MAIN DRIVING FACTORS INFLUENCING PASSAGE KINETICS IN RUMINANTS
(Principais fatores que influenciam a taxa de passagem em ruminantes)

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ABSTRACT
Mean retention time (MRT) is a key step in feed evaluation. Passage rate (kp) and MRT can be affected by several factors. In general, they can be classified as animal or diet-dependent factors. Furthermore, the markers and methods are central aspects of discrepancy regarding the procedures used to estimate kp and MRT in ruminants. Therefore, the primary objective of this paper was to review the main factors and the current knowledge regarding markers and the direct method used to determine kp and MRT in ruminants.

Key-words: Markers, methods, ruminants, passage rate, mean retention time.

RESUMO
Tempo médio de retenção (TMR) é um ponto chave na avaliação de alimentos. Taxa de passagem (kp) e TMR pode ser afetada por diversos fatores. Em geral, pode ser classificada como fatores dependente do animal ou da dieta. Além disso, marcadores e métodos são aspectos centrais de divergência com relação ao procedimento para estimar kp e TMR em ruminantes. Portanto, o objetivo desse artigo foi revisar os principais fatores e o conhecimento atual relacionado aos marcadores e ao método direto usado para determinar kp e MRT em ruminantes.

Palavras-chave: Marcadores, métodos, ruminantes, taxa de passagem, tempo médio de retenção.

INTRODUCTION
Ruminants are unique because they can convert fibrous plant material into nutrients due to microbial degradation in their forestomachs (VAN SOEST, 1994). Frequently, forage is the major energy source in ruminant feedstuffs. In addition, digestibility of forage in ruminants varies mostly due to the concentration of cell wall carbohydrates, which can be determined by neutral detergent
fiber analysis (HUHTANEN et al., 2006). The digestive system of ruminants was developed to retain the undigested fibrous material selectively, aiming at maximizing ruminal fiber digestion (ALLEN & MERTENS, 1988). Feeds must be digested and exit the rumen via the reticulo-omasal orifice to enable further intake of new feed (MERTENS, 1994). Consequently, longer retention time of feed at rumen improves fiber degradation at least until reaching the maximum achievable level of digestion. However, longer retention time of feeds may also restrict the intake, because forage intake can be limited by rumen capacity (VAN SOEST, 1994). In order to predict forage utilization by ruminants, it is essential to understand the factors that influence mean retention time (MRT) of fiber, which may help to explain differences in animal performance (TITGEMEYER, 1997; KRIZSAN et al., 2010). To obtain an accurate and precise estimate of passage rate (kp) and MRT, it is necessary to be aware of the main driving factors influencing passage kinetics, and to make assumptions regarding methods and markers. Therefore, the primary objective of this paper was to review the main factors and the current knowledge regarding markers and the direct methods used to determine kp and MRT in ruminants.

DEVELOPMENT

Definition and factors influencing passage kinetics

Passage rate can be defined as the flow of undigested residues through the digestive tract (VAN SOEST, 1994). Mean retention time is inversely proportional to kp when an indigestible marker is used, and it is a measure of the time the digesta is exposed to processes of mixing, digestion and absorption in the gastrointestinal tract (GIT) or a given segment (FAICHNEY, 2005). It can be calculated using Eq. 1, as described by VAN SOEST et al. (1992): MRT= Q / F [1]; where mean retention time (MRT) is given in hours (h), Q is the amount of marker measured (g) and F is the marker administration rate (g/h).

Rumen retention time of particles consists of digestion and passage. The digestion process is based on mastication and fermentation (alteration of particle size, functional specific gravity, etc.). After digestion, the undigested particles are able to leave the reticulorumen (ELLIS et al., 1999).

Neutral detergent fiber is composed by indigestible NDF (iNDF) and potential digestible NDF (pdNDF), and these fractions of NDF (i.e., iNDF and pdNDF) have different digestion and passage rates. Thus, the NDF must be considered as a heterogeneous entity (HUHTANEN et al.,
Previous studies (TAMMINGA et al., 1989; BAYAT et al., 2010) have shown that small particles containing iNDF had faster kp compared to small particles containing pdNDF, which indicates that pdNDF is selectively retained in the rumen. In this regard, MRT of NDF and pdNDF are highly biased since the digestion occurs concurrently with the passage (LUND, 2002). Therefore, MRT and kp can only be estimated for entities like iNDF, which are indigestible and disappear only via passage (ELLIS et al., 1999).

Passage rate and MRT can be affected by several factors. In general, they can be classified as animal or diet-dependent. Animal-dependent factors involve animal species, body weight (BW), sex and physiological state (FAICHNEY e WHITE, 1988; WESTON, 1988). Diet-dependent factors involve physical characteristics (particle size, rate of particle size reduction, and functional specific gravity) and chemical composition of the feed (carbohydrate content, protein content, fat supplementation, among others) (ELLIS et al., 1994; HUHTANEN et al., 2006).

**Animals Characteristics**

Rumen kp has often been considered species specific (OWENS e GOETSCH, 1986; CSIRO, 1990; LESCOAT e SAUVANT, 1995) due to morphological (i.e., salivary glands, lips, tongue), body size, and digestive capacity differences between species (HOFMANN, 1989; VAN SOEST, 1994). Therefore, differences in kp and digestibility among species may be possible. However, no differences between sheep and cattle ruminal kp were found (CANNAS e VAN SOEST, 2000; CANNAS W et al., 2003). Krämer et al. (2013), studying dairy cattle fed with 50:50 ratio of corn silage and concentrate, observed feed intake level of 37.2 g/kg BW and MRT of 38.7 h (estimated by iNDF). Furthermore, Leite et al. (2015), evaluating feed kinetics in weaned goats fed with 45:55 ratio of whole maize plants hay and concentrate, observed feed intake of 31.5 g/kg BW and 36.1 h (estimated by iNDF). One possible reason for similar MRT between these species may be related to the relative feed intake level and diet characteristics. Taking these into account, the differences among species may not appear when the animals (cattle, sheep and goat) are fed at similar intake level (% BW) and similar diets.

Body weight has a positive influence on MRT and a correlation between BW and MRT has been reported in studies comparing different herbivorous species (DEMMENT e VAN SOEST, 1983; ILLIUS e GORDON, 1992; GORDON e ILLIUS, 1994). Consequently, large animals should have greater capacity to retain feed for longer time and digest it more extensively than smaller...
animals (HACKMANN e SPAIN, 2010; STEUER et al., 2011). However, MüllerB et al. (2013) analyzed datasets on captive herbivorous and stated that larger species had no digestive advantage; they are just able to eat more than smaller species, when comparing their requirements. In agreement, CLAUSS et al. (2007) concluded that MRT is not much dependent on BW, but rather on relative DMI.

Passage rate can also be influenced by the physiological state such as gestation and lactation (FAICHNEY e WHITE, 1988; WESTON, 1988). There is a high nutrient demand at the end of pregnancy, due to fetus development (CRONJE, 2000). Simultaneously, the increase in fetal size promotes compression in the rumen and other segments, leading to a decrease in feed intake. However, feed intake decreases less than GIT volume, because the kp increases (MACEDO JUNIOR et al., 2012). In this case, the mechanisms that control kp are different compared to growing or lactating animals. Apparently, the uterus compression in the GIT increases intra-ruminal pressure and stimulates motility, increasing kp, irrespective of lower intake. Whereas lactating animals increase particle kp compared to nonlactating ones (COFFEY et al., 1989), it is related to greater dry matter intake (DMI), due to high demand of nutrients to produce milk (OKINE e MATHISON, 1991).

Sex is an inherent animal characteristic that influences nutritional requirements (NRC, 2007). Previous studies have reported that intact males show greater DMI compared to castrated males and females, due to the different nutrient requirements related to higher weight gain, composition of weight gain and maintenance (NRC, 2007). Therefore, since DMI may change according to sex, and intake level is the factor with the greatest influence on MRT, sex could have a significant impact on MRT. However, Leite et al. (2015) did not find any effect of sex on MRT and fiber kinetics in weaned goat kids. One possible reason for theses results is related to animal maturity, as they were young (from 15 to 30 kg and 102 to 201 days old) and the main differences among sexes were not yet evident.

**Feed Characteristics**

Physical characterics are among the main factors driving the feed particles to leave the reticulorumen. Rumen kp is based on particle size (POPPI et al., 1980), specific gravity (HOOPER e WELCH, 1985) and particle density (SUTHERLAND, 1988). During the fermentation process, gases are formed from microbial degradation that modify the specific gravity of particles and increase their buoyancy due to gas entrapment in feed particles (SUTHERLAND, 1988), thus decreasing their
probability to escape of rumen. On the other hand, small particles can be denser due to low gas entrapment, which increases their probability of escaping from the reticulorumen. Additionally, particle shape (TROELSEN e CAMPBELL, 1968) and physical location of feed particles within the rumen (WELCH, 1982; POPPI et al., 2001) should be considered when estimating the probability of particles to escape the rumen (WARNER, 2013).

Passage kinetics is also affected by diet composition. It can be attributed to different dietary components, which can affect passage and digestion kinetics through unbalance of nutrients (HUHTAHEN et al., 2006). Dietary components have different effects on rumen microbes, and interactions between them may occur. Thus, we can state that unbalance of nutrients on the diet can affect the passage rate. For low quality forages, limitations in the rate and extent of digestion can be attributed to a deficiency in the supply of essential nutrients (HOOVER, 1986). In contrast, in high producing ruminants fed with mixed diets, the rate of cell wall digestion can be strongly retarded by substrates that inhibit the growth of rumen cellulolytic bacteria. All these factors impact on microbial efficiency and, consequently on digestibility. Forage to concentrate ratio may also affect kp (GOETSCH e GALYEAN, 1982) so that animals fed with a diet with small particle size and low level of fiber present a greater kp.

Feed intake can be comprehended as a combination between animal and diet-dependent factors, which modulates the amount of feed the animal ingest. In addition, feed intake is the main factor that influences kp and MRT of feeds. A negative relationship between feed intake and MRT of particles in the rumen of sheep, cattle, and goats has been reported (HUHTANEN e KUKKONEN, 1995; DIAS et al., 2011; LEITE et al., 2015). Therefore, all aspects that can influence feed intake may affect kp and MRT. The result of this interaction reflects on diet digestibility, where a decreased feed intake, without impairing microbial synthesis, generally leads to an increased diet digestibility (DOREAU et al., 2003, 2004), due to the increased MRT, until reaching the maximum achievable level of digestion. Level of intake and its consequences in diet digestibility have been the most studied mechanisms modulating MRT.

Measurement of passage kinetics

The markers and methods are the central aspect of discrepancy regarding the procedures used to estimate kp and MRT in ruminants. The markers are related to the type (external and internal), number of markers (simple, double and triple markers), marker dosing (pulse dose or continuous
infusion), and sampling site (rumen, abomasum, duodenum, and faeces). Furthermore, kp and MRT can be estimated by the slaughter method, the rumen evacuation or the compartmental model method. However, in this review the main focus was on determination of kp and MRT by direct method (slaughter or rumen evacuation).

Several markers have been investigated (FAHEY e JUNG, 1983; OWENS e HANSON, 1992; WARNER et al., 2014). According to OWENS e HANSON (1992), an ideal marker must not be absorbed nor affect or be affected by the digestive tract or its population of microbes; it must flow parallel with or be physically similar to or intimately associated with the labelled material; and must have a specific and sensitive method of estimation.

Digesta consist of a heterogeneous mixture of particulate and liquid matter (FAICHNEY, 2005). If only one marker is used, it is impossible to know whether samples are representative of total flow (TITGEMEYER, 1997). In agreement, Siddons et al. (1985) and HUHTANEN et al. (1994) stated that it is too simplistic to consider a digesta as a single homogeneous particulate phase due to different rates of passage for particle and liquids. Furthermore, there is no single marker that can give reliable values for digesta flow (FAICHNEY, 2005). Therefore, the use of the double marker system has been applied to determine three or more phases (FRANCE e SIDDONS, 1986; AHVENJÄRVI et al., 2003).

Rare earth and chromium are the most frequently used external markers to study particulate matter kp (CANNAS et al., 2003; HUHTANEN et al., 2006). However, it has been reported that chromium can alter density and digestibility of labelled feedstuffs (EHLE et al., 1984). In addition, it has been associated to migration of rare earths from labelled feedstuffs to liquid in the rumen and reported to bind mainly to small particles (SIDDONS et al., 1985; COMBS et al., 1992).

Internal markers are intrinsic to the feed and hence circumvent the inherent limitation of external markers. There are many indigestible inert components such as lignin, acid-insoluble ash (present in feed in low concentration), and indigestible fiber (higher concentration) to determine particulate matter (WARNER, 2013). The major advantage of internal markers is that no preparation of markers is needed. One of the most studied internal marker is lignin (FAHEY e JUNG, 1983), there are problems with incomplete and variable recovery though. Indigestible cell wall components such as cellulose, neutral detergent fiber and acid detergent fiber (ADF) have been evaluated as internal markers (PENNING e JOHNSON, 1983; KRIZSAN et al., 2015). HUHTANEN et al. (1994) concluded that iNDF is more uniformly distributed in the solid phase than external markers. In this regard, the use of iNDF as a particulate marker may decrease errors, which originate from
unrepresentative sampling from a duodenal cannula. In addition, those authors found that faecal recoveries of iNDF and indigestible ADF (iADF) were more acceptable when determined by 288 h ruminal incubation than by in vitro incubation. The iNDF marker has been extensively studied in the last years, and a great evolution of this marker has been achieved. Nevertheless, this method requires the use of in situ incubation and of many steps to obtain a reliable result (KRIZSAN et al., 2015).

Liquid in the rumen acts as a lubricant and provides a medium for microbes to access feed particles and buffer (SEO et al., 2007). Therefore, it is important to determine liquid MRT. Polyethylene Glycol (PEG) can be used as liquid marker in ruminants. However, it has been shown that PEG concentration is influenced by high tannin levels and there is adsorption to particulate matter, which decreases recovery in rumen fluid for feed with higher digestibility (SUTHERLAND, 1962). DOWNES e MCDONALD (1964) also reported that the method of analysis for PEG has not been specific, sensitive or accurate. Thus, they proposed Cr-EDTA as an alternative to PEG fluid marker. Another possibility was proposed by Udén et al. (1980), who suggested the use of Co-EDTA as fluid marker in combination with Cr-mordanted fiber as particulate marker, whereas Cr-EDTA and Cr-mordanted fiber could not be used together. Liquid markers generally present fewer problems, and tend to have a much lower marker migration than a particulate marker (UDÉN et al., 1980).

To determine kp and MRT, there are two types of dosing and two types of sampling procedures. For dosing, there is the possibility of administrating the marker either by a pulse-dose or continuously for a period of days in an attempt to reach steady state conditions. The pulse dosing procedures make possible to determine the retention time in specific parts of GIT and digesta volume. Then, taking the knowledge of retention time and volume into account, kp can be calculated. On the other hand, the continuous dosing procedure has been used to determine instantaneous flow at a specific point of GIT (OWENS e HANSON, 1992). Digesta samples can be obtained from a specific site at successive times of sampling to obtain the marker excretion curve. Alternatively, it is possible to determine marker concentration in the rumen and also in various segments of the digestive tract through evacuation, either by canula or from slaughtered animals (PALOHEIMO e MÄKELÄ, 1959; OWENS e HANSON, 1992).

ROBINSON et al. (1987) applied the rumen evacuation through ruminal cannula (adaptation of the slaughter technique) to estimate digestive and passage kinetics of cell wall. Henceforth, the rumen evacuation method has been widely used (HUHTANEN et al., 2007). Additionally, this technique is considered the standard method to measure MRT. The rumen evacuation and slaughter methods provide the advantage to be independent of mathematical
descriptions, when using continuous dose. Unfortunately, the slaughter technique demands terminating the life of the experimental animals, and it is also time-consuming, expensive and laborious. This clearly precludes its use as a routine method.

Table 1: Main steps and issues to perform a study to determine mean retention time (MRT) or passage rate (Kp) by using a direct method of measurement.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markers</td>
<td>It is required at least a dual-phase marker system to access the true digesta, due to problems associated with unrepresentative samples.</td>
</tr>
<tr>
<td>To determine liquid phase:</td>
<td>Cr-EDTA or Co-EDTA can be used</td>
</tr>
<tr>
<td>To determine solid phase</td>
<td>Internal markers or external markers can be used</td>
</tr>
<tr>
<td>Application system of markers</td>
<td>It is possible to use a pulse or continuous dosing</td>
</tr>
<tr>
<td>If you have used a pulse dose:</td>
<td>It is necessary to collect samples through the time to determine MRT or Kp</td>
</tr>
<tr>
<td></td>
<td>It is necessary to collect sample after four or five days, when the steady state condition is reached.</td>
</tr>
<tr>
<td>Collection of samples</td>
<td>If you have used a continuos dose:</td>
</tr>
<tr>
<td>In fistulated animals: it is recommended to do at least two evacuations at the minimum and maximum pool size moments to determine the average pool size.</td>
<td></td>
</tr>
<tr>
<td>In the slaughter technique: it is recommended to slaughter the animal around two hours after feeding, which can represent the average pool size.</td>
<td></td>
</tr>
<tr>
<td>If you have used an internal marker (iNDF):</td>
<td>It is necessary to use in situ method to determine the marker concentration, which is required 288 hours of ruminal incubation</td>
</tr>
<tr>
<td>Markers determination</td>
<td>If you have used an external marker:</td>
</tr>
<tr>
<td>It is necessary to use an analytic method to determine the marker concentration such as atomic absorption method</td>
<td></td>
</tr>
<tr>
<td>The rumen evacuation or slaughter methods and continuous dose provide the advantage to be independent of mathematical descriptions.</td>
<td>The rumen evacuation and pulse dose assume a distribution of residence times, based on external marker profiles and it is necessary to model the excretion curve to estimate the parameters related to passage kinetics.</td>
</tr>
</tbody>
</table>

The steady state condition is defined as a constant influx and efflux of an indigestible particle in a given segment, though it is a theoretical statement. Taking it into account, previous studies have shown that there is a variation in the indigestible marker pool size over a 24-h period, especially when animals are fed twice a day. Thus, to obtain reliable estimates of kp and MRT using
rumen evacuation or slaughter technique, it is crucial to estimate the average pool size (HUHTANEN et al., 2007). The average pool size can be determined by frequent evacuations during a day or by performing an evacuation at the moment the pool size is on average. For instance, HUHTANEN et al. (2007) demonstrated that the moments close to the morning feeding and four hours after feeding are the minimum and maximum pool size, respectively.

The main difficulties and assumptions, as well as the main papers published with description of methodology, type of marker and observed values are summarized to guide new experiments with passage kinects determined by direct method (Tables 1 and 2).

**Table 2:** Values of mean retention time (MRT) or passage rate (Kp) regarding the markers and the direct method of measurement.

<table>
<thead>
<tr>
<th>Papers</th>
<th>Mean body weight, kg</th>
<th>Specie and breed</th>
<th>Method</th>
<th>Marker</th>
<th>Rumen Kp (h)</th>
<th>Rumen MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huhtanen and Kukkonen, 1994</td>
<td>500</td>
<td>Friesian Bulls</td>
<td>Rumen evacuation</td>
<td>iNDF</td>
<td>0.0165</td>
<td>60.6*</td>
</tr>
<tr>
<td>Huhtanen and Kukkonen, 1994</td>
<td>500</td>
<td>Friesian Bulls</td>
<td>Rumen evacuation</td>
<td>iADF</td>
<td>0.0160</td>
<td>62.7*</td>
</tr>
<tr>
<td>Ellis et al., 2002</td>
<td>33</td>
<td>Wheter Lambs</td>
<td>Slaughter</td>
<td>iNDF</td>
<td>0.0166*</td>
<td>60.2</td>
</tr>
<tr>
<td>Cannas et al., 2003</td>
<td>55</td>
<td>Sheep</td>
<td>Rumen evacuation or slaughter</td>
<td>iNDF</td>
<td>0.0204*</td>
<td>49.0</td>
</tr>
<tr>
<td>Cannas et al., 2003</td>
<td>593</td>
<td>Cattle</td>
<td>Rumen evacuation or slaughter</td>
<td>iNDF</td>
<td>0.0246*</td>
<td>40.7</td>
</tr>
<tr>
<td>Walz et al., 2004</td>
<td>20.6</td>
<td>Crossbred wether kids</td>
<td>Slaughter</td>
<td>iNDF</td>
<td>0.0267*</td>
<td>37.4</td>
</tr>
<tr>
<td>Walz et al., 2004</td>
<td>20.6</td>
<td>Crossbred wether kids</td>
<td>Slaughter</td>
<td>Rare earth</td>
<td>0.0437*</td>
<td>22.9</td>
</tr>
<tr>
<td>Ahvenjärvi et al., 2010</td>
<td>645</td>
<td>Finish Ayshire Dairy Cows</td>
<td>Slaughter</td>
<td>iNDF</td>
<td>0.0223*</td>
<td>44.8</td>
</tr>
<tr>
<td>Kriezsan et al., 2010</td>
<td>606 and 405</td>
<td>Growing Cattle and Dairy Cows</td>
<td>Rumen evacuation</td>
<td>iNDF</td>
<td>0.0260</td>
<td>38.5*</td>
</tr>
<tr>
<td>Krämer et al., 2013</td>
<td>557</td>
<td>Danish Holstein cows</td>
<td>Rumen evacuation</td>
<td>iNDF</td>
<td>0.0239*</td>
<td>41.9</td>
</tr>
<tr>
<td>Leite et al., 2015</td>
<td>16</td>
<td>Saanen goat kids</td>
<td>Slaughter</td>
<td>iNDF</td>
<td>0.0271*</td>
<td>36.9</td>
</tr>
<tr>
<td>Leite et al., 2015</td>
<td>16</td>
<td>Saanen goat kids</td>
<td>Slaughter</td>
<td>Cr-EDTA</td>
<td>0.2421*</td>
<td>4.1</td>
</tr>
</tbody>
</table>

*Calculated by the equation MRT=1/Kp.
1 iNDF = indigestible neutral detergent fiber.
2 iADF = indigestible acid detergent fiber.
3 Mean body weight for growing cows and dairy cows, respectively.
FINAL CONSIDERATIONS

The kp and MRT are key points in feed evaluation. The major characteristic driving particles kinetics is feed intake, and it is comprehended as a combination between animal and diet-dependent factors. Indigestible markers such as iNDF should be used to determine kp and MRT, when rumen evacuation or slaughter technique is performed. Additionally, it is necessary to determine at least a dual-phase marker system in order to access the true digesta. The frequency or the time to perform the evacuation is crucial to obtain a reliable result for rumen evacuation and slaughter technique. Regarding the pulse dose, the use of an external marker is a condition to perform this method. In addition, it is necessary to model the excretion curve to estimate the parameters related to passage kinetics.

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