interaction as fixed effects, and study and residual error as random effects. Sex did not affect MRT_{iNDF} in any GIT segments. Females and intact males presented similar reticulorumen MRT_{Cr} (5.6 h; $P = 0.92$) and they presented lower reticulorumen MRT_{Cr} than castrated males (7.0; $P \leq 0.04$). Total GIT MRT_{Cr} was similar between castrated males and females (15.7 h; $P = 0.11$) and between females and intact males (14.2 h; $P = 0.76$). Body weight did not affect MRT_{iNDF} in reticulorumen and colon-rectum and total GIT MRT_{Cr} ($P \geq 0.11$). Reticulorumen and omasum MRT_{Cr} increased as BW increased ($P < 0.01$), and abomasum MRT_{Cr} decreased as BW increased ($P = 0.02$). Feed intake, and wet tissues and wet pool size of all GIT segments increased as BW increased, except abomasum wet pool size ($P \leq 0.01$). The mechanism related to sex effect on MRT has to be elucidated. Reticulorumen MRT_{iNDF} and total GIT MRT_{Cr} were modulated by intake and capacity of reticulorumen and GIT, respectively. On the other hand, reticulorumen MRT_{Cr} seemed to be regulated by reticulo-omasal orifice opening and saliva secretion.

Key words: dairy goat, digesta kinetic, sorting mechanism, digesta washing, gastrointestinal tract fill

INTRODUCTION

Digesta mean retention time (MRT) is an important factor on digestive efficiency (Okine et al., 1998) because it is related to digestibility of plant cell wall (Allen and Mertens, 1988), rate and extent of protein digestion (Ørskov and McDonald, 1979), amount of protein which escapes from degradation in the reticulorumen (Fox et al., 2004), efficiency of microbial growth (Harrison and McAllan, 1980; Evans, 1981), and extent of methane losses (Okine et al., 1998). In this sense, MRT has an important role in compartmental models of feeding systems to predict the ruminal digestibility of carbohydrate and protein fractions (Cannas et al., 2004; Fox et al., 2004).

Studies carried out in the past reported relationship between body weight (BW) and MRT in herbivores (Demment, 1983; Robbins, 1983; Gordon and Illius, 1994). These studies justified this relationship because the gastrointestinal tract (GIT) capacity in herbivores increases in the same proportion of BW ($BW^{1.0}$) (Demment and Van Soest, 1985; Illius and Gordon, 1992), whereas the energy requirements/feed intake increases in the range of $BW^{0.75}$ (Kleiber, 1932; Bourlière, 1975; Blaxter et al., 1982). However, currently, the relationship between BW and MRT is considered a controversial subject. Previous studies with several species of herbivores did not find a significant relationship between MRT and BW for browsing and grazing ruminants (Clauss et al., 2007b; Steuer et al., 2011). On the other hand, recent studies also studying several species of herbivores, reported that the relationship between MRT and BW was confirmed for ruminants (Müller et al., 2013; Dittmann et al., 2015). Importantly, few studies in the literature have evaluated this relationship accounting for possible sex effects on MRT in ruminants.

Sex and BW are accounted into the species-specific physiological responses of MRT because they are related to ingested feed, feed intake, GIT capacity, and feed digestibility (Gross et al., 1995b). Feed intake and ingested feed are considered the driving force of MRT, and both are dependent on energy requirements (Clauss et al., 2007b; Meyer et al., 2010; Müller et al., 2013). In addition, recent meta-analytical studies with growing Saanen goats have shown sex and BW effects on net energy requirements for maintenance and growth (Souza et al., 2017, 2020). Therefore, considering a possible BW and sex effect on MRT, and the lack of studies with Saanen goats, the aim of this study was to evaluate the effects of BW and sex on MRT of particles and solutes in Saanen goats throughout the growing phase.

MATERIALS AND METHODS

Dataset

A data set including 103 individual records of castrated males (n = 36), females (n = 34), and intact males (n = 33) Saanen goats from 15 to 45 kg BW was analyzed. This dataset was combined from two studies in which goats were slaughtered at 15, 22, and 30 kg BW (Leite et al., 2015a; 2015b) and at 30, 37, and 45 kg BW (Silva, 2013). Males were castrated when they were around 1 and 5 months old for Leite et al. (2015a; 2015b) and Silva (2013) studies, respectively. Goats were weaned when they were around 2 months old and 12 kg BW, for all studies (Silva, 2013; Leite et al., 2015a; 2015b). And the experiments started when goats were 3.2 ± 0.67 and 10.2 ± 1.76 months old for Leite et al. (2015a; 2015b) and Silva (2013) studies, respectively. All procedures used across studies were reviewed by the University’s Animal Care Committee (Comissão de Ética e Bem-Estar Animal, CEBEA; Universidade Estadual Paulista, Jaboticabal, Brazil).

Experimental Procedures and Calculations

All goats were housed in individual 0.5 m^2 pens with free access to water. Goats were fed with similar diets ad libitum for all experiments (Table 1) and the diets were formulated to meet the daily requirements of goat kids. The whole diet was milled to pass a 10- mm screen (Figure 1). The daily feed intake was calculated by subtracting orts from the offered diet during the whole experiments (139 d), but only the feed intake in the last 5 d before slaughter was used to calculate the MRT and, therefore, only these data are presented. During the same experimental period (i.e., last 5 days) Cr-EDTA was administrated to determine the MRT of solutes. In the offered diet and orts, the dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and indigestible NDF (iNDF) concentration were determined, and posteriorly intake of DM (DMI), OM (OMI), NDF (NDFI), iNDF (iNDFI), potentially degradable NDF (pdNDFI) were calculated. In addition, we calculated the feeding level as multiples of metabolizable energy (ME) requirements for maintenance intake (L), using ME requirements for maintenance of growing Saanen goats (Souza et al., 2020). The ME in the diet was estimated when goats were around 22 and 37 kg BW for study (Leite et al., 2015ab) and (Silva, 2013), respectively. The ME concentration in the diet (kcal/ kg of DM) was estimated from gross energy intake, total energy losses from feces, urine, and gaseous products of digestion. Fecal and urinary excretions were obtained from their total collection (Souza et al., 2020). Energy loss from gaseous products of digestion was predicted according to Blaxter and Clapperton (1965) equation, as described by Souza et al. (2020). The feed intake was expressed as g and % of BW.

Goats were slaughtered as they reached approximately 15, 22, 30, 37, and 45 kg BW. Castrated males goats were 82 ± 12, 195 ± 52, 220 ± 80, 253 ± 31, 291 ± 77 days old at slaughter weight 15, 22, 30, 37, and 45 kg BW, respectively, females goats were 116 ± 12, 178 ± 24, 263 ± 75, 361 ± 21, 503 ± 54 days old at slaughter weight 15, 22, 30, 37, and 45 kg BW, respectively, and intact males were 92 ± 20, 214 ± 44, 237 ± 75, 249 ± 25, 280 ± 72 days old at slaughter weight 15, 22, 30, 37, and 45 kg BW, respectively.

After goats were slaughtered (2.2 ± 0.8 h after morning feeding), GIT was removed and separated into reticulorumen, omasum, abomasum, small intestine, cecum, and colon-rectum (colon and rectum free of fat and mesenteries). The segments were weighed before and after emptying to determine the mass of wet digesta and the wet weight of each segment tissues.

For all studies, the reticulorumen digesta was separated into solid and liquid fractions by straining the contents through 4 layers of cheesecloth. These fractions were weighed and sampled according to the proportions determined to obtain a representative sample. The digesta from omasum, abomasum, small intestine, and cecum were individually placed into trays and mixed/homogenized before sampling. Colon and rectum digesta were collected separately and placed into trays. The rectum digesta was minimally and manually broken up and then mixed with colon digesta until we got a very homogeneous colon-rectum paste, then samples were taken.

Table 1. Ingredients and chemical composition of the experimental diets

Item	(Leite et al., 2015a; 2015b)	(Silva, 2013)
Dietary ingredient, % DM		
Dehydrated corn plant[1]	45.40	44.70
Cracked corn grain	26.60	30.50
Soybean meal	22.30	15.10
Soybean oil	1.60	2.50
Limestone	1.00	1.30
Mineral supplement[2]	2.20	6.00
Ammonium chloride	0.90	0.00
Diet chemical composition[3], g/kg of DM ± SD		
DM	854 ± 10.9	865 ± 3.13
OM	935 ± 2.00	902 ± 3.27
CP	204 ± 5.40	154 ± 6.57
Crude fat	80 ± 4.90	51 ± 0.79
NDF	355 ± 25.00	313 ± 7.54
iNDF[4]	108 ± 10.50	113 ± 8.97
Lignin	57 ± 3.40	n.a.

[1] 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).

[2] Composition, per kg, as-fed basis: 190 g of Ca; 92 g of Cl; 73 g of P; 62 g of Na; 44 g of Mg; 1.35 g of Zn; 1.06 g of Fe; 0.94 mg of Mn; 0.73 g of F; 0.34 g of Cu; 18 mg of Se; 16 mg of I; 3 mg of Co.

[3] Mean and standard deviation of 10 composite samples. The chemical composition of the diet was calculated from the individual ingredients.

[4] iNDF= Indigestible NDF.

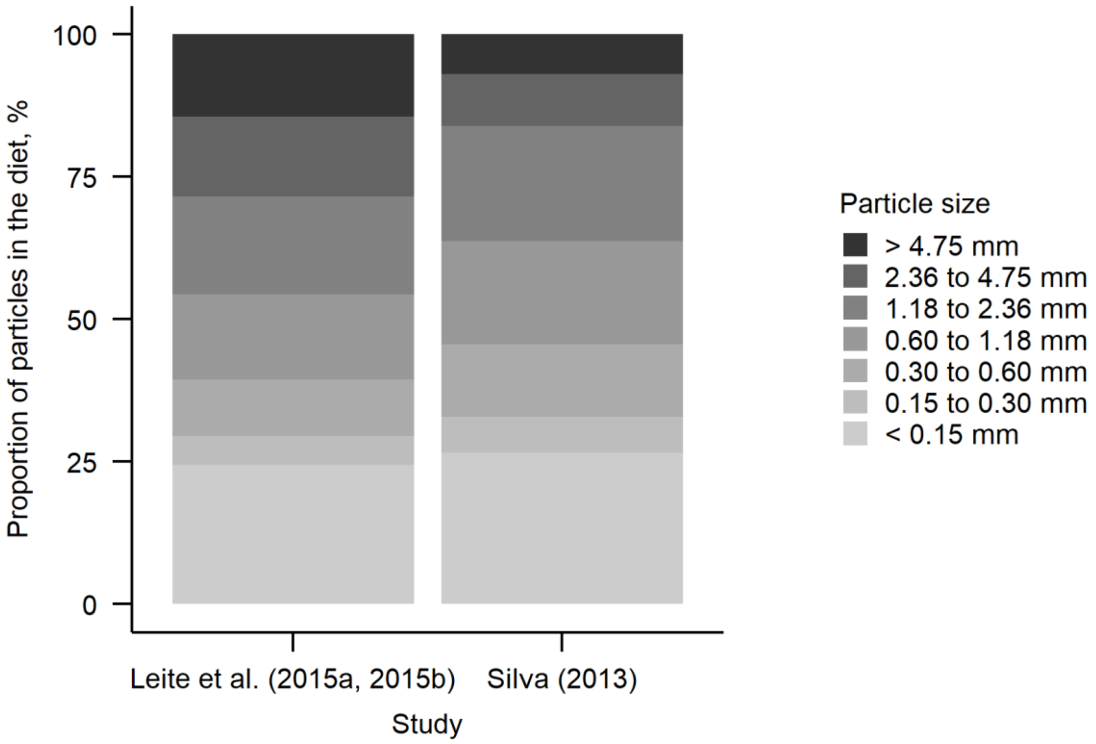

Figure 1. Particle size of diet offered to the goats during the studies (Silva, 2013; Leite et al., 2015a; 2015b)

In the digesta of each segment, the DM, OM, NDF, iNDF, and Cr concentrations were determined. We considered the wet weight of the total GIT tissues as the sum of the wet weight of individual tissues of GIT segments, and total GIT pool size (wet digesta and DM) as the sum of individual pool size of GIT segments. We expressed the wet pool size and the wet weight of tissues as grams.

The MRT was determined as the inverse of the digesta passage rate (k_p) (i.e., MRT = $1/k_p$). The k_p was assessed by using the method of emptying GIT compartments in slaughtered animals and using iNDF and Cr-EDTA as markers for particles (MRT_{iNDF}) and solutes (MRT_{Cr}), respectively. The details about preparation, administration, and analysis of both markers were previously published (Leite et al., 2015a; 2015b). The k_p of particles and solutes in different segments of the GIT were determined by the flux/compartmental pool method using the Eq. [1](Ellis et al., 1994):

k_p of indigestible entity (IE) = intake rate of IE/compartmental mass of IE, [1] where k_p is the fractional rate of IE escape per hour, intake rate of IE is expressed in grams per hour, and compartmental mass in the segment is expressed in grams.

The total MRT in the GIT was calculated by the sum of MRT in the reticulorumen, omasum, abomasum, small intestine, cecum, and colon-rectum. Moreover, the reticulorumen selectivity factors of particles and solutes (particles:solutes), that indicate digesta washing was calculated as the quotient between MRT estimated by iNDF and Cr-EDTA (Lechner et al., 2010; Müller et al., 2011).

Statistical Analyses

The data was analyzed using PROC MIXED of SAS (v 9.4, SAS Inst. Inc., Cary, NC) by the model (Eq. [2])

$$Y_{ijkl} = \mu + S_i + W_j + S_i \times W_j + T_k + e_{ijkl} \qquad [2]$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of sex i, W_j is the fixed effect of BW j, $S_i \times W_j$ is the interaction between sex i and BW j, $T_k \sim \text{iidN}(0, \sigma_T^2)$ is the random effect of study k, and $e_{ijkl} \sim \text{iidN}(0, \sigma_e^2)$ is the random residual error. Moreover, residual variances were modeled using distinct grouping (i.e., no grouping, study, sex, BW, or interaction between sex and BW) using the REPEATED/GROUP function of PROC MIXED. The best grouping for each variable was chosen using the lowest Akaike information criterion (Akaike, 1974), corrected for small samples (AICc) (Sugiura, 1978). Residuals were plotted against the predicted means to check the model assumptions regarding homoscedasticity, independence, and normality of the errors. Outliers were removed when their Studentized residuals were >|3|. For cecum wet tissues, abomasum wet digesta, iNDF:NDF ratio of cecum content, MRT of particles in cecum, and MRT of solutes in abomasum, 1 data point each was removed. For small intestine wet tissues, iNDF:NDF ratio of colon-rectum content, and MRT of solutes in reticulorumen, omasum, and cecum, 2 data points each were removed. For iNDF:NDF of ingested diet and abomasum wet tissues 3 data points each were removed.

Orthogonal polynomial contrasts were used to determine linear and quadratic effects of BW when it was significant ($P \leq 0.05$) using the CONTRAST statement of PROC MIXED of SAS (v 9.4, SAS Inst. Inc., Cary, NC). The effects of sex and sex within BW were compared by Tukey’s test. When the interaction between sex and BW was significant ($P \leq 0.05$), polynomial regressions were used to determine linear or quadratic effects of BW within sex using the PROC MIXED of SAS (v 9.4, SAS Inst. Inc., Cary, NC), by the model (Eq. [3]) as follow:

$$Y_{ijkl} = \mu + S_i + W^1(S_c) + ... + W^j(S_c) + W^1(S_f) + ... + W^j(S_f) + W^1(S_m) + ... + W^j(S_m) + T_k + e_{ijkl} \quad [3]$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of sex i, $W^1(S_c) + ... + W^j(S_c)$ are the fixed effects of BW raised by the exponent j (1 to 4) within castrated males, $W^1(S_f) + ... + W^j(S_f)$ are the fixed effects of BW raised by the exponent j (1 to 4) within females, $W^1(S_m) + ... + W^j(S_m)$ is the fixed effect of BW raised by the exponent j (1 to 4) within intact males, $T_k \sim iidN(0, \sigma_T^2)$ is the random effect of study k, and $e_{ijkl} \sim iidN(0, \sigma_e^2)$ is the random residual error with a variance σ_e^2. Statistical significance was set at $P \leq 0.05$.

RESULTS

Castrated males had greater DM intake, relative DM intake, and L than females (915 vs. 809 g, 3.19 vs. 2.91% BW, and 3.24 vs. 2.93, respectively; $P \leq 0.05$), and both were similar to intact males (912 g, 3.11% BW and 3.18, respectively; $P \geq 0.07$; Table 2). Moreover, DM intake (Figure 2) increased at a decreasing rate as BW increased ($P < 0.01$, Table 2). The relative DM intake decreased linearly as BW increased ($P < 0.01$) and L decreased at an increasing rate as BW increased, with the lowest means observed at 45 kg BW ($P \leq 0.03$; Table 2). There was a significant interaction between sex and BW ($P < 0.01$) in NDF content and iNDF:NDF ratio of the ingested diet (Table 2; Figure 2).

Table 2. Body weight, feed intake, and total NDF digestibility of castrated males (C), females (F), and intact males (I) Saanen goats slaughtered at five different BW

Item	BW (kg) and sex															SEM	*P*-Value[1]			
	15			22			30			37			45							
	C	F	I	C	F	I	C	F	I	C	F	I	C	F	I		Sex[2]	BW_L	BW_Q	Sex*BW[3]
BW, kg	15.7	16.2	15.8	22.2	21.5	23.4	30.4	29.3	31.7	39.0	38.7	38.4	45.2	44.4	44.9	1.020	0.11	<0.01	0.68	0.06
DM intake[4], g	613	552	601	780	792	813	945	893	941	1148	949	1120	1090	862	1085	91.40	0.03	<0.01	<0.01	0.79
relative DM intake, % BW	3.85	3.4	3.7	3.4	3.6	3.4	3.1	3.0	2.9	3.1	2.6	3.0	2.5	2.0	2.5	0.3	0.05	<0.01	0.38	0.66
NDF, % DM intake	29.4b	36.9a	29.5b	36.2	34.6	34.4	33.6	34.7	36.2	29.9	29.8	31.2	30.7	29.8	31.4	3.58	-	-	-	<001
																	C	0.46	<0.01	-
																	F	<0.01	0.99	-
																	I	0.91	<0.01	-
Intake iNDF[5]:NDF ratio	0.34	0.26	0.33	0.28	0.33	0.28	0.34	0.35	0.34	0.44	0.47	0.44	0.43a	0.46a	0.33b	0.059	-	-	-	<0.01
																	C	<0.01	0.01	-
																	F	<0.01	0.77	-
																	I	0.01	0.02	-
L[6]	3.4	2.97	3.32	3.32	3.47	3.31	3.23	3.06	3.12	3.36	2.86	3.32	2.88	2.3	2.86	0.28	0.05	0.06	0.03	0.73

[a-b]Means in the same row with different superscripts are different according to Tukey's test within of BW (*P* ≤ 0.05).

[1]Main effects and interaction sex × BW (Sex× BW). BW_L= linear effect of BW; BW_Q = quadratic effect of BW.

[2]Castrated males had greater DM intake, relative DM intake, and L than females (915 vs. 809 g, 3.19 vs. 2.91% BW, and 3.24 vs. 2.93, respectively; *P* ≤ 0.05), and both were similar to intact males (912 g, 3.11% BW and 3.18, respectively; *P* ≥ 0.07).

[3]When the interaction between sex and BW was significant the effect of BW was evaluated within each sex.

[4]Intake = ingested diet (offered diet – orts).

[5]iNDF= Indigestible NDF.

[6]L = feeding level as multiples of ME requirements for maintenance intake.

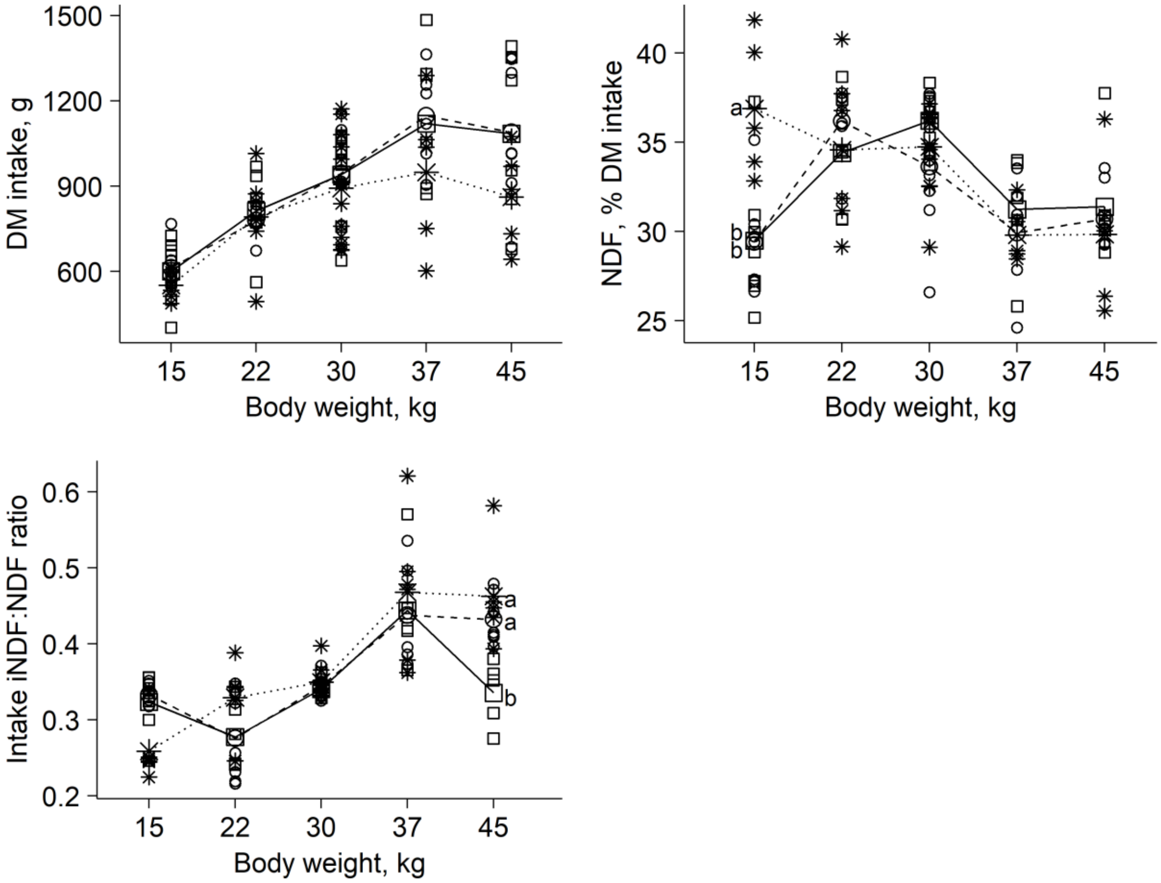

Figure 2. Castrated males had greater DM intake than females (915 vs. 809 g; *P* = 0.05), and both were similar to intact males (912 g; *P* ≥ 0.07). Body weight quadratically affected DM intake (*P* < 0.01). Means in the same body weight with different letters (a and b) are different according to Turkeys' test (*P* ≤ 0.05). Body weight linearly affected NDF content and the ratio between indigestible NDF (iNDF) and NDF (iNDF:NDF ratio) of the ingested diet of females (*P* ≤ 0.01). Body weight quadratically affected NDF content of ingested diet of males and iNDF:NDF ratio of the ingested diet of males (*P* ≤ 0.02). The symbol ○ and dashed line represent castrated males, ∗ and dotted line represent females, □ and solid line represent intact males.

Sex did not affect wet tissues, wet pool size, DM and NDF contents of reticulorumen and omasum, and the ratio between DM intake and reticulorumen wet pool size (DM intake:RR wet pool size ratio) (*P* ≥ 0.07; Table 3; Figure 3). The ratio between DM intake and reticulorumen wet tissues (DM intake:RR wet tissues ratio) was greater for males than females (*P* ≤ 0.04), and it was similar between males (*P* = 0.95; Figure 3). Reticulorumen and omasum wet tissues and wet pool size increased at a decreasing rate as BW increased (*P* < 0.01; Table 3). Reticulorumen DM content increased at an increasing rate as BW increased (P = 0.01; Table 3). The DM intake:RR wet tissues ratio linearly decreased as BW increased (*P* ≤ 0.05; Figure 3). Body weight did not affect reticulorumen and omasum NDF contents, omasum DM content, and the DM intake:RR wet pool size ratio (*P* ≥ 0.11; Table 3; Figure 3).

Sex did not affect reticulorumen iNDF:NDF ratio and $\mathrm{MRT}_{\mathrm{iNDF}}$ (Figure 3) and omasum $\mathrm{MRT}_{\mathrm{Cr}}$ (*P* ≥ 0.30; Table 3). However, females and intact males presented similar reticulorumen $\mathrm{MRT}_{\mathrm{Cr}}$ (5.6 h; *P* = 0.92) and they presented lower reticulorumen $\mathrm{MRT}_{\mathrm{Cr}}$ than castrated males (7.0; *P* ≤ 0.04; Table 3; Figure 3). The reticulorumen iNDF:NDF ratio increased from 15 to 30 kg BW and then decreased from 30 to 45 kg BW (*P* < 0.01; Table 3). Body weight did not affect reticulorumen $\mathrm{MRT}_{\mathrm{iNDF}}$ (*P* ≥ 0.11; Table 3; Figure 3). However, reticulorumen and omasum $\mathrm{MRT}_{\mathrm{Cr}}$ increased linearly as BW increased (*P* < 0.01; Table 3; Figure 3). Reticulorumen selectivity factor of particles:solutes in females decreased at a decreasing rate as BW increased (*P* < 0.01) and remained similar in males as BW increased (*P* ≥ 0.41; Table 3).

Even though castrated males demonstrated greater abomasum wet tissues than females and intact males only at 37 kg BW (*P* < 0.01 for interaction sex and BW; Table 3), castrated males had greater abomasum wet pool size (563 g) and $\mathrm{MRT}_{\mathrm{Cr}}$ (0.66 h) than females and intact males at all evaluated BW (*P* < 0.01; Table 3). Females and intact males had similar abomasum wet pool size and $\mathrm{MRT}_{\mathrm{Cr}}$ (429 g, and 0.46 h, respectively; *P* ≥ 0.99; Table 3). Regardless the interaction between sex and BW, abomasum wet tissues increased as BW increased, for all sexes (*P* < 0.01); different from abomasum wet pool size that did not present clear pattern with the increase of BW. Abomasum $\mathrm{MRT}_{\mathrm{Cr}}$ decreased linearly as BW increased (*P* = 0.02; Table 3).

Table 3. Wet tissues, pool size (wet digesta and composition), and digesta mean retention time (MRT) of indigestible NDF (iNDF) (MRT_{iNDF}) and MRT of Cr (MRT_{Cr}) of forestomaches segments of castrated males (C), females (F), and intact males (I) Saanen goats slaughtered at five different BW

Item	BW (kg) and sex															SEM	*P-Value*[1]			
	15			22			30			37			45							
	C	F	I	C	F	I	C	F	I	C	F	I	C	F	I		Sex[2]	BW_L	BW_Q	Sex*BW[3]
Reticulorumen																				
Wet tissues, g	399.3	390.7	400.8	512.5	543.8	529.2	646.3	617.5	638.2	806.9	715.0	741.5	779.7	791.4	749.8	35.25	0.67	<0.01	<0.01	0.86
Wet pool size, g	2444.3	2451.0	2326.7	3393.9	3478.7	3411.6	4157.3	3582.2	4348.6	4654.3	4007.9	4402.1	4591.9	4053.7	4527.2	258.76	0.10	<0.01	<0.01	0.61
DM, % wet pool size	16.0	13.6	14.8	14.7	13.7	13.7	14.2	15.0	14.4	16.6	14.7	16.7	19.0	16.8	16.0	1.74	0.07	0.03	0.01	0.42
NDF, % DM	49.6	53.6	49.9	48.2	54.2	50.8	50.4	50.3	51.3	50.3	48.1	51.2	46.9	47.8	53.2	4.12	0.09	0.42	0.66	0.09
iNDF:NDF ratio	0.48	0.52	0.50	0.57	0.52	0.54	0.60	0.58	0.54	0.61	0.54	0.53	0.51	0.48	0.51	0.098	0.20	0.81	<0.01	0.12
MRT_{iNDF}, h	35.44	45.79	34.05	47.41	38.87	40.94	40.60	37.39	35.02	44.76	40.49	41.93	44.85	45.33	46.02	4.48	0.56	0.11	0.42	0.66
MRT_{Cr}, h	5.04	2.81	4.39	5.10	4.55	4.97	6.52	7.22	5.34	8.52	5.57	6.25	9.81	8.58	6.75	0.78	<0.01	<0.01	0.79	0.08
$\text{MRT}_{\text{iNDF}}/\text{MRT}_{\text{Cr}}$	6.51b	17.56a	6.75b	6.70	7.01	8.47	6.18	5.41	6.07	6.11	6.17	6.66	5.22	5.44	6.69	1.20	-	-	-	<0.01
																	C	0.41	0.64	-
																	F	<0.01	<0.01	-
																	I	0.61	0.96	-
Omasum																				
Wet tissues, g	39.3	41.5	41.2	67.0	49.8	62.4	88.8	81.9	89.1	103.1	93.0	98.8	109.1	107.9	104.7	5.88	0.20	<0.01	<0.01	0.87
Wet pool size, g	39.3	37.7	47.1	92.8	52.4	94.4	136.1	117.1	133.8	176.7	146.1	166.2	141.7	162.3	176.1	20.50	0.17	<0.01	<0.01	0.80
DM, % wet pool size	25.7	23.8	26.6	22.6	22.5	25.9	23.9	23.7	23.6	26.2	23.3	23.0	24.9	23.8	25.1	1.45	0.26	0.71	0.15	0.67
NDF, % DM	43.8	48.1	42.9	48.8	52.6	43.8	41.9	40.6	47.9	34.7	40.3	37.3	38.2	40.7	39.8	3.44	0.38	0.66	0.73	0.44
MRT_{Cr}, h	0.12	0.07	0.12	0.18	0.11	0.15	0.20	0.25	0.18	0.26	0.33	0.20	0.42	0.16	0.33	0.0540	0.30	<0.01	0.79	0.34
Abomasum																				
Wet tissues, g	124.3	124.6	120.6	145.6	135.3	150.2	168.4	144.0	160.6	243.3a	187.9b	183b	187.0	195.1	181.5	29.96	-	-	-	<0.01
																	C	<0.01	<0.01	-
																	F	<0.01	0.39	-
																	I	<0.01	0.08	-
Wet pool size, g	474.1	459.1	433.7	537.9	477.0	486.3	568.0	375.5	416.2	830.6	525.0	442.0	405.8	298.8	377.9	120.24	<0.01	0.61	<0.01	0.07
DM, % wet pool size	14.5	13.7	14.4	12.2	11.1	9.9	11.5	12.9	10.6	16.7	12.9	11.6	16.9	12.6	14.8	1.25	-	-	-	0.04
																	C	0.03	<0.01	-
																	F	0.94	0.52	-
																	I	0.54	<0.01	-
NDF, % DM	30.7	35.2	34.4	42.7	44.9	43.4	37.2	37.3	45.4	33.5	38.7	37.8	34.1	40.5	39.2	5.15	0.03	0.86	0.01	0.63
MRT_{Cr}, h	0.67	0.59	0.67	0.73	0.72	0.49	0.62	0.48	0.43	0.74	0.23	0.34	0.55	0.28	0.36	0.130	<0.01	0.02	0.94	0.15

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

[1] Main effects and interaction sex × BW (Sex × BW). BW_L = linear effect of BW; BW_Q = quadratic effect of BW.

Table 3. Continued

[2] Females and intact males presented similar reticulorumen MRT_{Cr} (5.6 h; *P* = 0.92) and they presented higher reticulorumen MRT_{Cr} than castrated males (7.0; *P* ≤ 0.04). Castrated males had greater abomasum wet pool size and MRT_{Cr} than females and intact males at all evaluated BW (563 g vs. 427 and 431 g, and 0.66 vs 0.46 and 0.46 h, respectively; *P* < 0.01). Females and intact males had similar abomasum wet pool size and MRT_{Cr} (429 g, and 0.46 h, respectively; *P* ≥ 0.99). Intact males had greater abomasum NDF content than castrated males (40.0 vs. 35.6 % DM; *P* = 0.04). Females had similar abomasum NDF content than males (39.3 vs. 37.8 % DM; *P* ≥ 0.10).

[3] When the interaction between sex and BW was significant the effect of BW was evaluated within each sex.

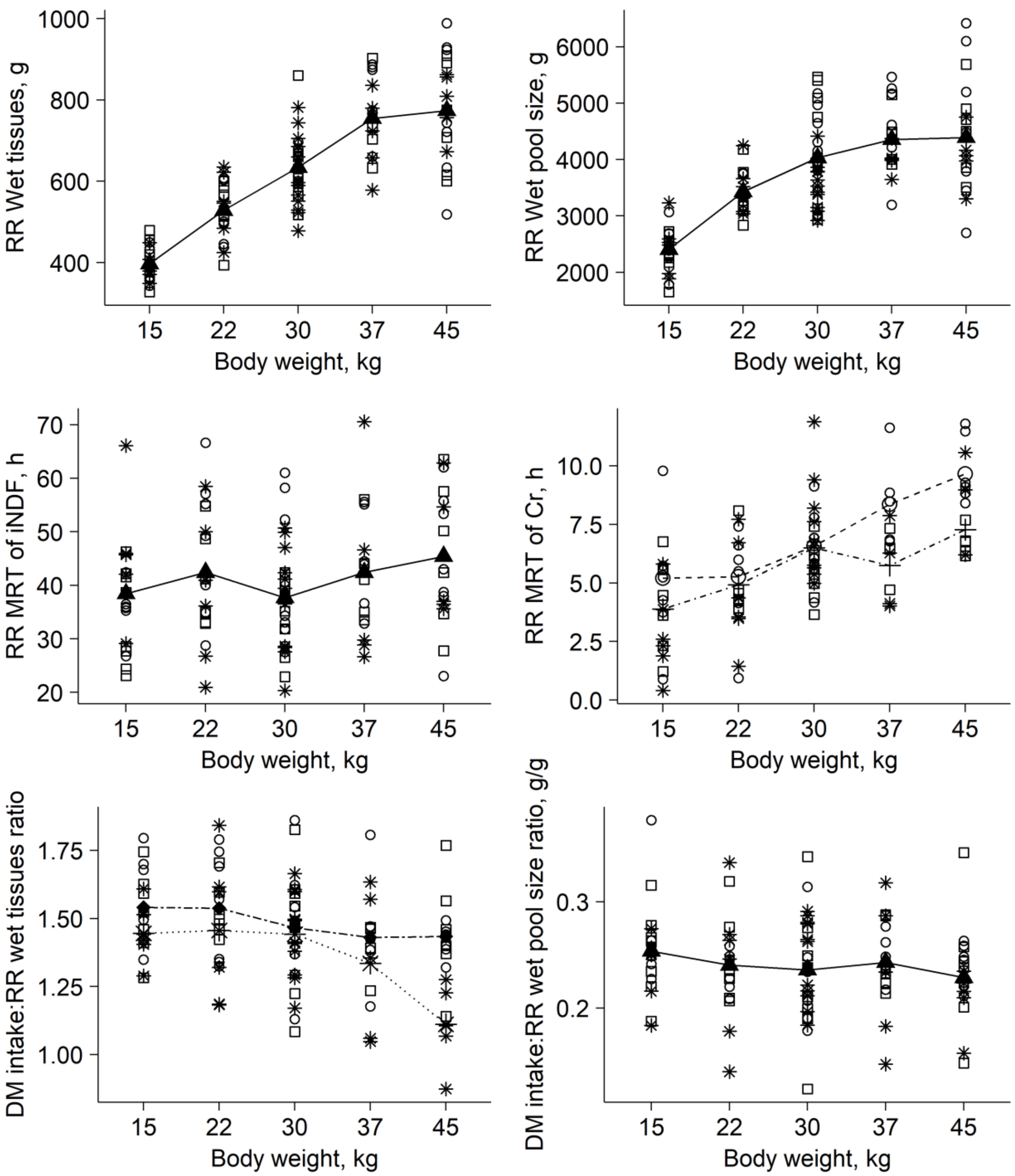

Figure 3. Sex did not affect wet tissues, wet pool size, DM and NDF contents of reticulorumen (RR), and the ratio between DM intake and reticulorumen wet pool size (DM intake:RR wet pool size ratio) (*P* ≥ 0.07). Females and intact males presented similar reticulorumen digesta mean retention time (MRT) of solutes (MRT_{Cr}) (5.6 h; *P* = 0.92) and they presented greater reticulorumen MRT_{Cr} than castrated males (7.0; *P* ≤ 0.04). The ratio between DM intake and reticulorumen wet tissues (DM intake:RR wet tissues ratio) was greater for males than females (*P* ≤ 0.04), and it was similar

between males ($P = 0.95$). Body weight quadratically affected reticulorumen wet tissues and wet pool size ($P < 0.01$). Body weight linearly affected reticulorumen MRT_{Cr} and the DM intake:RR wet tissues ratio ($P \leq 0.05$). Body weight did not affect reticulorumen MRT of particles (MRT_{iNDF}) and the DM intake:RR wet pool size ratio ($P \geq 0.11$). The symbol ○ and dashed line represent castrated males, ∗ and dotted line represent females, □ and solid line represent intact males, + and dash-dotted line represent females and intact males mean, ♦ and dash-short-dashed line represent males mean, and ▲ and solid line represent all sexes mean.

Small intestine wet tissues were greater for castrated males than females (681 vs. 599 g; $P \leq 0.04$; Table 4). Small intestine wet tissues were similar between castrated males and intact males (668 g) and between females and intact males (627 g; $P \geq 0.09$). On the other hand, small intestine wet pool size, DM content, and MRT_{Cr} were not affected by sex ($P \geq 0.69$). Small intestine wet tissues and wet pool size increased at a decreasing rate as BW increased ($P < 0.01$), and small intestine DM content and MRT_{Cr} remained similar as BW increased ($P \geq 0.12$; Table 4). Moreover, small intestine NDF content decreased linearly as BW increased for males ($P \leq 0.04$) and remained similar as BW increased for females ($P \geq 0.06$; Table 4). Females had lower small intestine NDF content than intact males at 15 kg BW ($P < 0.01$; Table 4). Castrated males and intact males had similar small intestine NDF content for all evaluated BW ($P \geq 0.77$; Table 4).

Sex did not affect the variables evaluated in cecum ($P \geq 0.17$; Table 4). Cecum wet tissues, wet pool size, NDF content, and iNDF:NDF ratio increased linearly as BW increased ($P \leq 0.01$; Table 4). Cecum MRT_{iNDF} increased at an increasing rate as BW increased ($P = 0.02$). Cecum MRT_{Cr} increased linearly as BW increased for females ($P = 0.02$) and increased at an increasing rate as BW increased for intact males ($P = 0.01$). Cecum MRT_{Cr} remained similar as BW increased for castrated males ($P \geq 0.42$; Table 4).

Table 4. Wet tissues, pool size (wet digesta and composition), and digesta mean retention time(MRT) of indigestible NDF (iNDF) ($\mathrm{MRT_{iNDF}}$) and MRT of Cr ($\mathrm{MRT_{Cr}}$) of intestine segments and total GIT of castrated males (C), females (F), and intact males (I) Saanen goats slaughtered at five different BW

Item	BW (kg) and sex															SEM	*P*-Value[1]			
	15			22			30			37			45				Sex[2]	$\mathrm{BW_L}$	$\mathrm{BW_Q}$	Sex*BW[3]
	C	F	I	C	F	I	C	F	I	C	F	I	C	F	I					
Small intestine																				
Wet tissues, g	533.4	467.4	463.2	707.9	629.7	618.8	731.5	665.8	696.5	772.8	605.6	773.7	661.0	626.1	721.5	67.66	0.01	<0.01	<0.01	0.40
Wet pool size, g	280.2	291.4	336.3	333.0	320.1	332.2	449.2	372.9	470.0	503.0	704.0	499.4	462.3	391.0	499.7	61.33	0.78	0.01	0.01	0.09
DM, % wet pool size	9.9	9.5	10.1	8.8	9.7	9.5	9.3	9.8	9.0	10.2	9.3	9.8	10.8	10.2	9.2	0.61	0.74	0.67	0.12	0.49
NDF, % DM	27.5ab	20.5b	29.8a	21.5	23.8	23.6	22.0	22.8	24.9	20.9	20.9	20.4	21.8	18.1	16.5	1.81	-	-	-	0.02
																	C	0.04	0.07	-
																	F	0.21	0.06	-
																	I	<0.01	0.85	-
$\mathrm{MRT_{Cr}}$, h	1.3	1.2	1.1	1.0	1.0	1.1	1.3	1.0	1.4	1.0	1.0	0.9	1.1	1.2	1.1	0.19	0.69	0.80	0.49	0.76
Cecum																				
Wet tissues, g	24.2	27.0	25.8	33.2	35.1	34.1	34.4	36.5	37.5	47.6	36.5	40.4	39.9	45.9	48.2	2.63	0.71	<0.01	0.18	0.10
Wet pool size, g	98.6	144.0	121.1	179.6	137.2	108.1	174.3	168.5	167.0	217.0	199.8	197.2	223.9	245.8	268.6	25.75	0.87	0.01	0.34	0.29
DM, % wet pool size	15.1	13.1	14.9	12.9	13.2	13.3	13.5	13.2	12.6	12.9	14.6	15.2	13.9	13.3	14.5	0.79	0.25	0.93	0.03	0.10
NDF, % DM	45.7	42.6	44.8	44.1	44.7	45.3	45.3	42.8	45.2	35.7	41.1	39.7	40.1	43.0	39.1	2.44	0.71	0.01	0.61	0.09
iNDF:NDF ratio	0.57	0.54	0.55	0.65	0.59	0.58	0.67	0.66	0.62	0.74	0.58	0.66	0.60	0.64	0.68	0.047	0.32	0.01	0.09	0.63
$\mathrm{MRT_{iNDF}}$, h	1.3	2.2	1.8	2.4	1.4	1.3	1.7	1.4	1.2	1.7	1.9	1.6	1.6	2.6	2.4	0.33	0.54	0.67	0.02	0.06
$\mathrm{MRT_{Cr}}$, h	1.3	1.5	1.6	1.7	1.4	0.9	1.2	1.6	1.2	1.2	2.2	1.5	1.2	2.3	1.9	0.40	-	-	-	0.02
																	C	0.42	0.92	-
																	F	0.02	0.35	-
																	I	0.16	0.01	-
Colon-rectum																				
Wet tissues, g	204.9	234.0	215.6	313.3	284.4	304.8	330.2	345.2	331.4	478.9	313.4	415.9	414.5	351.8	406.6	29.72	0.08	0.56	0.01	0.12
Wet pool size, g	231.0	276.1	253.8	416.7	315.7	437.3	467.4	444.6	470.3	539.6	564.0	552.5	534.6	529.9	545.0	61.92	0.76	0.02	0.01	0.94
DM, % wet pool size	19.6	19.0	21.7	16.8	17.4	18.7	21.8	21.4	18.0	22.2	27.1	21.9	22.2	25.0	25.3	1.37	-	-	-	0.04
																	C	0.01	0.95	-
																	F	<0.01	0.97	-
																	I	0.02	<0.01	-
NDF, % DM	45.3	43.7	45.5	46.3	44.2	44.9	45.7	43.3	47.7	42.3	43.8	43.3	44.2	42.2	40.0	4.89	0.48	0.22	0.16	0.61
iNDF:NDF ratio	0.57	0.60	0.53	0.65	0.59	0.68	0.63	0.66	0.62	0.61	0.70	0.81	0.65	0.64	0.59	0.041	0.69	0.04	<0.01	0.08
$\mathrm{MRT_{iNDF}}$, h	5.3	8.4	6.3	8.8	4.5	9.7	7.2	7.3	6.3	4.4	10.2	6.5	7.0	8.7	7.0	1.21	-	-	-	0.01
																	C	0.82	0.55	-
																	F	0.11	0.33	-
																	I	0.62	0.70	-
$\mathrm{MRT_{Cr}}$, h	5.4	5.4	5.1	6.0	4.5	5.3	6.4	6.9	4.9	6.1	7.6	3.9	7.0	7.4	4.5	1.49	<0.01	0.33	0.95	0.22

Table 4. Continued

Item	BW (kg) and sex															SEM	*P*-Value[1]			
	15			22			30			37			45				Sex[2]	BW_L	BW_Q	Sex*BW[3]
Total GIT																				
Wet tissues, g	1344.7	1304.4	1286.4	1798.7	1697.3	1718.9	2006.0	1887.7	1962.9	2430.1	1932.2	2235.2	2171.8	2099.0	2205.1	92.40	0.02	<0.01	<0.01	0.47
Wet pool size, g	3510.7	3602.3	3462.0	4897.1	4724.2	4813.1	5933.3	5070.2	5977.5	6978.0	6203.6	6316.3	6271.5	5738.5	6451.3	326.70	0.09	<0.01	<0.01	0.61
DM, % wet pool size	15.6	13.6	14.9	14.2	13.3	13.7	14.5	14.9	13.8	17.0	15.7	16.5	18.8	17.0	16.3	1.45	0.06	<0.01	<0.01	0.62
NDF, % DM	45.6	47.4	46.3	46.1	50.5	45.8	47.5	46.7	48.7	44.5	43.1	47.1	44.9	44.8	47.1	3.83	0.35	0.41	0.29	0.21
MRT_{Cr}[5], h	14.3	14.4	14.8	17.3	12.6	13.2	17.7	17.0	14.9	16.6	15.4	12.1	18.1	13.6	14.1	3.09	<0.01	0.69	0.45	0.47

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

[1] Main effects and interaction sex × BW (Sex × BW). BW_L = linear effect of BW; BW_Q = quadratic effect of BW.

[2] Small intestine wet tissues were greater for castrated males than females (681 vs. 599 g; $P \leq 0.04$). Small intestine wet tissues were similar between castrated males and intact males (668 g) and between females and intact males (627 g; $P \geq 0.09$). Colon-rectum MRT_{Cr} was lower for intact males than females and castrated males (4.76 vs. 6.37 and 6.17 h; $P \leq 0.02$). Females and castrated males had similar colon-rectum MRT_{Cr} ($P = 0.91$). Total GIT wet tissues were greater for castrated males than females (1950 vs 1784 g; P = 0.01). Total GIT wet tissues were similar between females and intact males (1833 g; $P = 0.24$) and between castrated males and intact males (1916 g; $P = 0.47$). Total GIT MRT_{Cr} was similar between castrated males and females (15.7 h; $P = 0.11$) and between females and intact males (14.2 h; $P = 0.76$).

[3] When the interaction between sex and BW was significant the effect of BW was evaluated within each sex.

[4] Total GIT MRT_{Cr}= Sum of MRT_{Cr} of reticulorumen, omasum, abomasum, small intestine, cecum, and colon-rectum tissues

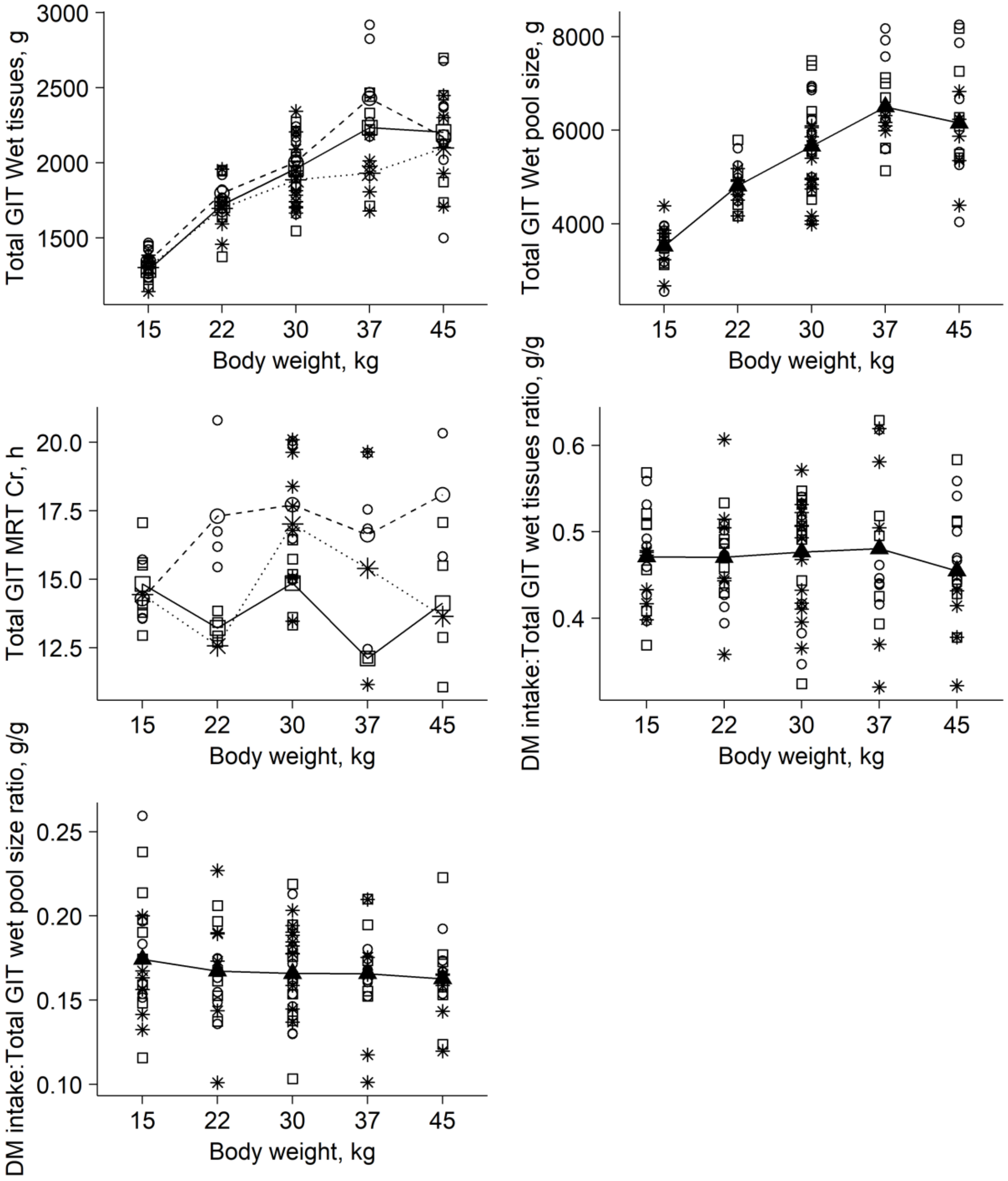

Figure 4. Total gastrointestinal tract (GIT) wet tissues were greater for castrated males than females (1950 vs 1784 g; $P = 0.01$). Total GIT wet tissues were similar between females and intact males (1833 g; $P = 0.24$) and between castrated males and intact males (1916 g; $P = 0.47$). Total GIT digesta mean retention time (MRT) of solutes (MRT_{Cr}) was similar between castrated males and females (15.7 h; $P = 0.11$) and between females and intact males (14.2 h; $P = 0.76$). Total GIT MRT_{Cr} was greater to castrated males than intact males (16.8 vs. 13.8 h; $P < 0.01$). Sex and body weight did

not affect the ratio between DM intake and total GIT wet tissues (DM intake:Total GIT wet tissues ratio) and the ratio between DM intake and total GIT wet pool size (DM intake:Total GIT wet pool size ratio) ($P \geq 0.30$). Body weight quadratically affected total GIT wet tissues and wet pool size ($P < 0.01$). Body weight did not affect total GIT MRT_{Cr} ($P \geq 0.45$). The symbol ○ and dashed line represent castrated males, ∗ and dotted line represent females, □ and solid line represents intact males. The symbol ▲ and solid line represent all sexes mean.

In the colon-rectum, sex only affected MRT_{Cr} ($P < 0.01$; Table 4). The lowest colon-rectum MRT_{Cr} was observed for intact males (4.76 h; $P \leq 0.02$). Females and castrated males had similar colon-rectum MRT_{Cr} ($P = 0.91$; Table 4). Colon-rectum wet tissues, wet pool size, and iNDF:NDF ratio increased at a decreasing rate as BW increased ($P \leq 0.01$; Table 4). Colon-rectum DM content increased linearly as BW increased in castrated males and females ($P \leq 0.01$) and increased at an increasing rate as BW increased in intact males ($P < 0.01$). Colon-rectum MRT_{iNDF} and MRT_{Cr} were not affected by BW growth ($P \geq 0.11$; Table 4).

Total GIT wet tissues were greater for castrated males than females (1950 vs 1784 g; $P = 0.01$; Table 4; Figure 4). Total GIT wet tissues were similar between females and intact males (1833 g; $P = 0.24$) and between castrated males and intact males (1916 g; $P = 0.47$; Table 4; Figure 4). Total GIT MRT_{Cr} was similar between castrated males and females (15.7 h; $P = 0.11$) and between females and intact males (14.2 h; $P = 0.76$). Sex and BW did not affect the ratio between DM intake and total GIT wet tissues (DM intake:Total GIT wet tissues ratio) and the ratio between DM intake and total GIT wet pool size (DM intake:Total GIT wet pool size ratio) ($P \geq 0.30$; Figure 4). Total GIT wet tissues and wet pool size increased at a decreasing rate as BW increased ($P < 0.01$). Total GIT DM content increased at an increasing rate as BW increased ($P < 0.01$). Total GIT MRT_{Cr} was greater to castrated males than intact males (16.8 vs. 13.8 h; $P < 0.01$; Table 4). Total GIT MRT_{Cr} was not affected by BW ($P \geq 0.45$; Table 4).

DISCUSSION

Sex effect on MRT

The effect of sex on MRT_{iNDF} and MRT_{Cr} in total GIT and GIT segments was tested in goats. Sex did not affect MRT_{iNDF} in any GIT segments. However, sex affected MRT_{Cr} in reticulorumen, abomasum, and total GIT.

Previous study indicated that MRT can be modulated by feed intake and GIT capacity, in which whether intake increases and GIT capacity remains constant MRT decreases, or whether GIT capacity increases and intake remains constant MRT increases (Clauss et al., 2007a). Our similar results on reticulorumen MRT_{iNDF} between sexes do not fully support this, since castrated males showed greater DM intake than females and similar reticulorumen capacity, thus one would expect a lower reticulorumen MRT_{iNDF} in castrated males. On the other hand, the DM intake:RR wet pool size ratio was similar among sexes, that agrees with the absence of effect of sex on reticulorumen MRT_{iNDF}. Our findings indicate that MRT_{iNDF} is modulated by intake and capacity (Clauss et al., 2007a). Composition of ingested diet was not consistently different among sex in our study, differences between sexes were observed only at 15 and 45 kg BW. Thus, this suggests composition of ingested diet may have contributed to the absence effect of sex on reticulorumen MRT_{iNDF}.

Females and intact males had similar reticulorumen MRT_{Cr} and lower than castrated males. Therefore, the assumption made by Clauss et al. (2007a) also does not fully support that reticulorumen MRT_{Cr} is modulated by intake and capacity. This demonstrates that other factors are affecting reticulorumen MRT_{Cr}. Saliva secretion has been shown negatively related to reticulorumen MRT_{Cr} (Seo et al., 2007). Gross et al. (1995a; 1995b) found that females Nubian ibexes (*Capra ibex nubiana*) had lower reticulorumen MRT_{Cr} than males, because females were around 50% lesser efficient chewers than males, and chewing stimulates saliva secretion. On the other hand, we do not think that chewing efficiency in females was the unique reason for the differences on reticulorumen MRT_{Cr} among sexes in our study. Our goats had the opportunity of diet searching and they were fed with diet that did not challenge the goats for having high chewing investment. Thus, sex effect on reticulorumen MRT_{Cr} is not clearly understood and must be further investigated with diets with high fiber content, that would require great chewing investment, and with feeding levels that would not allow diet searching.

Omasum is one of the GTI segments responsible by reducing digesta moisture (Holtenius and Björnhag, 1989). The greatest $\mathrm{MRT_{Cr}}$ observed in reticulorumen of castrated males was not observed in omasum $\mathrm{MRT_{Cr}}$, that would indicate that omasum fulfilled its function on reducing digesta moisture. Abomasum also demonstrated greater $\mathrm{MRT_{Cr}}$ for castrated males than females and intact males, however, in this case, it must be related to greater abomasum capacity in castrated males. Moreover, small intestine was not affected by great $\mathrm{MRT_{Cr}}$ in the abomasum of castrated males, as demonstrated by the absence effect of sex on small intestine wet pool size and $\mathrm{MRT_{Cr}}$. On the other hand, even though sex did not affect cecum capacity and $\mathrm{MRT_{Cr}}$, castrated males and females had great $\mathrm{MRT_{Cr}}$ in colon-rectum than intact males. Thus, our data did not show a clear pattern on the effect of sex on wet tissues, wet pool size, and $\mathrm{MRT_{Cr}}$ of GTI segments, and further studies may have to elucidate how sex would affect MRT in GTI segments.

As observed in the GTI sections, total GIT $\mathrm{MRT_{Cr}}$ was also different among sexes. This does not fully agree with the assumption that GIT $\mathrm{MRT_{Cr}}$ is modulated by feed intake and GIT capacity, because sex did not affect DM intake:Total GIT wet tissues ratio and DM intake:Total GIT wet pool size ratio. On the other hand, total GIT represents the sum of all GIT sections, and the differences among GIT segments have to be considered. The greater castrated males $\mathrm{MRT_{Cr}}$ in reticulorumen, abomasum, and colon-rectum than intact males were also observed in total GIT. However, females, that presented lower $\mathrm{MRT_{Cr}}$ in reticulorumen and abomasum than castrated males, presented total GIT $\mathrm{MRT_{Cr}}$ similar to castrated males. This similarity between females and castrated males total GIT $\mathrm{MRT_{Cr}}$ was also observed in colon-rectum, that is associated with water absorption (Clauss et al., 2016a). This suggests females had greater water absorption in colon-rectum section even though the absence effect of sex on colon-rectum wet tissues. Therefore, our results suggest females may have naturally greater water absorption capacity in colon-rectum than males as a mechanism to compensate great chewing investment and saliva secretion and low $\mathrm{MRT_{Cr}}$ and consequently avoid great fecal water losses (Gross et al., 1995b; a).

Moreover, our results also suggest that differences of sex on water absorption among GIT segments affect total GIT MRT_{Cr}.

BW effect on MRT

The effect of BW on MRT_{iNDF} and MRT_{Cr} in total GIT and GIT segments was tested in goats. Body Weight affected only cecum MRT_{iNDF}. However, BW affected MRT_{Cr} in reticulorumen, omasum, abomasum, and cecum of females and intact males.

Reticulorumen wet tissues and wet pool size increased similar to DM intake as BW increased (i.e., increased at a decreasing rate). On the other hand, the DM intake:RR wet pool size ratio was not affected by BW, while the DM intake:RR wet tissues ratio was great in goats slaughtered at 15 kg BW and linearly decreased as BW increased. This demonstrates the distention capacity of reticulorumen (Clauss et al., 2016b) and supports the assumptions that MRT_{iNDF} is modulated by intake and capacity (Clauss et al., 2007a). Moreover, the reticulorumen MRT of particles has been related to the composition of ingested diet (Seo et al., 2009). Composition of ingested diet affects changes on functional specific gravity of particles (FSH) (Seo et al., 2009) because of its relationship to rate of fermentation and hydration of the particles (Hooper and Welch, 1985). However, our results do not support this, because BW affected the composition of ingested diet.

Additionally, reticulorumen MRT_{Cr} was lower at young goats (i.e., 15 kg BW), and linearly increased as BW increased. This indicates that other factors than intake and capacity (Clauss et al., 2007a) must also be considered on reticulorumen MRT of solutes. Reticulorumen MRT of solutes has been positively related to frequency and duration of reticulo-omasal orifice opening and the amount of solutes in the reticulorumen, that comes mainly from water intake and saliva secretion (Seo et al., 2007). Water intake was not recorded in the studies of (Leite et al., 2015a; b) and (Silva, 2013). However, our results demonstrated that young goats, slaughtered at 15 kg BW, had low DM content in reticulorumen and it increased at an increasing rate as BW increased. Thus, this suggests great input of water in the reticulorumen of young goats that would come from saliva secretion (Seo et al., 2007). The major factor for secretory responses of salivary glands is chewing movements (Bartley, 1976), that is positively related to feed intake level (Galvani et al., 2010; Grimaud et al., 2010) and negatively related to BW (i.e., young ruminants are lesser efficient chewers than old ruminants, spending more time chewing per kilogram of ingested diet; Bae and Welch, 1983; Grandl et al., 2016, 2017). Moreover, frequency and duration of reticulo-omasal orifice opening depend on duration, amplitude, and frequency of primary reticular contractions, that extremally depends on intake level (Okine and Mathison, 1991; Seo et al., 2007). Our results demonstrated intake level (DM and L) was great at young goats, slaughtered at 15 kg BW, and decreased as BW increased. Thus, our results suggest, reticulorumen MRT of solutes is linearly and positively related to BW growth because frequency and duration of reticulo-omasal orifice opening and saliva secretion probably were great in young goats and decreased with aging.

Reticulorumen and omasum are related segments and it was very evident in our results. Wet tissues and wet pool size of reticulorumen and omasum increased similarly as BW increase. However, different than that observed for reticulorumen, BW did not affect omasum DM content. Water absorption is the main function of omasum (Holtenius and Björnhag, 1989). Thus, this demonstrated omasum absorbed solutes from the digesta that escape from reticulorumen. The omasum absorption in our study was around 12%, smaller than the absorption found by Holtenius and Björnhag (1989), that was around 18%. On the other hand, omasum MRT_{Cr} linearly increased as BW increased. Thus, even though omasum reduced the moisture content of digesta that escape from reticulorumen, the low MRT_{Cr} observed in reticulorumen of young goats and increase as BW increased was also observed in omasum and abomasum. Additionally, in the past omasum was not accounted in total NDF digestibility (Holtenius and Björnhag, 1989), however more recent studies have suggested that the omasum plays a role on fiber digestion, that may contribute to around 7% of total NDF digestibility in dairy cows (Ahvenjärvi et al., 2000, 2001). Our results demonstrated that NDF content decreased around 19% from reticulorumen to omasum. Thus, this may indicate NDF digestion in the omasum of growing goats.

Abomasum is one of the first GIT segment to reach the maximum growth rate, that is around 15 days of life in goats (Andrade et al., 2020). This happens because abomasum is responsible by enzymatic digestion during the suckling phase. Its importance in feed digestion begins to decrease at the weaning phase, that started for our goats when they were around 2 months old. According to Andrade et al. (2020), after goats start the transition period and eat solid feed diet more effectively they become functional ruminants in 15 days, that is the period to reticulorumen reaches the maximum growth rate. Our goats started at the experiment when they were around 3 months old, 30 days after the beginning of solid feed diet, and 15 days after they become functional ruminants. The abomasum wet tissues are able to distend in young ruminants and the abomasum capacity may increase without changes on wet tissues size (Ortigues and Doreau, 1995). However, the abomasum distention ability decreases with aging by the increase on tissues thickness (Ortigues and Doreau, 1995). Our results demonstrated young goats may had great digesta content in abomasum even though they did not have great wet tissues. The abomasum distention ability and great abomasum wet pool size in young goats and decrease as BW increase led to great abomasum MRT_{Cr} at 15 kg BW and linear decrease as BW increased even though omasum MRT_{Cr} was low at 15 kg BW and linearly decreased as BW increased. Thus, we suggest abomasum of young is able to distend to avoid the influence of omasum MRT_{Cr} on abomasum MRT_{Cr}.

Small intestine, similar to abomasum, has early development in goats, and it reaches the maximum growth rate when goats are around 15 days old (Andrade et al., 2020). The early development of small intestine is mainly explained by its function of enzymatic digestions, that is basically the main site for nutrient digestion during the suckling phase. Thus, because our goats were early waned, they started the experiment when their small intestine growth rate was decreasing, as demonstrated by the non-linear relationship between small intestines wet tissues and BW.

Small intestine is considered tubular segment and its flow has been considered laminar (Ellis et al., 1994; Faichney, 2005). However, based on iNDF:NDF ratio in small intestine, studies have questioned the general assumption of laminar flow in small intestine (Hristov et al., 2019). Our results demonstrated BW did not affect small intestine MRT_{Cr} even though abomasum MRT_{Cr} was greater in young goats (i.e., at 15 kg BW) and decreased as BW increased. The differences on MRT_{Cr} between abomasum and small intestine were possible because small intestine wet pool size increased as BW increased, indicating increase on digestion retention as BW increased and supporting the hypothesis that small intestine may not have laminar flow.

Additionally, small intestine MRT of solutes and particles have been demonstrated similar (Leite et al., 2015a; b). Thus, due to the absence of BW on small intestine MRT_{Cr}, we suggest BW did not affect small intestine MRT_{iNDF}. Based on that and the similarity on the increase of cecum and small intestine wet pool size as BW increased, the absence of effect of BW on cecum MRT_{iNDF} would be expected. However, cecum MRT_{iNDF} increased as BW increased. Mean retention time of particles is positively related to NDF digestibility (Allen and Mertens, 1988), and cecum is known by digesting fiber. The iNDF:NDF ratio in cecum increased as BW increased while reticulorumen iNDF:NDF ratio did not. This suggests fiber digestion in the segments between reticulorumen and cecum increased a BW increased, and it would be related to the increase on MRT_{iNDF} as BW increased, as demonstrated in cecum. Thus, even though BW did not affect small intestine MRT_{Cr}, the results of MRT_{iNDF} and iNDF:NDF ratio in cecum suggest mixing of digesta and NDF digestion may occurred in small intestine, and small intestine MRT_{Cr} and MRT_{iNDF} were not similar.

Despite cecum MRT increased as BW increased and colon-rectum wet tissues and wet pool size increased as BW increased, BW did not affect colon-rectum MRT_{iNDF} and MRT_{Cr}. Colon and rectum, specially distal colon and rectum, are associated with water absorption (Clauss et al., 2016a). Thus, we would expect great colon-rectum DM content in young goats and decrease as BW increased as a way to reduce digesta volume in young goats and keep constant colon-rectum MRT. However, our data demonstrated colon-rectum DM content increased as BW increased, indicating decrease on fecal water losses as BW increased. Thus, our results indicate colon-rectum efficiency on water absorption is low in young goats and increase as BW increased, that demonstrates the importance of ad libitum drinking water, especially for young goats.

Despite BW affected MRT_{Cr} in reticulorumen, omasum, abomasum, and cecum of females and intact males, BW did not affect total GIT MRT_{Cr}. Dry matter intake, total GIT wet tissues and wet pool size demonstrated similar growth as BW increased. This was also demonstrated by the absence effect of BW on the DM intake:Total GIT wet tissues ratio and the DM intake:Total GIT wet pool size ratio. This demonstrates that despite the differences on GIT segments capacity, compensations on MRT_{Cr} occur among the GIT segments. Therefore, in general terms our results support that for total GIT of growing goats the MRT_{Cr} is modulated by feed intake and GIT capacity.

CONCLUSIONS

Sex did not affect MRT_{iNDF} in any evaluated GIT segments and affected MRT_{Cr} in reticulorumen, abomasum, and total GIT. However, the mechanism related to sex effect on MRT must be elucidated. Body weight was positively related to reticulorumen MRT_{Cr}, but it was not related to reticulorumen MRT_{iNDF} and total GIT MRT_{Cr} in growing Saanen goats. Reticulorumen MRT_{iNDF} and total GIT MRT_{Cr} were modulated by intake and capacity of reticulorumen and GIT, respectively. On the other hand, reticulorumen MRT_{Cr} seemed to be regulated by reticulo-omasal orifice opening and saliva secretion.

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CHAPERT 3 - Inferring goats' growth, feeding level, and sex effects on latent variables underlying gastrointestinal tract digesta passage rate

ABSTRACT: Digesta passage rate (k_p) in ruminants is a very dynamic and complex process and is related to many other processes, such as digestion, methane emission, and microbial growth. Thus, the objectives of this study were to identify variables related to k_p of gastrointestinal tract (GIT) segments using multivariate statistical techniques and access the important source of variation (i.e., goats' growth, sex, and feeding level) using a meta-analytical approach. We used a database from two studies with Saanen goats that was split into two different data sets: database Growth, composed by individual records of castrated males (n = 36), females (n = 34), and intact males (n = 33) fed ad libitum and slaughtered at 15, 22, 30, 37, and 45 kg BW; and, database Feeding Level, composed by individual records of castrated males (n = 38), females (n = 33), and intact males (n = 36) fed ad libitum (26.82 ± 2.54 g/kg body weight (BW)), 75 (22.90 ± 1.99 g/kg BW) or 50% (20.44 ± 2.05 g/kg BW) of ad libitum over 109 ± 10 days. The multivariate factor analysis (MFA) was performed with each of these databases and then analysis of variance was performed to estimate the sources of variation related to each of the latent factors extracted with MFA from the databases. The four orthogonal latent factors, extracted from each database, explained around 60% of the original variability of each database while at the same time drastically reduced the data space into variables that explained changes on GIT k_p in growing goats or goats fed with feeding levels over long-term. Our results demonstrated that the digesta passage rate has different responses whether evaluated in growing goats or goats fed with feeding levels over long-term. Growing goats had k_p of solutes in reticulorumen, omasum, and colon-rectum positively related to DM intake level and chewing investment, and all of them were negatively related to GIT sections capacity and growth. Moreover, DM intake level, reticulorumen k_p of large particles, and large intestine k_p of solutes and particles were all positively related in growing goats. Goats fed with feeding levels over long-term had k_p of solutes in all GIT segments positively related to feeding level and capacity, and all of them were negatively related to chewing investment. Reticulorumen k_p of particles was not associated with any of the selected factors on the Feeding Level database, suggesting that the reticulorumen k_p of particles was constant in goats fed with feeding levels over long-term. Total NDF digestively was mainly related to ingested diet composition other than k_p. In conclusion, w our analysis suggest that feed intake level is the driving force of GIT k_p of solutes in growing goats and goats fed with feeding level over long-term, and capacity negatively affected k_p only in growing goats. Sex effect on GIT k_p seemed to depend on chewing investment, however, further investigation is needed to fully understand its effects on digesta passage rate.

Key words: passage rate, feed intake, pool size, rumen, Saanen

INTRODUCTION

Digesta passage rate (k_p) in ruminants is a dynamic and complex process that affect many other processes, such as digestion, methane emission, and microbial growth (Okine et al., 1998; Goopy et al., 2014; Clauss and Hummel, 2017). Many factors have been described to affect gastrointestinal tract (GIT) k_p, such as body weight (BW), intake level, sex, and diet. Body weight is related to GIT k_p because of its relationship with GIT capacity and feed intake (Müller et al., 2013) which in turn are driving forces of k_p (Clauss et al., 2007a). Capacity is partially limited by tissues size, especially in intestines (Ortigues and Doreau, 1995), that grows differently with ageing and sex (Andrade et al., 2020). Moreover, feed intake level is related to BW because both of them are related to maintenance requirements (Almeida et al., 2019) which differs according to sex (Souza et al., 2017, 2020). Feed intake also has been related to chewing investment, that in turn affects saliva secretion, reticulorumen contractions, reticulo-omasal orifice opening, and especially k_p of solutes (Seo et al., 2007, 2009). However, in reticulorumen, feed intake has not been related to k_p in sheep fed with feeding levels (Munn et al., 2015; Clauss et al., 2016a). Moreover, reticulorumen k_p of particles has been mainly related to ingested diet composition and k_p of solutes other than particles size and intake, because of its effect on particles' functional specific gravity (Seo et al., 2009). Moreover, the k_p in the GIT segments are inter-related compensations between sections on diet digestibility may also exist (Leite et al., 2015a).

In this context, the k_p is a complex process and not well understood in growing goats or goats fed with feeding levels over long-term. For a better understanding of complex processes, the large number of variables related to the process can be reduced to fewer variables, potentially explained through linear functions (Dadousis et al., 2018) that describe the biological concepts of interest. Multivariate factor analysis belongs to the framework of useful techniques for data dimension reduction based on linear functions. Moreover, these linear functions may be misleading when the data originate from different studies because the observations within a study have more in common than observations across studies (St-Pierre, 2001; Sauvant et al., 2008). Thus, these differences across studies must not be ignored in the multivariate meta-analytical analysis.

Thus, the objectives of this study were i) to identify variables related to k_p of GIT segments utilizing multivariate statistical techniques and ii) to understand the sources of variation (i.e. goats' growth, sex, and feeding level) underlying the multivariate analysis using a meta-analytical approach.

MATERIAL AND METHODS

Dataset

All procedures used across studies were reviewed by the University's Animal Care Committee (Comissão de Ética e Bem-Estar Animal, CEBEA; Universidade Estadual Paulista, Jaboticabal, Brazil).

We used two databases with records of castrated males, females, and intact males Saanen goats from three studies (Silva, 2013; Leite et al., 2015a; b). Database Growth was composed by individual records of castrated males (n = 36), females (n = 34), and intact males (n = 33) slaughtered at 15, 22, 30, 37, and 45 kg BW. Database Feeding Level was composed by individual records of castrated males (n = 38), females (n = 33), and intact males (n = 36) that were, within study and sex, randomly distributed in blocks of three goats, according to the feeding level (fed ad libitum (26.82 ± 2.54 g/kg BW), 75 (22.90 ± 1.99 g/kg BW) or 50% (20.44 ± 2.05 g/kg BW) of ad libitum). On average, studies lasted 109 ± 10 days. Goats fed with 75 or 50 % of ad libitum were slaughtered when goats fed ad libitum within the same block reached the target final BW (30 or 45 kg BW). Goats were fed with similar diets that were formulated to meet the daily requirements of goat kids (Table 1). The studies adopted similar procedures and chemical analyses (Gindri et al., 2020). The intake of DM (DMI), OM (OMI), NDF (NDFI), indigestible NDF (iNDF) (iNDFI), and potentially degradable NDF (pdNDFI) were calculated by subtracting orts from the offered diet. We considered for estimating k_p the intake during the 5-d period before slaughter day. During the same experimental period, Cr-EDTA was administrated to determine the k_p of solutes. The iNDF was used to determine the k_p of large particles. The details about preparation, administration, and analysis of both markers were previously published (Leite et al., 2015a; b).

Table 1. Ingredients and chemical composition (on a DM basis) of the experimental diets

Item	(Leite et al., 2015a; b)	(Silva, 2013)
Dietary ingredient (% DM)		
Dehydrated corn plant[1]	45	45
Cracked corn grain	27	31
Soybean meal	22	15
Soybean oil	1.6	2.5
Limestone	1.0	1.3
Mineral supplement[2]	2.2	6.0
Ammonium chloride	0.90	0.0
Diet chemical composition[3] (% DM ± SD)		
DM (%)	85 ± 1.09	87 ± 0.31
OM	95 ± 0.20	90 ± 0.33
CP	20 ± 0.54	15 ± 0.66
Crude fat	8.0 ± 0.49	5.1 ± 0.08
NDF	36 ± 2.5	31 ± 0.75
iNDF[4]	11 ± 1.05	11 ± 0.90
Lignin	5.7 ± 0.34	n.a.

[1] 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).

[2] Composition, per kg, as-fed basis: 190 g of Ca; 92 g of Cl; 73 g of P; 62 g of Na; 44 g of Mg; 1.35 g of Zn; 1.06 g of Fe; 0.94 mg of Mn; 0.73 g of F; 0.34 g of Cu; 18 mg of Se; 16 mg of I; 3 mg of Co.

[3] Mean and standard deviation (SD) of 10 composite samples. The chemical composition of the diet was calculated from the individual ingredients.

[4]iNDF= Indigestible NDF.

After slaughter (2.2 ± 0.8 h after morning feeding), GIT was removed and separated into reticulorumen, omasum, abomasum, small intestine, cecum, and colon-rectum, and reticulorumen pH was recorded. The segments were weighed before and after emptying to determine the pool size of wet digesta and wet weight of each segment tissues. In the digesta of each segment were determined the DM, OM, NDF, iNDF, and Cr concentration.

The k_p of solutes and k_p of particles in different GIT segments were determined by the flux/compartmental pool method using the Eq. [1] (Ellis et al., 1994):

k_p of indigestible entity (IE) = intake rate of IE/compartmental mass of IE, [2]

where k_p is the fractional rate of IE escape per hour, intake rate of IE is expressed in grams per hour, and compartmental mass in the segment expressed in grams.

The reticulorumen digesta particle size was determined using Endecotts sieve shaker, model wet sieving EFL 2000/1 (Endecotts Limited, Lombard Road, London). Reticulorumen wet digesta samples (60 g) were sieved by 10 minutes with water flow of 1.5 l per minute (Silva, 2013). The material retained in each sieve was dried at 60°C for 48 hours. The particle size retained in each sieve was taken as the percentage of the initial sample corrected to DM. The proportion of particles smaller than 0.15 mm was calculated by the difference between the sum of particles bigger than 0.15 mm and the initial sample corrected to DM.

The total digestibility of NDF (NDFD) was calculated using the ratio between iNDF and NDF contents (iNDF:NDF) of ingested diet and digesta in colon-rectum segment (Eq. [2]) (Leite et al., 2015b):

NDFD = {1 – [(iNDF:NDF of ingested diet)/ (iNDF:NDF of digesta in colon-rectum segment)]} [2]

Chewing investment (chews/g DMI) was estimated by the empirical model (Eq. [3]; n = 101; RMSPE = 1.34 chews/g DMI; $R^2 = 0.55$):

$\text{Chews/g DMI} = (66.4 \times 1.96^{\pm 1}) \times \text{NDFI}^{-1.094 \pm 0.107} \times \text{pdNDFI}^{0.93 \pm 0.110}$ [3]

where NDFI is NDF intake expressed as g/day and pdNDFI is the potentially digestible NDF intake expressed as g/kg BW. The equation was developed through a stepwise selection procedure and the model with the lowest AICc was chosen. The data used for this was from goats of database Feeding Level, when goats fed ad libitum were 22.4 ± 1.31 kg BW and 38.9 ± 1.45 kg BW in study Leite et al. (2015a) and study Silva (2013), respectively.

Statistical Analyses

Adjust for random effect

The data used in this paper is from three similar studies, however, each study has its peculiarities. Thus, the variance of the variables of each database associated with study and block was removed before performing the multivariate analyses. For database Growth, the variance accounted by study was computed using the mixed model (Eq. [4]):

$$Y_{ijkl} = \mu + S_i + W_j + S_i \times W_j + T_k + e_{ijkl} \quad [4]$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of sex i, W_j is the fixed effect of BW j, $S_i \times W_j$ is the interaction between sex i and BW j, $T_k \sim iidN(0, \sigma_T^2)$ is the random effect of study k, and $e_{ijkl} \sim iidN(0, \sigma_e^2)$ is the random residual error.

For database Feeding Level, the variance accounted by study and block were computed using the mixed model (Eq. [5]):

$$Y_{injk} = \mu + S_i + B_{n(i*k)} + F_j + S_i \times F_j + T_k + e_{injk} \quad [5]$$

where Y_{injk} is the dependent variable, μ is the overall mean, S_i is the fixed effect of sex i, $B_{n(i*s)} \sim iidN(0, \sigma_B^2)$ is the random effect of block n built according to sex and initial BW and nested in the interaction between sex i and study k, F_j is the fixed effect of feeding level j, $S_i \times F_j$ is the fixed effect of the interaction between sex i and feeding level j, $T_k \sim iidN(0, \sigma_T^2)$ is the random effect of study, and $e_{injk} \sim iidN(0, \sigma_e^2)$ is the residual random error.

Therefore, for each of those databases, the adjusted database was assembled by computing the observed value as the sum of the predicted value (predictions from the fixed effect part of the model only) and the calculated error for each observation. From database Feeding Level, the variance accounted by block was also removed because block is considered random effect and is not reproducible. The mixed models were fitted using lmer function of lme4 R package (Bates et al., 2015). These models were also used for identifying outliers. Outliers were removed when their studentized residuals were >|3|.

Missing values imputation

Results from a data set with missing data may be misleading if missing observations were not randomly produced (Little and Rubin, 2002). The iterative PCA method (Kiers, 1997) was used to impute missing values at this study. MissMDA R package (Josse and Husson, 2016), whose contain imputePCA function, was used to perform the iterative PCA method. The number of dimensions that should be used at the beginning of the iterative PCA algorithm is an issue about this technique. However, Josse and Husson (2012) suggested the method based on cross-validation to estimate the initial number of dimension. This method is available in the missMDA R package as estim_ncpPCA function and was used in this study.

Factor (Fa) analysis

The multivariate Fa analysis (MFA) was equally performed with each of those adjusted and imputed databases. However, the variable BW was removed from database Growth and variable DM intake level was removed from database feeding level because they were the direct generating source of variation of database Growth and database Feeding Level, respectively (Table 2). The psych R package (Revelle, 2018) was used for all steps of Fa analysis. In the first step, a correlation matrix was computed using the corr.test function and the correlation coefficients were recorded. We established the linear relationships among the variables using the correlation matrix because our data set was composed of variables that have a different scale(i.e., kg, g/kg, %, g, /h). In the second step, Bartlett’s test and the Kaiser–Meyer–Olkin (KMO) test were applied on the correlation matrix to assess the database adequacy to MFA (i.e., identify if there was a latent structure of dependency among the variables of our databases). In the third step, a scree plot was built and parallel analysis was computed with the correlation matrix using fa.parallel function (Figure 1). The scree plot was used to determine the number of Fa to retain in MFA. In the parallel analysis, the eigenvalues derived from the original data were compared to eigenvalues derived from a random data with the same size of the original data, and the ideal number of principal components were retained as long as the i^{th} eigenvalue from the original data was greater than the i^{th} eigenvalue from the random data. However, the biological interpretation of each Fa was also used to select the Fa. Finally, the MFA was performed using the fa function with the correlation matrix using maximum likelihood as the factoring method. Varimax rotation was used to redistribute the variance retained by the Fa and improve their interpretation. Varimax rotation improved the interpretation of the Fa because ii orthogonally transforms the eigenvectors making them as large as possible. Moreover, to improve the structure of dependency among the variables and the interpretation of the Fa, after MFA performed, variables with loadings < |0.3| were removed from the databases and then MFA was performed again. To interpret the Fa, variables with loadings > |0.3| were considered linked to the Fa.

Sources of variation

To estimate the sources of variation related to the Fa, analyses of variance were performed using lm and Anova functions of stats and car packages of software R (Fox and Weisberg, 2010). Orthogonal polynomial contrasts were used to determine the effects of BW and feeding level using emmeans and contrast functions of emmeans package of software R (Lenth, 2019). In particular, the analysis of variance with the samples scores of database Growth was performed with the following model (Eq. [6]):

$$Y_{ijl} = \mu + S_i + W_j + S_i \times W_j + e_{ijl} \qquad [6]$$

where Y_{ijl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of sex i, W_j is the fixed effect of BW j, $S_i \times W_j$ is the interaction between sex i and BW j, and $e_{ijl} \sim iidN(0, \sigma_e^2)$ is the random residual error. The analysis of variance with the samples scores of the database Feeding Level was performed considering the following model (Eq. [7]):

$$Y_{ijn} = \mu + S_i + F_j + S_i \times F_j + e_{ijn} \qquad [7]$$

where Y_{inj} is the dependent variable, μ is the overall mean, S_i is the fixed effect of sex i, F_j is the fixed effect of feeding level j, $S_i \times F_k$ is the fixed effect of the interaction between sex i and feeding level j, and $e_{ijn} \sim iidN(0, \sigma_e^2)$ is the residual random error. Sex effect was tested by the Tukey's test in both databases. The significance was declared at $P \leq 0.05$.

RESULTS

Descriptive statistics of the database Growth and database Feeding Level are shown in Table 2.

Table 2. Summary of statistics related to digestive variables of castrated males, females, and intact males growing Saanen goats slaughtered at 15, 22, 30, 37, and 45 kg BW (database Growth) or fed with three feeding levels (fed ad libitum (26.82 ± 2.54 g/kg BW), 75 (22.90 ± 1.99 g/kg BW) or 50% (20.44 ± 2.05 g/kg BW) of ad libitum; Database Feeding Level) used in this study

Variable	Database Growth					Database Feeding Level				
	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
BW (kg)	103	30	9.7	14.8	47.4	107	32	7.8	17	47
DM intake level (g/kg BW)	103	31	7.5	14	48	107	23	6.3	12	38
OM (% DM intake)	103	93	0.7	92	95	107	93	0.65	92	94
NDF (% DM intake)	103	33	5.9	21	45	107	34	6.1	22	43
pdNDF (% DM intake)	103	23	6.5	10	37	107	23	5.8	11	31
Total NDF digestibility	93	0.49	0.145	0.069	0.87	104	0.51	0.104	0.17	0.70
Chewing investment (chews/g DM intake)	103	64	28	30	143	106	58	18	30	107
Reticulorumen										
wet tissues (g)	103	609	162.04	328	989	107	591	139.8	276	989
wet pool size (g)	103	3692	989.3	1650	6418	106	3974	929.3	2141	6418
DM (% wet pool size)	103	15	2.5	6.9	21	106	14	3.1	5.3	20
k_p^3 of Cr (/h)	96	0.23	0.285	0.052	2.4	100	0.13	0.0507	0.055	0.28
k_p of iNDF (/h)	103	0.026	0.00785	0.0075	0.049	106	0.025	0.00926	0.013	0.065
pH	102	5.7	0.30	5.01	6.5	104	5.9	0.34	5.1	7.0
PS^4 < 0.15 mm (% DM)	100	45	11.1	0.49	63	100	47	12.7	4.4	84
PS 0.15 <> 0.35 mm (% DM)	101	6.0	1.96	2.3	14	100	6.1	2.1	1.6	13
PS 0.35 <> 0.60 mm (% DM)	101	14	3.9	4.2	31	100	13	3.5	4.2	23
PS 0.60 <> 1.18 mm (% DM)	101	13	3.9	7.0	33	100	12	3.8	3.1	28
PS 1.18 mm <> 2.36 mm (% DM)	101	12	3.9	5.6	31	100	10	3.4	2.8	18
PS 2.36 mm <> 4.75 mm (% DM)	101	6.4	2.2	2.5	13	100	6.5	2.3	0.33	12
Omasum										
wet tissues (g)	103	78	28.2	31	148	107	83	23.2	40	148

Table 2. Continued

Variable	Database Growth					Database Feeding Level				
	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
wet pool size (g)	103	113	58.3	18	261	106	131	46.2	36	261
DM (% wet pool size)	102	24	3.7	14	35	105	24	3.5	13	38
k_p of Cr (/h)	83	6.7	4.053	1.6	19	94	4.2	2.3	1.1	12
Abomasum										
wet tissues (g)	103	158	61.01	80	352	107	151	48.6	73	282
wet pool size (g)	103	460	201.2	118	1209	106	388	163.9	87	867
DM (% wet pool size)	103	13	3.2	5.2	23	106	12	2.8	6.5	22
k_p of Cr (/h)	90	2.1	0.87	0.48	4.5	90	2.0	0.81	0.74	4.3
Small intestine										
wet tissues (g)	103	650	128.1	380	1005	107	599	123.3	379	1005
wet pool size (g)	102	412	145.0	154	921	105	378	139.8	48	727
DM (% wet pool size)	99	10	1.4	5.1	13	104	9.2	1.46	6.7	14
k_p of Cr (/h)	91	0.97	0.359	0.27	1.99	95	0.92	0.381	0.34	2.02
Cecum										
wet tissues (g)	103	36	9.30	20	66	107	35	8.4	15	60
wet pool size (g)	103	173	74.09	47	389	106	192	71.8	50	409
DM (% wet pool size)	101	14	1.74	10	19	104	13	1.6	10	16
k_p of Cr (/h)	91	0.86	0.441	0.25	2.2	96	0.65	0.353	0.18	1.8
k_p of iNDF (/h)	99	0.72	0.380	0.10	2.07	102	0.53	0.307	0.065	2.1
Colon-rectum										
wet tissues (g)	103	327	101.6	120	688	107	301	84.2	134	661
wet pool size (g)	103	434	160.8	75	900	106	447	132.3	189	900
DM (% wet pool size)	102	21	4.2	13	34	105	22	3.7	11	34
k_p of Cr (/h)	87	0.21	0.0720	0.073	0.39	94	0.16	0.0570	0.055	0.32
k_p of iNDF (/h)	94	0.17	0.0955	0.031	0.65	104	0.12	0.0562	0.052	0.36

[1] pdNDF = potentially digestible NDF

[2] iNDF = indigestible NDF.

[3] k_p = digesta passage rate.

[4] PS = particle size.

The Pearson correlation of the 40 variables analysed in MFA is presented in Figure 1 and Figure 2. Bartlett's test ($P < 0.01$) and KMO value (0.79 for database Growth and 0.81 for database Feeding Level) indicated there was a latent structure of dependency among the variables within both databases. According to scree plot (Figure 3), the first four Fa, for both databases, should be interpreted because they retained a high proportion of total databases variances (63.47% for database Growth and 57.12% for database Feeding Level; Tables 3 and 4) and each of those retained Fa can potentially represent a biological process.

Factor 1 of database Growth (Table 3; Figure 4) was negatively related to DM intake level, OM content of ingested diet, chewing investment, k_p of solutes in reticulorumen, omasum, and colon-rectum, and reticulorumen concentration of particles with size from 0.35 to 1.18 mm, and positively related to wet tissues and wet pool size of all evaluated GIT segments, except abomasum wet pool size, reticulorumen concentration of particles with size from 2.36 to 4.75 mm, and abomasum k_p of solutes. According to analysis of variance, sex and BW affected Fa1 ($P \leq 0.01$; Table 4). The scores of Fa 1 were similar between males (0.12; $P = 0.94$) and between females and intact males ($P = 0.12$), and castrated males had positive and higher scores than females, that had negative scores (014 vs. -0.14; $P = 0.05$; Table 4; Figure 5). The scores of Fa 1 increased at a decreasing rate as BW increased ($P < 0.01$; Table 4; Figure 5). Factor 1 of database growth reveals k_p of solutes in reticulorumen, omasum, and colon-rectum are positively related to each other and decrease as goats age. Moreover, F1 of database growth demonstrates that the k_p of solutes in reticulorumen, omasum, and colon-rectum are positively related to DM intake level and chewing investment, and negatively related to wet tissues and the capacity of segments.

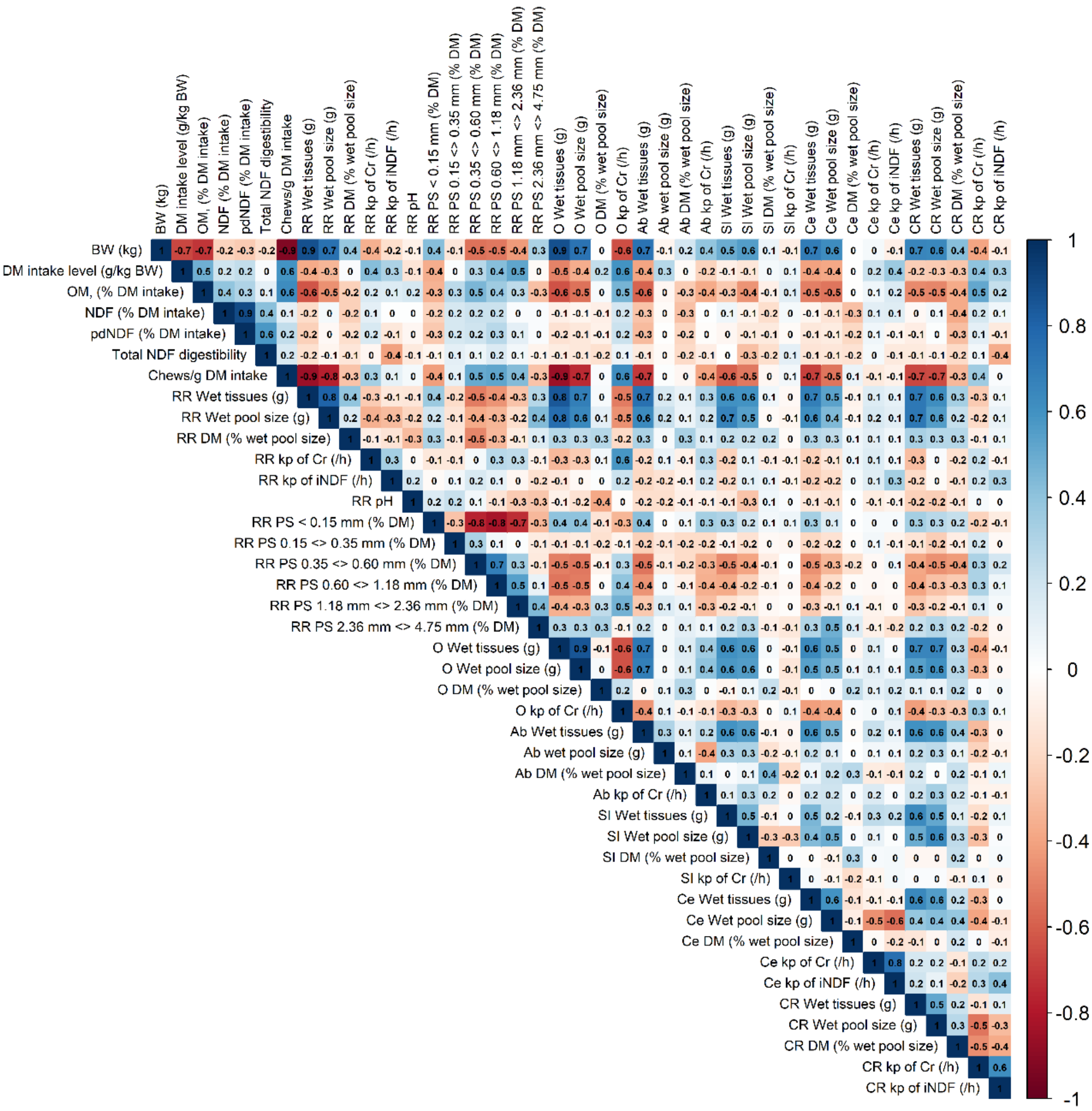

Figure 1. Correlation plot based on the variables of database Growth (castrated males, females, and intact males Saanen goats were slaughtered at 15, 22, 30, 37, and 45 kg body weight (BW)). Red colour represents negative correlation and blue colour represents positive correlation. The strength of the correlation is given by colour intensity. pdNDF = potentially digestible NDF; RR = reticulorumen; k_p = digesta passage rate; iNDF = indigestible NDF; PS = particle size; O = Omasum; Ab = Abomasum; SI = Small intestine; Ce = Cecum; CR = Colon-rectum.

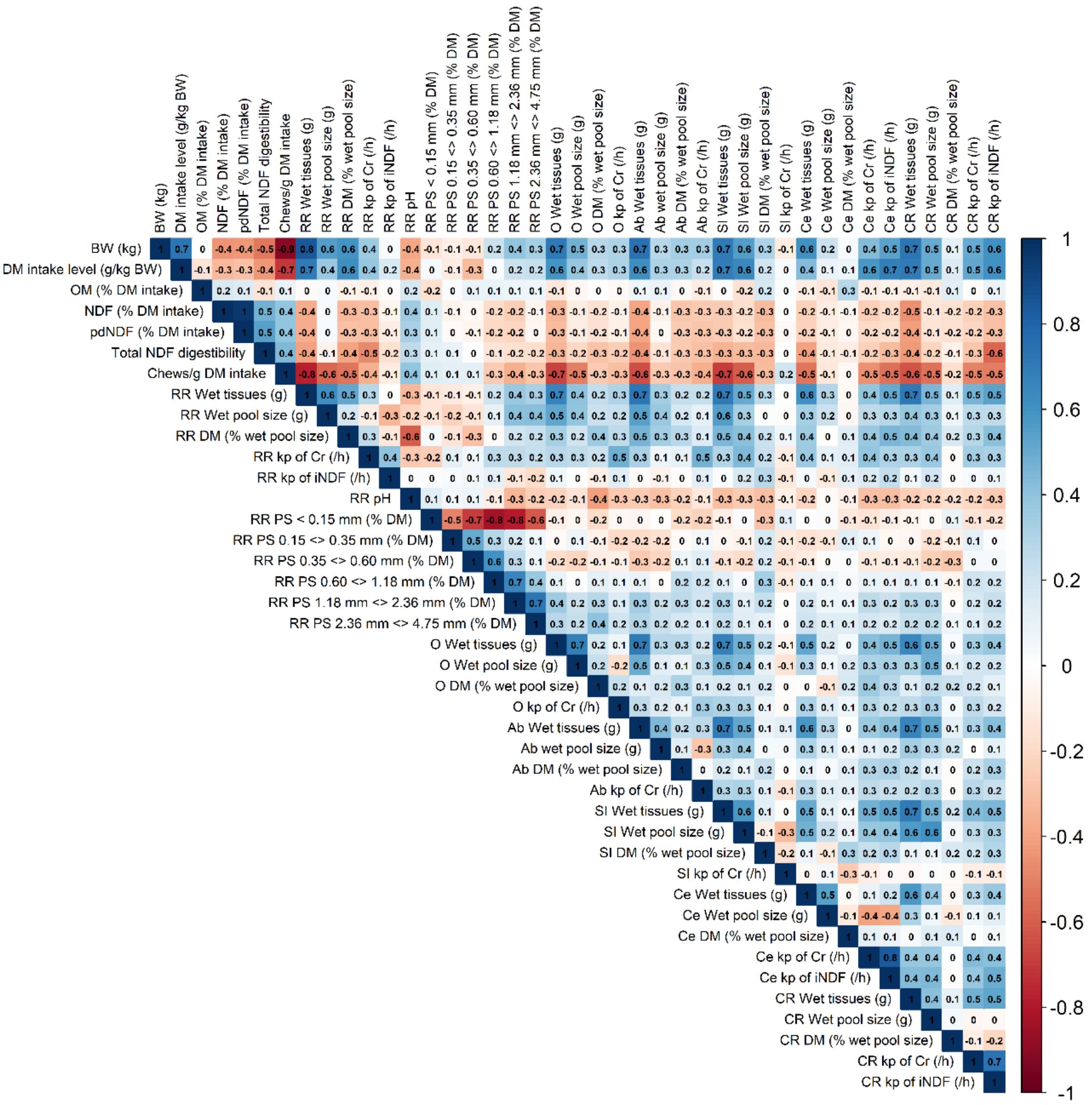

Figure 2. Correlation plot based on the variables of database Feeding Level (castrated males, females, and intact males Saanen goats were fed ad libitum (26.82 ± 2.54 g/kg body weight (BW)), 75 (22.90 ± 1.99 g/kg BW) or 50% (20.44 ± 2.05 g/kg BW) of ad libitum). Red colour represents negative correlation and blue colour represents positive correlation. The strength of the relationship is given by the colour intensity. pdNDF = potentially digestible NDF; RR = reticulorumen; k_p = digesta passage rate; iNDF = indigestible NDF; PS = particle size; O = Omasum; Ab = Abomasum; SI = Small intestine; Ce = Cecum; CR = Colon-rectum.

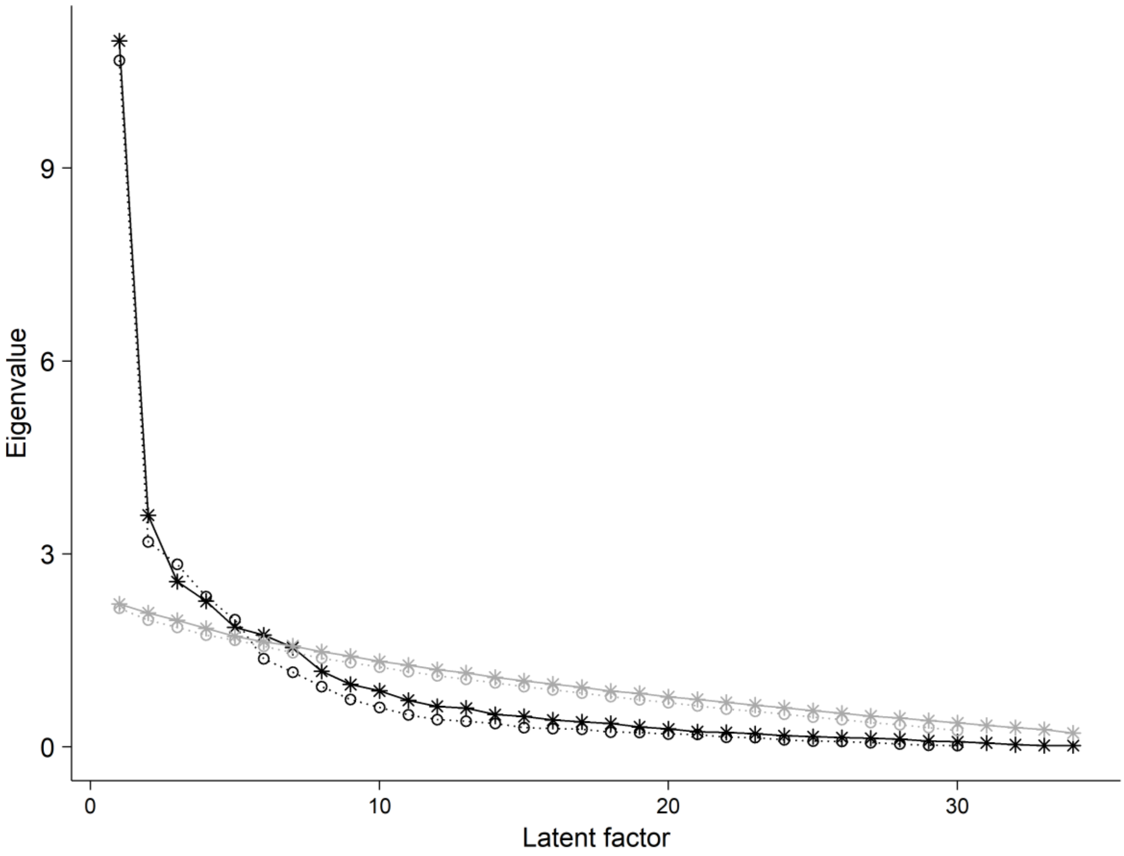

Figure 3. Scree plot shows the actual eigenvalues (i.e., total variance in the actual database accounted by each factor) and the simulated eigenvalues (i.e., total variance in a simulated database, with the same number of observation than the actual database, accounted by each factor). The first four factors of database Growth (castrated males, females, and intact males Saanen goats were slaughtered at 15, 22, 30, 37, and 45 kg body weight (BW)) and database Feeding Level (castrated males, females, and intact males Saanen goats were fed ad libitum (26.82 ± 2.54 g/kg body weight (BW)), 75 (22.90 ± 1.99 g/kg BW) or 50% (20.44 ± 2.05 g/kg BW) of ad libitum) were chosen based on the results of parallel analysis (actual eigenvalues higher than simulated eigenvalues) and biological interpretation. The black symbol ○ and dotted line represent actual eigenvalues of database Growth and the black ∗ and solid line represent actual eigenvalues of database Feeding Level. The grey symbol ○ and dotted line represent simulated eigenvalues of database Growth and the grey symbol ∗ and solid line represent simulated eigenvalues of database Feeding Level.

Factor 3 of database Growth (Table 3) was positively related to DM intake level, k_p of particles in reticulorumen, and k_p of solutes and particles in cecum and colon-rectum, and negatively related to cecum wet pool size. Sex did not affect Fa 3 (P = 0.49; Table 4). The scores of Fa 3 decreased at an increasing rate as BW increased (P < 0.01; Table 4; Figure 5). Factor 3 of database Growth demonstrates that the reticulorumen k_p of particles and large intestine k_p of solutes and particles are positively related among them and negatively related to animal growth. Moreover, this Fa demonstrates reticulorumen and colon-rectum k_p of particles are not related to the segment's wet tissues and capacity but with DM intake level. Factor 3 of database Growth also demonstrates cecum`s distention ability, because wet pool size was linked to the Fa but cecum`s wet tissues.

Factor 1 of database Feeding Level (Table 5; Figure 6) was positively related to BW, wet tissues and wet pool size of all evaluated GIT segments, reticulorumen DM content, k_p of solutes in reticulorumen, omasum, and large intestine, reticulorumen concentration of particles with size from 1.18 to 4.75 mm, and k_p of particles in large intestine. Total NDF digestibility, chewing investment, and reticulorumen pH were negatively related to Fa 1 on the database Feeding Level. According to the analysis of variance of Fa of database Feeding Level, sex and feeding level affected Fa 1 (P < 0.01; Table 6 and Figure 7). The scores of Fa 1 were similar between males (0.11; P = 0.37) and between females and castrated males (P = 0.09), and intact males had positive and higher scores than females, that had negative scores (0.20 vs. -0.24; P < 0.01; Table 6; Figure 7). The scores of F1 increased linearly as feeding level increased (P < 0.01). Factor 1 of database Feeding Level reveals that k_p of solutes in reticulorumen, omasum and large intestine and k_p of particles in large intestine are positively related to each other increase with long-term feeding level, despite the increase on GIT segments capacity with feeding level.

Factor 4 of database Growth and Fa 3 of database Feeding level were related to ingested diet composition (OM, NDF, and pdNDF contents) and total NDF digestibility (Table 3 and 5). These two factors were affected by sex, BW, and feeding level ($P \leq$ 0.04; Figure 5 and 7). However, the important information from these factors is that total NDF digestibility is mainly dependent on diet composition other than k_p of digesta in growing goats or goats fed with feeding levels.

Table 3. Rotated factors (Fa) loadings, communality and cumulative variance explained by the first five Fa of database Growth composed by individual observations of castrated males, females, and intact males Saanen goats slaughtered at 15, 22, 30, 37, and 45 kg BW

Item	Fa loadings[1]				Communality
	F1	F2	F3	F4	
DM intake level (g/kg BW)	**-0.40**	0.25	**0.43**	0.20	0.45
OM (% DM intake)	**-0.62**	0.18	0.25	0.25	0.53
NDF (% DM intake)	-0.01	0.11	0.13	**0.90**	0.85
pdNDF2 (% DM intake)	-0.09	0.13	-0.05	**0.98**	1.00
Total NDF digestibility	-0.12	-0.03	-0.12	**0.57**	0.35
Chew/g DM intake	**-0.89**	0.21	0.05	0.14	0.86
Reticulorumen					
wet tissues (g)	**0.89**	-0.16	-0.01	-0.13	0.84
wet pool size (g)	**0.87**	-0.01	0.02	0.05	0.76
k_p[3] of Cr (/h)	**-0.30**	0.04	0.10	0.13	0.12
k_p of iNDF[4] (/h)	-0.17	-0.01	**0.36**	-0.12	0.17
PS[5] < 0.15 mm (% DM)	0.22	**-0.97**	-0.05	-0.12	1.00
PS 0.35 <> 0.60 mm (% DM)	**-0.42**	**0.67**	0.16	0.09	0.67
PS 0.60 <> 1.18 mm (% DM)	**-0.37**	**0.75**	0.07	0.15	0.72
PS 1.18 mm <> 2.36 mm (% DM)	-0.21	**0.71**	0.03	0.00	0.55
PS 2.36 mm <> 4.75 mm (% DM)	**0.45**	**0.45**	-0.21	0.00	0.44
Omasum					
wet tissues (g)	**0.89**	-0.21	-0.09	-0.04	0.85
wet pool size (g)	**0.79**	-0.24	-0.05	-0.02	0.68
k_p of Cr (/h)	**-0.56**	0.20	0.21	0.15	0.42
Abomasum					
wet tissues (g)	**0.75**	-0.23	-0.02	-0.22	0.66
k_p of Cr (/h)	**0.31**	-0.28	-0.03	0.05	0.18
Small intestine					
wet tissues (g)	**0.67**	-0.18	0.15	0.05	0.51
wet pool size (g)	**0.64**	-0.04	-0.06	-0.05	0.42
Cecum					
wet tissues (g)	**0.71**	-0.17	-0.17	-0.04	0.56
wet pool size (g)	**0.56**	0.03	**-0.64**	-0.09	0.73
k_p of Cr (/h)	0.19	-0.05	**0.75**	0.12	0.61
k_p of iNDF (/h)	0.12	-0.06	**0.97**	0.02	0.97
Colon-rectum					
wet tissues (g)	**0.74**	-0.18	0.10	0.00	0.59
wet pool size (g)	**0.71**	-0.13	-0.01	0.10	0.53
k_p of Cr (/h)	**-0.34**	0.14	**0.35**	0.08	0.26
k_p of iNDF (/h)	0.02	0.11	**0.44**	-0.11	0.21
Cumulative variance (%)	35.58	46.22	55.68	63.47	-

[1] Bold values indicate loadings ≥ |0.3|

[2] pdNDF = potentially digestible NDF

[3] k_p = digesta passage rate

[4] iNDF = indigestible NDF

[5] PS = particle size

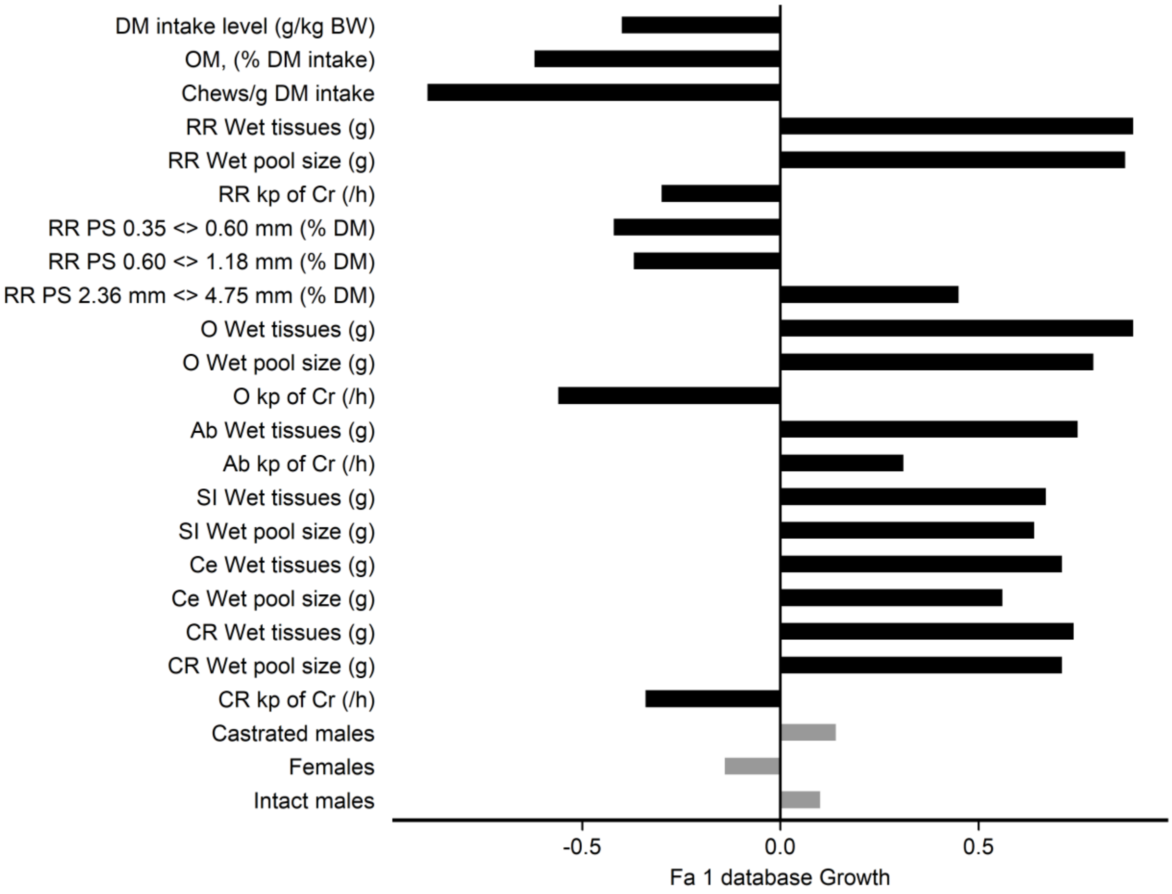

Figure 4. Variables with loadings greater than |0.3| and samples scores of castrated males, females, and intact males on factor (Fa) 1 of database Growth (castrated males, females, and intact males Saanen goats were slaughtered at 15, 22, 30, 37, and 45 kg body weight (BW)). The scores of Fa 1 were similar between males (0.12; P = 0.94) and between females and intact males (P = 0.12), and castrated males had positive and higher scores than females, that had negative scores (014 vs. -0.14; P = 0.05). RR = reticulorumen; k_p = digesta passage rate; iNDF = indigestible NDF; PS = particle size; O = Omasum; Ab = Abomasum; SI = Small intestine; Ce = Cecum; CR = Colon-rectum.

Table 4. Effect of sex, body weight (BW), and interaction between sex and BW (Sex × BW) on the scores of the first five orthogonal latent factors (Fa) of database Growth composed by individual observations of castrated males (C), females (F), and intact males (I) Saanen goats slaughtered at 15, 22, 30, 37, and 45 kg BW

Item[1]	*P*-value fixed effects						
	Sex	BW	Orthogonal polynomial contrast[2]				Sex × BW[3]
			BW_{L}	BW_{Qd}	BW_{C}	BW_{Qt}	
Fa 1	0.04	<0.01	<0.01	<0.01	0.81	0.68	0.54
Fa 2	0.49	0.23	0.02	0.99	0.78	0.53	0.96
Fa 3	0.38	<0.01	<0.01	<0.01	0.89	0.93	0.12
Fa 4	-	-	-	-	-	-	<0.01
	C	-	0.47	0.04	<0.01	0.54	-
	F	-	<0.01	0.27	0.83	0.07	-
	I	-	0.95	0.04	0.03	0.89	-

[1] Fa 1 = Factor related to DM intake level, chewing investment, wet tissues and wet pool size of all evaluated gastrointestinal tract (GIT) segments, k_p of solutes in reticulorumen, omasum, abomasum, and colon-rectum. Fa 2 = Factor related to reticulorumen particle size concentration. Fa 3 = Factor related to DM intake level, reticulorumen k_p of particles, and k_p of solutes and particles in large intestine. Fa 4 = Factor related to diet composition.

[2] BW_{L}= linear effect of BW; BW_{Qd} = quadratic effect of BW; BW_{C}= cubic effect of BW; BW_{Qt} = quartic effect of BW.

[3] The scores of Fa 1 were similar between males (0.12; $P = 0.94$) and between females and intact males ($P = 0.12$), and castrated males had positive and higher scores than females, that had negative scores (014 vs. -0.14; $P = 0.05$). The scores of Fa 4 were similar between males and females at 22, 30, 37, 45 kg BW ($P \geq 0.07$), however, females had positive and higher scores than males, that had negative scores, at 15 kg BW (1.25 vs -0.73; $P < 0.01$).

DISCUSSION

Four orthogonal latent variables were extracted from both databases, out of 40 individual goats' digestive variables. The four orthogonal latent variables explained around 60% of the original variability while at the same time drastically reduced the data space. Furthermore, even though k_p in ruminants is a dynamic process and related to many factors that depend on diet and animal, the MFA model was able to capture variables that explain changes on GIT k_p in growing goats and goats fed with feeding levels over long-term. The first FA of both databases captures more than half (34%) of total variance explained by the four selected Fa. This indicates Fa 1 of both databases were the most important Fa.

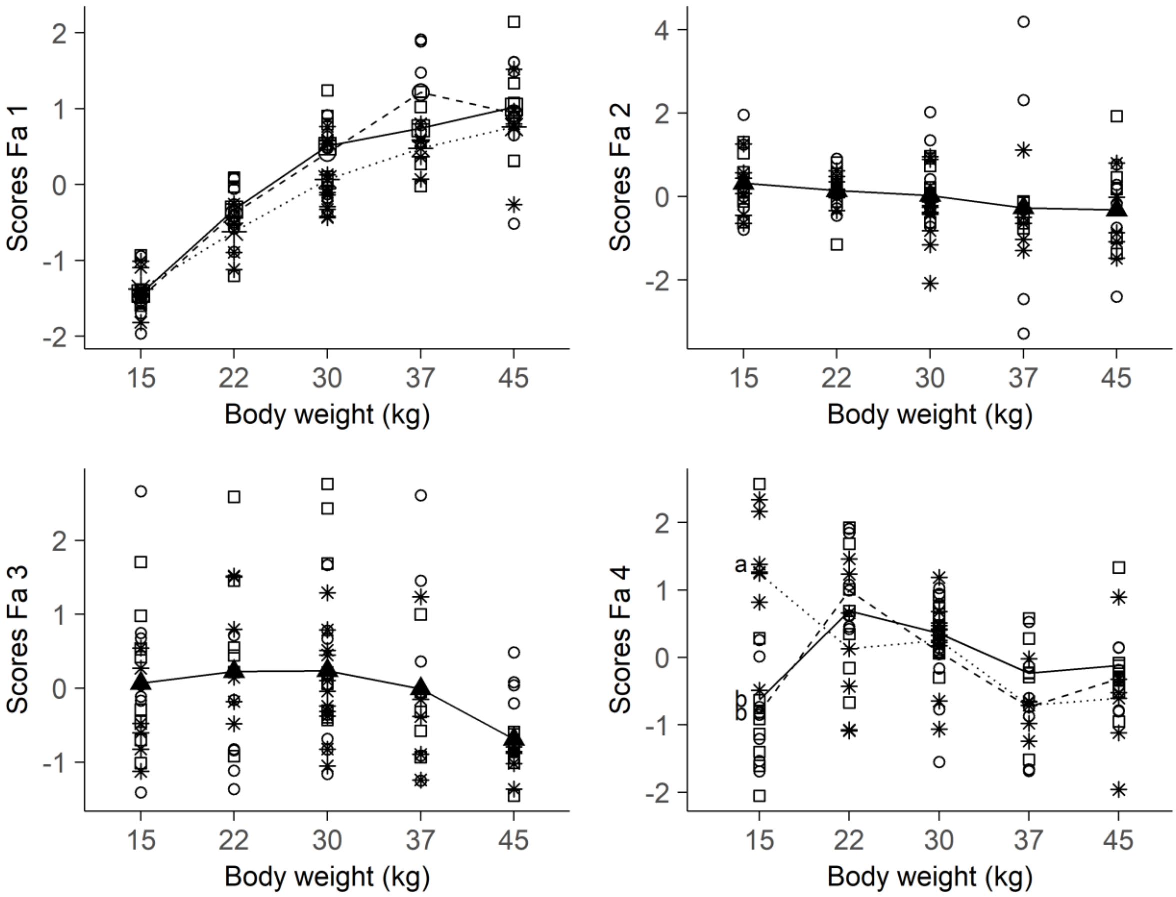

Figure 5. Least-squares (LS) means and observed values of the scores of four factors (Fa) across sex and body weight (BW) of database Growth (castrated males, females, and intact males Saanen goats were slaughtered at 15, 22, 30, 37, and 45 kg BW). Description: Fa 1 = Factor related to DM intake level, chewing investment, wet tissues and wet pool size of all evaluated gastrointestinal tract (GIT) segments, k_p of solutes in reticulorumen, omasum, abomasum, and colon-rectum. Fa 2 = Factor related to reticulorumen particle size concentration. Fa 3 = Factor related to DM intake level, reticulorumen k_p of particles, and k_p of solutes and particles in large intestine. Fa 4 = Factor related to diet composition. Means in the BW with different letters (a and b) are different according to Tukey's' test (P ≤ 0.05). The symbol ○ and dashed line represent observed and LS means of castrated males, ⁎ and dotted line represent observed and LS means of females, □ and solid line represent observed and LS means of intact males. The symbol ▲ and solid line represent LS means of sex across body weight at Fa 2 and 3.

Our results demonstrated that the digesta passage rate was differently related to gut capacity and chewing investment whether evaluated in growing goats or goats fed with feeding levels over long-term. On the other hand, DM intake level positively affected solutes and particles passage rate in growing goats or goats fed with feeding levels over long-term. Growing goats had k_p of solutes in reticulorumen, omasum, and colon-rectum positively related to DM intake level and chewing investment, however, they were negatively related to the segment's wet tissues and capacity. Conversely, goats fed with feeding levels over long-term had k_p of solutes in reticulorumen, omasum, and large intestine positively related to feeding level and the segment's wet tissues and capacity. Moreover, differently than observed for growing goats, goats fed with feeding levels over long-term had chewing investment negatively related to feeding level and k_p of solutes in reticulorumen, omasum, and large intestine. Growing goats had k_p of particles in reticulorumen and large intestine positively related among them and positively related to DM intake level. However, in goats fed with feeding levels over long-term, k_p of particles was constant in reticulorumen but positively related to feeding levels and segment's capacity, and negatively related to chewing investment in the large intestine.

Intake and GIT capacity have been related to digesta passage rate in ruminants (Clauss et al., 2007a). Growth positively affects intake because of their mutual relationship with maintenance requirements (Almeida et al., 2019). Additionally, intake has a positive effect on GIT tissues growth (Wardrop and Coombe, 1960; Kabbali et al., 1992), that also follow animal growth (Andrade et al., 2020). In this context, one would not expect a relationship between growth and digesta passage rate, since intake and capacity increase with ageing. However, our results on Fa 1 of database Growth demonstrated that k_p of solutes only in reticulorumen, omasum, and colon-rectum was high in young goats and decreased with ageing. Thus, factors other than intake and capacity would be related to k_p of solutes in these segments.

Gastrointestinal tract segments have different tissue growth rates, according to the type of digestion performed in each segment (Andrade et al., 2020), and despite the segment's distention capacity their tissues size works limiting their capacity (Ortigues and Doreau, 1995). Abomasum and small intestines, related to milk digestion in young ruminants, have earlier development than reticulorumen, omasum, and large intestines, related to digestion of solid diets after suckling phase (Andrade et al., 2020). Thus, taking into consideration that capacity is limited by tissues size, the differences in tissue's growth rate among GIT segments may affect the k_p in GIT segments. The k_p of solutes in abomasum and small intestine was not related to goat's growth because of their capacity that was not full filled in young goats as a consequence of their early development and great capacity in young goats. On the other hand, the k_p of solutes in reticulorumen, omasum, and colon-rectum was affected by growth because of their capacity that was full filled in young goats as a consequence of their late development and low capacity in young goats. Therefore, even though cecum was not related to Fa 1 on database Growth, we suggest the k_p of solutes in growing goats depends on GIT capacity affected by GIT tissues growth.

Additionally, growth has also been demonstrated to affect chewing investment (chews/g DM intake) (Gross et al., 1995a, 1996). Chewing activity (total daily chews) is positively related to reticular contractions and saliva secretion (Bartley, 1976), contributing to increasing on k_p of solutes (Seo et al., 2007, 2009). Young ruminants have higher chewing investment than old ruminants, chewing more per kilogram of ingested diet (Bae and Welch, 1983; Grandl et al., 2016, 2017). Conversely, chewing investment is negatively related to total daily DM intake (Galvani et al., 2010), that increases with ageing (Almeida et al., 2019). This would indicate that chewing activity increases with ageing. However, we are evaluating growing goats that are not efficient chewers at young age. Thus, based on that, our results suggest that chewing activity decreased with ageing and growing goats had not only their k_p of solutes in reticulorumen, omasum, and colon-rectum affected by capacity but also by chewing activity.

The DM intake level was related to Fa 1 on database Growth because it summarizes all the factors related to k_p of solutes in GIT that were related to ageing. We previously discussed that GIT capacity and chewing investment changes with ageing and affect k_p of solutes in reticulorumen, omasum, and colon-rectum. Growth also affects DM intake level because both of them are related to maintenance requirements (Almeida et al., 2019; Souza et al., 2020). However, maintenance requirements decrease with ageing, when it is expressed as kcal per kg of BW (Kleiber, 1932; Blaxter et al., 1982; Souza et al., 2020), and DM intake has demonstrated the same pattern when it is scaled to BW (Kleiber, 1932). Even the disagreement regarding a proper exponent that scales DM intake to BW, all the proposed allometric exponent are smaller than one and range from 0.66 to 0.85 (Clauss et al., 2007b; Müller et al., 2013; Almeida et al., 2019). This demonstrates DM intake expressed as level of BW (i.e. g/kg BW) decreases with ageing, as observed in Fa 1 of database Growth. Thus, this demonstrates that DM intake level would be a great predictor for k_p of solutes in growing goats because it summarizes chewing investment and capacity that are also related to ageing.

Additionally, when growth is not considered and goats are fed with feeding levels over long-term, 109 ± 10 days, feeding level was positively related to k_p of solutes in reticulorumen, omasum, cecum, and colon-rectum and k_p of particles in cecum and colon-rectum. However, these k_p were also positively related to capacity of all evaluated GIT segments and negatively related to chewing investment. One would not expect this because an increase in intake, followed by increasing on capacity, would not result in k_p increase (Clauss et al., 2007a). However, our results suggest that the increase on GIT capacity was not proportional to intake increase resulting in distention of segments, as reticulorumen, and/or increase on k_p (Clauss et al., 2016b). In addition to reticulorumen distention, chewing activity also contributes to the increase in reticulorumen k_p of solutes (Seo et al., 2007, 2009). Chewing investment was negatively related to reticulorumen k_p of solutes on Fa 1 of database Feeding Level, because of its negative relationship with feeding level, when age is not considered (Galvani et al., 2010). On the other hand, studies have demonstrated that even when chewing investment decreases with feeding level, chewing activity increases with feeding level (Galvani et al., 2010). Thus, Fa 1 of database Feeding Level indicates that feeding level positively affects reticulorumen k_p of solutes because of its positive relationship with chewing activity and reticulorumen distention.

Table 5. Rotated factors (Fa) loadings, communality and cumulative variance explained by the first five Fa of database Feeding Levels composed by individual observations of castrated males, females, and intact males Saanen goats fed with three feeding levels (fed ad libitum (26.82 ± 2.54 g/kg BW), 75 (22.90 ± 1.99 g/kg BW) or 50% (20.44 ± 2.05 g/kg BW) of ad libitum)

Item	Fa loadings[1]				Communality
	F1	F2	F3	F4	
BW (kg)	**0.88**	0.08	**0.31**	0.04	0.87
NDF (% DM intake)	-0.16	-0.08	**-0.96**	-0.02	0.95
pdNDF[2] (% DM intake)	-0.15	-0.08	**-0.98**	0.01	0.99
Total NDF digestibility	**-0.39**	-0.07	**-0.48**	-0.04	0.39
Chew/g DM intake	**-0.83**	-0.08	-0.27	-0.10	0.79
Reticulorumen					
wet tissues (g)	**0.85**	0.09	0.23	-0.01	0.79
wet pool size (g)	**0.69**	0.12	-0.16	-0.08	0.53
DM (% wet pool size)	**0.55**	-0.09	0.23	0.19	0.40
K_p[3] of Cr (/h)	**0.32**	0.21	0.25	0.18	0.24
pH	**-0.33**	-0.09	-0.28	-0.14	0.22
PS[4] < 0.15 mm (% DM)	-0.04	**-0.99**	-0.05	-0.05	1.00
PS 0.15 <> 0.35 mm (% DM)	-0.19	**0.50**	0.03	0.13	0.30
PS 0.35 <> 0.60 mm (% DM)	-0.25	**0.70**	0.04	-0.03	0.56
PS 0.60 <> 1.18 mm (% DM)	0.10	**0.83**	0.12	-0.02	0.72
PS 1.18 mm <> 2.36 mm (% DM)	**0.35**	**0.77**	0.11	0.02	0.72
PS 2.36 mm <> 4.75 mm (% DM)	**0.37**	**0.60**	-0.06	-0.05	0.50
Omasum					
wet tissues (g)	**0.75**	0.04	0.21	0.07	0.61
wet pool size (g)	**0.54**	0.02	0.02	0.08	0.30
k_p of Cr (/h)	**0.35**	0.02	0.04	0.09	0.13
Abomasum					
wet tissues (g)	**0.78**	0.00	0.28	-0.04	0.70
wet pool size (g)	**0.42**	-0.01	-0.02	-0.09	0.18
Small intestine					
wet tissues (g)	**0.79**	0.04	0.14	0.13	0.66
wet pool size (g)	**0.65**	-0.06	0.08	0.07	0.44
Cecum					
wet tissues (g)	**0.68**	-0.02	0.13	-0.27	0.55
wet pool size (g)	0.29	0.00	0.06	**-0.72**	0.60
k_p of Cr (/h)	**0.43**	0.08	0.14	**0.78**	0.82
k_p of iNDF[5] (/h)	**0.54**	0.03	0.09	**0.74**	0.85
Colon-rectum					
wet tissues (g)	**0.73**	0.01	**0.33**	0.00	0.63
wet pool size (g)	**0.58**	-0.01	0.05	0.14	0.37
k_p of Cr (/h)	**0.46**	0.11	0.15	0.21	0.29
k_p of iNDF (/h)	**0.53**	0.11	0.22	0.19	0.38
Cumulative variance, %	32.30	42.90	50.46	57.12	-

[1] Bold values indicates loadings ≥ |0.3|.

[2] pdNDF = potentially digestible NDF.

[3] k_p = digesta passage rate.

[4] PS = particle size

[5] iNDF = indigestible NDF

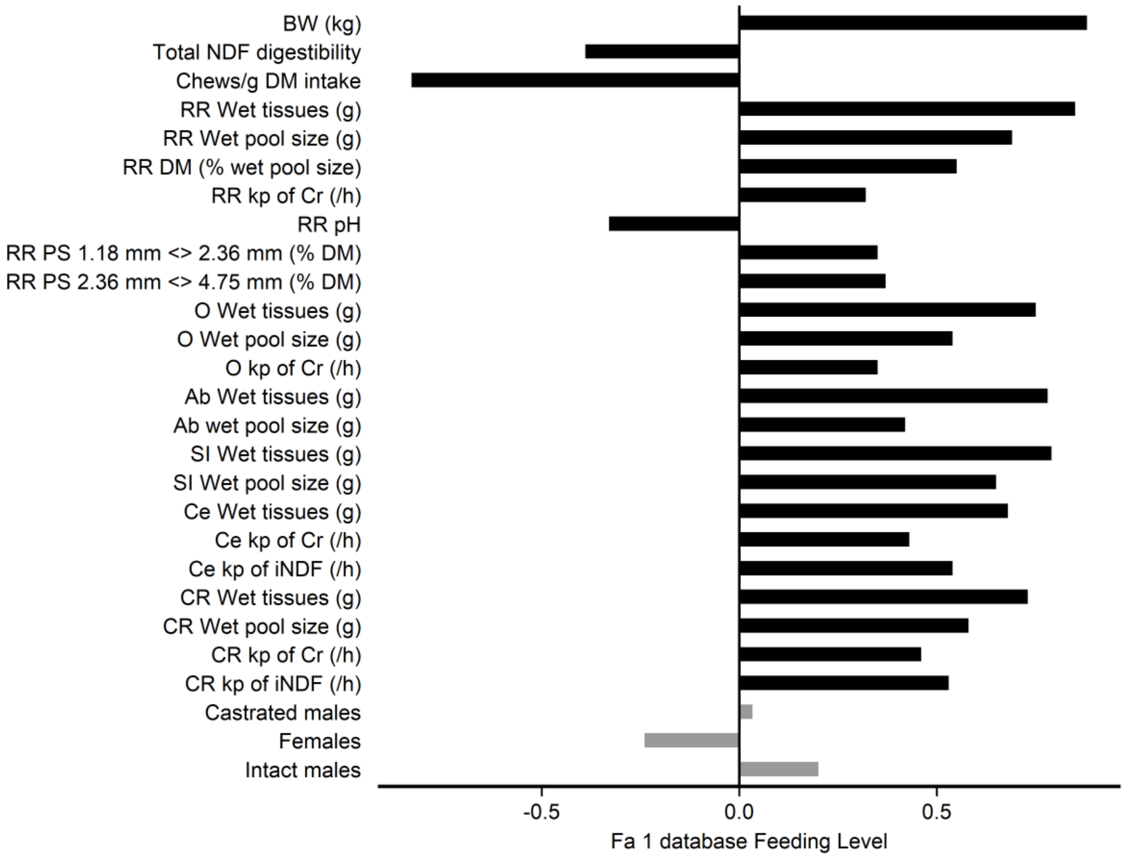

Figure 6. Variables with loadings greater than |0.3| and samples scores of castrated males, females, and intact males on the factor (Fa) 1 of database Feeding Level (castrated males, females, and intact males Saanen goats were fed ad libitum (26.82 ± 2.54 g/kg body weight (BW)), 75 (22.90 ± 1.99 g/kg BW) or 50% (20.44 ± 2.05 g/kg BW) of ad libitum). The scores of Fa 1 were similar between males (0.11; $P = 0.37$) and between females and castrated males ($P = 0.09$), and intact males had positive and higher scores than females, that had negative scores (0.20 vs. -0.24; $P < 0.01$). RR = reticulorumen; k_p = digesta passage rate; iNDF = indigestible NDF; PS = particle size; O = Omasum; Ab = Abomasum; SI = Small intestine; Ce = Cecum; CR = Colon-rectum.

Table 6. Effect of sex, feeding level (FL), and interaction between sex and FL (Sex × FL) on the scores of the first five orthogonal latent factors (Fa) of database Feeding Levels composed by individual observations of castrated males, females, and intact males Saanen goats fed with three feeding levels (FL; fed ad libitum (26.82 ± 2.54 g/kg BW), 75 (22.90 ± 1.99 g/kg BW) or 50% (20.44 ± 2.05 g/kg BW) of ad libitum)

Item[1]	*P*-value fixed effects				
	Sex	FL	Orthogonal polynomial contrast[2]		Sex × FL[3]
			FL_L	FL_{Qd}	
Fa 1	<0.01	<0.01	<0.01	0.10	0.30
Fa 2	0.38	0.70	0.77	0.43	0.53
Fa 3	0.01	<0.01	<0.01	<0.01	0.14
Fa 4	0.05	0.12	0.42	0.06	0.07

[1] Fa 1: Factor related to body weight (BW), total NDF digestibility, chewing investment, wet tissues and wet pool size of all evaluated gastrointestinal tract (GIT) segments, k_p of solutes in reticulorumen, omasum, and large intestine, and k_p of particles in large intestine. Fa 2 = Factor related to reticulorumen particle concentration. Fa 3 = Factor related to diet composition. Fa 4 = Factor related to cecum wet pool size and k_p of solutes and particles.

[2] FL_L= linear effect of FL; FL_{Qd} = quadratic effect of FL;

[3] The scores of Fa 1 were similar between males (0.11; *P* = 0.37) and between females and castrated males (*P* = 0.09), and intact males had positive and higher scores than females, that had negative scores (0.20 vs. -0.24; P < 0.01). The scores of Fa 3 were similar between castrated males and females (*P* = 0.76) and between males (*P* = 0.06), and females had positive and higher scores than intact males, that had negative scores (0.26 vs -0.33; *P* = 0.01). The scores of Fa 4 were similar between intact males and females (*P* = 0.16) and between males (*P* = 0.85), and castrated males had positive and higher scores than females, that had negative scores (0.19 vs -0.32; *P* = 0.05).

Following the GIT segments, omasum had k_p of solutes related to reticulorumen k_p of solutes. One of the main omasum functions is to reduce the moisture of digesta that escapes from reticulorumen (Holtenius and Björnhag, 1989). Moreover, omasum distention has been related to reticulorumen motility and scape of digesta suggesting that the omasum may have a regulatory role in reticulorumen scape of digesta (Tsuda et al., 1991). Thus, the relationship between reticulorumen and omasum k_p is expected.

Furthermore, abomasum and small intestine k_p of solutes were not related to Fa 1 on database Feeding level, however, large intestine k_p of solutes and particles was related to it. Previous studies have demonstrated that distention of the rumen markedly increases motility and flow of digesta of large intestine mediated by the vagus nerves (Grovum and Phillips, 1978; Fioramonti and Ruckebusch, 1979). On the other hand, there are no records in the literature demonstrating this effect on abomasum and small intestine motility, however, the opposite has been demonstrated (Tsuda et al., 1991).

Thus, the k_p of particles and solutes in large intestine was related to Fa 1 of database Feeding Level instead of abomasum and small intestine because of its relationship with reticulorumen distention. Moreover, solutes and particles have comparable k_p after the abomasum because the digesta flow is mainly tubular (Grovum and Williams, 1973; Huhtanen and Kukkonen, 1995; Leite et al., 2015a). Thus, the large intestine k_p of particles and solutes was regulated by reticulorumen distention in goats fed with feeding levels. This corroborates to the idea that reticulorumen controls around 70% of the total k_p of particulate material in GIT (Ahvenjärvi et al., 2010; Leite et al., 2015b) and influences k_p of far segments, as large intestine (Huhtanen et al., 2006).

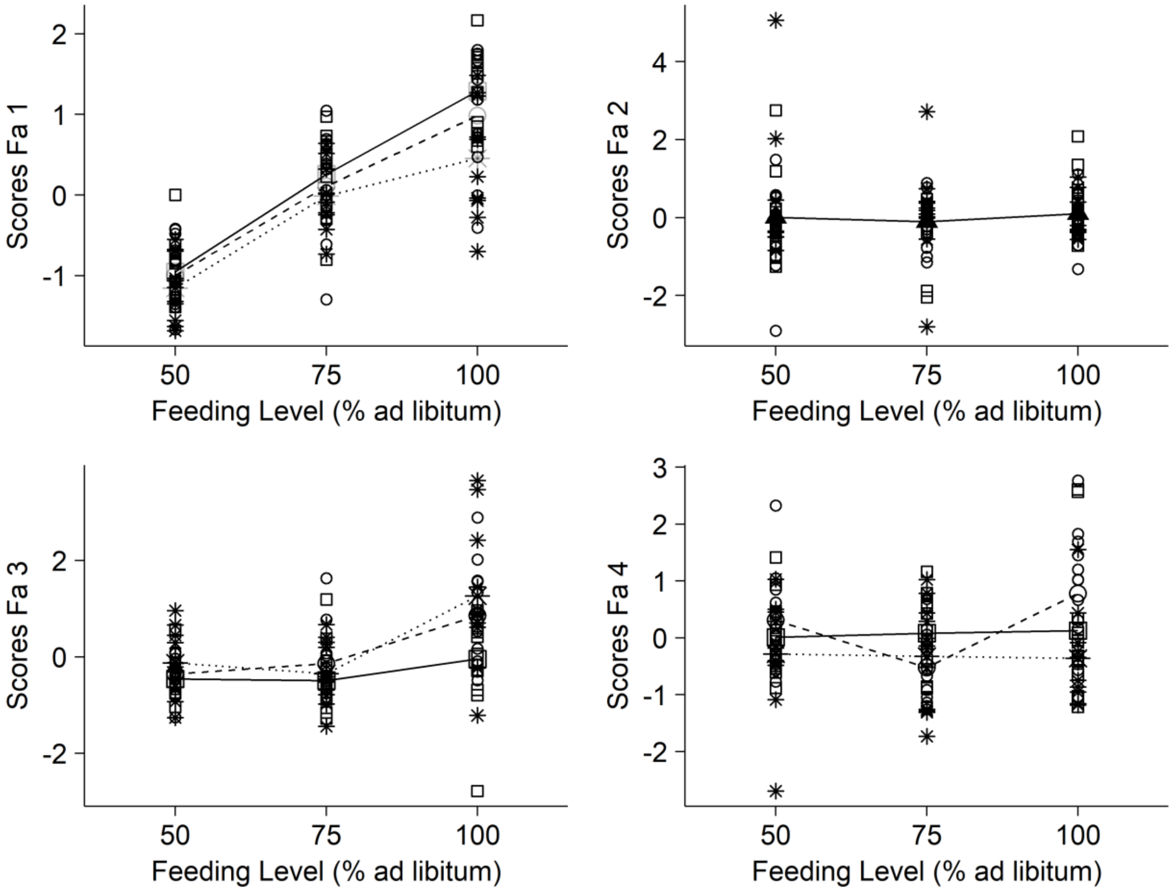

Figure 7. Least-squares (LS) means and observed values of the scores of four factors (Fa) across sex and feeding levels of database Feeding Level (castrated males, females, and intact males Saanen goats were fed ad libitum (26.82 ± 2.54 g/kg body weight (BW)), 75 (22.90 ± 1.99 g/kg BW) or 50% (20.44 ± 2.05 g/kg BW) of ad libitum). Description: Fa 1: Factor related to body weight (BW), total NDF digestibility, chewing

investment, wet tissues and wet pool size of all evaluated gastrointestinal tract (GIT) segments, k_p of solutes in reticulorumen, omasum, and large intestine, and k_p of particles in large intestine. Fa 2 = Factor related to reticulorumen particle concentration. Fa 3 = Factor related to diet composition. Fa 4 = Factor related to cecum wet pool size and k_p of solutes and particles. The symbol ○ and dashed line represent observed and LS means of castrated males, ∗ and dotted line represent observed and LS means of females, □ and solid line represent observed and LS means of intact males. The symbol ▲ and solid line represent LS means of sex across body weight at Fa 2 and 3.

In contrast, reticulorumen k_p of particles, in Fa 3 on database Growth, was positively related to large intestine k_p of particles and solutes and both of them were positively related to DM intake level. However, any variable that could indicate reticulorumen distention was related to Fa 3 on database Growth. Thus, our results suggest that factors other than reticulorumen distention affect the relationship between reticulorumen k_p of particles and large intestine k_p of particles and solutes in growing goats.

Reticulorumen distention has not itself been related to k_p of particles because of adaptations on reticulorumen to maintain a variety of functions related to digestive physiology (Clauss et al., 2016b). This corroborates to the fact that total NDF digestibility was mainly related to diet composition in both evaluated database other than reticulorumen k_p of particles. Reticulorumen k_p of particles has been related to ingested diet composition (Seo et al., 2009). Ingested diet composition affects functional specific gravity and flotation capacity of the particles (Huhtanen et al., 2006). Functional specific gravity is considered one of the most important factors controlling particle sedimentation to escapable pool at the floor of the ventral rumen (Seo et al., 2009). However, ingested diet composition was also not related to reticulorumen k_p of particles. Thus, our results suggest other factors than ingested diet chemical composition modulated reticulorumen k_p of particles.

Reticulorumen k_p of particles was also considered mainly dependent on particle size (Poppi et al., 1980). However, the content of particles in reticulorumen was not related to reticulorumen k_p of particles in both evaluated databases. The content of particles in reticulorumen also depends on k_p of solutes in reticulorumen and chewing investment. Solutes flow is responsible for carrying out small particles from reticulorumen (Seo et al., 2007, 2009). Particles with small size and high functional specific gravity are taken to reticulum by ruminal mixing movements and by the end of the second phase of reticular contraction they flow to omasum through reticulo-omasal orifice, carried out by solutes (Seo et al., 2007, 2009). Our results of both databases agree with this process because the content of small particles was negatively related to reticulorumen k_p of solutes. For Fa 1 of database Growth, the content of reticulorumen particles smaller than 0.15 mm was negatively related to reticulorumen k_p of solutes. For Fa 1 of database Feeding Level, despite the loadings smaller than |0.3|, the content of reticulorumen particles smaller than 0.60 mm was negatively related to reticulorumen k_p of solutes. Chewing investment positively affects the comminution process (Pérez-Barbería and Gordon, 1998). The Fa 1 of both evaluated databases agree with this because the reticulorumen content of particles with size greater than 4.75 mm and 1.18 mm, for database Growth and Feeding Level, respectively, were positively related to both Fa 1, and chewing investment was also negatively related to both Fa 1. This indicates comminution process was more intense in young goats and decreased with ageing and more intense in goats fed with 50% of ad libitum and decreased as feeding level increased. Therefore, even reticulorumen k_p of particles was not related to the content of particles in reticulorumen, our results demonstrated changes on the escape of small particles from reticulorumen and also reduction of particles size by the comminution process. Moreover, this indicates iNDF marker was not able to determine changes in the escape of small particles from reticulorumen.

Sex was identified as a source of variation of Fa 1 on database Growth and database Feeding Level. The samples' scores of Fa 1 of database Growth increased from females to castrated males. This indicates that, based on the variables' loadings on Fa 1 o database Growth, castrated males had greater GIT wet tissues and capacity than females, and females had greater DM intake level, chewing investment, and k_p of solutes in reticulorumen, omasum, and colon-rectum than castrated males. On the other hand, the samples scores of Fa 1 of database Feeding Level increased from females to intact males. This indicates that, based on the variables' loadings on Fa 1 of database Feeding Level, intact males had greater GIT wet tissues, capacity, and k_p of solutes, and k_p of particles in large intestine than females and females had greater chewing investment, reticulorumen content of water and particles with size smaller than 0.60 mm than intact males. Our results corroborate to the idea that k_p is modulated by intake and capacity (Clauss et al., 2007a) and partially suggest that females may have higher chewing investment and k_p of solutes than males (Gross et al., 1995a, 1996).

Capacity is partially related to tissues size (Ortigues and Doreau, 1995). A recent meta-analytical study with 237 growing Saanen goats has demonstrated no effect of sex on GIT tissues growth over time (Andrade et al., 2020). Andrade et al. (2020) also demonstrated that males Saanen goats, when adequately fed, have greater BW than females Saanen goats at the same age, because of male's faster growth rate. Thus, based on our study where growth was addressed by BW, one would expect females Saanen goats have greater GIT tissues and capacity than males Saanen goats at the same BW and under similar environment. However, our results presented the opposite.

Additionally, chewing investment was also greater for females than castrated males, in Fa 1 of database Growth, and than intact males, in Fa 1 of database Feeding Level. This partially agrees with previous studies where females' Nubian ibexes (*Capra ibex nubiana*) chew 50% more per kilogram of ingested diet than males, because of their 15% smaller molar surface area (Gross et al., 1995b; a). Chewing investment affects saliva secretion, reticulo-omasal orifice opening, and comminution process, and in turn, it is related to k_p (Seo et al., 2007, 2009; Grandl et al., 2018). The Fa 1 of database Growth partially agrees with this because females had greater k_p of solutes in reticulorumen, omasum, and colon-rectum than castrated males. On the other hand, Fa 1 of database Feeding Level demonstrated that females had greater reticulorumen content of water and particles with size smaller 0.60 mm, but GIT k_p of solutes. This suggests that females had greater saliva secretion and particles' comminution, and smaller particles in reticulorumen. Water in reticulorumen is essential for the density-dependent sorting mechanism (Clauss et al., 2016b). Moreover, the content of proteins in saliva and saliva viscosity, that negatively contributes to particles stratification (Clauss et al., 2006), decreases as saliva secretion increases (Bailey, 1961). Thus, we suggest that females fed with feeding levels had smaller particles in reticulorumen that were densely packed in the ventral rumen, leading to lower volume of reticulorumen digesta. Thereafter, because of the lower volume of reticulorumen, the escape rate of digesta was avoided in females.

Our results suggested differences among sexes on k_p of solutes in reticulorumen, omasum, and large intestine and k_p of particles in large intestine, however, they did not demonstrate any effect of sex on reticulorumen k_p of particles. Therefore, considering sex effect on k_p may depend on chewing investment (Gross et al., 1995b; a), further studies with different diets and particles size, that would challenge chewing investment, should be conducted directly investigating the effect of sex on k_p of digesta.

CONCLUSIONS

Our results suggested feed intake level is the driving force of GIT k_p of solutes in growing goats and goats fed with feeding level over long-term, and capacity negatively affected k_p only in growing goats. Sex effect on GIT k_p seemed to depend on chewing investment, however, further investigation is needed to fully understand its effects on digesta passage rate.

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CHAPTER 4 - Prediction models of reticulorumen particle and solute kinetics in growing goats

ABSTRACT: The objective of the present study was to propose empirical models for predicting the reticulorumen passage rate (k_p) of particles and solutes in goats and evaluate sex effect on the parameters. Our database involved 175 individual records of castrated male (n = 61), female (n = 57), and intact male (n = 57) growing Saanen goats from two studies. The data set involved goats fed ad libitum and slaughtered at 15, 22, 30, 37, and 45 kg BW and goats fed with feeding levels (ad libitum, 75 or 50% of ad libitum). Akaike's information criterion was used to select the best prediction models. K-fold cross-validation was performed to evaluate the predictive ability of the selected models using Lin's concordance correlation coefficient (CCC) and root mean square prediction error (RMSPE). Sex was not selected as a predictor variable for none of the selected models. Two models were selected for each reticulorumen k_p, including or not reticulorumen wet tissues and/or wet pool size. The DM intake (kg/day), potentially digestible NDF (pdNDF) intake level (g/kg BW), and reticulorumen wet pool size (kg) demonstrated similar and important role on predicting reticulorumen k_p of solutes (CCC = 0.59; RMSPE = 0.050 /h), however, when reticulorumen wet pool size was not included in the model, reticulorumen k_p of solutes could still be precisely and accurately predicted only using DM intake level (g/kg BW) (CCC = 0.47; RMSPE = 0.053 /h). The reticulorumen wet pool size (kg), NDF intake (kg/day), and iNDFI:NDFI ratio of ingested diet had similar and the greatest impact on the output of the model of reticulorumen k_p of particles when reticulorumen wet tissues and wet pool size were in the model (CCC = 0.51; RMSPE = 0.0064 /h). On the other hand, iNDFI:NDFI ratio of ingested diet and NDF intake level (g/kg BW) had similar and the greatest impact on the output of the model of reticulorumen k_p of particles when reticulorumen wet tissues and wet pool size were not in the model and the reticulorumen k_p of solutes had the lowest impact (CCC = 0.20; RMSPE = 0.0074 /h). Reticulorumen k_p of solutes demonstrated to be more dependent on feed intake level than reticulorumen k_p of particles, that demonstrated to be more dependent on diet composition and k_p of solutes. Moreover, our models seem precise and accurate for predicting reticulorumen

k_p of solutes (CCC = 0.57 and 0.47; RMSPE = 0.051 and 0.054 /h) and k_p of particles (CCC = 0.48 and 0.17; RMSPE = 0.0066 and 0.0076 /h) after k-fold cross-validation.

Key words: passage rate, feed intake, sex, equations, Saanen

INTRODUCTION

Digesta passage rate (k_p) in ruminants is a dynamic process and is accounted for in digestion, methane emission, and microbial growth (Okine et al., 1998). Knowledge about reticulorumen k_p of solutes is very important because solutes in reticulorumen act a lubricant and provide a medium for the density-dependent sorting mechanism and microbes to access particles (Seo et al., 2007; Clauss et al., 2016). Moreover, reticulorumen k_p of solutes is positively related to efficient production of microbial protein and methane emission per unit of DM intake (Clauss and Hummel, 2017; Grandl et al., 2018). Reticulorumen k_p of solutes also contributes to the outflow of soluble nutrients and particles from reticulorumen (Seo et al., 2009). Reticulorumen k_p of particles has an important role on rate and extend of total NDF digestibility (Fox et al., 2004). Reticulorumen is responsible by at least 90% of total NDF digestibility (Leite et al., 2015a). Moreover, reticulorumen controls 70% of the total k_p of particulate material in the gastrointestinal tract (GIT) (Leite et al., 2015a) and influences k_p of far segments, as distal tract (Huhtanen et al., 2006). Thus, the knowledge about reticulorumen k_p of particles and solutes is essential to optimize the use of ingested feed by ruminants.

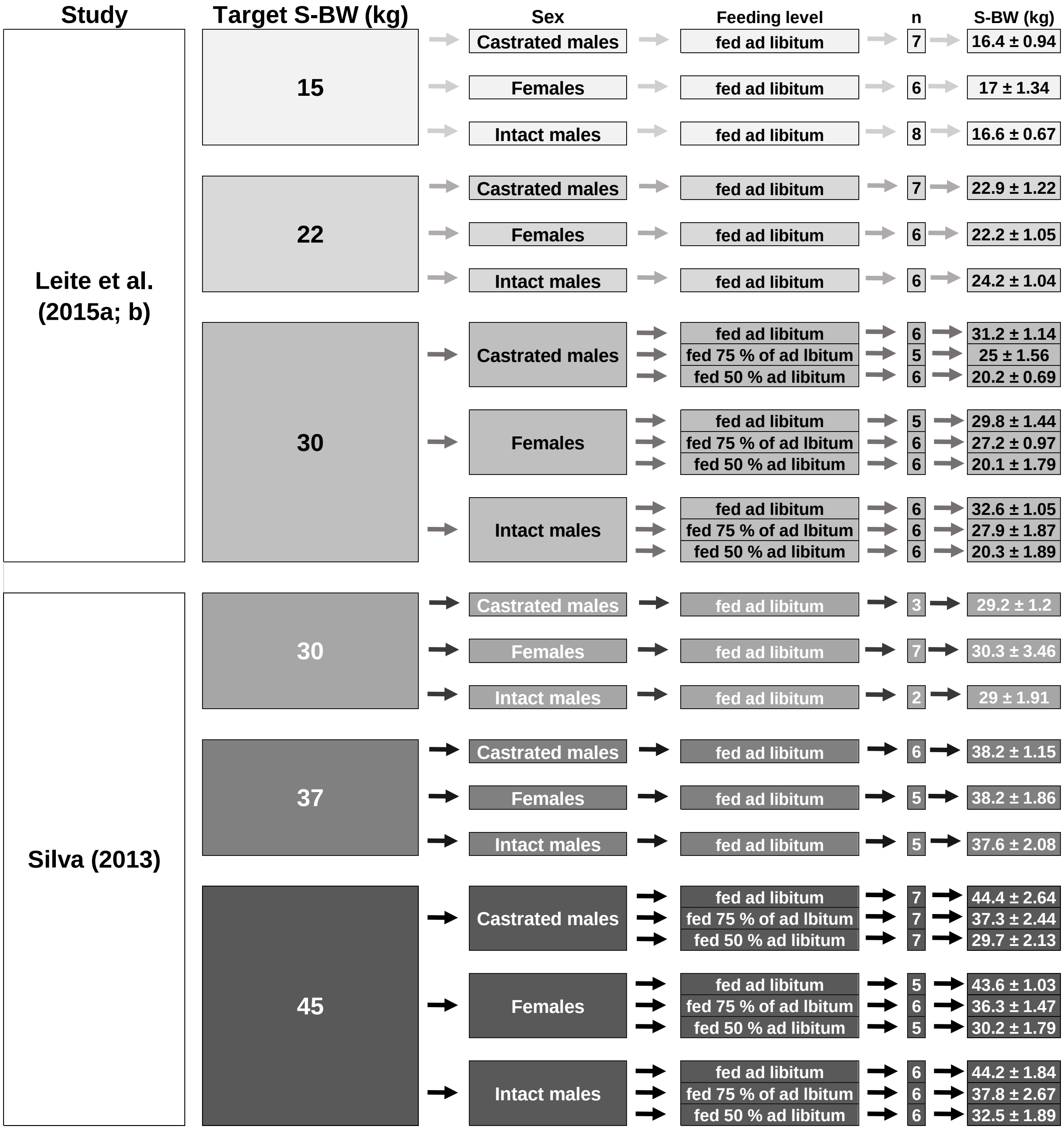
Additionally, it has been demonstrated that despite both reticulorumen k_p of solutes and particles being regulated by reticulorumen capacity, they are also regulated by such specific factors (Clauss et al., 2007b; Seo et al., 2007, 2009). Diet chemical composition has been demonstrated the driver force of reticulorumen k_p of particles, because of its relationship to functional specific gravity (Seo et al., 2009). Reticular contraction and saliva secretion, both dependent on chewing investment (Seo et al., 2007), have been related to reticulorumen k_p of solutes (Seo et al., 2007). Moreover, sex may account on reticulorumen k_p of particles and solutes by its effect on chewing investment, intake, diet composition (Gross et al., 1996), and reticulorumen tissues size, that affects reticulorumen capacity (Andrade et al., 2020).

Therefore, the objective of this study was to develop empirical models for predicting reticulorumen k_p of particles and solutes for goats and also evaluate sex effect on the parameters.

MATERIAL AND METHODS

Data set

A data set, from two studies (Silva, 2013; Leite et al., 2015a; b), including 175 individual records of castrated males (n = 61), females (n = 57), and intact males (n = 57) growing Saanen goats from 15 to 45 kg BW fed with three feeding levels (ad libitum, 75, and 50% of ad libitum) was used (Figure 1). Goats fed ad libitum were slaughtered at 15, 22, 30, 37, and 45 kg BW and they were denominated growing goats. Moreover, goats fed ad libitum and slaughtered at 30 and 45 kg BW, for study Leite et al. (2015a; b) and Silva (2013), respectively, were also into blocks of equal sex, study, and similar initial BW with goats fed with 75 or 50% of ad libitum, that were denominated goats fed with feeding levels. Goats fed with feeding levels were slaughtered when goats fed ad libitum within the same block were slaughtered. Goats from both studies were fed with a similar diet, which was formulated to meet the daily requirements of goat kids (Table 1).



Study	Target S-BW (kg)	Sex	Feeding level	n	S-BW (kg)
Leite et al. (2015a; b)	15	Castrated males	fed ad libitum	7	16.4 ± 0.94
		Females	fed ad libitum	6	17 ± 1.34
		Intact males	fed ad libitum	8	16.6 ± 0.67
	22	Castrated males	fed ad libitum	7	22.9 ± 1.22
		Females	fed ad libitum	6	22.2 ± 1.05
		Intact males	fed ad libitum	6	24.2 ± 1.04
	30	Castrated males	fed ad libitum	6	31.2 ± 1.14
			fed 75 % of ad lbitum	5	25 ± 1.56
			fed 50 % ad libitum	6	20.2 ± 0.69
		Females	fed ad libitum	5	29.8 ± 1.44
			fed 75 % of ad lbitum	6	27.2 ± 0.97
			fed 50 % ad libitum	6	20.1 ± 1.79
		Intact males	fed ad libitum	6	32.6 ± 1.05
			fed 75 % of ad lbitum	6	27.9 ± 1.87
			fed 50 % ad libitum	6	20.3 ± 1.89
Silva (2013)	30	Castrated males	fed ad libitum	3	29.2 ± 1.2
		Females	fed ad libitum	7	30.3 ± 3.46
		Intact males	fed ad libitum	2	29 ± 1.91
	37	Castrated males	fed ad libitum	6	38.2 ± 1.15
		Females	fed ad libitum	5	38.2 ± 1.86
		Intact males	fed ad libitum	5	37.6 ± 2.08
	45	Castrated males	fed ad libitum	7	44.4 ± 2.64
			fed 75 % of ad lbitum	7	37.3 ± 2.44
			fed 50 % ad libitum	7	29.7 ± 2.13
		Females	fed ad libitum	5	43.6 ± 1.03
			fed 75 % of ad lbitum	6	36.3 ± 1.47
			fed 50 % ad libitum	5	30.2 ± 1.79
		Intact males	fed ad libitum	6	44.2 ± 1.84
			fed 75 % of ad lbitum	6	37.8 ± 2.67
			fed 50 % ad libitum	6	32.5 ± 1.89

Figure 1. Description of factors (slaughter body weight (S-BW), feeding level, and sex) evaluated in the original studies that compose the data set used for developing the models

Experimental Procedures and Calculations

All procedures used across studies were reviewed by the University's Animal Care Committee (Comissão de Ética e Bem-Estar Animal, CEBEA; Universidade

Estadual Paulista, Jaboticabal, Brazil). Both studies adopted similar procedures and chemical analyses that are described in Leite et al. (2015a; b) and Silva (2013). The intake of DM (DMI), OM (OMI), NDF (NDFI), indigestible NDF (iNDFI), and potentially digestible NDF (pdNDF) (pdNDFI) were calculated by subtracting orts from the offered diet during the 5-d period before slaughter day. During the same experimental period, Cr-EDTA was administrated to determine the k_p of solutes. The iNDF was used to determine the k_p of particles.

Table 1. Ingredients and chemical composition of the experimental diets

Item	(Leite et al., 2015a; 2015b)	(Silva, 2013)
Dietary ingredient, % DM		
Dehydrated corn plant[1]	45.40	44.70
Cracked corn grain	26.60	30.50
Soybean meal	22.30	15.10
Soybean oil	1.60	2.50
Limestone	1.00	1.30
Mineral supplement[2]	2.20	6.00
Ammonium chloride	0.90	0.00
Diet chemical composition[3], g/kg of DM ± SD		
DM	854 ± 10.9	865 ± 3.13
OM	935 ± 2.00	902 ± 3.27
CP	204 ± 5.40	154 ± 6.57
Crude fat	80 ± 4.90	51 ± 0.79
NDF	355 ± 25.00	313 ± 7.54
iNDF[4]	108 ± 10.50	113 ± 8.97
Lignin	57 ± 3.40	n.a.

[1] 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).

[2] Composition, per kg, as-fed basis: 190 g of Ca; 92 g of Cl; 73 g of P; 62 g of Na; 44 g of Mg; 1.35 g of Zn; 1.06 g of Fe; 0.94 mg of Mn; 0.73 g of F; 0.34 g of Cu; 18 mg of Se; 16 mg of I; 3 mg of Co.

[3] Mean and standard deviation of 10 composite samples. The chemical composition of the diet was calculated from the individual ingredients.

[4] iNDF= Indigestible NDF.

After slaughter (2.2 ± 0.8 h after morning feeding), reticulorumen was removed and weighed before and after emptying to determine the pool size of wet digesta and the wet weight of segment tissues. In the digesta, the iNDF and Cr concentrations were determined. The k_p in reticulorumen was determined by the flux/compartmental pool method using the Eq. [1] (Ellis et al., 1994):

$$k_p \text{ of indigestible entity (IE)} = \frac{\text{intake rate of IE}}{\text{compartmental mass of IE}} \qquad [1]$$

where k_p is the fractional rate of IE (iNDF or Cr) escape per hour, the intake rate of IE is expressed in grams per hour, and compartmental mass in the segment expressed in grams. Potentially digestible NDF intake was calculated by subtracting iNDFI from NDFI.

Feeding behaviour was recorded from goats fed ad libitum and slaughtered at 30 and 45 kg BW of study one and two, respectively, and goats fed with 75 and 50% ad libitum. Feeding behaviour was recorded when goats fed ad libitum were around 22.4 ± 1.31 kg BW and 38.9 ± 1.45 kg BW in study one and two, respectively. 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. Chewing (mastication of ingested material) while feeding and ruminating was recorded during the 24 h of observation from at least 18 focal samples during 41 ± 5 seconds each. The observers were strategically positioned to avoid disturbing the daily activities of the animals. Chewing investment was calculated using the Eq. [2]:

$$\text{Chewing investment} = \frac{\text{chews during feeding} + \text{chews during ruminating}}{\text{DMI}} \qquad [2]$$

where chews during feeding is the chews per minute during feeding × total feeding time expressed in minutes, chews during ruminating is chews per minute during ruminating × total ruminating time expressed in minutes, and DMI is expressed in grams per day.

For goats fed ad libitum and slaughtered at 15 and 22 kg BW in study one and goats fed ad libitum and slaughtered at 30 and 37 kg BW in study two, chewing investment was estimated using an empirical model fitted with the data of those goats that had it recorded. The model was developed through a stepwise selection procedure and the following model (Eq. [3]; n = 101; RMSPE = 1.34 chews/g DMI; R^2 = 0.55), that presented the lowest AICc was chosen:

$$\text{Chewing investment}_{\text{chews/g DMI}} = (66.4 \times 1.96^{\pm 1}) \times \text{NDFI}_{\text{g/day}}^{-1.094 \pm 0.107} \times \text{pdNDFI}_{\text{g/kg BW}}^{0.93 \pm 0.110} \quad [3]$$

Previous analysis to model selection

Before running the model selection, outliers were removed from the data set according to Gindri et al. (2020), who previously evaluated the growing goats with the following model (Eq. [4]):

$$Y_{ijkl} = \mu + S_i + W_j + S_i \times W_j + T_k + e_{ijkl} \quad [4]$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the fixed effect of sex i, W_j is the fixed effect of BW j, $S_i \times W_j$ is the interaction between sex i and BW j, $T_k \overset{i.i.d}{\sim} N(0, \sigma_T^2)$ is the random effect of study k, and $e_{ijkl} \overset{i.i.d}{\sim} N(0, \sigma_e^2)$ is the random residual error. Gindri et al. (2020) considered outliers when their student 98ized residuals were >|3|.

Additionally, the goats fed with feeding levels were analysed for identifying outliers with the following model (Eq. [5]):

$Y_{injk} = \mu + S_i + B_{n(i*k)} + F_j + S_i \times F_j + T_k + e_{injk}$ [5]

where Y_{injk} is the dependent variable, μ is the overall mean, S_i is the fixed effect of sex i, $B_{n(i*s)} \overset{i.i.d}{\sim} N(0, \sigma_B^2)$ is the random effect of block n built according to sex and initial BW and nested in the interaction between sex i and study k, F_j is the fixed effect of feeding level j, $S_i \times F_j$ is the fixed effect of the interaction between sex i and feeding level j, $T_k \overset{i.i.d}{\sim} N(0, \sigma_T^2)$ is the random effect of study, and $e_{injk} \overset{i.i.d}{\sim} N(0, \sigma_e^2)$ is the residual random error. Outliers were removed when their studentized residuals were >|3|.

Moreover, we plotted (not shown) the relationship among candidates' predictor variables and the relationship between candidates' predictor variables and dependent variables. These plots indicated the candidates' predictor variables were linearly related to reticulorumen k_p of particles and solutes and they were also used to avoid collinearity among candidate's' predictor variables.

Model selection

The variables individual goat's BW (kg), sex, DMI, NDFI, and pdNDFI, expressed either as % DM intake, daily intake (kg/day), or as daily intake level (g/kg BW), iNDF:NDF ratio of ingested diet, reticulorumen wet tissues and wet pool size (kg), and chewing investment were used as candidates' predictor variables for models of reticulorumen k_p of particles and solutes (Table 2). Reticulorumen k_p of solutes was also included as a candidate predictor variable of reticulorumen k_p of particles (Seo et al., 2009). Aiming to increase residual homoscedastic variance and the predictive ability of the models, the reticulorumen k_p of particles and solutes were used untransformed and transformed to the natural logarithm.

Table 2. Descriptive statistics of variables used to develop prediction models of reticulorumen passage rate of particles and solutes in growing goats

Variable	n	Mean	SD	Minimum	Maximum
BW (kg)	149	29	8	15	47
Intake[1] (kg/day)					
DM	149	0.77	0.25	0.34	1.4
OM	149	714	226	315	1327
NDF	149	0.26	0.08	0.10	0.47
pdNDF[2]	149	176	65	64	333
Intake level (g/kg BW)					
DM	149	27	8	12	48
OM	149	25	8	11	45
NDF	149	9.5	3.6	3.5	21
pdNDF	149	6.5	3.0	2.1	16
Intake composition (% DM intake)					
OM	149	93	0.7	92	95
NDF	149	34	6.0	21	45
pdNDF	149	23	6.1	10	37
iNDF[3]:NDF ratio	149	0.33	0.07	0.17	0.58
Reticulorumen					
Wet tissues (kg)	149	0.58	0.14	0.33	0.99
Wet pool size (kg)	149	3.8	0.9	1.8	6.4
k_p[4] Cr (/h)	149	0.15	0.066	0.052	0.43
k_p iNDF (/h)	149	0.026	0.008	0.013	0.056
Chewing investment (chews/g DM intake)	149	0.88	0.32	0.41	1.6

[1] Intake = ingested diet (offered diet – orts)

[2] pdNDF = potentially digestible NDF

[3] iNDF = Indigestible NDF

[4] k_p = passage rate

The predictor variables for each model were selected by fitting linear mixed models that had all possible combinations of candidates' predictor variables. The linear mixed models were fitted using lmer function from lme4 package (version 1.1-17) of R (R Core Team), considering a random effect of study $s \overset{i.i.d}{\sim} N(0, \sigma^2_s)$ and $\varepsilon \overset{i.i.d}{\sim} N(0, \sigma^2_\varepsilon)$. The model that minimized the Akaike information criterion, corrected for small samples (AICc) (Sugiura, 1978), was selected as the best model for each response variable. The AICc was computed using the function AICc from MuMIn package (Barton, 2020) of R (R Core Team). We selected models that included or not included reticulorumen wet tissues and wet pool size because they can only be recorded in slaughtered animals or predicted by models (Munn et al., 2015; Andrade et al., 2020). Variance inflation factor (VIF) was also computed using vif function from car package (Fox et al., 2020) of R (R Core Team), and all the selected models had VIF smaller than 10.

Model evaluation

The magnitude of the predictor variables in the models was used to determine the ranking of the predictor variables according to their sensitivity. This was performed by fitting the models with the lowest AICc, however, the variables were standardized to remove dimensional effects (Kutner et al., 2004). Correlation transformation was used to standardize the variables (Kutner et al., 2004), as follow Eq. [6 and 7]:

$$Y^{*} = \frac{1}{\sqrt{n-1}} \left(\frac{Y - \widehat{Y}}{SD_{Y}}\right) \qquad [6]$$

$$X_{ik}^{*} = \frac{1}{\sqrt{n-1}} \left(\frac{X_{ik} - \widehat{X_{k}}}{SD_{X_{k}}}\right) \qquad [7]$$

where, Y^{*} and X_{ik}^{*} are the respective standardized Y and X_k variables, $\widehat{Y}$ and $\widehat{X_k}$ are the respective means of the Y and X_k variables, and SD_Y and SD_{X_k} are the respective standard deviation of the Y and X_k variables.

Additionally, the predictive power of the selected models for each reticulorumen k_p was assessed using K-fold cross-validation using a function we coded. The data set was randomly folded a thousand times and in each time four folds were created with the same number of observations. The mean square prediction error (MSPE; defined as the averaged squared differences between observations and predictions from the fixed effect part of the model only) was used to decompose the uncertainty of prediction into error associated with mean bias, error due to systematic bias, and error caused by random errors. The positive square root of MSPE (RMSPE) was used as an indicator of the average uncertainty of prediction (accuracy). Lin's concordance correlation coefficient (CCC) was used as a measure of goodness of fit and agreement between observations and predictions (Lin, 1989). The coefficient of determination (R^2) was used as an indicator of precision of predictions by factors evaluated in the studies used in the data set (i.e. growing goats and goats fed with feeding levels). The CCC was computed using the function epi.ccc from epiR package (Telmo Nunes et al., 2020) of R (R Core Team). The R^2 was computed using the function r.squaredGLMM from MuMIn package (Barton, 2020) of R (R Core Team). The MSPE, RMSPE, CCC, and R^2 were calculated for each created fold and at the end of the cross-validation procedure, they were averaged.

RESULTS

Model selection and development of models

For reticulorumen k_p of solutes, DM intake (kg/day), pdNDF intake level (g/kg BW), and reticulorumen wet pool size (kg) were selected as predictors variables by the stepwise selection procedure, regardless transformation (Table 3). However, when the models with reticulorumen wet tissues and wet pool size were not considered, the model with only DM intake level (g/kg BW) as predictor variable had the lowest AICc, regardless transformation (Table 3). The models of the untransformed k_p of solutes produced similar CCC, RMSPE, R^2, and residual homoscedastic variance than models of the transformed k_p of solutes (Table 3). Thus, the models of the untransformed k_p of solutes were selected for further evaluation.

Table 3. Selected models for reticulorumen passage rate of solutes (k_p Cr)

Full model

			Selected predictor variables by the stepwise selection procedure[1]						n	CCC[2]	RMSPE$_{/h}$[3]	RMSPE$_{ln(/h)}$	R^2[4]
	β_0		DMI $_{kg/day}$		pdNDFI $_{g/kg\ BW}$		RRwetPS $_{kg}$						
	Mean	SE	Mean	SE	Mean	SE	Mean	SE					
k_p Cr $_{/h}$	0.12	0.023	0.082	0.021	0.0091	0.0015	-0.025	0.0057	149	0.59	0.050	-	0.42
ln[5](k_p Cr $_{/h}$)	-2.20	0.14	0.78	0.13	0.055	0.010	-0.20	0.036	149	0.66	-	0.31	0.49

Simple model

			Selected predictor variables by the stepwise selection procedure[1]						
	β_0		DMI $_{g/kg\ BW}$						
	Mean	SE	Mean	SE					
k_p Cr $_{/h}$	0.027	0.017	0.0044	0.00059	149	0.47	0.053	-	0.30
ln(k_p Cr $_{/h}$)	-2.9	0.10	0.034	0.0036	149	0.56	-	0.34	0.39

[1] Parameters estimates: DMI = DM intake, pdNDFI = potentially digestible NDF intake, RRwetPS = reticulorumen wet pool size

[2] CCC = Lin's concordance correlation coefficient

[3] RMSPE = Root mean square prediction error

[4] R^2 = Coefficient of determination

[5] ln = natural logarithm

For reticulorumen k_p of particles, NDFI (kg/day), iNDFI:NDFI ratio, and reticulorumen wet tissues and wet pool size (kg) were selected as predictor variables by the stepwise selection procedure, regardless transformation (Table 4). However, when the models with reticulorumen wet tissues and wet pool size were not considered the model with NDF intake level (g/kg BW), iNDFI:NDFI ratio and k_p of solutes had the lowest AICc, regardless transformation (Table 4). The models of the untransformed k_p of particles produced similar CCC, RMSPE, R^2, and residual homoscedastic variance than models of the transformed k_p of particles (Table 4). Thus, the models of the untransformed k_p of particles were selected for further evaluation.

Model evaluation

According to sensitive analysis, pdNDF intake level (g/kg BW), reticulorumen wet pool size (kg), and DM intake (g/day) had a similar impact on the output of the model of k_p of solutes when reticulorumen wet pool size was in the model (Figure 2). For the model of k_p of particles (Figure 3), reticulorumen wet pool size (kg), NDF intake (kg/day), and iNDFI:NDFI ratio of ingested diet had similar and the greatest impact on the output of the model of k_p of particles when reticulorumen wet tissues and wet pool size were in the model. On the other hand, iNDFI:NDFI ratio of ingested diet and NDF intake level (g/kg BW) had similar and the greatest impact on the output of the model of k_p of particles when reticulorumen wet tissues and wet pool size were not in the model. The effect of k_p of solutes on the output of the model of k_p of particles was smaller than the other predictor variables.

Table 4. Selected models for reticulorumen passage rate of particles (k_p iNDF)

	Full model														
			Selected predictor variables by the stepwise selection procedure[1]								n	CCC[2]	RMSPE $_{/h}$ [3]	RMSPE $_{ln(/h)}$	R^2 [4]
	β_0		NDFI $_{kg/day}$		iNDFI:NDFI		RRwettissues $_{kg}$		RRwetPS $_{kg}$						
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE					
k_p iNDF $_{/h}$	0.019	0.0037	0.056	0.0090	0.063	0.012	-0.016	0.0070	-0.0051	0.00079	149	0.51	0.0064	-	0.34
ln[5](k_p iNDF $_{/h}$)	-4.0	0.13	2.2	0.3	2.3	0.4	-0.52	0.25	-0.19	0.03	149	0.54	-	0.23	0.37

	Simple model												
			Selected predictor variables by the stepwise selection procedure[1]										
	β_0		iNDFI:NDFI		NDFI $_{g/kg\ BW}$		k_p Cr $_{/h}$						
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	n	CCC	RMSPE $_{/h}$	RMSPE $_{ln(/h)}$	R^2
k_p iNDF $_{/h}$	0.0040	0.0063	0.036	0.013	0.00062	0.00027	0.028	0.011	149	0.20	0.0074	-	0.11
ln(k_p iNDF $_{/h}$)	-4.6	0.2	1.5	0.5	0.028	0.010	0.94	0.41	149	0.23	-	0.27	0.13

[1] Parameters estimates: NDFI = NDF intake, iNDFI:NDFI = the ratio between indigestible NDF and NDF of ingested diet, RRwettissues = reticulorumen wet tissues, RRwetPS = reticulorumen wet pool size, k_p Cr = reticulorumen k_p of solutes

[2] CCC = Lin's concordance correlation coefficient

[3] RMSPE = Root mean square prediction error

[4] R^2 = Coefficient of determination

[5] ln = natural logarithm

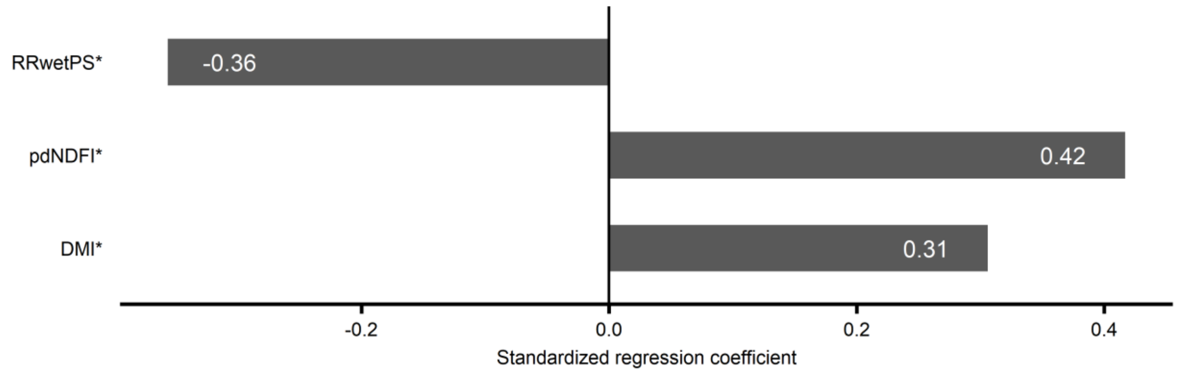


Figure 2. Standardized regression coefficients obtained from standardized developed model of reticulorumen k_p of solutes ($k_{p\ /h} = 0.12 \pm 0.023 + 0.082 \pm 0.021 \times DMI_{kg/day} + 0.0091 \pm 0.0015 \times pdNDFI_{g/kg\ BW} - 0.025 \pm 0.0057 \times RRwetPS_{kg}$). RRwetPS* = standardized reticulorumen wet pool size (kg), pdNDFI* = standardized potentially digestible NDF intake level (g/kg BW), DMI* = standardized DM intake (kg/day)

According to k-fold cross-validation, the model of k_p of solutes, when reticulorumen wet pool size was in the model, presented CCC of 0.57, the root of MSPE of 0.051 /h, and the decomposition of the MSEP indicated that 0.036 of this error was associated with mean bias, 0.031 was due to systematic bias, and 0.93 was caused by random errors (Figure 4). Moreover, the precision (R^2) of this model was higher for goats fed with feeding levels than growing goats (0.43 vs. 0.26, respectively). However, when reticulorumen wet pool size was not in the model of k_p of solutes presented CCC of 0.47, the root of MSPE of 0.054 /h, the decomposition of the MSEP indicated that 0.037 of this error was associated with mean bias, 0.029 was due to systematic bias, and 0.93 was caused by random errors (Figure 4). Moreover, the precision (R^2) of this model was higher for goats fed with feeding levels than growing goats (0.30 vs. 0.15, respectively).

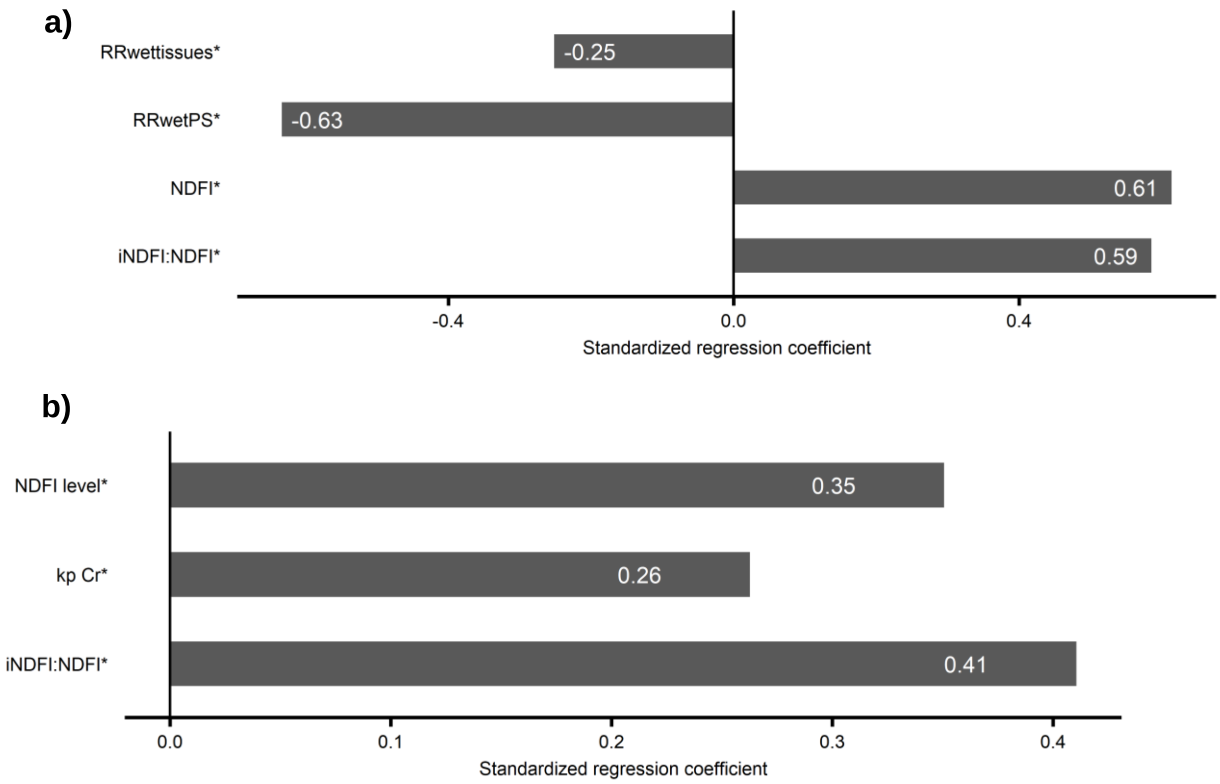


Figure 3. Standardized regression coefficients obtained from standardized developed models of reticulorumen k_p of particles (a: $k_{p\ /h}$ = 0.019 ± 0.0037 + 0.056 ± 0.0090 × $NDFI_{kg/day}$ + 0.063 ± 0.012 × iNDFI:NDFI - 0.016 ± 0.0070 × $RRwettissues_{kg}$ – 0.0051± 0.00079 × $RRwetPS_{kg}$; b: $k_{p\ /h}$ = 0.0040 ± 0.0063 + 0.036 ± 0.013 × iNDFI:NDFI + 0.00062 ± 0.00027 × $NDFI_{g/kg\ BW}$ + 0.028 ± 0.011 × k_p $Cr_{/h}$). RRwettissues* = standardized reticulorumen wet tissues (kg), RRwetPS* = standardized eticulorumen wet pool size (kg), NDFI* = standardized NDF intake (kg/day), iNDFI:NDFI* = standardized ratio between indigestible NDF and NDF of ingested diet, NDFI level* = standardized NDF intake level (g/kg BW), kp Cr* = standardized passage rate of solutes

For the model of k_p of particles, when reticulorumen wet tissues and wet pool size were in the model, the model presented CCC of 0.48, the root of MSPE of 0.0067 /h, and the decomposition of the MSEP indicated that 0.036 of this error was associated with mean bias, 0.040 was due to systematic bias, and 0.92 was caused by random errors (Figure 5). Moreover, the precision of this model (R^2) was similar between growing goats and goats fed with feeding levels (0.32 vs. 0.31, respectively). However, when reticulorumen wet tissues and wet pool size were not in the model of k_p of particles presented CCC of 0.17, the root of MSPE of 0.0076 /h, and the decomposition of the MSEP indicated that 0.038 of this error was associated with mean bias, 0.049 was due to systematic bias, and 0.91 was caused by random errors (Figure 5). Moreover, the precision (R^2) of this model was higher for goats fed with feeding levels than growing goats (0.13 vs. 0.07, respectively).

DISCUSSION

Model development

Empirical models for predicting reticulorumen k_p of particles and solutes were developed using a database with growing goats, from 15 to 45 kg BW, fed with feeding levels (ad libitum, 75%, or 50% of ad libitum). Reticulorumen k_p of particles and solutes demonstrated dependent on intake (kg/day) when capacity was also accounted in the models. Intake and capacity have been related to k_p in ruminants (Clauss et al., 2007a; Gindri et al., 2020). Moreover, when capacity was not in the models just intake level (g/kg BW) was selected as predictor variables. This indicates intake level may summarize intake and capacity into one variable. These results were expected because of the presence of growing goats in our database. Studies have demonstrated intake and capacity increase with ageing, however, they increase at a different rate because of their relationship to maintenance energy requirements and tissues size (Andrade et al., 2020; Gindri et al., 2020). Therefore, our results support reticulorumen k_p is modulated by intake and capacity in growing goats or they can also be summarized by intake level.

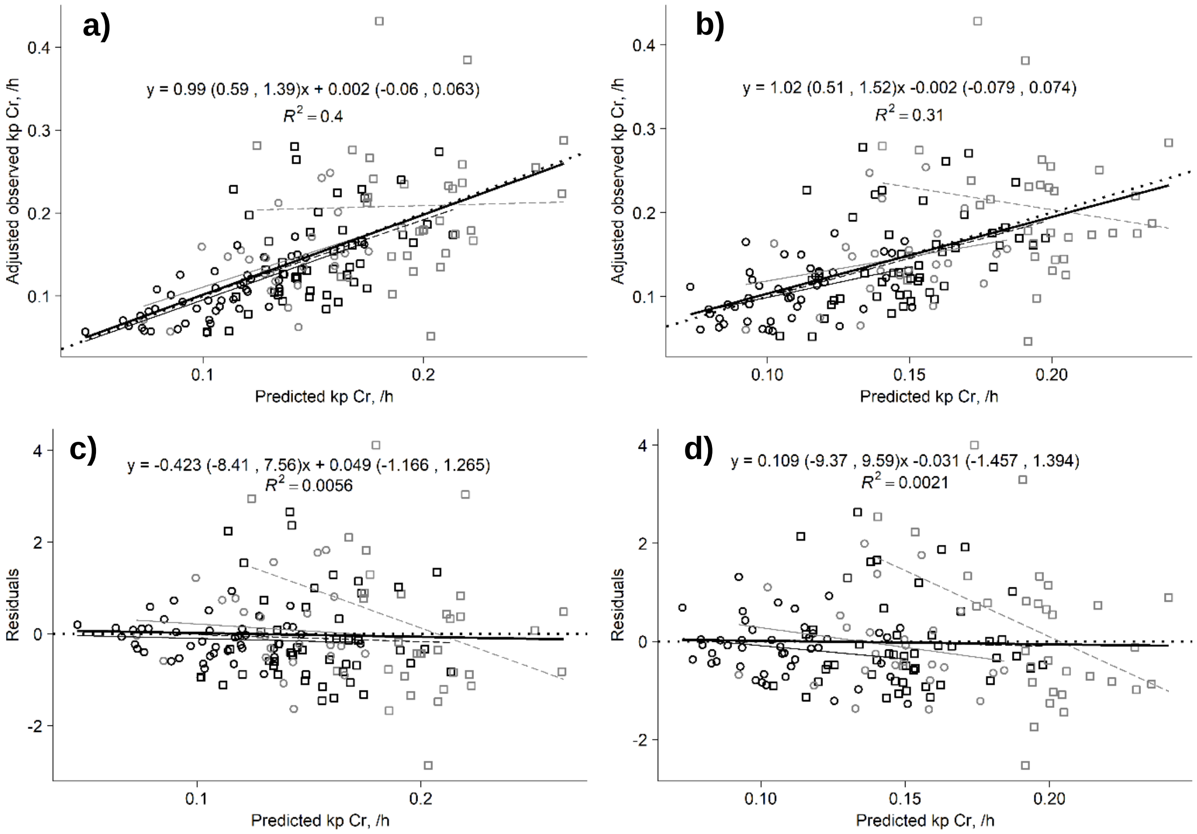


Figure 4. Cross-validation results. Regression between adjusted observed reticulorumen passage rate (k_p) of solutes, in the top part of the graphics (a and b), or studentized residuals, in the bottom part of the graphics (c and d), and predicted k_p of solutes using the developed models (a and c: $k_{p\ /h} = 0.12 \pm 0.023 + 0.082 \pm 0.021 \times DMI_{kg/day} + 0.0091 \pm 0.0015 \times pdNDFI_{g/kg\ BW} - 0.025 \pm 0.0057 \times RRwetPS_{kg}$; b and d: $k_{p\ /h} = 0.027 \pm 0.017 + 0.0044 \pm 0.00059 \times DMI_{g/kg\ BW}$). The grey dashed thin line and grey block are goats fed ad libitum and slaughtered at around 15, 22, and 30 kg BW from study Leite et al. (2015b), and the grey solid thin line and grey circle are goats fed with feeding levels from study Leite et al. (2015a). The black dashed thin line and black block are goats fed ad libitum and slaughtered at around 30, 37, and 45 kg BW from study Silva (2013), and the black solid thin line and black circle are goats fed with feeding levels from study Silva (2013). The black thick solid line is the regression line. The black thick dotted line is the Y=X line.

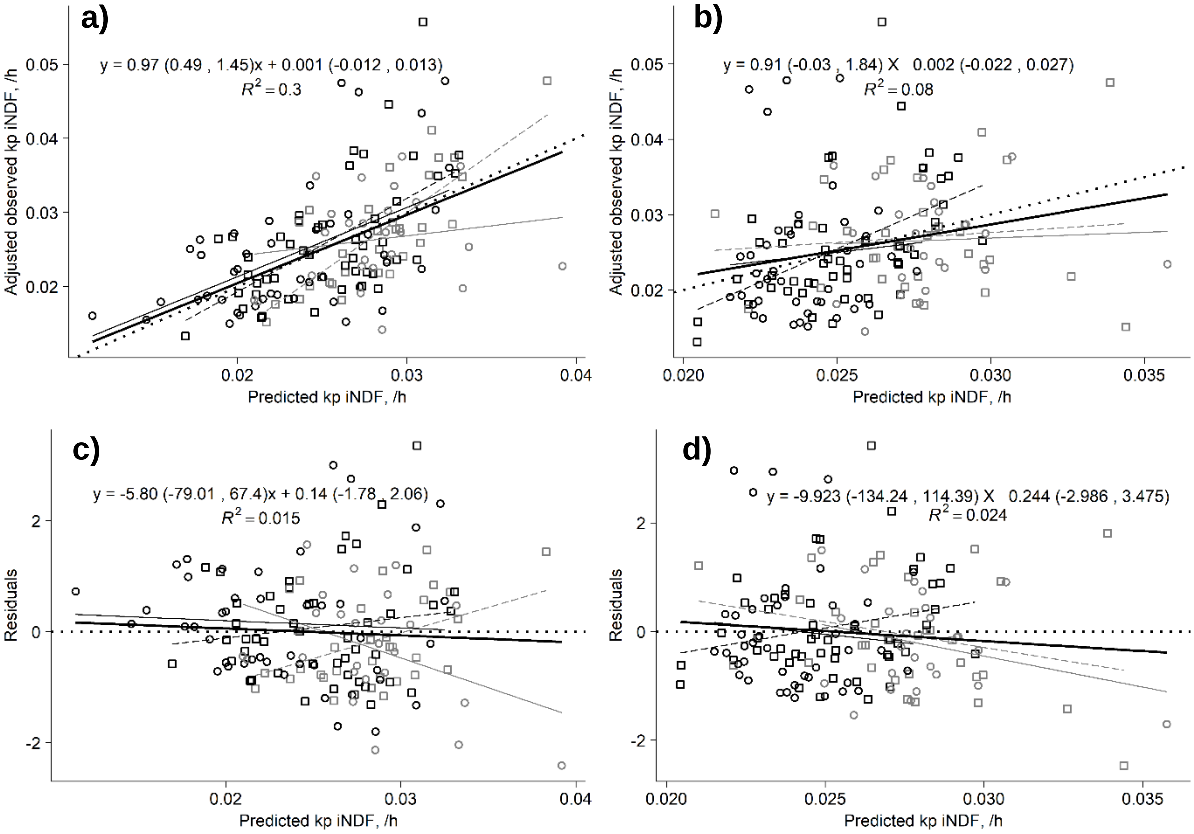


Figure 5. Cross-validation results. Regression between adjusted observed reticulorumen passage rate (k_p) of particles, in the top part of the graphics (a and b), or studentized residuals, in the bottom part of the graphics (c and d), and predicted k_p of particles using the developed models (a and c: $k_{p\ /h}$ = 0.019 ± 0.0037 + 0.056 ± 0.0090 × $NDFI_{kg/day}$ + 0.063 ± 0.012 × iNDFI:NDFI - 0.016 ± 0.0070 × $RRwettissues_{kg}$ – 0.0051± 0.00079 × $RRwetPS_{kg}$; b and d: $k_{p\ /h}$ = 0.0040 ± 0.0063 + 0.036 ± 0.013 × iNDFI:NDFI + 0.00062 ± 0.00027 × $NDFI_{g/kg\ BW}$ + 0.028 ± 0.011 × k_p $Cr_{/h}$). The grey dashed thin line and grey block are goats fed ad libitum and slaughtered at around 15, 22, and 30 kg BW from study Leite et al. (2015b), and the grey solid thin line and grey circle are goats fed with feeding levels from study Leite et al. (2015a). The black dashed thin line and black block are goats fed ad libitum and slaughtered at around 30, 37, and 45 kg BW from study Silva (2013), and the black solid thin line and black circle are goats fed with feeding levels from study Silva (2013). The black thick solid line is the regression line. The black thick dotted line is the Y=X line.

Additionally, the effect of ageing in reticulorumen k_p has been related to chewing investment because young ruminants chew more per kg of ingested diet than old ruminants (Grandl et al., 2018). Chewing investment would affect the reticulorumen k_p of particles and solutes by increasing saliva secretion and comminution (Seo et al., 2007). Comminution is important on reticulorumen k_p of particles because it is related to changes on particles functional specific gravity, that is considered one of the most important factors controlling particle sedimentation to the escapable pool at the floor of the ventral rumen (Seo et al., 2009). However, chewing investment was not selected as a predictor variable for none of the selected models. This probably happened because chewing investment is also affected by particles resistance to comminution, that is related to the content of NDF in the diet and physical and chemical properties of the NDF, such as tissue origin, shape and size, maturity stage, and forage species (Jung and Allen, 1995). And, our database was not composed by diets with different ingredients and forage species.

On the other hand, recent studies have demonstrated that particles functional specific gravity is mostly affected by diet chemical composition than comminution (Seo et al., 2009). The models proposed in this study had as predictor variables iNDFI:NDFI ratio of the ingested diet and NDF intake level, demonstrating k_p is very responsive to diet composition even in a database in which goats were not fed with diets of different ingredients and forage species. Diets composed of particles with a high content of iNDF have been related to fast reticulorumen k_p of particles (Krizsan et al., 2010). This happens because these particles have reduced fermentation activity, and consequently high functional specific gravity (Huhtanen et al., 2006). Moreover, NDF concentration has been positively related to particles resistance to comminution

(PÉREZ-BARBERIA, 1998). However, our models demonstrated that a diet with high iNDF concentration produces fast escape of particles from reticulorumen, regardless of NDF intake. Therefore, our models confirm diet composition is extremely related to reticulorumen k_p.

According to Seo et al. (2009), changes on functional specific gravity is not the only step for the scape of particles from reticulorumen. As soon as the particles reach the escapable pool in the ventral rumen, they depend on the flow of solutes out of reticulorumen to be carried out. This was demonstrated in our model by the presence of reticulorumen k_p of solutes in the model of reticulorumen k_p of particles. Therefore, our proposed models for reticulorumen k_p of particles work in the two steps of scape of particles out of reticulorumen, particle sedimentation and escape out of reticulorumen.

Despite the expected differences among sex on reticulorumen k_p, for none of the selected models, sex was considered as a predictor variable. One of our hypothesis about sex affecting reticulorumen k_p was that differences among sex on intake but not on reticulorumen capacity (Gindri et al., 2020) would affect the reticulorumen k_p. However, this was not observed in this study probably because intake and capacity were in the models, or also because intake level was in the models, and intake level is less affected by sex than intake (Gindri et al., 2020). Moreover, we also expected sex effect on reticulorumen k_p because females' goats chew 50% more per kilogram of ingested diet than males' goats because of their 15% smaller molar surface area (Gross et al., 1996). However, most of our database is composed of goats fed ad libitum, consequently, our goats had the opportunity of diet searching. Moreover, females search diet with higher quality than males for compensating their chew's inefficiency (Gross et al., 1996) and diet composition was selected as predictor variables in our models. Thus, our models suggest that diet searching compensated for differences in chewing efficiency among sex avoiding sex effect on reticulorumen k_p of particles and solutes.

Model evaluation

Our proposed model for predicting k_p of particles with reticulorumen wet tissues and wet pool size as predictor variables presented much better accuracy and precision (CCC = 0.48; RMSPE = 0.0066 /h; RMSPEp (RMSPE as a proportion of independent variable mean) = 25.43 %) than other empirical models in the literature (Cannas and Soest, 2000; Tedeschi et al., 2012; Regadas Filho et al., 2014). However, they presented similar accuracy and precision when compared to our model for predicting reticulorumen k_p of particles without reticulorumen wet tissues and wet pool size (CCC = 0.17; RMSPE = 0.0076 /h; RMSPEp = 29.55%). The model proposed by Cannas and Soest (2000) and improved by Tedeschi et al. (2012), presented RMSPE = 0.00625/h (RMSPEp ≈ 27.4%) and CCC = 0.39. The model proposed by Tedeschi et al., (2012) presented RMSPE = 0.0122/h (RMSPEp ≈ 53.55%) and CCC = 0.22. The proposed model by Regadas Filho et al. (2014), also presented values of RMSPE higher than that observed in our models (RMSPE = 0.024/h/ RMSPEp ≈ 31.6%). Additionally, both literature's models, described above, included diet composition, as included in our models, and feed intake level. However, reticulorumen k_p of solutes was included in our models for predicting reticulorumen k_p of particles, and it may have contributed to improve the accuracy and precision of our models. The reticulorumen k_p of solutes was also accounted in mechanistic model for predicting reticulorumen k_p of particles proposed by Seo et al. (2009). This model presented RMSPE = 0.0090/h (RMSPEp ≈ 19.3%), that is better than the models presented above.

Additionally, due to the lack of models for prediction k_p of solutes for goats, the empirical model for predicting reticulorumen k_p of solutes proposed by Seo et al. (2006) for dairy cattle presented RMSPE = 0.0326/h (RMSPEp ≈ 31.0%), that is similar to our models for predicting reticulorumen k_p of solutes for goats (CCC = 0.57 and 0.47; RMSPE = 0.051 and 0.054 /h; RMSPEp = 34.43 and 36.58%). The model proposed by Seo et al. (2006) was considered suitable for predicting reticulorumen k_p of solutes in dairy cattle. Moreover, Seo et al. (2006), evaluating prediction behaviour of previous models for predicting k_p of solutes in cattle and sheep, demonstrated the model with the lowest RMSPE had RMSPE of 0.0340/h and RMSPEp ≈ 32.4% (Evans 1981), that is similar to that presented by our models. Moreover, the mechanistic model, developed by Seo et al. (2007) for predicting reticulorumen k_p of solutes of dairy cows, presented quite better behaviour (RMSPE = 0.013/h; RMSPEp ≈ 10.1%) than the empirical models presented by Seo et al. (2006) and our model.

CONCLUSIONS

The proposed empirical models demonstrated intake and capacity are important in predicting reticulorumen k_p, and they could be summarized by intake level. Moreover, our proposed models demonstrated the importance of diet composition and k_p of solutes on predicting k_p of particles. Despite our proposed models were built with database compose by growing goats fed with similar diets, the predictor variables of the models suggest the models can be used with different diets. However, to confirm this, the ability of the models to predict k_p of goats fed with different diets must be further evaluated.

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CHAPTER 5 – Implications

The knowledge about digesta passage rate in goats is essential to formulate diets and we focused on evaluating the effect of growth, feeding level, and sex on digesta passage rate of reticulorumen, omasum, abomasum, small intestine, cecum, and colon-rectum segments of Saanen goats. The main contribution of the results obtained in this study is that passage rate of particles and solutes are not constant in gastrointestinal tract segments of growing goats and goats fed with feeding levels over long-term and that they are mostly regulated by intake and capacity.

Regarding growth, we found reticulorumen passage rate of solutes is high in young goats and decreases with aging, however, passage rate of particles is not affected by aging. We found passage rate of particles and solutes in total gastrointestinal tract is regulated by intake and capacity in growing goats and passage rate of solutes in reticulorumen is also regulated by chewing investment.

Regarding feeding levels, we found passage rate of solutes in all evaluated gastrointestinal tract sections increases with feeding levels, regardless of capacity. Passage rate of particles is constant in stomachs of goats fed with feeding levels over long-term but intestines.

Regarding sex, it was unclear and must be further investigated in growing goats and goats fed with feeding levels.

Regarding the developed models and the used statistic techniques, reticulorumen passage rate of solutes demonstrated to be more dependent on feed intake and capacity or feed intake level than reticulorumen passage rate of particles, that demonstrated to be more dependent on diet composition and passage rate of solutes. Models are a great tool for understanding biological processes. Multivariate factor analysis is very useful for understanding factors related to biological processes and must be used before the development of biological models.

APPENDIX

APPENDIX I

Chapter 3 – R code

The code below was used for analyzing the data used in the third chapter of this dissertation. The steps are import data, data imputation, adjust for study effect, factor analysis, and ANOVA analysis.

Used library

```
library(ggplot2) #plot

library(psych) #multivariate analysis

library("PerformanceAnalytics") #chart.correlation

library(lme4) # fit models

library(missMDA)  #imputation

library(car)   #anova linear non-mix models

library(corrplot)  #correlation matrix

library(lmerTest) #anova for linear mix models

library(lsmeans)  #lsmeans

library(keep)   #subset
library(dplyr) #mutate

library(bibtex)
```

Import data

```
setwd("C:/")
Full_database_g <- read.table("Database.txt")
```

PCA dataset

```
PCA_Dataset <- subset(Full_database_g, select = c(Study, Animal, Experiment, Feed_restriction, Sex,  BW_ref, Block,
BW,  DMI_g_kg_BW,  OMI_conc ,NDFI_conc,  pdNDFI_conc, NDF_digestibility, Chew_g_DMI, Reticulo_rumen__Wet_tissues_g, Reticulo_rumen__Wet_pool_size_g,  Reticulo_rumen_DM_conc,  kp_iNDF_RR,  Kp_RR_yb,  Kp_RR_Cr,  RR_pH, RR_lesser_150_mic_perc, RR_150_mic_perc,RR_350_mic_perc,RR_600_mic_perc,RR_1.18_mm_perc,RR_2.36_mm_perc, Omasum__Wet_tissues_g,  Omasum__Wet_pool_size_g,  Omasum_DM_conc,  O_Kp_Cr, Abomasum__Wet_tissues_g,  Abomasum__Wet_pool_size_g,  Abomasum_DM_conc,  Kp_Ab_Cr, Small_intestine__Wet_tissues_g,  Small_intestine__Wet_pool_size_g,  Small_intestine_DM_conc,  Kp_SI_Cr, Cecum__Wet_tissues_g,  Cecum__Wet_pool_size_g,  Cecum_DM_conc,  kp_iNDF_Ce,
```

```
Kp_Ce_Cr, Colon_rectum__Wet_tissues_g, Colon_rectum__Wet_pool_size_g, Colon_rectum_DM_conc, kp_iNDF_CR, Kp_CR_Cr ))
```

Impute dataset with PCA

```
PCA_Dataset_Impute <- imputePCA(PCA_Dataset[,-(1:7)], ncp=8, scale = TRUE)

PCA_Dataset_Imputed <- cbind.data.frame(PCA_Dataset[,(1:7)],PCA_Dataset_Impute$completeObs)
```

Split dataset

```
PCA_Dataset_adjusted_Experiment_1 <- subset(PCA_Dataset_Imputed, Feed_restriction=="0")
PCA_Dataset_adjusted_Experiment_2 <- subset(PCA_Dataset_Imputed, Experiment=="2")
```

Adjust for study effect - Experiment growth

```
data = PCA_Dataset_adjusted_Experiment_1

for (i in 8:ncol(data)){
  names <- colnames(data)
  col_plot <- data[,i]
  Mean <- aggregate(col_plot ~ Sex*BW_ref*Study, data = data, FUN = mean)
  Mean <- ggplot(Mean, aes(x = as.numeric(BW_ref), y = col_plot, color=Sex, shape=Study)) +
  ylab(names[i]) +   theme_minimal(base_size = 14) +
  geom_point(size = 3, alpha = 0.5, aes(color = Sex)) + geom_line(aes(linetype=Sex), size=1) +
  scale_x_discrete(name = "Body weight, kg", limits = c("15","22","30", "37","45"))

  dat <- data
  id <- dat[,c(1:2)]
  col <- dat[,i]
  model <- lmer(col ~ Sex*BW_ref + (1 |Study), data=dat)

  pred <- data.frame(predict(model, newdata=dat, re.form=NA))
  pred <- cbind(id, pred)

  res <- data.frame(residuals (model))
  res_id <- data.frame(id, dat[,i])
  res_id <- na.omit(res_id)
  res <- cbind (res_id[,-3], res)

  adj <- merge(pred, res, by = c("Study","Animal"), all = TRUE)

  adj$adj <- adj[,3] + adj[,4]

  adj <- adj[order(adj$"Study",adj$"Animal"),]
```

```
  data <- data[order(data$"Study",data$"Animal"),]

  data[,i] <- adj$adj

  col_plot <- data[,i]
  Mean_Yadj<- aggregate(col_plot ~ Sex*BW_ref*Study, data = data, FUN = mean)
Mean_Yadj <- ggplot(Mean_Yadj, aes(x = as.numeric(BW_ref), y = col_plot,color=Sex, shape=Study)) +
  ylab(names[i]) +   theme_minimal(base_size = 14) +
  geom_point(size = 3, alpha = 0.5, aes(color = Sex)) + geom_line(aes(linetype=Sex), size=1) +
  scale_x_discrete(name = "Body weight, kg", limits = c("15","22","30", "37","45"))
plot(Mean)
plot(Mean_Yadj)
}

PCA_Dataset_adjusted_Experiment_1 <- data
```

Adjust for study effect - Experiment feeding level

```
data = PCA_Dataset_adjusted_Experiment_2

for (i in 8:ncol(data)){
  names <- colnames(data)
  col_plot <- data[,i]
  Mean <- aggregate(col_plot ~ Sex*BW_ref*Feed_restriction*Study, data = data, FUN = mean)
  Mean <- ggplot(Mean, aes(x = as.numeric(BW_ref), y = col_plot, color=Sex, shape=Feed_restriction)) +
  ylab(names[i]) +   theme_minimal(base_size = 14) +
  geom_point(size = 3, alpha = 0.5, aes(color = Sex)) + geom_line(aes(linetype=Sex), size=1) +
  scale_x_discrete(name = "Body weight, kg", limits = c("15","22","30", "37","45"))

  dat <- data
  id <- dat[,c(1:2)]
  col <- dat[,i]
  model <- lmer(col ~ Sex*Feed_restriction + (1|Study:Sex:Block) + (1 |Study), data=dat)

  pred <- data.frame(predict(model, newdata=dat, re.form=NA))
  pred <- cbind(id, pred)

  res <- data.frame(residuals (model))
  res_id <- data.frame(id, dat[,i])
  res_id <- na.omit(res_id)
  res <- cbind (res_id[,-3], res)
```

```
  adj <- merge(pred, res, by = c("Study","Animal"), all = TRUE)

  adj$adj <- adj[,3] + adj[,4]

  adj <- adj[order(adj$"Study",adj$"Animal"),]

  data <- data[order(data$"Study",data$"Animal"),]

  data[,i] <- adj$adj

  col_plot <- data[,i]
  Mean_Yadj<- aggregate(col_plot ~ Sex*BW_ref*Feed_restriction*Study, data = data, FUN = mean)
Mean_Yadj <- ggplot(Mean_Yadj, aes(x = as.numeric(BW_ref), y = col_plot,color=Sex, shape=Feed_restriction)) +
  ylab(names[i]) +   theme_minimal(base_size = 14) +
  geom_point(size = 3, alpha = 0.5, aes(color = Sex)) + geom_line(aes(linetype=Sex), size=1) +
  scale_x_discrete(name = "Body weight, kg", limits = c("15","22","30", "37","45"))
plot(Mean)
plot(Mean_Yadj)
}

PCA_Dataset_adjusted_Experiment_2 <- data
```

PCA experiment growth

- **Corr plot exp growth**

```
corr_plot_exp_1 <- PCA_Dataset_adjusted_Experiment_1[,-c(1:7)]

names(corr_plot_exp_1) <- c(
 "BW (kg)","DM intake level (g/kg BW)","OM, (% DM intake)", "NDF (% DM intake)","pdNDF (% DM intake)","Total NDF digestibility","Chews/g DMI",
  "RR Wet tissues (g)" , "RR Wet pool size (g)" , "RR DM (% wet pool size)" , "RR kp of iNDF (/h)" , "RR kp of Yb (/h)" , "RR kp of Cr (/h)", "RR pH",
  "RR PS < 0.15 mm (% DM)","RR PS 0.15 <> 0.35 mm (% DM)" , "RR PS 0.35 <> 0.60 mm (% DM)" , "RR PS 0.60 <> 1.18 mm (% DM)" , "RR PS 1.18 mm <> 2.36 mm (% DM)" , "RR PS 2.36 mm <> 4.75 mm (% DM)",
  "O Wet tissues (g)" , "O Wet pool size (g)" , "O DM (% wet pool size)" , "O kp of Cr (/h)",
  "Ab Wet tissues (g)" ,"Ab wet pool size (g)", "Ab DM (% wet pool size)" , "Ab kp of Cr (/h)",
  "SI Wet tissues (g)" , "SI Wet pool size (g)" , "SI DM (% wet pool size)" , "SI kp of Cr (/h)",
  "Ce Wet tissues (g)" , "Ce Wet pool size (g)" , "Ce DM (% wet pool size)" , "Ce kp of iNDF (/h)" , "Ce kp of Cr (/h)",
  "CR Wet tissues (g)" , "CR Wet pool size (g)" , "CR DM (% wet pool size)" , "CR kp of iNDF (/h)" , "CR kp of Cr (/h)")
```

```
corr_plot_exp_1 <- cor(corr_plot_exp_1)
tiff(file="corrplot_exp_1_adjust_full_model_diet_composition_2.tiff",
width=10, height=10, units="in", res=700)
corrplot(corr_plot_exp_1, method="color", tl.cex = 0.7, type="upper", addCoef.col = "black", number.cex = 0.5,
  number.font = 2, number.digits = 1,tl.pos = "td", tl.col="black", cl.cex=1, cl.ratio=0.1)
dev.off()
```

- **Correlation**

```
Data_correlation <- corr.test(PCA_Dataset_adjusted_Experiment_1[,-c(1:8)], use="pairwise", method= "pearson")
correlation <- as.data.frame(Data_correlation$r)
p_value <- as.data.frame(Data_correlation$p)
```

- **Teste de esfericidade de Bartlett**

```
cortest.bartlett(correlation, n = 103,diag=TRUE)
```

- **Medida de adequacidade da amostra de Kaiser-Meyer-Olkin (KMO)**

```
KMO(correlation)
```

- **Scree plot**

```
FA <- fa.parallel(correlation, n.obs=103, fm="ml", fa="both", show.legend=T, main="",
                  ylabel="Eigen values of principal components", plot= T)
```

- **Factor analysis**

```
Fatores <- fa(correlation, nfactors = 5, rotate = "varimax", fm="ml")
Fatores
```

- **I have deleted variables with loadings smaller than |0.3|**

```
write.table(Fatores$loadings,"FL.txt")
Loadings_FA <- read.table("FL.txt")
PCA_Dataset_Imputed_t <- data.frame(t(PCA_Dataset_adjusted_Experiment_1[,-c(1:8)]))
PCA_Dataset_Imputed_t$x <- 1:41   #change dimension
Loadings_FA_ <- (Loadings_FA)
Loadings_FA_$x <- 1:41 #change dimension
Loadings_FA_[(Loadings_FA_ >= - 0.294 & Loadings_FA_ <= 0.294)] <- 0
Loadings_FA_$na <- rowSums(Loadings_FA_[,c(1:5)])#change dimension
Loadings_FA_ <- subset(Loadings_FA_, na!=0)
Loadings_FA_ <- Loadings_FA_[,-7]  #dois numeros a mais em relação ao numero de fatores
PCA_Dataset_Imputed_load = merge(PCA_Dataset_Imputed_t, Loadings_FA_, by = c("x"), all = TRUE)
PCA_Dataset_Imputed_load <- na.omit(PCA_Dataset_Imputed_load)
rownames(PCA_Dataset_Imputed_load) <- rownames(Loadings_FA_)
PCA_Dataset_Imputed_load <- t(PCA_Dataset_Imputed_load[,c(2:104)])
```

- **Correlation after variables deletion**

```
Data_correlation <- corr.test(PCA_Dataset_Imputed_load, use="pairwise", method=
"pearson")
correlation <- as.data.frame(Data_correlation$r)
p_value <- as.data.frame(Data_correlation$p)
```

- **Kaiser-Meyer-Olkin (KMO) test**

```
KMO(correlation)
```

- **Scree plot after variables deletion**

```
FA <- fa.parallel(correlation, n.obs=103, fm="ml", fa="both", show.legend=T, main=""
,
                ylabel="Eigen values of principal components", plot= T)
```

- **Factor analysis after variables deletion**

```
Fatores <- fa(correlation, nfactors = 5, rotate = "varimax", fm="ml")
Fatores
```

- **I have deleted variables with loadings smaller than |0.3|**

```
write.table(Fatores$loadings,"FL.txt")
Loadings_FA <- read.table("FL.txt")
PCA_Dataset_Imputed_t <- data.frame(t(PCA_Dataset_Imputed_load))
PCA_Dataset_Imputed_t$x <- 1:37   #change dimension
Loadings_FA_ <- (Loadings_FA)
Loadings_FA_$x <- 1:37 #change dimension
Loadings_FA_[(Loadings_FA_ >= - 0.294 & Loadings_FA_ <= 0.294)] <- 0
Loadings_FA_$na <- rowSums(Loadings_FA_[,c(1:5)])#change dimension
Loadings_FA_ <- subset(Loadings_FA_, na!=0)
Loadings_FA_ <- Loadings_FA_[,-7]  #dois numeros a mais em relação ao numero d
e fatores
PCA_Dataset_Imputed_load = merge(PCA_Dataset_Imputed_t, Loadings_FA_, by =
c("x"), all = TRUE)
PCA_Dataset_Imputed_load <- na.omit(PCA_Dataset_Imputed_load)
rownames(PCA_Dataset_Imputed_load) <- rownames(Loadings_FA_)
PCA_Dataset_Imputed_load <- t(PCA_Dataset_Imputed_load[,c(2:104)])
```

- **Correlation after variables deletion**

```
Data_correlation <- corr.test(PCA_Dataset_Imputed_load, use="pairwise", method=
"pearson")
correlation <- as.data.frame(Data_correlation$r)
p_value <- as.data.frame(Data_correlation$p)
```

- **Bartlett test**

```
cortest.bartlett(correlation, n = 103,diag=TRUE)
```

- **Medida de adequacidade da amostra de Kaiser-Meyer-Olkin (KMO)**

```
KMO(correlation)
```

- **Scree plot after variables deletion**

```
FA <- fa.parallel(correlation, n.obs=103, fm="ml", fa="both", show.legend=T, main=""
,
                  ylabel="Eigen values of principal components", plot= T)
```

- **Factor analysis after variables deletion**

```
Fatores <- fa(correlation, nfactors = 5, rotate = "varimax", fm="ml")
Fatores

write.table(cbind(Fatores$loadings,Fatores$communality),"FL_BW.txt")
write.table(cbind(FA$pc.values, FA$pc.sim), "e.values_BW.txt")
Scores <- fa(PCA_Dataset_Imputed_load, nfactors = 5, rotate = "varimax", fm="ml")
write.table(Scores$scores,"Scores_BW.txt")
```

- **ANOVA**

```
Scores <- read.table("Scores_BW.txt")
ANOVA_data <- cbind(PCA_Dataset_adjusted_Experiment_1[,c(1:7)], Scores)
names(ANOVA_data)[8:12] <- c("F1", "F2", "F3","F4","F5")
for(i in 1:7){
ANOVA_data[,i] = as.factor(ANOVA_data[,i])
}
write.table(ANOVA_data,"ANOVA_data_BW.txt")


#t(contr.poly(3,c(0, 25, 50)))
t(contr.poly(5,c(15, 22, 30, 37, 45)))

#F1
F1 <- lm(F1 ~ Sex*BW_ref , data = ANOVA_data, contrasts=list(Sex="contr.sum",B
W_ref="contr.sum"))
summary(F1)

BW_cont <- lsmeans(F1, ~ BW_ref,  lmer.df = "satterth")

contrast(BW_cont,
       list("Linear"=c(-0.6238565, -0.3287892,  0.008430493,  0.3034977, 0.6407174)
,
           "Quadratic"=c(0.5266992, -0.2344936, -0.534596700, -0.2985844, 0.54097
54),
           "Cubic"=c(-0.3411849,  0.6468316,  0.019286943, -0.6163605, 0.2914268),
           "^4"=c(0.1303223, -0.4674605,  0.716772843, -0.4887088, 0.1090741)))

Sex_cont <- lsmeans(F1, ~ Sex,  lmer.df = "satterth")

pairs(Sex_cont, adjust="none")

F1_Mean<- aggregate(F1 ~ BW_ref, data = ANOVA_data, FUN = mean)

F1_plot_BW <- ggplot(NULL, aes(x = as.numeric(BW_ref), y = F1)) +
     geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.leg
end = FALSE) +
```

```
     geom_line(data = F1_Mean,size=1,show.legend = FALSE, alpha=1)+
     ylab("Scores Fa 1") +
     geom_point(data = F1_Mean, size = 6, alpha = 1, show.legend = FALSE) +
  theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
  axis.text.x = element_text(size=28),
  axis.text.y = element_text(size=28),
  axis.title.x=element_text(size=28),
  axis.title.y=element_text(size=28)
  )+
    scale_x_discrete(name = "Body weight (kg)", limits = c("15","22","30", "37","45"))
+
  scale_color_manual(values=c("green4","red", "blue"))

tiff(file="F1_plot_BW.tiff",
width=10, height=7, units="in", res=700)
plot(F1_plot_BW)
dev.off()

#F2
F2 <- lm(F2 ~ Sex*BW_ref , data = ANOVA_data, contrasts=list(Sex="contr.sum",B
W_ref="contr.sum"))
summary(F2)

Anova(F2, type="III")

BW_cont <- lsmeans(F2, ~ BW_ref,  lmer.df = "satterth")

contrast(BW_cont,
        list("Linear"=c(-0.6238565, -0.3287892,  0.008430493,  0.3034977, 0.6407174)
,
            "Quadratic"=c(0.5266992, -0.2344936, -0.534596700, -0.2985844, 0.54097
54),
            "Cubic"=c(-0.3411849,  0.6468316,  0.019286943, -0.6163605, 0.2914268),
            "^4"=c(0.1303223, -0.4674605,  0.716772843, -0.4887088, 0.1090741)))

F2_Mean<- aggregate(F2 ~ BW_ref, data = ANOVA_data, FUN = mean)

F2_plot_BW <- ggplot(NULL, aes(x = as.numeric(BW_ref), y = F2)) +
     geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.leg
end = FALSE) +
     geom_line(data = F2_Mean, size=1,show.legend = FALSE)+
     ylab("Scores Fa 2") +
     geom_point(data = F2_Mean, size = 6, alpha = 1, show.legend = FALSE) +
  theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
  axis.text.x = element_text(size=28),
  axis.text.y = element_text(size=28),
```

```
  axis.title.x=element_text(size=28),
  axis.title.y=element_text(size=28)
  )+
    scale_x_discrete(name = "Body weight (kg)", limits = c("15","22","30", "37","45"))
+
  scale_color_manual(values=c("green4","red", "blue"))

tiff(file="F2_plot_BW.tiff",
width=10, height=7, units="in", res=700)
plot(F2_plot_BW)
dev.off()

#F3
F3 <- lm(F3 ~ Sex*BW_ref , data = ANOVA_data, contrasts=list(Sex="contr.sum",B
W_ref="contr.sum"))
summary(F3)

Anova(F3, type="III")

BW_cont <- lsmeans(F3, ~ BW_ref,  lmer.df = "satterth")

contrast(BW_cont,
         list("Linear"=c(-0.6238565, -0.3287892,  0.008430493,  0.3034977, 0.6407174)
,
              "Quadratic"=c(0.5266992, -0.2344936, -0.534596700, -0.2985844, 0.54097
54),
              "Cubic"=c(-0.3411849,  0.6468316,  0.019286943, -0.6163605, 0.2914268),
              "^4"=c(0.1303223, -0.4674605,  0.716772843, -0.4887088, 0.1090741)))

F3_Mean<- aggregate(F3 ~ BW_ref, data = ANOVA_data, FUN = mean)

F3_plot_BW <- ggplot(NULL, aes(x = as.numeric(BW_ref), y = F3)) +
      geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.leg
end = FALSE) +
      geom_line(data = F3_Mean, size=1,show.legend = FALSE)+
      ylab("Scores Fa 3") +
      geom_point(data = F3_Mean, size = 6, alpha = 1,show.legend = FALSE) +
  theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
  axis.text.x = element_text(size=28),
  axis.text.y = element_text(size=28),
  axis.title.x=element_text(size=28),
  axis.title.y=element_text(size=28)
  )+
    scale_x_discrete(name = "Body weight (kg)", limits = c("15","22","30", "37","45"))
+
  scale_color_manual(values=c("green4","red", "blue"))

tiff(file="F3_plot_BW.tiff",
```

```
width=10, height=7, units="in", res=700)
plot(F3_plot_BW)
dev.off()

#F4
F4 <- lm(F4 ~ Sex*BW_ref , data = ANOVA_data, contrasts=list(Sex="contr.sum",BW_ref="contr.sum"))
summary(F4)

Anova(F4, type="III")

BW_cont <- lsmeans(F4, ~ BW_ref,  lmer.df = "satterth")

contrast(BW_cont,
       list("Linear"=c(-0.6238565, -0.3287892,  0.008430493,  0.3034977, 0.6407174),
           "Quadratic"=c(0.5266992, -0.2344936, -0.534596700, -0.2985844, 0.5409754),
           "Cubic"=c(-0.3411849,  0.6468316,  0.019286943, -0.6163605, 0.2914268),
           "^4"=c(0.1303223, -0.4674605,  0.716772843, -0.4887088, 0.1090741)))

Sexo_BW_cont <- lsmeans(F4, ~ Sex*BW_ref, lmer.df = "satterth")

cont_BW_Castrated <- contrast(Sexo_BW_cont,
       list("Linear"=c(-0.6238565, 0,0,-0.3287892, 0,0,  0.008430493, 0,0,  0.3034977, 0,0, 0.6407174,0,0),
           "Quadratic"=c(0.5266992,0,0, -0.2344936,0,0, -0.534596700,0,0, -0.2985844,0,0, 0.54097540,0,0),
           "Cubic"=c(-0.3411849,0,0,  0.6468316,0,0,  0.019286943,0,0, -0.6163605,0,0, 0.2914268,0,0),
           "^4"=c(0.1303223,0,0, -0.4674605,0,0,  0.716772843,0,0, -0.4887088,0,0, 0.1090741,0,0)))
cont_BW_Castrated

cont_BW_Females <- contrast(Sexo_BW_cont,
                        list("Linear"=c(0,-0.6238565,0,0,-0.3287892, 0,0,  0.008430493, 0,0,  0.3034977, 0,0, 0.6407174,0),
                            "Quadratic"=c(0,0.5266992,0,0, -0.2344936,0,0, -0.534596700,0,0, -0.2985844,0,0, 0.54097540,0),
                            "Cubic"=c(0,-0.3411849,0,0,  0.6468316,0,0,  0.019286943,0,0, -0.6163605,0,0, 0.2914268,0),
                            "^4"=c(0,0.1303223,0,0, -0.4674605,0,0,  0.716772843,0,0, -0.4887088,0,0, 0.1090741,0)))
cont_BW_Females

cont_BW_Intact <- contrast(Sexo_BW_cont,
                       list("Linear"=c(0,0,-0.6238565,0,0,-0.3287892, 0,0,  0.008430493, 0,0,  0.3034977, 0,0, 0.6407174),
                           "Quadratic"=c(0,0,0.5266992,0,0, -0.2344936,0,0, -0.534596700,0,0, -0.2985844,0,0, 0.54097540),
```

```
                          "Cubic"=c(0,0,-0.3411849,0,0,  0.6468316,0,0,  0.019286943,0, 0, -0.6163605,0,0, 0.2914268),
                          "^4"=c(0,0,0.1303223,0,0, -0.4674605,0,0,  0.716772843,0,0, -0.4887088,0,0, 0.1090741)))
cont_BW_Intact

pairs(Sexo_BW_cont, adjust="none")

F4_Mean<- aggregate(F4 ~ Sex*BW_ref, data = ANOVA_data, FUN = mean)

F4_plot_BW <- ggplot(NULL, aes(x = as.numeric(BW_ref), y = F4)) +
     geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.legend = FALSE) +
     geom_line(data = F4_Mean,aes(color = Sex),size=1,show.legend = FALSE)+
     ylab("Scores Fa 4") +
     geom_point(data = F4_Mean, size = 6, alpha = 1, aes(color = Sex),show.legend = FALSE) +
  theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
  axis.text.x = element_text(size=28),
  axis.text.y = element_text(size=28),
  axis.title.x=element_text(size=28),
  axis.title.y=element_text(size=28)
  )+
    scale_x_discrete(name = "Body weight (kg)", limits = c("15","22","30", "37","45")) +
  annotate(geom="text", x=c(0.8,0.8,0.8), y=c(1.4,-0.6,-0.8), label=c("a","b","b"),
            color=c("red","blue","green4"),size = 8) +
  scale_color_manual(values=c("green4","red", "blue"))

tiff(file="F4_plot_BW.tiff",
width=10, height=7, units="in", res=700)
plot(F4_plot_BW)
dev.off()

#F5
F5 <- lm(F5 ~ Sex*BW_ref , data = ANOVA_data, contrasts=list(Sex="contr.sum",BW_ref="contr.sum"))
summary(F5)

Anova(F5, type="III")

BW_cont <- lsmeans(F5, ~ BW_ref,  lmer.df = "satterth")

## NOTE: Results may be misleading due to involvement in interactions

contrast(BW_cont,
        list("Linear"=c(-0.6238565, -0.3287892,  0.008430493,  0.3034977, 0.6407174),
```

```
          "Quadratic"=c(0.5266992, -0.2344936, -0.534596700, -0.2985844, 0.5409754),
          "Cubic"=c(-0.3411849,  0.6468316,  0.019286943, -0.6163605, 0.2914268),
          "^4"=c(0.1303223, -0.4674605,  0.716772843, -0.4887088, 0.1090741)))

F5_Mean<- aggregate(F5 ~ BW_ref, data = ANOVA_data, FUN = mean)

F5_plot_BW <- ggplot(NULL, aes(x = as.numeric(BW_ref), y = F5)) +
    geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.legend = FALSE) +
    geom_line(data = F5_Mean, size=1,show.legend = FALSE)+
    ylab("Scores Fa 5") +
    geom_point(data = F5_Mean, size = 6, alpha = 1, show.legend = FALSE) +
 theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
 panel.grid.major = element_blank(),
 panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
 axis.text.x = element_text(size=28),
 axis.text.y = element_text(size=28),
 axis.title.x=element_text(size=28),
 axis.title.y=element_text(size=28)
 )+
  scale_x_discrete(name = "Body weight (kg)", limits = c("15","22","30", "37","45"))
+
 scale_color_manual(values=c("green4","red", "blue"))

tiff(file="F5_plot_BW.tiff",
width=10, height=7, units="in", res=700)
plot(F5_plot_BW)
dev.off()
```

PCA experiment feeding level

- **Corr plot exp feeding level**

```
corr_plot_exp_2 <- PCA_Dataset_adjusted_Experiment_2[,-c(1:7)]

names(corr_plot_exp_2) <- c(
 "BW (kg)","DM intake level (g/kg BW)","OM (% DM intake)", "NDF (% DM intake)","pdNDF (% DM intake)","Total NDF digestibility","Chews/g DMI",
 "RR Wet tissues (g)" , "RR Wet pool size (g)" , "RR DM (% wet pool size)" , "RR kp of iNDF (/h)" , "RR kp of Yb (/h)" , "RR kp of Cr (/h)", "RR pH",
 "RR PS < 0.15 mm (% DM)","RR PS 0.15 <> 0.35 mm (% DM)" , "RR PS 0.35 <> 0.60 mm (% DM)" , "RR PS 0.60 <> 1.18 mm (% DM)" , "RR PS 1.18 mm <> 2.36 mm (% DM)" , "RR PS 2.36 mm <> 4.75 mm (% DM)",
 "O Wet tissues (g)" , "O Wet pool size (g)" , "O DM (% wet pool size)" , "O kp of Cr (/h)",
 "Ab Wet tissues (g)" ,"Ab wet pool size (g)", "Ab DM (% wet pool size)" , "Ab kp of Cr (/h)",
 "SI Wet tissues (g)" , "SI Wet pool size (g)" , "SI DM (% wet pool size)" , "SI kp of Cr (/h)",
```

```
  "Ce Wet tissues (g)" , "Ce Wet pool size (g)" , "Ce DM (% wet pool size)" , "Ce kp of
iNDF (/h)" , "Ce kp of Cr (/h)",
  "CR Wet tissues (g)" , "CR Wet pool size (g)" , "CR DM (% wet pool size)" , "CR kp
of iNDF (/h)" , "CR kp of Cr (/h)")

corr_plot_exp_2 <- cor(corr_plot_exp_2)
tiff(file="corrplot_exp_2_adjust_full_model_diet_composition_2.tiff",
width=10, height=10, units="in", res=700)
corrplot(corr_plot_exp_2, method="color", tl.cex = 0.7, type="upper", addCoef.col = "
black", number.cex = 0.5,
  number.font = 2, number.digits = 1,tl.pos = "td", tl.col="black", cl.cex=1, cl.ratio=0.1)
dev.off()
```

- **Correlation**

```
Data_correlation <- corr.test(PCA_Dataset_adjusted_Experiment_2[,-c(1:7,9)], use=
"pairwise", method= "pearson")
correlation <- as.data.frame(Data_correlation$r)
p_value <- as.data.frame(Data_correlation$p)
```

- **Bartlett test**

```
cortest.bartlett(correlation, n = 107,diag=TRUE)
```

- **Kaiser-Meyer-Olkin (KMO) test**

```
KMO(correlation)
```

- **Scree plot**

```
FA <- fa.parallel(correlation, n.obs=107, fm="ml", fa="both", show.legend=T, main=""
,
                ylabel="Eigen values of principal components", plot= T)
```

- **Factor analysis**

```
Fatores <- fa(correlation, nfactors = 6, rotate = "varimax", fm="ml")
Fatores
```

- **I have deleted variables with loadings smaller than |0.5|**

```
write.table(Fatores$loadings,"FL.txt")
Loadings_FA <- read.table("FL.txt")
PCA_Dataset_Imputed_t <- data.frame(t(PCA_Dataset_adjusted_Experiment_2[,-c(
1:7,9)]))
PCA_Dataset_Imputed_t$x <- 1:41   #change dimension
Loadings_FA_ <- (Loadings_FA)
Loadings_FA_$x <- 1:41 #change dimension
Loadings_FA_[(Loadings_FA_  >= - 0.2944 & Loadings_FA_ <= 0.2944)] <- 0
Loadings_FA_$na <- rowSums(Loadings_FA_[,c(1:6)])#change dimension
Loadings_FA_ <- subset(Loadings_FA_, na!=0)
Loadings_FA_ <- Loadings_FA_[,-8]  #change dimension
PCA_Dataset_Imputed_load = merge(PCA_Dataset_Imputed_t, Loadings_FA_, by =
c("x"), all = TRUE)
PCA_Dataset_Imputed_load <- na.omit(PCA_Dataset_Imputed_load)
```

```
rownames(PCA_Dataset_Imputed_load) <- rownames(Loadings_FA_)
PCA_Dataset_Imputed_load <- t(PCA_Dataset_Imputed_load[,c(2:108)])
```

- **Correlation after variables deletion**

```
Data_correlation <- corr.test(PCA_Dataset_Imputed_load, use="pairwise", method= "pearson")
correlation <- as.data.frame(Data_correlation$r)
p_value <- as.data.frame(Data_correlation$p)
```

- **Kaiser-Meyer-Olkin (KMO) test**

```
KMO(correlation)
```

- **Scree plot after variables deletion**

```
FA <- fa.parallel(correlation, n.obs=107, fm="ml", fa="both", show.legend=T, main="",
                 ylabel="Eigen values of principal components", plot= T)
```

- **Factor analysis after variables deletion**

```
Fatores <- fa(correlation,nfactors = 5, rotate = "varimax", fm="ml")
Fatores
```

- **I have deleted variables with loadings smaller than |0.3|**

```
write.table(Fatores$loadings,"FL.txt")
Loadings_FA <- read.table("FL.txt")
PCA_Dataset_Imputed_t <- data.frame(t(PCA_Dataset_Imputed_load))
PCA_Dataset_Imputed_t$x <- 1:35   #change dimension
Loadings_FA_ <- (Loadings_FA)
Loadings_FA_$x <- 1:35 #change dimension
Loadings_FA_[(Loadings_FA_ >= - 0.294 & Loadings_FA_ <= 0.294)] <- 0
Loadings_FA_$na <- rowSums(Loadings_FA_[,c(1:5)])#change dimension
Loadings_FA_ <- subset(Loadings_FA_, na!=0)
Loadings_FA_ <- Loadings_FA_[,-7]  #dois numeros a mais em relação ao numero de fatores
PCA_Dataset_Imputed_load = merge(PCA_Dataset_Imputed_t, Loadings_FA_, by = c("x"), all = TRUE)
PCA_Dataset_Imputed_load <- na.omit(PCA_Dataset_Imputed_load)
rownames(PCA_Dataset_Imputed_load) <- rownames(Loadings_FA_)
PCA_Dataset_Imputed_load <- t(PCA_Dataset_Imputed_load[,c(2:108)])
```

- **Correlation after variables deletion**

```
Data_correlation <- corr.test(PCA_Dataset_Imputed_load, use="pairwise", method= "pearson")
correlation <- as.data.frame(Data_correlation$r)
p_value <- as.data.frame(Data_correlation$p)
```

- **Kaiser-Meyer-Olkin (KMO) test**

```
KMO(correlation)
```

- **Scree plot after variables deletion**

```
FA <- fa.parallel(correlation, n.obs=107, fm="ml", fa="both", show.legend=T, main=""
,
                  ylabel="Eigen values of principal components", plot= T)
```

- **Factor analysis after variables deletion**

```
Fatores <- fa(correlation, nfactors = 5, rotate = "varimax", fm="ml")
Fatores

write.table(cbind(Fatores$loadings,Fatores$communality),"FL_FR.txt")
write.table(cbind(FA$pc.values, FA$pc.sim), "e.values_FR.txt")
Scores <- fa(PCA_Dataset_Imputed_load, nfactors = 5, rotate = "varimax", fm="ml")
write.table(Scores$scores,"Scores_FR.txt")
```

- **ANOVA**

```
Scores <- read.table("Scores_FR.txt")
ANOVA_data <- cbind(PCA_Dataset_adjusted_Experiment_2[,c(1:7)], Scores)
names(ANOVA_data)[8:12] <- c("F1", "F2", "F3", "F4","F5")
for(i in 1:7){
ANOVA_data[,i] = as.factor(ANOVA_data[,i])
}
write.table(ANOVA_data,"ANOVA_data_FR.txt")

t(contr.poly(3,c(0, 25, 50)))

ANOVA_data$Feed_restriction_2 <- as.numeric(ANOVA_data$Feed_restriction)

ANOVA_data$Feed_restriction_2[ANOVA_data$Feed_restriction_2 == 1 ] <- 100

ANOVA_data$Feed_restriction <- (ANOVA_data$Feed_restriction_2)
for(i in 1:7){
ANOVA_data[,i] = as.factor(ANOVA_data[,i])
}

ANOVA_data <- ANOVA_data[,-13]

#F1
F1 <- lm(F1 ~ Sex*Feed_restriction , data = ANOVA_data, contrasts=list(Sex="contr.
sum", Feed_restriction="contr.sum"))
summary(F1)

Anova(F1, type="III")

FR_cont <- lsmeans(F1, ~ Feed_restriction,  lmer.df = "satterth")

contrast(FR_cont,
       list("Linear"=c(-0.7071068,0.0000000,0.7071068),
           "Quadratic"=c(0.4082483,-0.8164966,0.4082483)))

Sex_cont <- lsmeans(F1, ~ Sex,  lmer.df = "satterth")
```

```
pairs(Sex_cont, adjust="none")

F1_Mean<- aggregate(F1 ~ Sex*Feed_restriction, data = ANOVA_data, FUN = mea
n)

F1_plot_FR <- ggplot(NULL, aes(x = as.numeric(Feed_restriction), y = F1)) +
     geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.leg
end = FALSE) +
     geom_line(data = F1_Mean,aes(color = Sex),size=1,show.legend = FALSE)+
     ylab("Scores Fa 1") +
     geom_point(data = F1_Mean, size = 6, alpha = 1, aes(color = Sex),show.legend
= FALSE) +
  theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
  axis.text.x = element_text(size=28,colour = "black"),
  axis.text.y = element_text(size=28,colour = "black"),
  axis.title.x=element_text(size=28,colour = "black"),
  axis.title.y=element_text(size=28,colour = "black")
  )+
   scale_x_discrete(name = "Feed intake", limits = c("50% ad libitum","75% ad libitu
m","ad libitum")) +
  scale_color_manual(values=c("green4","red", "blue"))

tiff(file="F1_plot_FR.tiff",
width=10, height=7, units="in", res=700)
plot(F1_plot_FR)
dev.off()

#F2
F2 <- lm(F2 ~ Sex*Feed_restriction , data = ANOVA_data, contrasts=list(Sex="contr.
sum", Feed_restriction="contr.sum"))
summary(F2)

Anova(F2, type="III")

FR_cont <- lsmeans(F2, ~ Feed_restriction,  lmer.df = "satterth")

contrast(FR_cont,
        list("Linear"=c(-0.7071068,0.0000000,0.7071068),
            "Quadratic"=c(0.4082483,-0.8164966,0.4082483)))

F2_Mean<- aggregate(F2 ~ Feed_restriction, data = ANOVA_data, FUN = mean)

F2_plot_FR <- ggplot(NULL, aes(x = as.numeric(Feed_restriction), y = F2)) +
     geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.leg
end = FALSE) +
     geom_line(data = F2_Mean,size=1,show.legend = FALSE)+
     ylab("Scores Fa 2") +
     geom_point(data = F2_Mean, size = 6, alpha = 1, show.legend = FALSE) +
```

```
  theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
  axis.text.x = element_text(size=28,colour = "black"),
  axis.text.y = element_text(size=28,colour = "black"),
  axis.title.x=element_text(size=28,colour = "black"),
  axis.title.y=element_text(size=28,colour = "black")
  )+
    scale_x_discrete(name = "Feed intake", limits = c("50% ad libitum","75% ad libitum","ad libitum"))+
  scale_color_manual(values=c("green4","red", "blue"))

tiff(file="F2_plot_FR.tiff",
width=10, height=7, units="in", res=700)
plot(F2_plot_FR)
dev.off()

#F3
F3 <- lm(F3 ~ Sex*Feed_restriction , data = ANOVA_data, contrasts=list(Sex="contr.sum",Feed_restriction="contr.sum"))
summary(F3)

Anova(F3, type="III")

FR_cont <- lsmeans(F3, ~ Feed_restriction,  lmer.df = "satterth")

contrast(FR_cont,
        list("Linear"=c(-0.7071068,0.0000000,0.7071068),
            "Quadratic"=c(0.4082483,-0.8164966,0.4082483)))

Sex_cont <- lsmeans(F3, ~ Sex,  lmer.df = "satterth")

pairs(Sex_cont, adjust="none")

F3_Mean<- aggregate(F3 ~ Sex*Feed_restriction, data = ANOVA_data, FUN = mean)

F3_plot_FR <- ggplot(NULL, aes(x = as.numeric(Feed_restriction), y = F3)) +
     geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.legend = FALSE) +
     geom_line(data = F3_Mean,aes(color = Sex),size=1,show.legend = FALSE)+
     ylab("Scores Fa 3") +
     geom_point(data = F3_Mean, size = 6, alpha = 1, aes(color = Sex),show.legend = FALSE) +
  theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
  axis.text.x = element_text(size=28,colour = "black"),
  axis.text.y = element_text(size=28,colour = "black"),
  axis.title.x=element_text(size=28,colour = "black"),
```

```
  axis.title.y=element_text(size=28,colour = "black")
  )+
    scale_x_discrete(name = "Feed intake", limits = c("50% ad libitum","75% ad libitu
m","ad libitum"))+
  scale_color_manual(values=c("green4","red", "blue"))

tiff(file="F3_plot_FR.tiff",
width=10, height=7, units="in", res=700)
plot(F3_plot_FR)
dev.off()

#F4
F4 <- lm(F4 ~ Sex*Feed_restriction, data = ANOVA_data, contrasts=list(Sex="contr.
sum",Feed_restriction="contr.sum"))
summary(F4)

Anova(F4, type="III")

FR_cont <- lsmeans(F4, ~ Feed_restriction,  lmer.df = "satterth")

contrast(FR_cont,
         list("Linear"=c(-0.7071068,0.0000000,0.7071068),
              "Quadratic"=c(0.4082483,-0.8164966,0.4082483)))

F4_Mean<- aggregate(F4 ~ Feed_restriction, data = ANOVA_data, FUN = mean)

F4_plot_FR <- ggplot(NULL, aes(x = as.numeric(Feed_restriction), y = F4)) +
      geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.leg
end = FALSE) +
      geom_line(data = F4_Mean,size=1,show.legend = FALSE)+
      ylab("Scores Fa 4") +
  geom_point(data = F4_Mean, size = 6, alpha = 1,show.legend = FALSE) +
  theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
  axis.text.x = element_text(size=28,colour = "black"),
  axis.text.y = element_text(size=28,colour = "black"),
  axis.title.x=element_text(size=28,colour = "black"),
  axis.title.y=element_text(size=28,colour = "black")
  )+
    scale_x_discrete(name = "Feed intake", limits = c("50% ad libitum","75% ad libitu
m","ad libitum"))+
  scale_color_manual(values=c("green4","red", "blue"))

tiff(file="F4_plot_FR.tiff",
width=10, height=7, units="in", res=700)
plot(F4_plot_FR)
dev.off()
```

```
#F5
F5 <- lm(F5 ~ Sex*Feed_restriction , data = ANOVA_data, contrasts=list(Sex="contr.sum",Feed_restriction="contr.sum"))
summary(F5)

Anova(F5, type="III")

Sex_FR_cont <- lsmeans(F5, ~ Sex*Feed_restriction,  lmer.df = "satterth")
contrast(FR_cont,
        list("Linear"=c(-0.7071068,0.0000000,0.7071068),
             "Quadratic"=c(0.4082483,-0.8164966,0.4082483)))

cont_BW_Castrated <- contrast(Sex_FR_cont,
        list("Linear"=c(-0.7071068,0,0,    0.0000000,0,0,      0.7071068,0,0),
             "Quadratic"=c(0.4082483,0,0,  -0.8164966,0,0,     0.4082483,0,0)))
cont_BW_Castrated

cont_BW_Females <- contrast(Sex_FR_cont,
        list("Linear"=c(0,-0.7071068,0,      0,0.0000000,0,    0,0.7071068,0),
             "Quadratic"=c(0,0.4082483,0,    0,-0.8164966,0,   0,0.4082483,0)))

cont_BW_Females

cont_BW_Intact <- contrast(Sex_FR_cont,
        list("Linear"=c(0,0,-0.7071068,      0,0,0.0000000,    0,0,0.7071068),
             "Quadratic"=c(0,0,0.4082483,    0,0,-0.8164966,   0,0,0.4082483)))
cont_BW_Intact

pairs(Sex_FR_cont, adjust="none")

F5_Mean<- aggregate(F5 ~ Sex*Feed_restriction, data = ANOVA_data, FUN = mean)

F5_plot_FR <- ggplot(NULL, aes(x = as.numeric(Feed_restriction), y = F5)) +
     geom_point(data = ANOVA_data, size = 6, alpha = 1, aes(color = Sex),show.legend = FALSE) +
     geom_line(data =F5_Mean,aes(color = Sex),size=1,show.legend = FALSE)+
     ylab("Scores Fa 5") +   theme_minimal(base_size = 14) + theme(panel.border = element_blank(),
  panel.grid.major = element_blank(),
  panel.grid.minor = element_blank(),axis.line = element_line(colour = "black"),
  axis.text.x = element_text(size=28,colour = "black"),
  axis.text.y = element_text(size=28,colour = "black"),
  axis.title.x=element_text(size=28,colour = "black"),
  axis.title.y=element_text(size=28,colour = "black")
  )+
   scale_x_discrete(name = "Feed intake", limits = c("50% ad libitum","75% ad libitum","ad libitum")) +
  annotate(geom="text", x=c(3.1,3.1,3.1), y=c(0.8,-0.05,-0.35), label=c("a","b","b"),
            color=c("green4","blue","red"),size = 8)+
```

```
  scale_color_manual(values=c("green4","red", "blue"))


tiff(file="F5_plot_FR.tiff",
width=10, height=7, units="in", res=700)
plot(F5_plot_FR)
dev.off()
```

APPENDIX II

Chapter 4 – R code

The functions below were used for exploring the relationship among the variables and for k-fold cross-validation of reticulorumen k_p of particles and solutes. I developed the k-fold-cross-validation function and must be properly cited when used.

Used libraries

```
library(lme4)

library(groupdata2)

library(MuMIn)

library(epiR)
```

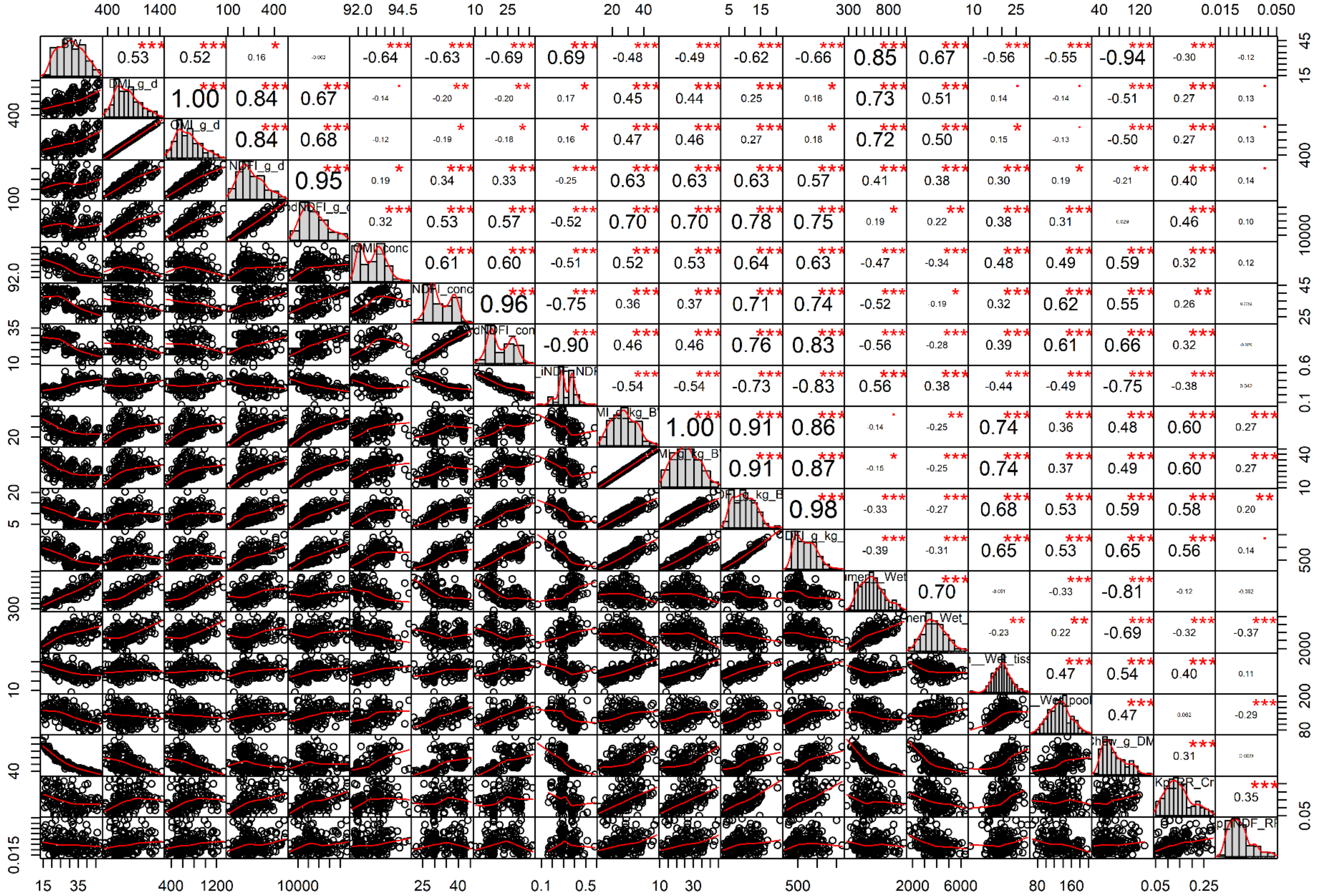
Import data

```
setwd("C:/")
Full_database_g <- read.table("Database.txt")
```

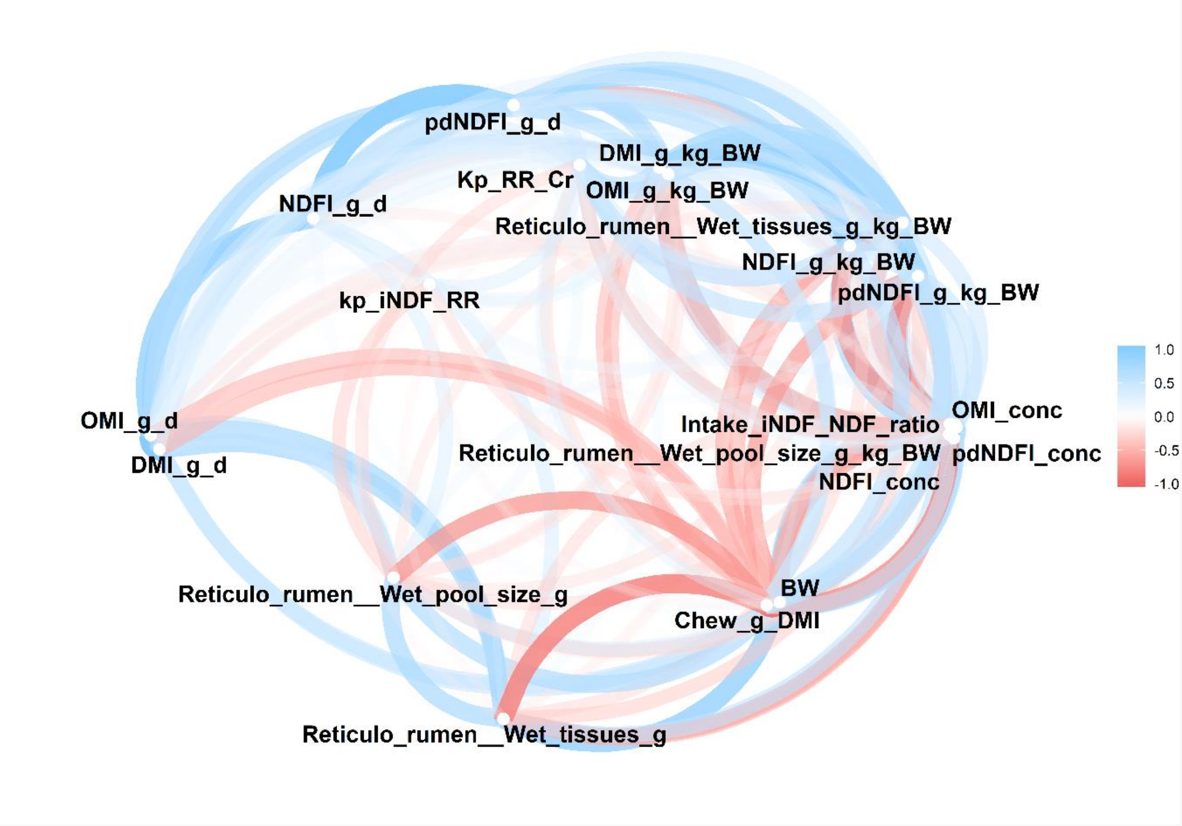
Explore the data

```
explore <- subset(Full_database_g, select = c(
BW,
DMI_g_d ,
OMI_g_d ,
NDFI_g_d ,
pdNDFI_g_d ,
OMI_conc,
NDFI_conc,
pdNDFI_conc,
Intake_iNDF_NDF_ratio,
DMI_g_kg_BW ,
OMI_g_kg_BW ,
NDFI_g_kg_BW ,
pdNDFI_g_kg_BW ,
Reticulo_rumen__Wet_tissues_g,
Reticulo_rumen__Wet_pool_size_g,
Reticulo_rumen__Wet_tissues_g_kg_BW,
Reticulo_rumen__Wet_pool_size_g_kg_BW,
Chew_g_DMI,
Kp_RR_Cr,
kp_iNDF_RR))

tiff(file="corrplot_thesis1.tiff",
width=10, height=7, units="in", res=700)
```

```
chart.Correlation(explore, histogram=T, pch="°",tl.pos='n')
dev.off()
```


BW
DMI_g_d
OMI_g_d
NDFI_g_d
dNDFI_g_d
OMI_conc
NDFI_conc
dNDFI_con
iNDF_NDF
MI_g_kg_B
MI_g_kg_B
DFI_g_kg_B
DFI_g_kg_
Wet
Wet_
Wet_tiss
Wet_pool
Chew_g_DM
Kp_RR_Cr
p_NDF_RF
0.53 0.52 0.16 -0.002 -0.64 -0.63 -0.69 0.69 -0.48 -0.49 -0.62 -0.66 0.85 0.67 -0.56 -0.55 -0.94 -0.30 -0.12
1.00 0.84 0.67 -0.14 -0.20 -0.20 0.17 0.45 0.44 0.25 0.16 0.73 0.51 0.14 -0.14 -0.51 0.27 0.13
0.84 0.68 -0.12 -0.19 -0.18 0.16 0.47 0.46 0.27 0.18 0.72 0.50 0.15 -0.13 -0.50 0.27 0.13
0.95 0.19 0.34 0.33 -0.25 0.63 0.63 0.63 0.57 0.41 0.38 0.30 0.19 -0.21 0.40 0.14
0.32 0.53 0.57 -0.52 0.70 0.70 0.78 0.75 0.19 0.22 0.38 0.31 0.46 0.10
0.61 0.60 -0.51 0.52 0.53 0.64 0.63 -0.47 -0.34 0.48 0.49 0.59 0.32 0.12
0.96 -0.75 0.36 0.37 0.71 0.74 -0.52 0.19 0.32 0.62 0.55 0.26
-0.90 0.46 0.46 0.76 0.83 -0.56 -0.28 0.39 0.61 0.66 0.32
-0.54 -0.54 -0.73 -0.83 0.56 0.38 -0.44 -0.49 -0.75 -0.38
1.00 0.91 0.86 -0.14 -0.25 0.74 0.36 0.48 0.60 0.27
0.91 0.87 -0.15 -0.25 0.74 0.37 0.49 0.60 0.27
0.98 -0.33 -0.27 0.68 0.53 0.59 0.58 0.20
-0.39 -0.31 0.65 0.53 0.65 0.56 0.14
0.70 -0.051 -0.33 -0.81 -0.12 -0.082
-0.23 0.22 -0.69 -0.32 -0.37
0.47 0.54 0.40 0.11
0.47 0.062 -0.29
0.31
0.35
400 1400 100 400 92.0 94.5 10 25 20 40 5 15 300 800 10 25 40 120 0.015 0.050
15 35 400 1200 10000 25 40 0.1 0.5 10 30 500 2000 6000 80 160 0.05 0.25
400 100 92.0 35 10 20 20 5 300 10 40 0.015
45 15 400 10000 45 25 0.6 0.1 40 10 500 2000 200 80 0.05

```
tiff(file="corrplot_thesis2.tiff",
width=10, height=7, units="in", res=700)
network_plot(correlate(explore), min_cor=0)

dev.off()
```



Function k-fold-cross-validation

```
 cross <- function(data, k, rept, model, dependent){
  datt <- as.list(rep("",rept))
  performances_RMSE_prop <- matrix(NA, ncol=k, nrow=rept)
  performances_CCC <- matrix(NA, ncol=k, nrow=rept)
  performances_RMSE_h <- matrix(NA, ncol=k, nrow=rept)
  performances_R2 <- matrix(NA, ncol=k, nrow=rept)
  performances_a_yx_line <- matrix(NA, ncol=k, nrow=rept)
  performances_UCI_a_yx_line <- matrix(NA, ncol=k, nrow=rept)
  performances_LCI_a_yx_line <- matrix(NA, ncol=k, nrow=rept)
  performances_b_yx_line <- matrix(NA, ncol=k, nrow=rept)
  performances_UCI_b_yx_line <- matrix(NA, ncol=k, nrow=rept)
  performances_LCI_b_yx_line <- matrix(NA, ncol=k, nrow=rept)
  performances_R2_yx_line <- matrix(NA, ncol=k, nrow=rept)
  performances_mean_bias <- matrix(NA, ncol=k, nrow=rept)
  performances_UCI_mean_bias <- matrix(NA, ncol=k, nrow=rept)
  performances_LCI_mean_bias <- matrix(NA, ncol=k, nrow=rept)
  performances_slope_bias <- matrix(NA, ncol=k, nrow=rept)
```

```
  performances_UCI_slope_bias <- matrix(NA, ncol=k, nrow=rept)
  performances_LCI_slope_bias <- matrix(NA, ncol=k, nrow=rept)
  performances_R2_bias <- matrix(NA, ncol=k, nrow=rept)
  matrix_predicted <- matrix(NA, ncol = k*rept+4, nrow = nrow(data))

  for (i in 1:rept) {
    datt[[i]] <- fold(data, k = k, cat_col = 'Sex')

    for (fold in 1:k){
      training_set <- datt[[i]][datt[[i]]$.folds != fold,]
      testing_set <- datt[[i]][datt[[i]]$.folds == fold,]
      model <-  lmer(model, training_set, REML=FALSE)
      predicted <- predict(model, testing_set, allow.new.levels=TRUE, re.form=NA)
      testing_set$predicted <- predicted
      data_pred <-  merge(data, testing_set, by = c("Study","Animal"), all = TRUE)
      RMSE_prop <- 100*(sqrt(sum((testing_set[[dependent]] -predicted)^2)/length(testing_set[[dependent]]))/mean(testing_set[[dependent]]))
      CCC <- epi.ccc(testing_set[[dependent]], predicted)$rho.c$est
      RMSE_h <- (sqrt(sum((testing_set[[dependent]] - predicted)^2)/length(testing_set[[dependent]])))
      R2 <- r.squaredGLMM(model)[1]
      a_yx_line <- summary(lm(testing_set[[dependent]]~predicted))$coef[[1]]
      UCI_a_yx_line <- confint(lm(testing_set[[dependent]]~predicted))[1,2]
      LCI_a_yx_line <- confint(lm(testing_set[[dependent]]~predicted))[1,1]
      b_yx_line <- summary(lm(testing_set[[dependent]]~predicted))$coef[[2]]
      UCI_b_yx_line <- confint(lm(testing_set[[dependent]]~predicted))[2,2]
      LCI_b_yx_line <- confint(lm(testing_set[[dependent]]~predicted))[2,1]
      R2_yx_line <- summary(lm(testing_set[[dependent]]~predicted))$adj.r.squared
      mean_bias <- summary(lm((testing_set[[dependent]]-predicted)~predicted))$coef[[1]]
      UCI_mean_bias <- confint(lm((testing_set[[dependent]]-predicted)~predicted))[1,2]
      LCI_mean_bias <- confint(lm((testing_set[[dependent]]-predicted)~predicted))[1,1]
      slope_bias <- summary(lm((testing_set[[dependent]]-predicted)~predicted))$coef[[2]]
      UCI_slope_bias <- confint(lm((testing_set[[dependent]]-predicted)~predicted))[2,2]
      LCI_slope_bias <- confint(lm((testing_set[[dependent]]-predicted)~predicted))[2,1]
      R2_bias <- summary(lm((testing_set[[dependent]]-predicted)~predicted))$adj.r.squared

      performances_RMSE_prop[i, fold] <- RMSE_prop
      performances_CCC[i, fold] <- CCC
      performances_RMSE_h[i, fold] <- RMSE_h
      performances_R2[i, fold] <- R2
      performances_a_yx_line[i, fold] <- a_yx_line
```

```
      performances_UCI_a_yx_line[i, fold] <- UCI_a_yx_line
      performances_LCI_a_yx_line[i, fold] <- LCI_a_yx_line
      performances_b_yx_line[i, fold] <- b_yx_line
      performances_UCI_b_yx_line[i, fold] <- UCI_b_yx_line
      performances_LCI_b_yx_line[i, fold] <- LCI_b_yx_line
      performances_R2_yx_line[i, fold] <- R2_yx_line
      performances_mean_bias[i, fold] <- mean_bias
      performances_UCI_mean_bias[i, fold] <- UCI_mean_bias
      performances_LCI_mean_bias[i, fold] <- LCI_mean_bias
      performances_slope_bias[i, fold] <- slope_bias
      performances_UCI_slope_bias[i, fold] <- UCI_slope_bias
      performances_LCI_slope_bias[i, fold] <- LCI_slope_bias
      performances_R2_bias[i, fold] <- R2_bias
      matrix_predicted[,fold+i*4] <- data_pred[,length(data_pred)]

    }
  }
  list(RMSE = performances_RMSE_h, RMSEp = performances_RMSE_prop, CCC = performances_CCC,
       R2 = performances_R2,matrix_predicted=matrix_predicted,
       a_yx_line=performances_a_yx_line,LCI_a_yx_line=performances_LCI_a_yx_line,UCI_a_yx_line=performances_UCI_a_yx_line,
       b_yx_line=performances_b_yx_line,LCI_b_yx_line=performances_LCI_b_yx_line,UCI_b_yx_line=performances_UCI_b_yx_line,
       R2_yx_line=performances_R2_yx_line,
       mean_bias=performances_mean_bias,LCI_mean_bias=performances_LCI_mean_bias,UCI_mean_bias=performances_UCI_mean_bias,
       slope_bias=performances_slope_bias,LCI_slope_bias=performances_LCI_slope_bias,UCI_slope_bias=performances_UCI_slope_bias,
       R2_bias=performances_R2_bias)
}
```

k-fold-cross-validation

```
data= Full_database_g

model_kp_iNDF = kp_iNDF_RR ~ OMI_g_kg_BW + NDFI_conc + pdNDFI_conc + Kp_RR_Cr + (1|Study)

cross_1000 <- cross(data=Full2, k=4, rept=1000,model=model, dependent ='kp_iNDF_RR')

predicted_matrix <- cross_1000$matrix_predicted

Index_1000 <- matrix(NA, ncol=1, nrow=18)
rownames(Index_1000) <- c("RMSPE", "RMSPEp","CCC","R2","a y=x line","LCI 95% a y=x line","UCI 95% a y=x line","b y=x line","LCI 95% b y=x line","UCI 95% b y=x line" ,"R2 y=x line", "mean bias","LCI 95% mean bias","UCI 95% mean bias","slope bias","LCI 95% slope bias","UCI 95% slope bias","R2 bias")
```

```
RMSE_1000 = apply(cross_1000$RMSE, 1, mean)
plot(cumsum(RMSE_1000)/seq_along(RMSE_1000), type="b")

Index_1000[1] <- mean(RMSE_1000)

RMSEp_1000 = apply(cross_1000$RMSEp, 1, mean)
plot(cumsum(RMSEp_1000)/seq_along(RMSEp_1000), type="b")

Index_1000[2] <- mean(RMSEp_1000)

CCC_1000 = apply(cross_1000$CCC, 1, mean)
plot(cumsum(CCC_1000)/seq_along(CCC_1000), type="b")

Index_1000[3] <- mean(CCC_1000)

R2_1000 = apply(cross_1000$R2, 1, mean)
plot(cumsum(R2_1000)/seq_along(R2_1000), type="b")

Index_1000[4] <- mean(R2_1000)

a_yx_line_1000 = apply(cross_1000$a_yx_line, 1, mean)
plot(cumsum(a_yx_line_1000)/seq_along(a_yx_line_1000), type="b")

Index_1000[5] <- mean(a_yx_line_1000)

LCI_a_yx_line_1000 = apply(cross_1000$LCI_a_yx_line, 1, mean)
plot(cumsum(LCI_a_yx_line_1000)/seq_along(LCI_a_yx_line_1000), type="b")

Index_1000[6] <- mean(LCI_a_yx_line_1000)

UCI_a_yx_line_1000 = apply(cross_1000$UCI_a_yx_line, 1, mean)
plot(cumsum(UCI_a_yx_line_1000)/seq_along(UCI_a_yx_line_1000), type="b")

Index_1000[7] <- mean(UCI_a_yx_line_1000)

b_yx_line_1000 = apply(cross_1000$b_yx_line, 1, mean)
plot(cumsum(b_yx_line_1000)/seq_along(b_yx_line_1000), type="b")

Index_1000[8] <- mean(b_yx_line_1000)

LCI_b_yx_line_1000 = apply(cross_1000$LCI_b_yx_line, 1, mean)
plot(cumsum(LCI_b_yx_line_1000)/seq_along(LCI_b_yx_line_1000), type="b")

Index_1000[9] <- mean(LCI_b_yx_line_1000)

UCI_b_yx_line_1000 = apply(cross_1000$UCI_b_yx_line, 1, mean)
plot(cumsum(UCI_b_yx_line_1000)/seq_along(UCI_b_yx_line_1000), type="b")

Index_1000[10] <- mean(UCI_b_yx_line_1000)
```

```
R2_yx_line_1000 = apply(cross_1000$R2_yx_line, 1, mean)
plot(cumsum(R2_yx_line_1000)/seq_along(R2_yx_line_1000), type="b")

Index_1000[11] <- mean(R2_yx_line_1000)

mean_bias_1000 = apply(cross_1000$mean_bias, 1, mean)
plot(cumsum(mean_bias_1000)/seq_along(mean_bias_1000), type="b")

Index_1000[12] <- mean(mean_bias_1000)

LCI_mean_bias_1000 = apply(cross_1000$LCI_mean_bias, 1, mean)
plot(cumsum(LCI_mean_bias_1000)/seq_along(LCI_mean_bias_1000), type="b")

Index_1000[13] <- mean(LCI_mean_bias_1000)

UCI_mean_bias_1000 = apply(cross_1000$UCI_mean_bias, 1, mean)
plot(cumsum(UCI_mean_bias_1000)/seq_along(UCI_mean_bias_1000), type="b")

Index_1000[14] <- mean(UCI_mean_bias_1000)

slope_bias_1000 = apply(cross_1000$slope_bias, 1, mean)
plot(cumsum(slope_bias_1000)/seq_along(slope_bias_1000), type="b")

Index_1000[15] <- mean(slope_bias_1000)

LCI_slope_bias_1000 = apply(cross_1000$LCI_slope_bias, 1, mean)
plot(cumsum(LCI_slope_bias_1000)/seq_along(LCI_slope_bias_1000), type="b")

Index_1000[16] <- mean(LCI_slope_bias_1000)

UCI_slope_bias_1000 = apply(cross_1000$UCI_slope_bias, 1, mean)
plot(cumsum(UCI_slope_bias_1000)/seq_along(UCI_slope_bias_1000), type="b")

Index_1000[17] <- mean(UCI_slope_bias_1000)

R2_bias_1000 = apply(cross_1000$R2_bias, 1, mean)
plot(cumsum(R2_bias_1000)/seq_along(R2_bias_1000), type="b")

Index_1000[18] <- mean(R2_bias_1000)

Index_1000 <- as.data.frame(t(Index_1000))
```