Use of faecal components as markers to estimate intake and digestibility of grazing sheep

E.B. Azevedo a,b,d, C.H.E.C. Poli b, D.B. David b,c, G.A. Amaral b,c, L. Fonseca b, P.C.F. Carvalho b, V. Fischer b, S.T. Morris d

a Federal University of Pampa (UNIPAMPA), Itaqui, RS, CEP 97650-000, Brazil
b Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, CEP 91540-000, Brazil
c State Foundation of Agricultural Research (FEPAGRO), São Gabriel, RS, CEP 97300-000, Brazil
d Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11-222, Palmerston North, New Zealand

ARTICLE INFO

Article history:
Received 29 September 2013
Received in revised form 10 April 2014
Accepted 17 April 2014

Keywords:
Crude protein
Faecal crude protein
Italian Ryegrass
Lolium multiflorum Lam
Phenological stage

ABSTRACT

This research was carried out to evaluate the use of faecal components as markers to estimate intake and digestibility of Italian Ryegrass (Lolium multiflorum Lam.) of grazing sheep. The research had two phases. In Phase 1 seven indoor experiments were carried out using individual metabolic cages with 16 lambs in each experiment (four treatments with four animals each). Three phenological stages of the pasture (vegetative, pre-flowering and flowering) were evaluated and four different allowances of Italian Ryegrass, collected by grab sampling daily, of 1.5, 2.0, 2.5 kg of dry matter/100 kg of live weight and ad libitum. The indoor experimental design was completely randomized and the experiments were grouped according to the phenological stages. Organic matter intake (OMI, g/day), total daily production of faeces, faecal crude protein amount (fCP, g/day), faecal crude protein concentration (fCPc, g/kg of organic matter), faecal acid detergent fibre amount (fADF, g/day), faecal acid detergent concentration (fADFc, g/kg of organic matter) and organic matter digestibility (OMD) were assessed in the indoor experiment. In Phase 2, male sheep were used in two grazing experiments to graze Italian Ryegrass under different management conditions, which were herbage allowance, pasture phenological stage and rotational or continuous system. This phase was designed to validate the equations previously obtained. In Phase 1 significant linear regression equations were found between OMI and fCP in each phenological stage (P < 0.05). The intake equations were compared by contrasts analysis and found to be different (P < 0.001) between phenological stages, confirming the need to use the data separately by maturity stage. Two equations (simple and multiple hyperbolic) were tested for the relationship between OMD and fCPc, and the multiple hyperbolic, which includes fCPc and fADFc showed best accuracy. In Phase 2 the regression between the actual and estimated had a correlation coefficient of 0.94 and relative prediction error of 9.28%, showing the feasibility of using the generated equations.

© 2014 Elsevier B.V. All rights reserved.

Abbreviations: ADF, ash free acid detergent fibre; CP, crude protein; DM, dry matter; FADF, faecal acid detergent fibre amount; FADFc, faecal acid detergent fibre concentration; fCP, faecal crude protein amount; fCPc, faecal crude protein concentration; MSPE, mean square prediction error; N, nitrogen; NDF, ash free neutral detergent fibre; OMD, organic matter digestibility; OMI, organic matter intake; RPE, relative prediction error

* Corresponding author at: Federal University of Pampa (UNIPAMPA), Itaqui, RS, CEP 97650-000, Brazil. Tel.: +55 55 3433 1669.
E-mail address: eazevedo@yahoo.com.br (E.B. Azevedo).

http://dx.doi.org/10.1016/j.livsci.2014.04.018
1871-1413/© 2014 Elsevier B.V. All rights reserved.
1. Introduction

Pasture intake is critical in determining animal performance in grazing systems. However the measurement of herbage intake of grazing animals is difficult because there are no easy and precise methodologies (Penning, 2004). Many techniques have been used to estimate herbage intake and nutritional parameters in grazing ruminants such as external (n-Alkanes, chromium oxide, rare earths) and/or internal (faecal protein and fibre compound) markers.

Results from external marker techniques are variable, when different experiments with the same marker were compared. Smit et al. (2005) comparing techniques for measuring herbage intake concluded that use of n-Alkanes was the better technique; however Ferri et al. (2008) considered that this external marker overestimates the intake, compared to the faecal protein method. The external markers methods depend on the herbage hand-plucking, to estimate the digestibility (ytterbium, chromic oxide) or to establish a relation between compounds present in the pasture and dosed (n-Alkane) and it needs several attention to collect a representative sample, especially if the sward is heterogeneous (Peyraud, 1997). It is important due to the difficulty in sampling the exact portion of herbage that is ingested by the ruminant. Often it is difficult to reach a consensus between the various researchers (Carvalho et al., 2007), as stated above, analysing Smit et al. (2005) and Ferri et al. (2008) results. Even Smit et al. (2005) concluded that n-Alkanes technique can be used to estimate herbage intake, considering that in different years tested, there was difference in the intake estimation depending on the pair of alkane used.

To overcome the problem of herbage sample, several studies have evaluated faecal crude protein as an index to estimate intake (David et al., 2014; Peripolli et al., 2011) and digestibility (Boval et al., 2003; Fanchone et al., 2009; Lukas et al., 2005). The faecal protein technique is based on the direct relationships between the amount of faecal crude protein (fCP, grams/day) and the organic matter intake (OMI, g/d) (Lancaster, 1949). There is also a relationship between the concentration of faecal crude protein (fCPc, grams/kg of organic matter) and organic matter digestibility (OMD) that is based on the increased relationship between faecal protein and faecal organic matter due to increase in digestibility. If the digestibility decreases, the concentration of fCPc in the OM is diluted by the increasing amount of faecal OM and is, therefore, an indicator of digestibility (Lukas et al., 2005).

The advantages of using faecal crude protein, in comparison with other markers, are that it allows an intake measure through just the amount of protein in the faeces and most of the external marker methods used to assess herbage intake require complex method of analysis, not always available in all research centres (Berchielli et al., 2005). The use of the faecal protein method depends only on equipment and techniques commonly available in most laboratories that perform routine tests of forage quality. In addition, estimations of intake and digestibility can be done without a dosing or sampling of the forage and, just for organic matter digestibility estimation, only the protein concentration in a spot faecal sample is needed; hence there is no need to use sheep fitted with a faecal collection harness.

In order to establish the correlations between protein in the faeces and digestibility and intake it is important to carry out experiments in metabolic cages with similar forage to that offered to grazing animals as this relationship can change depending on herbage species and season (Coates and Penning, 2000). Results using the protein faecal index technique have been promising compared with other markers used to estimate herbage intake in grazing animals (Ferri et al., 2008; Schneider et al., 2011).

The aim of this experiment was establish the relationships between chemical content of faeces and intake and digestibility at different phenological stages of Italian Ryegrass and then to assess these nutritional parameters at pasture based on the equations previously obtained.

2. Material and methods

2.1. Location and experimental design

The indoor and grazing experiments were conducted at the Agronomic Experimental Station of Federal University of Rio Grande do Sul (UFRGS), located in Eldorado do Sul (30°05’S, 51°40’W), Brazil. The climate was humid subtropical (Cfa) according to the Köppen classification (Moreno, 1961). The local mean precipitation was 1440 mm and the mean temperature varied between 9 and 25°C, according to the season (Bergamaschi et al., 2003). The research was divided into two phases: Phase 1 – generation of the equations to estimate intake and digestibility of Italian Ryegrass (Lotium multiflorum Lam.) through indoor trials with sheep in metabolic cages in a completely randomized experimental design with four replicates (animals), four forage allowances and three phenological stages of the herbage; Phase 2 – the equations were then evaluated with sheep grazing Italian Ryegrass under different management conditions. All the procedures were in accordance with accepted principles for the care and welfare animals of the UFRGS guidelines.

2.2. Phase 1: indoor experiments

Seven experiments with sheep fed with Italian Ryegrass in metabolic cages were carried out during the years 2007–2010. All seven experiments had a similar experimental design and sampling schedule. In each experiment 16 male sheep (Texel, 12 months old, average live weight year 2007: 39.8 ± 4 kg; 2008: 36.5 ± 3.3 kg; 2009: 31.1 ± 4.1 kg and 2010: 29.6 ± 4.7 kg) were randomly allocated to one of the four levels of forage allowance: 1.5, 2, 2.5 kg of dry matter (DM)/100 kg of live weight (LW) or ad libitum allowing for 20% refusals, of the total offered. Three phenological stages were studied in seven experiments: vegetative (2009 and 2010), pre-flowering (2008 and 2009) and flowering (2007, 2008 and 2010). The phenological stages were defined as vegetative – leaf growth and development in most of the plants; pre-flowering – stem elongation and inflorescences begin to appear; and flowering – inflorescences completely exposed in most of the plants. Three phenological stages were studied to determine
if specific equations in each phenological cycle for organic matter digestibility (OMD) and organic matter intake (OMI) are required. The use of four levels of feed and three different plant phenological cycles was to create contrasts and therefore, to obtain equations that are more widely applicable. All the animals received a vermifuge (Nitroxinil 34%, 1.5 ml/50 kg LW) five days before each experiment began and ad libitum water was available at all the times while the animals were caged. Six animals were removed from the study because two showed signs of foot rot disease and four because of diarrhea; hence 106 animals were used in the experiments. Three animals were removed in the vegetative stage in 2009 (1.5 and 2 kg of DM/100 kg of LW and ad libitum forage allowance) and three animals in the vegetative stage in 2010 (1.5 and 2.5 kg of DM/100 kg of LW and ad libitum forage allowance).

The Italian Ryegrass was cut from a pasture with natural reseeding in an area previously used for soybean during the summer. The forage was kept at a height of 25–35 cm using a mower and the top half of the plants was harvested by hand-plucking with the aid of a sickle, in order to simulate material that would be ingested by the animals. Representative samples (500 g) of the harvested pasture were taken daily to determine dry matter content by drying in an oven at 55 °C for 72 h. One pooled sample from the test period was used for chemical composition and for morphological composition (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vegetative</th>
<th>Pre-flowering</th>
<th>Flowering</th>
<th>Phenological stage (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (^a) (g/kg)</td>
<td>145 ± 2.2</td>
<td>177 ± 15.8</td>
<td>248 ± 27.6</td>
<td>0.072</td>
</tr>
<tr>
<td>Values expressed as g/kg DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP (^b)</td>
<td>246 ± 9.9</td>
<td>167 ± 4.5</td>
<td>140 ± 12.3</td>
<td>0.006</td>
</tr>
<tr>
<td>OM (^c)</td>
<td>900 ± 2.0</td>
<td>904 ± 4.9</td>
<td>933 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>NDF (^d)</td>
<td>431 ± 54.1</td>
<td>548 ± 29.6</td>
<td>600 ± 16.2</td>
<td>0.044</td>
</tr>
<tr>
<td>Lignin (^e)</td>
<td>26 ± 2.7</td>
<td>47 ± 1.7</td>
<td>58 ± 2.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Leaf</td>
<td>809 ± 191.3</td>
<td>457 ± 2.6</td>
<td>153 ± 34.3</td>
<td>0.018</td>
</tr>
<tr>
<td>Stem</td>
<td>152 ± 15.2</td>
<td>405 ± 27.6</td>
<td>323 ± 9.1</td>
<td>0.174</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>39 ± 3.9</td>
<td>138 ± 30.2</td>
<td>524 ± 41.8</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^a\) Dry matter.  
\(^b\) Crude protein.  
\(^c\) Organic matter.  
\(^d\) Ash free neutral detergent fibre.  
\(^e\) Lignin determined with sulphuric acid technique.

2.2.1. Intake and digestibility measurements

The experiments were designed as a conventional digestibility experiment (Rymer, 2000), with an adaptation period of 10 days followed by five days for collection of faeces and herbage intake measurements. The herbage offered was harvested prior to feeding, in the morning (9 am) and afternoon (6 pm). Before feeding in the morning, the total refusals were collected and weighed. During the five days of collection, at 8 am the faeces were collected from the faecal bags attached to the animal and weighed. Twenty per cent of the daily feed offered, the refusal feed, and faeces were collected, dried in an oven at 55 °C for 72 h and thereafter they were pooled by animal, ground through a 1-mm screen, and stored until analysis. Intake was calculated by difference between feed offered and feed refusal. Digestibility was calculated as the difference between intake and faeces amount, divided by intake.

2.3. Phase 2: grazing experiments

The equations generated in the indoor experiments were tested to estimate OMI and OMD in two grazing experiments with sheep grazing Italian Ryegrass pastures. The grazing Experiment 1 started in August 2008 for a period of 68 days, divided into three periods: Period 1 (period of adaptation); Period 2 (Pre-flowering); Period 3 (Flowering). Three herbage allowances (5, 10 and 20 kg of DM/100 kg LW) were evaluated, following an experimental design of randomized block with two pasture phenological stages (Pre-flowering and Flowering), three herbage allowances with three replications (paddock). Each paddock was grazed by three test animals, totaling twenty-six Texel male sheep because one animal contracted foot rot disease and was excluded from the experiment. The animal was from the 5 kg of DM/100 kg LW treatment, and it was removed on period 3, been replaced by another sheep just to keep the herbage allowance, but this animal was not used for faecal collection. The animals were grouped by weight (three weight classes) and then each herbage allowance had three paddocks, so that each paddock received only animals from one weight class, which was a blocking criterion. The initial average live weights were 39.1 ± 1.6 kg (12 months old), 48.1 ± 6.7 kg (18 months old) and 61.6 ± 2.0 kg (24 months old). The animals were kept in the paddocks 24 h in the day with water offered ad libitum. Herbage mass and sward height were measured in the first day of each period. Herbage mass was estimated by cutting of four quadrants (0.50 × 0.50 m) in each paddock and the sward height was measured using a sward stick graded

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vegetative</th>
<th>Pre-flowering</th>
<th>Flowering</th>
<th>Phenological stage (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMI (g/day)</td>
<td>431 ± 27.1</td>
<td>640 ± 36.7</td>
<td>618 ± 21.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>OMD</td>
<td>0.84 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>0.66 ± 0.01</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>fCP (g/day)</td>
<td>22.59 ± 2.0</td>
<td>25.02 ± 1.6</td>
<td>27.4 ± 1.1</td>
<td>0.080</td>
</tr>
<tr>
<td>fCPC (g/kg OM)</td>
<td>268 ± 2.2</td>
<td>161.9 ± 2.5</td>
<td>113 ± 16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>fADFc (g/kg OM)</td>
<td>281 ± 5.0</td>
<td>334 ± 5.5</td>
<td>413 ± 7.6</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
in centimetres. The herbage mass was used to measure the stocking rate each 21 days, and it was controlled by the use of regulator animals using the put-ands-take methodology (Mott and Lucas, 1952). Samples of pasture were taken by grab sampling to evaluate herbage quality during the measurements. After collection, samples were oven dried at 55 °C for 72 h and used for chemical analysis.

In the grazing Experiment 2 (2010) grazing method (continuous and rotational) and herbage allowance (10 and 20 kg of DM/100 kg LW) were studied in a factorial arrangement (2 × 2). Each treatment was replicated three times, giving a total of 12 paddocks, with two test animals on each, a total of 24 Texel male sheep (six months old, 28 ± 4.5 kg live weight). The experiment started in July 2010 and continued for 85 days, divided into three periods, with the following length: Period 1 – 29 days, Period 2 – 28 days and Period 3 – 28 days. The Period 1 was used for animal adaptation to the experimental protocol and Periods 2 and 3 the pasture was in vegetative stage. In the paddocks with rotational method, the rotational frequency was of two days. Herbage allowance, stocking rate, herbage mass, sward height and chemical composition of the pasture were measured in the same way as Experiment 1, described above.

2.3.1. Evaluation of intake and digestibility regressions in the grazing experiments

Intake and digestibility measurements were made twice in each experiment (52 samples in grazing Experiment 1 and 48 samples in grazing Experiment 2). In both experiments a 21 day adaption period (Period 1) was used before intake and digestibility measurements commenced. In Experiment 1 the OMI intake equations were used according to the phenological stage of the pasture (Period 2 – Pre-Flowering and Period 3 – Flowering) and in Experiment 2 the equation used in Periods 2 and 3 was related to vegetative stage.

Faecal output was measured by collecting all faeces excreted by each animal in individual harness bags, for five days. The harnesses were emptied every day at 4 pm. The total faeces collected over the 5 days was weighed, mixed and homogenised. A subsample of 20% was taken, dried in an oven at 55 °C for 72 h and thereafter it was ground through a 1-mm screen, and stored until analysis as described below. The OMI and OMD were estimated from the chemical constituents of the faecal subsample taken for each animal, using the regression equations established in indoor experiments.

2.4. Chemical analysis

Samples of forage offered, refused, and faeces were analysed for organic matter (OM) by heating in an oven at 550 °C (AOAC, 1975), nitrogen content (N) by the Kjeldahl method (AOAC, 1975), and total crude protein (CP) content was obtained as N × 6.25. Neutral-detergent fibre (NDF) and acid detergent fibre (ADF) analyses were carried out following the procedure of Robertson and Van Soest (1981). All values were corrected for ash-free content and NDF was assayed with a heat stable amylase.

The determination of total CP, NDF and ADF excreted in the faeces was carried out by multiplying the measured concentration in the faecal sample by the daily faecal production. Faecal CP and ADF concentrations (g/kg OM) were used in the OMD estimations, while faecal CP daily excretion (g/day) was used in the OMI predictions.

2.5. Statistical analyses of indoor experiments

Indoor experiments were grouped according to morphological and chemical characteristics of the forage, using a cluster analysis (JMP software version 8, SAS Institute Inc., Cary, NC, USA).

Linear regression equations were established between OMI (g/d) and fCP (g/d) with the data grouped by stage of maturity of plants (vegetative, pre-flowering and flowering). The equations separated by maturity stage were compared by contrasts analysis ($P < 0.05$) testing parallelism and intercepts between them. To evaluate the use of faecal amount of acid detergent fibre (fADF, g/d) and neutral detergent fibre (fNDF g/d) in conjunction with fCP in a multiple equation, the stepwise method was used for variable selection in a multiple regression. The fCP values observed in the indoor trials were used in the equations generated to obtain estimated values of OMI. These data were compared with the values of OMI observed in the metabolic cages tests, in Phase 1; thus the variability of the average distance between the estimated and observed value was evaluated by mean square prediction error (MSPE) according to Fuentes-Pila et al. (1996). The fitness of the equations was evaluated by the relative prediction error (RPE) defined as the ratio between the positive root square of the MSPE and the mean of the actual intake values (Fuentes-Pila et al., 2003).

In order to evaluate the fCPC (g/kg OM) as a marker to estimate digestibility, regression equations were established between the OMD observed in the indoor experiments and fCPC, using the hyperbolic model. The inclusion of fADFc (g/kg OM) in a multiple hyperbolic equation with fCPC was also evaluated. fCPC values observed were used in the equations generated to obtain estimated values of OMD. These data were compared with the OMD values observed in the indoor experiments; thus the variability of the average distance between the estimated and observed OMD values was evaluated by mean square prediction error (MSPE) according to Fuentes-Pila et al. (1996).

To test for the effects of treatments on grazing experiments parameters, an analysis of variance was carried out ($P < 0.05$) to compare means. The OMI estimated by the equations, using this relation with fCP, was compared with the measured OMI obtained from OM faecal output and OMD estimated by multiple hyperbolic regression, using this relation with fCPC and fADFc.

3. Results

3.1. Forage characteristics, intake and digestibility in indoor experiments

The forage offered to the animals had a different ($P < 0.05$) chemical composition throughout the stages of
plant maturity, in which the CP decreased and NDF increased from the vegetative to flowering stage (Table 1). As expected, leaf content decreased and inflorescence increased ($P < 0.05$) as the plant matured. OMI increased ($P < 0.05$) from vegetative to pre-flowering stage and OMD was reduced ($P < 0.05$) from 0.84 (vegetative) to 0.66 (flowering). The fCP did not differ statistically between stages and had a large range of values (7.6–53.9 g/d). fCPc increased and fADFc decreased ($P < 0.05$) as the pasture reached the end of its cycle.

3.2. Organic matter intake and organic matter digestibility equations

The equation of regressions calculated from fCP, according to the phenological stage of pasture (Table 3), presented always a coefficient of determination ($R^2$) greater than 0.80 and a relative prediction error (RPE) lower than 10. There was a significant effect ($P < 0.001$) between phenological stages in a contrasts analysis that compared the equations intercept and parallelism between them. Total output (g/d) of fADF and fNDF was not included in the model to estimate organic matter intake by stepwise analysis ($P > 0.05$) at none of the maturity stages, leaving just fCP (g/d) as the index marker in the equations to estimate OMI.

The regressions between OMD and faecal components concentrations (fADFc and fCPC) indicated that the fCPC content was not linearly related to OMD (Fig. 1), and generated a simple hyperbolic equation with $R^2$ of 0.77% and 5.87% of RPE ($P < 0.001$; Table 4). To increase the $R^2$ value of the regression, a multiple hyperbolic equation was studied, including fADFc and fCPC ($R^2$ increased to 0.83% and the RPE decreased to 5.11%). When the relationship between fADFc and OMD was established, relationship was linear and negative ($P < 0.05$; Fig. 2).

3.3. Herbage characteristics and intake in grazing experiments

In the grazing Experiment 1 there were differences ($P < 0.05$) in herbage characteristics between herbage allowances (Table 5). Statistical effect in faeces measurements was verified when phenological stages were compared. The OMI did not differ ($P > 0.05$) in any effect, but the OMD was lower in the Period 3 (flowering) compared to Period 2 (pre-flowering). In the grazing Experiment 2, in herbage characteristics, in exception of sward height, all the other parameters were different ($P < 0.05$) between Periods (Table 6). In the faeces measures it was observed effect ($P < 0.05$) of herbage allowance in OM faeces output.
and of the grazing methods and herbage allowance in fCPc. OMI differed ($P < 0.05$) between grazing methods and OMD between herbage allowances.

The use of the equations generated from the indoor experiments and in the validation in grazing experiments (Fig. 3) showed a coefficient of correlation of 0.94 and a RPE of 9.28% between OMI estimated and measured.

### 4. Discussion

The chemical and morphological characteristics of the plants are changed due to the change of the maturity stage, as observed in the present study, which was verified by the reduction in crude protein and increase in the NDF as the plant reached the flowering stage. It happens when the participation of the leaf is reduced and is substituted by inflorescence. As the plant nears the maturity, the content of the cell wall increases, lignin accumulates, and, at the end of the cycle, the maturity of the pasture is reached more quickly (Minson, 1990). The same response was observed by Gerdes et al. (2005) in Italian ryegrass and Chaves et al. (2002) in perennial ryegrass. Minson (1980) observed that, in tropical pastures, the digestibility is reduced by 0.1 units by day, and the relation between chemical characteristics and phenological stage is similar than in temperate species. An effect from leaf content, and consequently protein and energy content, causes large reflections in the intake and digestibility, in which the intake is affected negatively by reducing the quality of the forage. An inverse effect was observed in OMI in this study, that the intake in flowering was higher than in vegetative stage. It can be explained by the expression form of the data, that used grams per day, which is variable not just for forage characteristics, but for the animal size. It was verified that the animals in vegetative stage were lighter than in flowering. In OMD, that is a ratio of the forage that is ingested and digested, a reduction along the maturity of the plant was verified, which clearly indicated a decrease in the pasture quality. The significant linear regressions with a high $R^2$ between the amounts of protein excreted in the faeces and the OMI of sheep indicate that it is a reliable variable to use for measuring intake of Italian Ryegrass in grazing sheep, in agreement with Boval et al. (1996) and David et al. (2014). The inclusion of fNDF and fADF did not improve the estimations, different from that observed by David et al. (2014), which establishes a relationship to use for measuring intake of Italian Ryegrass in grazing sheep. The chemical and morphological characteristics of the forage in the present study (Table 1) had a variation between
Table 6
Herbage characteristics, faeces chemical composition and total output and organic matter intake (OMI) and digestibility (OMD) estimated with equations (Vegetative equation for both Periods) with sheep grazing two herbage allowances (HA: 10 and 20 kg DM/100 kg LW)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Continuous Rotational</td>
<td>Treatments effect (P)</td>
</tr>
<tr>
<td>HA</td>
<td>GM</td>
<td>PS</td>
</tr>
<tr>
<td>10%</td>
<td>7</td>
<td>0.7</td>
</tr>
<tr>
<td>20%</td>
<td>7</td>
<td>0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Continuous Rotational</td>
</tr>
<tr>
<td>Herbage mass (kg/ha)</td>
<td>1110</td>
</tr>
<tr>
<td>Dry matter (g/kg)</td>
<td>281</td>
</tr>
<tr>
<td>NDF (g/kg DM)</td>
<td>435</td>
</tr>
<tr>
<td>OM (g/day)</td>
<td>962</td>
</tr>
<tr>
<td>OMD</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Faest measures

Faecal production (g of OM/day) 198

Crude protein (g/kg OM) 311

ADF (g/kg OM) 435

OMI (g/day) 962

OMD 0.77


Table 2, in that, the effect of phenological stage is verified in OMD and fCPc. Therefore, all the data together had a hyperbolic design, as proposed by the literature (Lukas et al., 2005) as a good model to be used in digestibility estimation based on faecal crude protein index.

The equation generated by the hyperbolic model using fCPC (Table 4) showed determination coefficient of 0.77, but the increase of the determination coefficient to 0.83 by phenological stages, where the leaf amount decreased, modifying the nutritional quality of the forage (NDF increased and CP decreased). Consequently, the OMI and faecal protein relationship changed depending upon the plant maturity stage, increasing the R² and decreasing the RPE when the data were separated by phenological stage.

There are variations between ruminant species and type of diet offered (Penning, 2004). Moreover, animal factors and pasture factors and its interactions influence the intake estimations by faecal protein when animals are grazing. In this framework, Peripolli et al. (2011) found a linear relationship between OMI and faecal nitrogen (FN) excretion (g/d), when they analysed 58 indoor experiments with sheep feed with 28 forages (pure or in mixtures: grass and/or legumes; tropical and temperate species). Data were also analysed separately according to the digestibility and type of forage production cycle, resulting in less variation and more precise equations in most groups.

The hyperbolic function (Fig. 1) describes the rapid increase in OMD per unit of fCPC, followed by a relatively sharp incline before reaching the maximum digestibility. The hyperbolic equations estimated the OMD with a RPE lower than 6%, similar to range classified as a satisfactory estimation by Fuentes-Pila et al. (1996). Other authors consider that the hyperbolic model is most suited for this technique (Boval et al., 1996; Fanchone et al., 2009). This model is based on a biological relationship between the digestibility and the faecal protein compounds (Lukas et al., 2005). Once the amount of protein excreted by faeces is directly related to intake, any change in the OMD will also modify the faecal protein concentration. In a different way than the intake equations, the digestibility equation with the data from phenological stages showed accurate results to estimate the OMD. It is possible to note in Fig. 1 that the data are grouped by stage, which is corroborated by the statistical differences shown in Table 2, in that, the effect of phenological stage is verified in OMD and fCPC. Therefore, all the data together had a hyperbolic design, as proposed by the literature (Lukas et al., 2005) as a good model to be used in digestibility estimation based on faecal crude protein index.

![Fig. 3. Relationship between measured and estimated organic matter intake (OMI) of sheep fed with Italian ryegrass at vegetative (♦), pre-flowering (♦) and flowering (×) stages. The solid line indicates the regression when y=x.](image-url)
the fADFc inclusion justified its use in a multiple hyperbolic model, and the negative relationship between digestibility and fADFc (Fig. 2) reinforces this statement. The use of other components in addition to faecal protein content, in the estimation of OMD, is discussed in the literature (Lukas et al., 2005; Wang et al., 2009), being the fADFc considered the best performing component (Boval et al., 2003). Ribeiro Filho et al. (2005) used multiple equations, including fCPc, fADFc and also CP content of pasture in OMD estimation. They used an equation calibrated from 31 experimental periods with cows fed indoors with fresh perennial ryegrass, Cook’s-foot or white clover (Peyraud et al., unpublished results). The authors did not comment about the use of herbage CP content effect in the digestibility data, but Lukas et al. (2005) observed that dietary CP did not affect the difference between estimated and measured results when included in an equation to estimate the OM digestibility. The problem of using components of the forage supposed to be ingested by the animal is compounded by difficulty in collecting a representative sample of what is actually ingested by the animal.

Validation showed the accuracy of using the equations in grazing sheep (Fig. 3), being in agreement with other studies (Boval et al., 1996, 2003; David et al., 2014). It is important to note that the validation was performed using a range of animals and treatments, and the equations used could cover all these situations. In the OMI estimation, according to the analysis that showed best accuracy in use of the data separated by phenological stage, the equation related to each herbage morphological character was verified in the grazing experiments. Therefore, it is crucial to know in which conditions the pasture is presented, mainly the leaf, stem and florescence participation in the forage.

The use of fCP to estimate the intake in a grazing situation needs a total faecal output value; therefore special attention should be given to avoid faecal losses in the faecal bags used. The results in the present study indicate that OMI and OMD can be accurately estimated without herbage samples being taken, which allows for the animal variation accounted in the diet selection. Even faecal collection using harness can cause some discomfort for the animals, but it does provide an estimate of intake without external markers thereby reducing the time for handling animals, because the external marker technique needs an equilibrium time of 6–7 days (Peyraud, 1997) to start the faeces collection, so more than ten days of handling the animals necessarily. Schneider et al. (2011), when comparing methodologies, considered that the fCP method has the largest potential because it is not based on assumptions such as appropriate forage sampling or external marker recovery rate.

It is important to consider that intake can be estimated directly by the equations proposed in this paper, but can also be estimated using the organic matter digestibility and faecal organic matter output measured by total collection in bags. An alternative for females, as it is not recommended to use the faecal bag collection in females due to urine contamination, a spot sample of faeces could be collected to estimate OMD by fCPc and fADFc and organic matter faecal output measured by an external marker. This option was used by Ribeiro Filho et al. (2005) with dairy cows, measuring the OM digestibility by fCPc-based-equation and the faecal output by dosing ytterbium oxide as an external marker assuming a complete recovery rate. Glindeemann et al. (2009) using female sheep estimated the OMD by the faecal crude protein equation given by Wang et al. (2009) and faecal production by dosing titanium oxide as an external marker which concluded that the methods applied to measure intake and digestibility of herbage in grazing experiment with sheep were suitable. To choose the fCP as a nutritional marker it is important to know your limitations, as discussed above, and consider the experimental objectives and conditions, especially the animal species, gender, herd size and herbage characteristics.

5. Conclusions

Faecal crude protein amount has potential use to estimate herbage intake in sheep grazing Italian Ryegrass; however it is recommend that separate equations by phenological stage of the plant be used and it is important to establish the pasture phenological condition to apply the correct equation to estimate the intake. It is necessary to be precise in estimating the faecal protein output by an external marker or faecal bags.

The multiple hyperbolic model for estimating OM digestibility by fCPc and fADFc is accurate for animals fed with Italian Ryegrass and for its use just a spot sample is required.

Conflict of interest statement

I wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgement

Special thanks to students from Grazing Ecology Research Group (UFRGS – Brazil) who assisted in the experiments and to Margalida Joy (CITA – Spain) for her important corrections in the manuscript. This study has been supported by National Council for Scientific and Technological Development (CNPq – Brazil) and Cerro Corado Ranch who provided the animals.

References


