JOSÉ GILSON LOUZADA REGADAS FILHO

STUDIES ON GROWTH OF BODY PROPER AND ON THE DYNAMICS OF FIBER IN THE GASTROINTESTINAL TRACT OF DAIRY GOATS: A QUANTITATIVE APPROACH

Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae.*

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This thesis is dedicated to my Family for their constant love and support over the years

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BIOGRAFY

José Gilson Louzada Regadas Filho was born February 27, 1982 in Fortaleza/Ce-Brazil. During his undergrad (2003-2007) in Agronomy Engineering at Universidade Federal do Ceará, he became interested in Animal Science. After graduating he pursued his Master's degree in Ruminant Nutrition at the same university. He studied nutritional requirements of sheep.

In 2007 an exchange program brought him to the Viçosa/MG-Br to work with Dr. Marcelo Teixeira Rodrigues at Universidade Federal de Viçosa. As a result of this program, he began a D.Sc degree program with the same adviser with major interest in ruminant nutrition modeling. During his D.Sc course he had the chance to spend six month (August 2012 to January 2013) as intern student at Texas A&M University under supervision of Dr. Luis Orlindo Tedeschi, where he developed an extra research on in vitro gas production systems and respirometry. On August 02th José Gilson L. Regadas Filho defend his thesis "STUDIES ON GROWTH OF BODY PROPER AND ON THE DYNAMICS OF FIBER IN THE GASTROINTESTINAL TRACT OF DAIRY GOATS: A QUANTITATIVE APPROACH" to obtain the Doctor Scientiae degree in Animal Science.

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ABSTRACT

REGADAS FILHO, José Gilson Louzada, D.Sc., Universidade Federal de Viçosa, august of 2013. **Studies on growth of body proper and on the dynamics of fiber in the gastrointestinal tract of dairy goats: a quantitative approach.** Adviser: Marcelo Teixeira Rodrigues. Co-adviser: Ricardo Augusto Mendonça Vieira.

This thesis was composed of four studies. The first study aimed to use nonlinear mixed-model methodology to compare the growth curves of two dairy goat genotypes (Alpine and Saanen). The nonlinear functions evaluated included the Brody, Von Bertalanffy, Richards, Logistic, and Gompertz functions. First, the random effects u_1, u_2, and u_3 were linked to the asymptotic body weight (β_1), constant of integration (β_2), and rate constant of growth (β_3) parameters, respectively. In addition to a traditional fixed-effects model, we evaluated four combinations of models using random variables. Second, we refined the fit of the best adjusted model by using the power variance and modeling the error structure. The Richards (u_1 and u_3) function had the best fit to the data. Different Richards growth-curve parameters should be used for the predominantly Alpine and Saanen genotypes. The nonlinear mixed-model methodology is an efficient tool to address growth-curve features. The second study aimed to evaluate rumen-fiber stratification and to develop a mathematical approach for predicting the mean retention time (MRT) of forage and concentrates considering this stratification for goats. A dataset from three studies was used that contained information regarding fiber and lignin intake as well as rumen content and the kinetics of fiber passage for forage and concentrates. The heterogeneous fiber pools in the rumen were evaluated using the Lucas' test assumptions. The equations used to predict the MRT for forage and concentrate fiber were developed using stepwise regression. A sensitivity analysis was conducted using a Monte Carlo simulation to investigate the relationships between the dependent and independent variables and between the forage- and concentrate-passage rates. The homogeneous fiber-pool approach could not be applied once that was detected a metabolic portion (intercept $\neq$ 0) for lignin content. The stepwise regression model for MRT for forage estimation had an approximate coefficient of determination and a root mean square error (RMSE) for the forage of 0.53 and 9.78 h, respectively, and for the concentrate of 0.49 and 5.86 h, respectively. The rates of passage of forage and concentrate in goats overlap and are closely related. A heterogeneous rumen fiber pool should be assumed for goats fed diets with high fiber contents. The objectives of the third study were to evaluate the use of the

equations previously estimated in a mechanistic nutritional model for goats (Small Ruminant Nutrition System – SRNS) and to compare with the homogeneous fiber-pool approach adopted currently. Moreover, an alternative approach to predict the dry matter intake (DMI) based on optimization process was evaluated. The metabolizable energy intake (MEI) and milk yield (MY) estimated using these scenarios were compared with the results of an independent dataset (n = 327). The alternative method to estimate DMI was compared with the results observed in the independent dataset. The evaluated scenarios presented the same ability to predict the MEI and MY. However, both of the scenarios presented a mean bias in the MY prediction. The alternative approach to estimate the DMI is reasonable and can be used to formulate diets. It is necessary to develop a submodel that accounts for tissue mobilization to be inserted in the SRNS model. The fourth experiment aimed to compare two computerized systems used for determining the fermentation kinetics, through pressure sensors, of in vitro anaerobic gas incubation of feedstuffs commonly fed to ruminant animals. The evaluated systems were the ANKOM^{RF} Gas Production Systems® (aIVGP), which uses a wireless system, and the *in* vitro anaerobic fermentation system (tIVGP) as described by Pell and Schofield (1993). The total gas production, fractional production rate of gas, pH of the solution, and hydrogen and methane concentrations in the bottle's headspace were used to compare the systems. A significant concentration of hydrogen was not found in the headspace of the tIVGP. The IVGP systems had similar values for total gas production, methane concentration, and solution pH. However, the estimated values of the fractional production rate of gas were different. The results suggest that both of the IVGP systems had similar fermentation patterns. The difference in the fractional production rate of gas between these IVGP systems may be due to differences in the headspace gas composition.

RESUMO

REGADAS FILHO, José Gilson Louzada, D.Sc., Universidade Federal de Viçosa, agosto de 2013. **Estudos sobre as propriedades do crescimento corporal e sobre a dinâmica da fibra no trato gastrointestinal de caprinos leiteiros: uma abordagem quantitativa**. Orientador: Marcelo Teixeira Rodrigues. Coorientador: Ricardo Augusto Mendonça Vieira.

Esta tese foi desenvolvida a partir de quatro estudos. O primeiro estudo teve por objetivo avaliar o uso da abordagem de modelos não lineares mistos na comparação de curvas de crescimento de dois genótipos de caprinos leiteiros (Alpina e Sannen). Os modelos não lineares avaliados foram Brody, Von Bertalanffy, Richards, Logístico e Gompertz. Efeitos aleatórios (u_1, u_2 *e* u_3) foram vinculados aos parâmetros que representam o peso assintótico (β_1), constante de integração (β_2) e taxa de crescimento (β_3), respectivamente. Quatro combinações de efeitos aleatórios foram avaliadas, além da abordagem tradicional considerando todos os parâmetros da função fixos. Uma correlação dos erros foi identificada a partir da analise de resíduos e uma modelagem da estrutura do erro foi necessária. A função de Richards (u_1 e u_3) com uma estrutura de erro do tipo SP(MATERN) apresentou melhor ajuste. Existe diferença no padrão de crescimento para os dois genótipos avaliados. A metodologia de modelos não lineares mistos é uma eficiente ferramenta para lidar com características intrínsecas de curvas de crescimento como correlação de erros e heterogeneidade de variância. O segundo estudo foi realizado com o objetivo de estudar a estratificação da fibra no rúmen e desenvolver uma abordagem matemática para estimação do tempo médio de retenção (TMR) de concentrados e forragens no rúmen de caprinos que considerasse tal estratificação. Para isto, um banco de dados que continha informações a respeito do consumo e da massa ruminal de NDF e lignina, bem como informações a respeito da cinética de passagem de forragem e concentrados através de indicadores foi montado. Utilizaram-se os pressupostos do Teste de Lucas para identificar compartimentos heterogêneos para a fibra e lignina no rúmen. Foi utilizada regressão stepwise para identificar variáveis preditoras significativas, posteriormente, uma metanálise foi realizada de forma a retirar o efeito de experimento na regressão. Foi realizada também uma avaliação da relação entre taxa de passagem de volumoso e concentrado através de simulação de Monte Carlo. A abordagem de um único compartimento ruminal para lignina não se aplica, uma vez que foi detectada uma significativa (intercepto $\neq 0$) porção metabólica para tal entidade. A regressão

stepwise para estimativa do TMR para forragens considerando os dois compartimentos ruminais rendeu um coeficiente de determinação aproximado (R^2) e uma raiz do quadrado médio do erro (RQME) de 0.53 e 9.78 h, respectivamente e para concentrado de 0.49 e 5.86 h, respectivamente. A taxa de passagem de forragens e concentrado em caprinos é relacionada. A estratificação da fibra ruminal deve ser assumida quando cabras são alimentadas com altos conteúdos de fibra. O terceiro estudo teve por objetivo avaliar a inserção das equações anteriormente desenvolvidas em um modelo nutricional mecanicista para caprinos (Small Ruminant Nutrition System – SRNS) e comparar com a atual abordagem de um único compartimento de fibra ruminal. Além disso, uma abordagem alternativa para estimação do consumo de matéria seca (CMS) foi avaliada. Um banco de dados (n = 327) que continha informações necessárias para as predições foi levantado. As variáveis avaliadas predição do consumo de energia metabolizável (CEM) e produção de leite (PL). A abordagem alternativa para estimação do consumo de matéria seca é baseada em métodos de otimização. O CMS estimado desta forma foi comparado com CMS do observado no banco de dados. O modelo adotando o compartimento heterogêneo de fibra ruminal teve o mesmo poder de predição da abordagem considerando um único compartimento, entretanto, ambos os cenários apresentaram um viés médio ao estimar a produção de leite. O CMS estimado pelo o método alternativo apresentou boas estimativas e pode ser utilizado em formulação de rações para ruminantes. É necessário o desenvolvimento de um submodelo que considere a mobilização de reservas corporais a ser introduzido no SRNS de forma a tornar as predições de PL mais verossímil. O quarto estudo teve por objetivo comparar dois sistemas que são usados para determinar a cinética de fermentação de alimentos para ruminantes através da técnica de produção de gases *in vitro*. Os sistemas avaliados foram o ANKOM^{RF} Gas Production Systems® (aIVGP) que utiliza um sistema se fio; e o outro foi sistema foi o descrito por Pell e Schofield (1993) (tIVGP). As variáveis utilizadas para comparar os sistemas foram a concentração de hidrogênio e metano no gás acumulado, o pH da solução após a fermentação, o total de gás produzido e a taxa de produção de gás. Não foi encontrado significativa concentração de hidrogênio no tIVGP, portanto não foi comparado entre os sistemas. Os sistemas apresentam similares resultados para produção total de gás, concentração de metano e pH da solução, entretanto, as taxas de produção de gás foram diferentes. Os resultados sugerem que os sistemas apresentam similar padrão de fermentação. A diferença na taxa de produção de gás pode se dar devido a diferentes composições iniciais na fase gasosa dos sistemas.

GENERAL INTRODUCTION

The demand for nutrients for animal maintenance and production is considered the primary factor for determining dry matter intake (DMI) in ruminants. Therefore, maximizing the DMI is a major concern for those involved with animal production. However, tropical forages are known to have a high fiber concentration and they undergo cell wall lignification very early. As a consequence, a reduction in the voluntary feed intake caused by alterations in the dynamic of fiber degradation and passage is expected, which affects the energy available for microbial growth, the efficiency of metabolizable energy and nutrient partitioning. Therefore, a recommendation for adequate levels of fiber that can maximize the energy intake and provide a suitable environment for microbial growth has been recognized as invaluable for formulating more economical and efficient diets.

A non-specific and comprehensive method is required to study how fiber interacts with other nutritional or productive parameters in ruminants. Nutritional models based on mechanistic approaches have encompassed theoretical aspects of ruminant production; hence, such models are the most appropriate method for addressing this issue. The Small Ruminant Nutrition System (**SRNS**) (Tedeschi et al., 2010) is the most recent nutritional model for sheep and goats; its set of equations regarding the dietary supply of energy and nutrients is based on the Cornell Net Carbohydrate and Protein System (**CNCPS**) for cattle (Fox et al., 2004) and sheep (Cannas et al., 2004). However, there are gaps in the information regarding some important factors. For example, ruminal fiber stratification is a phenomenon that can occur when ruminants are fed a considerable amount of fiber (Hungate, 1975, Sutherland, 1988), and it has not been considered by SRNS or any other

nutritional model. The lack of data supporting the use of a heterogeneous ruminal fiber approach for goats may explain why it is not used, so a simplification was assumed.

Another important issue not described in the literature is the mature body mass (MBM) of goats. The MBM is essential information used in the SRNS to predict goat maturity. An effective way to access this information is by using growth curve studies. However, certain factors have not been taken into account during goat growth curve analysis, including correlated errors and heteroscedasticity; consequently, the parameters and standard errors estimated in previous papers may be biased. Therefore, an appropriate approach must be used to obtain reliable parameters for nutritional models (e.g., asymptotic body mass, rate of growth and inflection point).

In a different context, the mechanistic nutritional models currently in use are dependent on accurate and precise information regarding the degradation rates of different nutrient fractions. The in vitro gas production technique (IVGP) is an important tool for meeting that requirement and can be used in concert with in vitro or in vivo fiber degradation studies to estimate/access the rate of degradation for difficult-to-obtain nutrient fractions (fractions A1 (soluble sugars) and B1 (starch and pectin) of carbohydrates) (Favoreto et al., 2008). However, there is a lack of standardization in this analysis among different laboratories that may result in biased parameters, even when analyzing exactly the same feed. At this time, there are some commercial apparatuses that can be used to analyze the IVGP; nevertheless, a comparison between traditional and reliable methods must be made.

The objectives of this thesis were to 1) study the goat growth curve by applying adequate statistical methods; 2) study ruminal fiber stratification in goats; 3) evaluate the SRNS model by considering an alternative approach to addressing ruminal fiber mass and dry matter intake; and 4) evaluate a commercial apparatus for IVGP estimation.

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Comparison of growth curves of two genotypes of dairy goats using nonlinear mixed models

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ABSTRACT

The objective of this study was to use a nonlinear mixed model methodology to fit the growth curves (weight vs. time) of two dairy goat genotypes (Alpine, +A; and Saanen, +S). The nonlinear functions evaluated included Brody, Von Bertalanffy, Richards, Logistic, and Gompertz. The growth curve fit was performed using two steps. First, random effects u_1, u_2, and u_3 were linked to the asymptotic body weight (β_1), constant of integration (β_2), and rate constant of growth (β_3) parameters, respectively. In addition to a traditional fixed-effects model, we evaluated four combinations of models using random variables: all parameters associated with random effects (u_1, u_2, and u_3), only β_1 and β_2 (u_1 and u_2), only β_1 and β_3 (u_1 and u_3), and only β_1 (u_1). Second, we refined the fit of the best adjusted model by using the power variance and modeling the error structure. Residual variance (σ_e^2) and the Akaike information criterion were used to evaluate the models. After the best fitting model was chosen, the genotype curve parameters were compared. The residual variance was reduced in all scenarios for which random effects were considered. The Richards (u_1 and u_3) function had the best fit to the data. This model was re-parameterized using two isotropic error structures for unequally spaced data, and the structure known in the literature as SP(MATERN) proved to be a better fit. The growth curve parameters differed ($P < 0.01$) between the two genotypes, with the exception of the constant that determines the proportion of the final size at which the inflexion point occurs (β_4) ($P = 0.14$). The nonlinear mixed model methodology is an efficient tool for evaluating growth curve features, and it is advisable to assign biologically significant parameters with random effects. Moreover, we recommend evaluating error structure modeling to account for possible correlated errors that may be present even when using random effects. Different Richard growth curve parameters should be used for the predominantly Alpine and Saanen genotypes because there are differences in their growth patterns.

Keywords: Alpine, correlated errors, random effect, Saanen

INTRODUCTION

Studies on the growth curves for livestock have been extensively used to examine how body weight and other characters of interest (fat deposition, organ size, etc.) develop over time and in relation to the environment, diet, genotype, and other factors. Normally, this relationship (weight *vs.* time) is assessed using nonlinear functions, such as $y_i = f(x_i, \beta_i) + e_i$, where y_i in which y_i is the average body weight of the animal at time x_i; y_i is estimated using a nonlinear function f with β_i parameters, whose estimates are obtained by using ordinary least squares regression; and e_i represents the unexplained error. The errors are assumed to be normally, identically, and independently distributed with a mean zero and a constant variance, or iid ~ N(0, σ^2). On the basis of these assumptions, the nonlinear regression model is considered a fixed-effects model (Craig and Schinckel, 2001).

However, when multiple observations are recorded in the same experimental unit, a parameter or coefficient that varies from one unit to another can be considered random (Peek et al., 2002). This proposition is based on the concept of random effects, in which an effect is considered random if its levels represent values of a larger population with a probability distribution (Littell et al., 2006), which is the case, for example, for the asymptotic body weight (a common parameter in most of the models used in livestock growth studies).

In addition, measurements taken on the same experimental unit are more closely related than those on other experimental units, and on the same experimental unit, measures at closely intervals are more correlated than measures at longer intervals (both within the same unit) (Littell et al., 2006). These relationships are inherent for this type of data, which may disrupt the basic assumptions of statistical analysis, such as independence

of errors and variance homogeneity (Craig and Schinckel, 2001, Littell et al., 2006, Strathe et al., 2010). Thus, if random effects are not added to the model, the estimated standard error of the parameters may be biased because the assumption of independence of errors might be violated (Peek et al., 2002).

Another factor that supports the use of the nonlinear mixed model methodology is the type of data normally obtained for use in growth studies. In general, the data are unbalanced, with different numbers of body weight measurements for different animals and a tendency for the number of animals in the study to decrease over time due to death, slaughter, disposal, and other factors. The unbalanced nature of this type of data can lead to bias in the estimated parameters when using the conventional method (Craig and Schinckel, 2001, Wang and Zuidhof, 2004).

However, there is still controversy about which growth curve parameters normally used in the literature should be considered as random. This decision should be based on the biological interpretation of the parameters, the significance of the estimated variance components, and, above all, common sense.

Given this context, the objectives of the study described in this paper were the following: 1) to identify the best commonly used nonlinear function to describe dairy goat growth, 2) to determine which growth curve parameters should be linked to random coefficients, 3) to compare the curves obtained from the nonlinear mixed model methodology to curves obtained with traditional methodology, and 4) to compare the curve parameters obtained for two genotypes of dairy goats.

MATERIALS AND METHODS

Animals and data set descriptions

The data used in this study were collected from the goat herd of the Goat Sector at the Federal University of Viçosa (Universidade Federal de Viçosa-UFV), Viçosa, MG, Brazil. We collected a total of 14,003 weighing records from female goats between 1992 and 2010. Only those goats with four or more recorded weight measurements were included. Data from animals without recorded birth dates were excluded. Then, the data were divided according to the genotype of the animals: Saanen (+S) and Alpine (+A). A graphical analysis was conducted to assess the consistency of the data and to identify the excluded end points, including data from animals of all other genotypes. The database is summarized in Table 1.

Animal management consisted of a free-stall milk production system in which animals had ad libitum access to corn silage and concentrate. There are normally two calving seasons per year for goats; however, each animal was allowed just one calving per year. During the growth phase, the animals were weaned, on average, when they were 18 kg (110-140 days of life), and the first pregnancy occurred, on average, at 35 kg of body weight (340-370 days of life). The goats lactated for approximately 300 days, and their average milk production was 2.6 kg/day. The number of lactations ranged from zero to seven.

The number of weighings by animal ranged from four to 21, (Table 1), and the minimum and maximum difference between two consecutive weighings were one and 1,190 days, respectively. These features make the data set extremely unbalanced, spatially and numerically. Furthermore, the initial graph analysis suggested that there was an increase in the variance of the data over time (the independent parameter). Thus, a special approach to modeling the growth curves was required.

Growth curve functions

We evaluated five nonlinear models commonly used in the literature for describing animal growth curves. In these models, shown in Table 2, y = animal weight at time t_i; β_1 = estimated body weight of the animal when $t \rightarrow \infty$, or asymptotic body weight; β_2 = constant of integration; β_3 = rate constant, which determines the spread of the curve along the time axis; and β_4 = constant that determines the proportion of the final size at which the inflexion point occurs. The inflexion point can be determined using the equation $IP_{(\%\beta_1)} = \beta_4^{1/(1-\beta_4)}$, and the instantaneous absolute growth rate can be calculated using the equation $IAGR = \beta_3 \times Y_{est}\left[(\beta_1/Y_{est})^{1-\beta_4} - 1\right]/(1-\beta_4)$, where Y_{est} is the body weight estimated at a given time (Richards, 1959).

Nonlinear mixed model

A nonlinear mixed model methodology was used to obtain the growth curve parameters according to the following expression:

$$\mathbf{y}_i = f\left(\mathbf{x}_{ij}, \boldsymbol{\beta}, \mathbf{u}_i\right) + \mathbf{e}_i \quad (1)$$

where $\mathbf{y}_i$ is a vector (n_i x 1) of recorded body weights for the subject i = 1, 2,…, m; m is the number of goats; f is a nonlinear function of the covariate matrix $\mathbf{x}_{ij}$; $\mathbf{x}_{ij}$ is a matrix (n_i x 2) of independent variables, in which the first column contains the i^{th} age of measurement and the second column contains the j^{th} subject (goat) whose body weight was measured; $\boldsymbol{\beta}$ is a vector (p_i x 1) of unknown fixed-effect parameters; $\mathbf{u}_i$ is a vector (q_i x 1) of unknown random-effect parameters unique to the subject goat i and assumed to follow a multivariate distribution with a mean of zero and a conditional unstructured covariance matrix, n x n (**G**); and $\mathbf{e}_i$ is a vector (n_i x 1) of the error term assumed to follow a multivariate random normal distribution with zero mean and conditional covariance matrix structure (**R**).

The dependent variable (y) in this case is influenced by quantitative (time) and qualitative (genotype) variables. The effects of the quantitative variables are analyzed via

nonlinear regression. However, it is also necessary to assess the effects of the qualitative variable on the dependent variable and the regression parameters; thus, we created *dummy* variables z_1 and z_2, where for the +S genotype, $z_1 = 0$ and $z_2 = 1$, and for the +A genotype, $z_1 = 1$ and $z_2 = 0$. With this approach, we were able to estimate growth curve parameters (β_1, β_2, β_3 and β_4) independently for each genotype.

The growth curve modeling consisted of two steps, as described below. First, we formulated a set of candidate nonlinear mixed models that were preliminarily analyzed to choose the function with the best fit. In this first step, we evaluated which curve parameters should have a random effect component and compared them with the traditional fixed-effect model. However, despite the high hardiness with which nonlinear mixed model methodology treats the correlated errors, it is important to note that heterogeneity and correlated errors can occur even with the inclusion of random effects (Meng and Huang, 2010, Yang and Huang, 2011). Thus, a refinement of the model chosen in the first step was necessary to accommodate the violated model assumptions (second step).

First step

A central phase in the model-building of mixed-effects models is to decide which of the parameters in the nonlinear model require random effects to account for their between-subject variation and which can be treated as purely fixed effects (Pinheiro and Bates, 2000). Thus, we created the random parameters u_1, u_2, and u_3 to account for between-subject variation for the β_1, β_2, and β_3 parameters, respectively, enabling curve parameters to be estimated for each goat (Littell et al., 2006). No random parameter was created for the β_4 parameter in the Richards function because it was difficult to achieve the convergence criterion and the high correlations between the estimated parameters (over-parameterized model). The following four combinations of random effects were evaluated:

all of the parameters related to random effects (u_1, u_2, and u_3), only parameters related to β_1 and β_2 (u_1 and u_2), only parameters related to β_1 and β_3 (u_1 and u_3), and only that related to β_1 (u_1). To avoid problems with floating-point errors and overflows, we rescaled β_3 in the models due the difference in magnitude of this parameter in relation the others.

First, the equations were fitted to each model and previously described combination of random effects through PROC NLMIXED (SAS 9.3) (see Appendix 1). The FIRO method was used to achieve convergence. The FIRO method uses the First-Order Method described by Beal and Sheiner (1982) to compute the integral over the random effects to the marginal maximum likelihood. The fixed-effects model was also estimated using PROC NLMIXED, except that, in this case, variance components were not included. The FIRO method does not support the model without random effects; thus, we used the Adaptive Gaussian Quadrature described by Pinheiro and Bates (1995). The estimated parameters, standard error, and residual variance for the fixed-effects model were similar to those of the model obtained using PROC NLIN.

The following criteria were adopted to select the function that best described the growth curve of the goats: 1) convergence (the iterative process in NLMIXED converges at the n^{th} iteration when $(g_k H_k^{-1} g_k)/(|l_k| + 1e^{-6}) < 1e^{-8}$) (SAS Institute Inc., 2008); 2) the final hessian matrix is nonsingular and positive definite; 3) the approach described by Burnham and Anderson (2002) and Vieira et al. (2012) using the Akaike information criterion (AIC), the difference among AIC values (Δ_r), the Akaike weights or likelihood probabilities (w_r), and the evidence ratio or relative likelihood (ER_r), which can be computed using the following equations:

$$AIC = 2f\left(\hat{\theta}\right) + 2p$$

$$\Delta_r = AIC_r - \min AIC_r$$

$$w_r = \frac{\exp(-\Delta_r/2)}{\sum_{r=1}^{R} \exp(-\Delta_r/2)}$$

$$ER_r = \frac{\max w_r}{w_r}$$

where *f()* is the negative of the marginal log-likelihood function, $\hat{\theta}$ is the vector of estimated parameters, and *p* is the number of parameters (SAS Institute Inc., 2008); and 4) a graphical analysis of the Pearson residuals against the predicted values was used to evaluate the model assumptions. The Pearson residuals were obtained for the fixed model and random model without a correlation structure as $r_i = e_i / \sqrt{\widehat{\sigma_e^2}}$ and for the model with a correlation structure (second step described below) as $\boldsymbol{r}_i = \widehat{\boldsymbol{C}}'^{-1}(\boldsymbol{y}_i - \widehat{\boldsymbol{y}}_i)$, where $\widehat{\boldsymbol{C}}$ denotes the Cholesky root of the estimated **R** matrix (SAS Institute Inc., 2008).

Second step

The model with the best goodness-of-fit (combination between nonlinear equation and random parameters) was selected for further analysis.

Despite there being no sign of heteroscedasticity in the residuals of the chosen model, it was easy to identify high Pearson residuals obtained even when using random effects in the model. The higher values found for the Pearson residuals, many of them above three, were an indication of problems in the model. The first remedy would be to exclude the outliers from the data set and re-parameterize the model, which would solve the problem but cause a loss of information.

Therefore, we decided to use two tools to address this problem and the remaining correlated errors. The residual variance was modeled using the power-of-the-mean variance (PV) function. Using the notation described by Littell et al. (2006), the variance matrix **R** is assumed to be of the form $\boldsymbol{R} = diag(\sigma^2 |x_i' \beta^*|^{\theta})$, where θ is the power to be estimated. To choose the value of θ, we found the power that minimized the approximate -2Log likelihood (Littell et al., 2006); this θ value was estimated to be 2.91.

As previously noted, correlated errors can occur even when random effects are used in the model. These errors primarily occur if there are parameters in the function that do not have a random parameter to account for their between-subject variability. An error structure matrix can be modeled to account for this interrelation. When the model is fit without modeling the serial correlation, the independently and identically distributed error structure is assumed to be $Var[e_i] = \sigma_e^2$ and $Cov[e_i, e_j] = 0$. However, there is one intrinsic characteristic of our data set that must be considered, namely, the unequally spaced measurement intervals for the animal subjects.

There is a range of structures available in the literature to address this issue. Structures that accept unequally spaced data have been described by Littell et al. (2006) and the SAS Institute Inc. (2008). Normally, the covariance is assumed to be a function of the distance between locations. If d_{ij} denotes the interval of time between the measurements made on the same animal, the covariance models have the general form $COV[e_i, e_j] = \sigma_e^2[f(d_{ij})]$ (Littell et al., 2006).

In the second step, we examined all possible isotropic candidate structures described in the literature; however, only two structures met our convergence criterion: the spatial power function (SP(POW)) and the MATERN function (SP(MATERN)).

The spatial power structure provides a direct generalization of the auto-regressive structure for equally spaced data and is assumed to be $f(d_{ij}) = \rho^{d_{ij}}$. The MATERN isotropic covariance function is given as $f(d_{ij}) = \frac{\left(\frac{d_{ij}}{2V}\right)^V 2K_v\left(\frac{d_{ij}}{\rho}\right)}{\Gamma(V)}$, where K_V is a modified Bessel function of the second kind of order V; Γ is the gamma function; ρ is a scalar parameter controlling the spatial range of correlation; and V is the 'smoothness' parameter, which allows great flexibility for modeling the local spatial covariance. When V is small ($V \rightarrow 0$), the spatial process is assumed to be rough, and when V is large ($V \rightarrow \infty$), the

process is assumed to be smooth (Littell et al., 2006, Minasny and McBratney, 2007, SAS Institute Inc., 2008).

The NLMIXED procedure does not support the modeling of error structure directly; thus, we used the SAS macro %NLINMIX to refine the model to account for the chosen error structure. The restricted maximum likelihood was used along with the two expansion methods available in the SAS macro %NLINMIX (ZERO and EBLUP) to attempt to fit the model (see Appendix 1). The model adjusted in this way was compared with the fixed model and the random parameter model that was estimated using the previously described approach.

After selecting the model that best described the growth curves and included the previously estimated variance and covariance matrices, we were able to test hypotheses regarding one or more growth curve parameters. Thus, we tested the difference between the growth curve parameters of the genotypes with the objective of simplifying the model ($\alpha = 0.05$).

Cross validation

A cross validation was performed to evaluate the goodness-of-fit of the chosen function. The original data set was randomly partitioned into two sub-data sets (training and test data), each containing 50% of the animals per genotype. The division of the original data set was conducted using PROC SURVEYSELECT from the SAS software (version 9.3). The first sub-data set (training data set) was used to fit the previously chosen best model (steps 1 and 2), and the second sub-data set (test data set) was used to evaluate the robustness of the model. Equation precision was measured with the coefficient of determination (r^2) between the observed and predicted values and the simultaneous F-test of the intercept and slope (intercept = 0 and slope = 1) whereas the accuracy of the model was determined based on the concordance correlation coefficient (CCC) and the root mean

square error prediction (RMSEP) and its decomposition into mean bias, systematic bias, and random errors (Tedeschi, 2006). The model evaluation was implemented using the Model Evaluation System v. 3.1.13 (http://nutritionmodels.tamu.edu/mes.html; verified May 28, 2013). Moreover, the parameters of the curve fit from the training data set were compared with the parameters of the curves fit from the original data set using 95% confidence intervals.

Impact of the number of records on random parameters

One of the major advantages of the nonlinear mixed model methodology is the possibility of having individual growth parameters adjusted for each animal and testing to determine how some parameters differ from the population mean. Using the chosen model and the individual deviation of asymptotic and constant rate parameters, we were able to evaluate the effect of the number of records on the parameters estimated for each goat using descriptive graphic analysis.

RESULTS

One function did not achieve the convergence criterion (Richards u_1, u_2, and u_3). The Richards (u_1 and u_2) model presented the singular Hessian matrix, which prevents its unique inversion. The Hessian matrix rendered by the Brody model was not positive definite, and the Gompertz (u_1, u_2, and u_3) model had at least one negative eigenvalue.

Table 3 shows the estimated parameters for all of the evaluated models and combinations of random variables. Although represented by the same Greek letters, except for asymptotic body weight (β_1), the estimated parameters cannot be compared among nonlinear functions (Forni et al., 2009). The only comparisons that are possible are between combinations of random variables or between assessed genotypes within each function. Among all of the scenarios that were assessed, the +S animals consistently had

higher asymptotic body weights. Estimates of asymptotic body weight varied from 43.20 to 58.11 kg for the +A genotype and from 48.00 to 67.38 kg for the +S genotype.

The calculated birth weight (calculated as time = 0) for the Logistic and Gompertz (all combinations of random effects) models produced unrealistic estimated birth weight values. Reasonable estimates were made by the Brody and Richards (all combination of random effects) models.

High β_3 parameter values indicate higher precocity, i.e., a higher fractional rate at which the animal approaches asymptotic body weight (Brown et al., 1976). The +A animals presented higher values (except for the Richards (u_1 and u_3)) for this parameter than the +S animals, possibly indicating earlier development.

Table 4 shows the values of the criteria used to select the model that best describes goat growth in addition to the estimated variance and covariance components. The fixed-effect models were found to always display higher residual variance than the random-effects models did. The Richards (u_1 and u_3) function had the best fit. The value of Δ_r to the nearest model was 86, which, according to Burnham and Anderson (2002), indicates essentially no support. The large Δ_r values obtained became a calculation of the likelihood probability and evidence ratio unnecessary, as these criteria are equal to 1 for the Richards (u_1 and u_3) model.

The random effects of the Richards (u_1 and u_3) model were significant ($P < 0.001$). The value found for the component that measures the population variability of parameter β_1 was 209.3, and for β_3, this value was 1.20. However, high Pearson residuals were observed when the Richards (u_1 and u_3) model (Figure 1) was used. Thus, this model was chosen for further analysis.

In the second step, the variance power and the modeled error structure matrix were included. Only the SP(POW) and SP(MATERN) structure achieved the convergence

criterion. The use of these tools produced low values of the Akaike information criterion when compared with the previous approach. The SP(MATERN) structure yielded better adjustments, as determined by the fact that Δ_r = 1,126 (Table 5).

Using the SP(MATERN) error structure, there were differences between estimated parameters β_1 (P < 0.0001), β_2 (P = 0.0025), and β_3 (P = 0.0026) of the genotypes evaluated; however, there was no difference in the β_4 parameter (P = 0.1430) (Table 5). Therefore, the model was re-parameterized to account for just one β_4 parameter. The final parameters adjusted for each genotype were as follows: β_1 = 52.76 (SE = 0.463), β_2 = 0.9439 (SE = 0.002), and β_3 = 2.8326 (SE = 0.066) for the +A animals, and β_1 = 55.99 (SE = 0.587), β_2 = 0.9501 (SE = 0.002), and β_3 = 2.6303 (SE = 0.079) for the +S animals. The common β_4 parameter was 0.9642 (SE = 0.013). The variance components were σ^2_{u1} = 47.48, σ^2_{u3} = 0.4604, and σ_{u1u3} = -1.9971 (P < 0.0001) (Table 6). The growth curves are shown in Figure 2.

The inflexion point was determined as a function of β_4 (Richards, 1959). This parameter did not differ between the evaluated genotypes; the inflexion point at the same proportions of asymptotic body weight for both genotypes (36% of β_1) yielded 19.05 kg and 20.22 kg for genotypes +A and +S, respectively.

Figure 1 shows the Pearson residuals for the Richards model plotted against the predicted values (kg) by genotype for the Fixed model (Panel 1), the Random model (u_1 and u_3) (Panel 2), and the Random (u_1 and u_3) + VP + SP(MATERN) model (Panel 3). When the function was fit using the traditional approach, heteroscedasticity and the correlated errors occurred. In contrast, when random effects were added to the model, there was an improvement in the quality of the fitting; however, there were high Pearson residuals in this case, and correlated errors are easy to identify by the shape of the plotted residuals. This increase in residuals occurred once there were two common parameters (β_2

and β_4) in the functions, even when the errors were assumed to be independent. The variance power and error structure modeling yielded a better fit, and the Pearson residuals were within an acceptable range.

The Richards (u_1 and u_3) + VP + SP(MATERN) modeling approach was used to fit the training data set in the cross-validation analysis. The parameters estimated (data not show) did not differ from the parameters fitted from the original data set ($P > 0.05$), indicating that the chosen approach was effective even when using a reduced data set. When using the test data set to evaluate the model, the simultaneous F-test for the intercept (1.18 ± 0.14) and slope (0.98 ± 0.0045) rejected H_0 ($r^2 = 0.90$). The CCC obtained was 0.95 (range from 0 to 1), indicating high accuracy. The RMSEP was 5.48 kg, and its decomposition indicated a high contribution from the random errors (98.37%), whereas the contributions of the mean bias (1.47%) and systematic bias (0.32%) were negligible. Figure 3 shows the relationship between the observed and predicted body weight values from the cross validation.

The Figure 4 shows that there was no apparent effect on the random parameter estimates from the number of weight measurements (Figure 4).

DISCUSSION

Growth curves and random effects

The nonlinear mixed model has as its main feature the partitioning of error variation into within- and between-subject variation. The fixed effects β_1, β_2, β_3, and β_4 represent the mean values of the parameters in the population of individuals. The individual deviations are represented by the random effects u_1, u_2 and u_3, which are assumed to be distributed normally with mean 0 and unstructured covariance matrix **G**.

For models with only the variance component linked to asymptotic body weight, the error variation was partitioned into variation within goats (σ_e^2) and the variation between goats (σ_{u1}^2). When two variance components were added to the variation between goats, the error variation was partitioned into variation due to asymptotic body weight, the sigmoidal-shaped curve of each animal, and the covariance between these terms (σ_{u1}^2, σ_{u2}^2, and σ_{u1u2}) or due to the asymptotic body weight, rate constant, and covariance between these terms ($\sigma_{u1}^2, \sigma_{u3,}^2$ and σ_{u1u3}), and so on, when 3 random effects were added; however, when three variance components were added in the growth curve model, there were indications of over-parameterization and more highly correlated parameters, causing problems with convergence and the hessian matrix.

In consequence, the estimate of the random effects (u_1, u_2, and u_3) of each goat represents the deviation of a determined parameter from its corresponding parameter for its population average. For example, the mean asymptotic body weight of the population of goats with +A genotype is 52.7 kg (Table 6), and the specific estimate of random effects for a specific goat is 5.9 kg (empirical best linear unbiased prediction – EBLUP). Therefore, the mean asymptotic body weight estimated for this specific animal is 58.6 kg, independent of short-term fluctuations in weight due to extraneous environmental effects such as climate and food supply as well as lactation and pregnancy. Thus, the mean estimated parameters are not greatly affected by these types of weight fluctuations. In fact, these parameters represent the mean value of the growth pattern of the goats on the production system.

It is possible that including parameters with biological significance with random variables in the model would reduce the residual variation, given that this tool reduces variability due to the parameters that have probability distributions and that can vary greatly from animal to animal, such as asymptotic body weight or rate constant. The

random effect estimate measures the difference between the value assigned to each individual and the average population value (Aggrey, 2009).

The high values observed for σ_{u1}^2 in most of the estimated models indicate the considerable contribution of this parameter in the residual variance component when this random variable is not added to the general model. Thus, it is important to incorporate this effect when using this methodology to estimate growth curves.

The σ_{u3}^2 component was almost always found to be significant. In addition, adding the random effects linked to the rate constant improved the estimated model fit, a fact that was validated by better selection criteria (residual variance and AIC) when including this component. Moreover, the significance of this parameter indicates that the rate constant varies among the goat population and is thus subject to breeding program selection.

A major advantage of selecting a nonlinear mixed model methodology to fit livestock growth curves is the possibility of including population variation measurements in stochastic models to predict animal performance. Normally, models predicting animal performance are deterministic, static, and empirical, and for the same *input*, there is only one *output* that is modeled from the averages, with little or no emphasis on population variation (Pomar et al., 2003). Therefore, when small samples are used, such as in the case of small farms, the errors become greater, especially when the model parameters vary greatly, as is the case for asymptotic body weight.

Understanding the variation among animals for the more widely variable parameters of the livestock system (dry matter intake, growth parameters, nutrient use efficiency, etc.) using stochastic components can be essential to understanding the mechanisms involved in population response to certain conditions (Pomar et al., 2003). Linking these variations to dynamic models, for which the phenomena are understood over time, and to mechanistic

models, for which the biological principles are understood and explained, can make the predictions of such models more credible.

The significant differences in the asymptotic body weight and constant rate parameters of the data are indicative of differences in the growth pattern between the genotypes. Alpine goats likely achieve their mature weight earlier than do Saanen goats, as indicated by the higher rate constant of growth and lower asymptotic body weight estimated for this genotype. This knowledge can be used by farmers to choose between genotypes depending on their interest. Additionally, the different asymptotic body weights might be useful in mechanistic models, such as the Small Ruminant Nutrition Systems (SRNS) (Tedeschi et al., 2010), which makes use of this parameter in its set of equations to predict the degree of maturity for goats. This information is already available for cattle and some sheep genotypes; however, the data set pertaining to goats needs to be expanded.

The Von Bertalanffy, Logistic, and Gompertz models have a fixed inflection point (IP) relative to asymptotic body weight, which limits the biological interpretation of these functions due to the lack of flexibility in the estimation of the trend in the instantaneous absolute growth rate. In the case of the Richards model, the inflection point is variable and is a function of the β_4 parameter. The best model had common parameter β_4 = 0.9642 [$IC_{95\%}$ 0.9394 – 0.9890], which yields a transitional function in form between the Brody (β_4 = 0) and the Gompertz (β_4 = 1) functions (Richards, 1959).

The flexibility of the β_4 parameter is the most important advantage of the Richards function. The inflexion point in goats (36% of asymptotic body weight) may differ from the fixed inflexion point adopted by the other functions. The inflexion point occurs where the estimated instantaneous absolute growth rate changes from an increasing to a decreasing function. This information might be useful for strategic plans for goat feeding; information regarding the timing for the greatest capacity of the animal growth could be

critical for plan optimization. Moreover, it is expected that from this point (~ 20 kg) onward, there is a decrease in growth rate, indicating a necessity for a change in feeding strategy.

Despite the fact that the genotypes have the same origin and are often considered similar in terms of productivity (only visibly different in their coats), differences have been found in their lactation curves (Guimarães et al., 2006), and now, in the present study, in their growth patterns. This result indicates the need to use separate models (growth, lactation, etc.) for these two genetic groups to predict animal response.

Modeling of the error structure

As noted by Littell et al. (2006), the first tool to be chosen when using the SAS software to fit a nonlinear mixed model is PROC NLMIXED, as it is more general because it accepts other distributions for the dependent variable; however, when it is necessary to model the error structure, the SAS macro %NLIMIX becomes more useful. Additional differences between the two approaches with relation to the estimation method can be found in Littell et al. (2006), Vonesh (2012).

The SP(MATERN) structure has been historically used in spatial studies and has two parameters: ρ = 411.02 (SE = 52.27) and V = 0.1471 (SE = 0.006) (P < 0.0001). The lower value found for the V parameter implies that the spatial process is rough; in other words, the correlation decreases abruptly between measurements. To illustrate this behavior, we plotted the correlation between the measurements made on the animal with the maximum number of weight values (21 data points, with the first measure made at birth and the last at 1,984 days of life) (Figure 5). The correlation decreases more rapidly over time, so that the correlation between two measurements with an interval of 23 days was 0.59. However, this finding is an indication that the correlation between two measurements was relatively well controlled.

The possible reason that the SP(MATERN) structure adjusted better to this data set is the difference between the measurements found here. Our data included between one and 1,190 days of difference between two consecutive measurements (lag). Obviously, two measurements taken at a close interval are typically more highly correlated than measurements taken at more distant time points (Littell et al., 2006). In our data set, 61% of the measurements were made before one year of life, which means that the data are temporally close; the inflection point calculated in the Richards models occurs at 19.05 kg and 20.22 kg for the +A and +S genotypes, respectively, which occurs at approximately 131 days of life. Measurements taken close to these points will be less correlated than measurements taken far from them due the higher instantaneous absolute growth rate observed around these inflection points. Figure 5 shows this relation.

Cross validation

Despite the fact that the simultaneous F-test for the intercept and slope rejected the null hypothesis, the high coefficient of determination indicates that the approach chosen is robust enough to estimate the body weight of goats. Furthermore, the RMSEP presented a relatively low value (5.48 kg) with a great contribution from random errors in the total error prediction. This finding indicates that effects that are not controlled are the main factors affecting the predictions. As shown in Figure 3, the predictions are more credible in the early phases of growth, at lower body weights; once there is a small variation around the average prediction, an increase in body weight leads to an occurrence of factors that are not controlled and operate to expand the variation around the estimated body weight; however, the average body weight estimated remains without bias, as indicated by the almost total overlap between the dashed line and the continuous lines.

Historically, the growth curve parameters for goats have not been evaluated using adequate statistical approaches, which may have created biases for the estimated values.

The equations presented here are expected to yield more credible predictions for estimated mean values and population variations for some parameters.

Effects of number of weight values on the random effect estimates

Nonlinear mixed models are strong tools for modeling and understanding population variability by incorporating random effects to account for between-subject variations. Apparently, the lack of influence of the numbers of weight measurements on random effects is another advantage of this method. Thus, this approach can be used to select animals that are different from the population average with regard to their rate of growth and those with asymptotic body weight.

CONCLUSION

The Richards model is adequate for describing the growth curve of dairy goats. The nonlinear mixed model methodology is an efficient tool to address growth curve features such as heteroscedasticity and correlated errors. We suggest assigning the parameters with biological significance with random effects, thus reducing the residual variance and making the estimates more credible. Moreover, we recommend evaluating the modeling structure error to account for possible errors correlated even using random effects. Different Richard growth curve parameters should be used for the predominantly Alpine and Saanen genotypes because there are differences in their growth patterns.

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Table 1 – Summary of the data set assessed

Genotype	nº of animals	nº of weights	Max. obs. per goat	Min. obs. per goat	Max. age (days)
+S	498	4,292	21	4	2,666
+A	658	5,707	18	4	3,180
Total	1,156	9,999	-	-	-

Table 2 – Evaluated growth curve functions

Model	General function
Brody	$\hat{y} = \beta_1\left(1 - \beta_2 e^{-\beta_3 t}\right)$
Von Bertalanffy	$\hat{y} = \beta_1(1 - \beta_2 e^{-\beta_3 t})^3$
Richards	$\hat{y} = \beta_1(1 - \beta_2 e^{-\beta_3 t})^{\beta_4}$
Logistic	$\hat{y} = \beta_1/(1 + \beta_2 e^{-\beta_3 t})$
Gompertz	$\hat{y} = \beta_1 e^{-\beta_2 e^{-\beta_3 t}}$

Table 3 – Estimated parameters for the different models, random variable combinations, and genotypes assessed

Model	Random variables	+A				+ S			
		β_1	β_2	$\beta_{3\ (x\ 1000)}$	$\beta 4$	β_1	β_2	$\beta_{3\ (x\ 1000)}$	$\beta 4$
Brody	*u1*	54.58	0.9270	2.675		60.28	0.9303	2.283	
	u1, u2	54.57	0.9253	2.680		60.04	0.9283	2.302	
	u1, u3	52.58	0.9284	2.771		54.67	0.9350	2.656	
	u1, u2, u3	52.94	0.9279	2.734		54.28	0.9350	2.670	
	Fixed	54.58	0.9160	2.622		58.57	0.9205	2.372	
Von Bertalanffy	*u1*	51.56	0.5131	4.161		55.93	0.5148	3.670	
	u1, u2	51.56	0.5201	4.188		56.46	0.5227	3.658	
	u1, u3	48.49	0.5147	4.412		50.71	0.5201	4.115	
	u1, u2, u3	49.25	0.8431	3.786		54.02	0.8555	3.547	
	Fixed	52.42	0.4954	3.863		55.76	0.4986	3.552	
Logistic	*u1*	48.50	4.9345	7.472		52.13	4.9252	6.675	
	u1, u2	48.07	5.6973	7.792		52.23	5.6423	6.750	
	u1, u3	45.75	4.8093	7.722		48.24	4.8525	7.007	
	u1, u2, u3	45.61	4.8515	7.609		48.00	4.9214	6.913	
	Fixed	50.30	4.4939	6.548		53.31	4.5172	6.016	
Gompertz	*u1*	50.55	2.0216	4.940		54.61	2.0273	4.385	
	u1, u2	50.52	2.1024	5.020		55.17	2.1123	4.394	
	u1, u3	47.45	2.0206	5.216		49.72	2.0429	4.830	
	u1, u2, u3	43.20	4.5335	5.615		48.11	4.6551	5.327	
	Fixed	51.72	1.9262	4.502		54.91	1.9393	4.148	
Richards	*u1*	57.36	0.9827	1.903	0.733	67.38	0.9921	1.290	0.669
	u1, u2	53.87	0.9817	2.196	0.750	55.45	0.9916	1.897	0.700
	u1, u3	56.38	0.9776	1.999	0.767	57.91	0.9781	2.044	0.791
	u1, u2, u3	Did not converge							
	Fixed	58.11	0.9899	1.649	0.652	64.65	0.9934	1.329	0.636

Table 4 – Variance and covariance components, residual variances, and information criteria of the fitted growth curves

Model	Random variables	σ^2_{u1}	σ_{u1u2}	σ_{u1u3}	σ^2_{u2}	σ_{u2u3}	σ^2_{u3}	σ^2_e	AIC	Δ_r
Brody	*u1*	74.91*						12.92	57040	2354
	u1, u2	80.01*	0.08*		-4.0E-05[ns]			12.96	56999	2313
	u1, u3	156.47*		-13.29*			1.83*	8.66	54992	306
	u1, u2, u3	160.53*	0.12*	-13.19*	-3.6E-04*	-7.6E-03*	1.81*	8.82	54772	86
	Fixed							33.46	63490	8804
Von Bertalanffy	*u1*	64.77*						16.62	59293	4607
	u1, u2	78.14*	0.17*		2.4E-03*			15.12	59083	4397
	u1, u3	119.17*		-16.87*			4.10*	10.79	57063	2377
	u1, u2, u3	167.69*	-6.68*	-19.63*	0.73*	1.52*	3.91*	7.66	57287	2601
	Fixed							37.69	64679	9993
Logistic	*u1*	56.26*						24.11	62627	7941
	u1, u2	69.37*	8.53*		5.02*			19.94	62005	7319
	u1, u3	93.07*		-21.95*			9.72*	17.02	60983	6297
	u1, u2, u3	106.51*	7.21*	-20.62*	0.37**	-1.87*	7.83*	16.72	60843	6157
	Fixed							45.73	66613	11927
Gompertz	*u1*	61.78*						18.65	60323	5637
	u1, u2	76.07*	1.12*		0.11*			16.32	59980	5294
	u1, u3	110.08*		-18.47*			5.43*	12.30	58207	3521
	u1, u2, u3[†]	208.47	-99.11	-54.93	70.08	35.42	19.10	8.85	58134	3448
	Fixed							39.88	65245	10559
Richards	*u1*	83.61*						11.91	56287	1601
	u1, u2[‡]	30.69	-1.0E-02		-1.7E-04			13.52	56900	2214
	u1, u3	209.30*		-13.01*			1.20*	8.50	54686	0
	u1, u2, u3	Did not converge								
	Fixed							31.72	62960	8274

** - P<0.01; * - P<0.001

† At least one eigenvalue was negative.

‡ Singular Hessian matrix.

ns – Not significant

Table 5 – Re-parameterized Richard model parameters with error structure

Model	+A				+ S			
	β_1	β_2	$\beta_{3\ (x\ 1000)}$	β_4	β_1	β_2	$\beta_{3\ (x\ 1000)}$	β_4
SP(POW)	52.91	0.9544	2.6608	0.8994	57.27	0.9671	2.2589	0.8581
SP(MATERN)	52.50	0.9397	2.9535	0.9813	56.47	0.9535	2.5413	0.9443

	σ^2_{u1}	σ^2_{u3}	σ_{u1u3}	σ^2_e	AIC	Δ_r
SP(POW)	71.79*	0.6633*	-3.946*	0.0719	50740	1126
SP(MATERN)	47.58*	0.4520*	-1.956*	0.0987	49613	0

Hypothesis test SP(MATERN)	DF	T value	P-value
$\beta_{1_{+S}} = \beta_{1_{+A}}$	7683	4.87	<0.0001
$\beta_{2_{+S}} = \beta_{2_{+A}}$	7683	3.02	0.0025
$\beta_{3_{+S}} = \beta_{3_{+A}}$	7683	-3.01	0.0026
$\beta_{4_{+S}} = \beta_{4_{+A}}$	7683	-1.46	0.1430

* - P<0.001

Table 6 – Re-parameterized Richard model with error structure and just one β_4 parameter

Model	+A			+ S			
	β_1	β_2	$\beta_{3\ (x\ 1000)}$	β_1	β_2	$\beta_{3\ (x\ 1000)}$	β_4 †
SP(MATERN)	52.76	0.9429	2.8326	55.99	0.9501	2.6303	0.9642
	σ^2_{u1}		σ^2_{u3}	σ_{u1u3}		σ^2_e	AIC
SP(MATERN)	47.80*		0.4604*	-1.9971*		0.0986	49609

* P < 0.001

† Common β_4 parameter

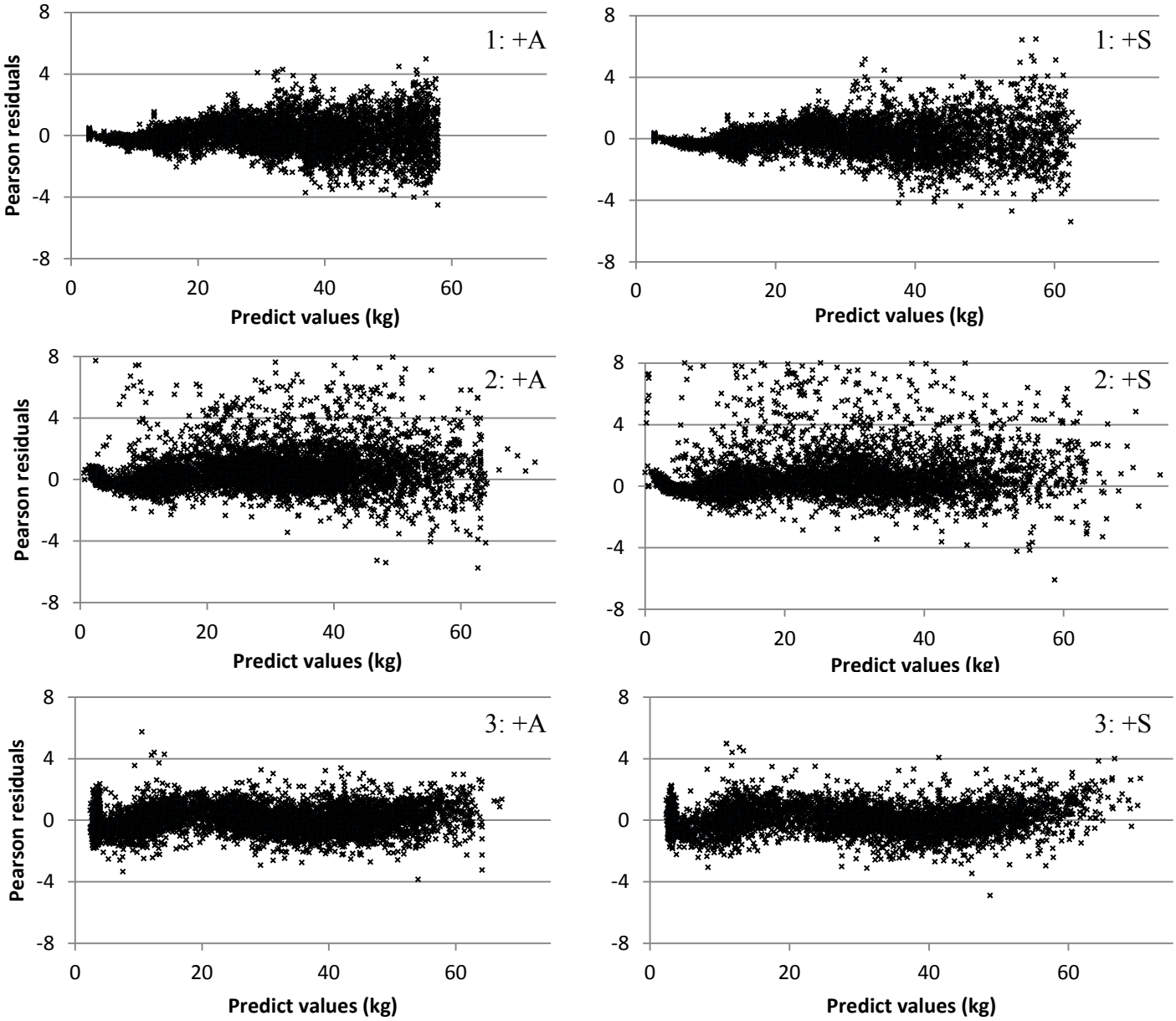

Figure 1 – Pearson residuals of the Richards functions versus predicted values (kg) using the following approaches: 1) Fixed-effects model; 2) random-effects model (u_1 and u_3); 3) random (u_1 and u_3) + VP + SP(MATERN).

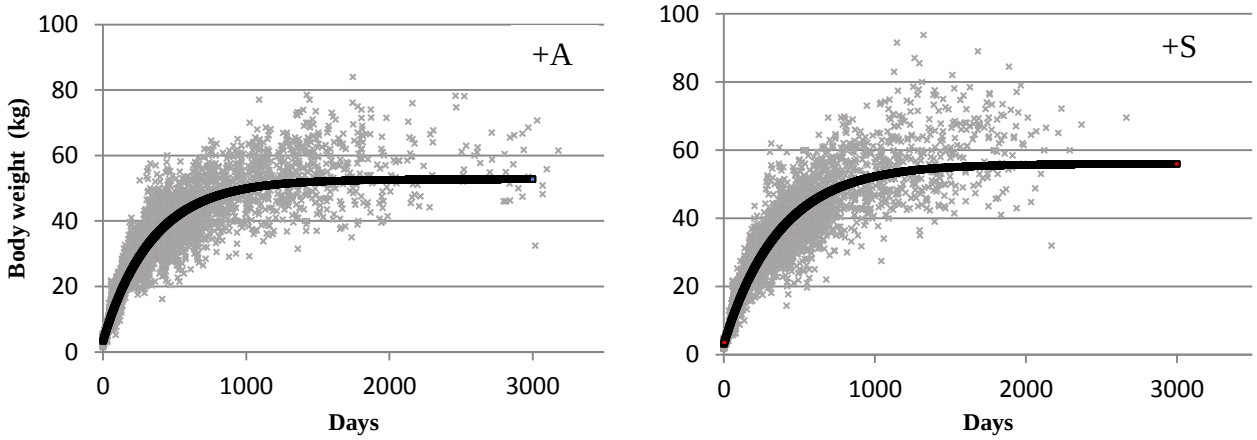

Figure 2 – Adjusted growth curve for the two genotypes (+A, Alpine; +S Saanen) evaluated.

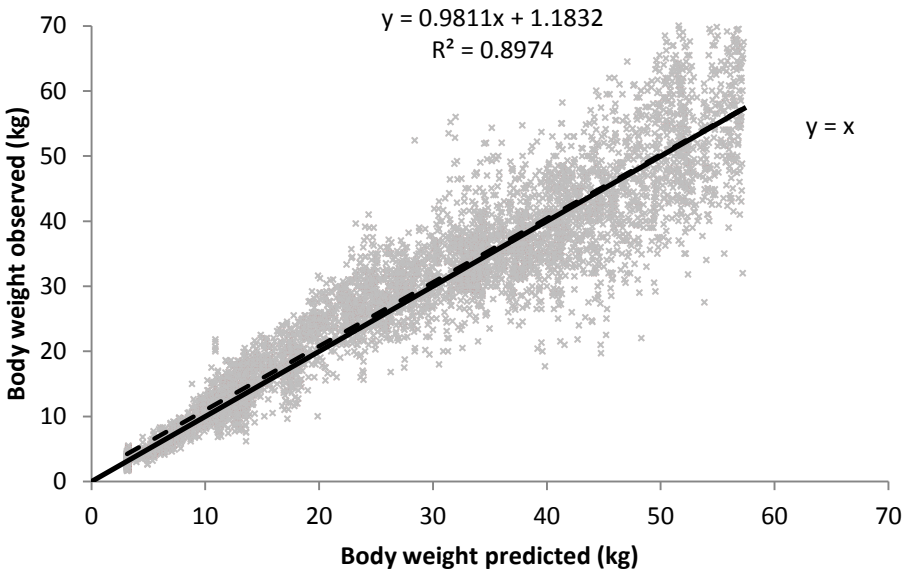

Figure 3 – Linear regression between observed and predicted body weights (dashed line). The continuous line is the Y = X line.

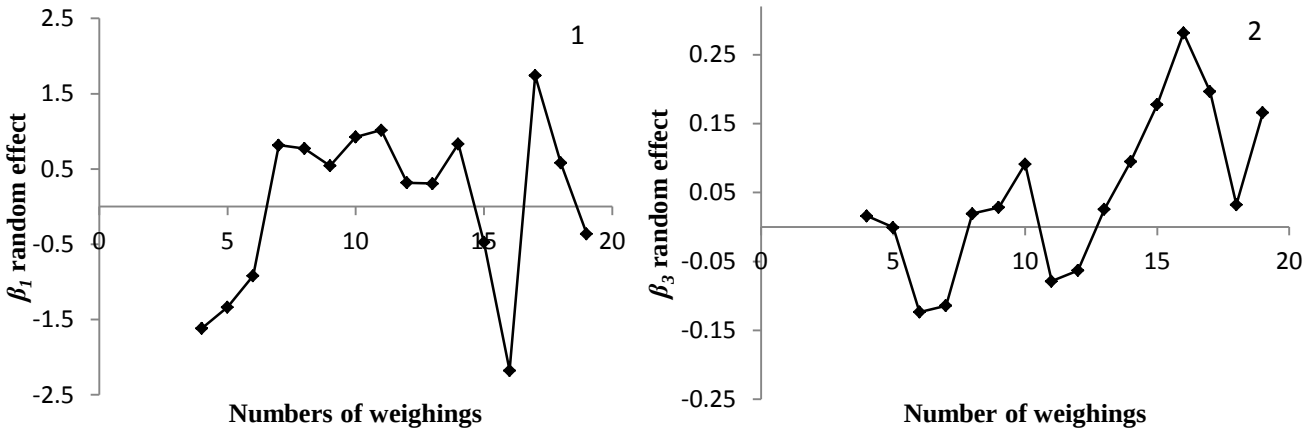

Figure 4 – Effect of the number of weight measurements on the random effects for the 1) u_1 random parameter and 2) u_3 random parameter.

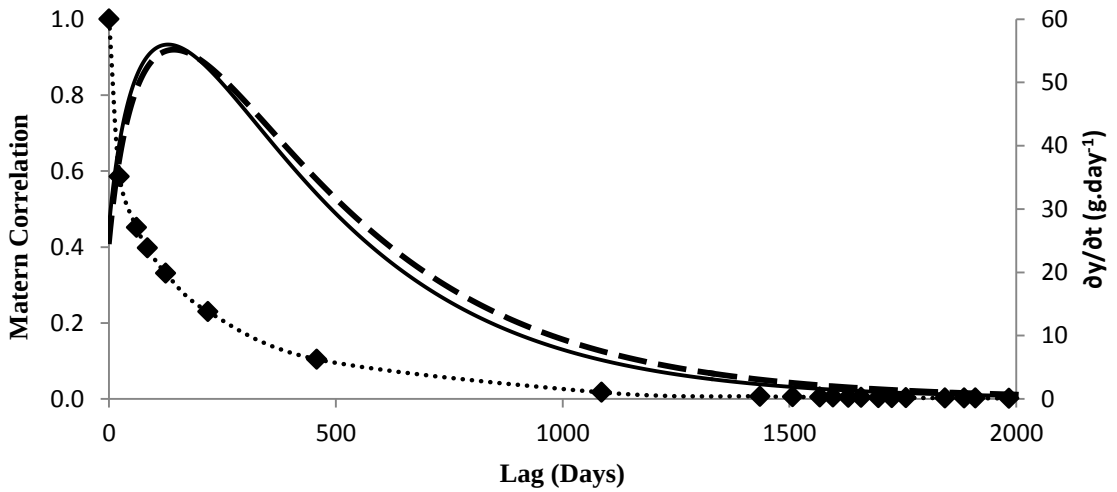

Figure 5 – The left axis shows the MATERN correlation function versus Lag (days) (point line). Black diamonds (♦) correspond the values for animals with 21 weight measurements during their lifetimes. The right axis shows the instantaneous absolute growth rate ($g.day^{-1}$) for +S animals (continuous line) and +A animals (dashed line).

Assessment of the heterogeneous rumen fiber pool and development of a mathematical approach for predicting the mean retention time of feeds in goats[1]

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ABSTRACT

The objectives of this study were to evaluate rumen fiber stratification and to develop a mathematical approach for predicting the mean retention time (**MRT**) of forage and concentrates in goats. A dataset from three studies was used that contained information regarding fiber and lignin intake as well as rumen content and the kinetics of fiber passage for forage and concentrates. The kinetic information was obtained through pulse dose and the fecal concentration measurement of forage and concentrate markers in the same animals that were used to measure rumen content. The evaluation of heterogeneous fiber pools in the rumen was performed using the Lucas' test assumptions, and the marker excretion profiles were interpreted using a model known in the literature as G*n*G1. The G*n*G1 model assumes an age-dependent fractional rate for the transfer of particles from the raft to the escapable pool in the rumen (λ_r, h^{-1}) and an age-independent fractional rate for the escape of particles from the escapable pool to the remaining parts of the stomach (k_e, h^{-1}). The equations used to predict the MRT for forage and concentrate fiber were developed using stepwise regression. A sensitivity analysis was conducted using Monte Carlo simulation to investigate the relationships between the dependent and independent variables and between forage and concentrate passage rates. The Lucas' test yields goodness-of-fit estimates for NDF analysis; however, the homogeneous fiber pool approach could not be applied because a positive intercept ($P<0.05$) was identified for lignin rumen content. The stepwise regression model for MRT for forage estimation had an approximate coefficient of determination and a root mean square error (RMSE) for forage of 0.53 and 9.78 h, respectively, and for concentrate of 0.49 and 5.86 h, respectively. The sensitivity analysis yielded a mean k_p value for forage of 0.0322 h^{-1} (0.0158 to 0.0556 h^{-1}) with 99% confidence interval. For the concentrate, the mean k_p value was of 0.0334 h^{-1} (0.0146 to 0.0570 h^{-1}). A heterogeneous rumen fiber pool should

be assumed for goats fed diets with considerable fiber contents. The results of the sensitivity analysis indicated that both λ_r and k_e are of similar importance to the rate of passage in goats. The rates of passage of forage and concentrate in goats overlap and are closely related.

Key words: fractional rate of passage, Lucas' test, sensitivity analysis, small ruminants.

INTRODUCTION

In applied ruminant nutritional models, the fiber pool has historically been assumed to be a compartment of uniform mass in the ruminoreticulum, regardless of the ruminant species considered. However, a uniform mass pool may not occur when animals are fed diets with considerable concentrations of fiber. Instead, in these cases, the digesta is stratified into two distinct solid phases, i.e., the floating mat (raft), which is selectively retained due to its buoyancy, and a solid phase containing smaller particles dispersed within the fluid phase that are able to escape from the rumen (Hungate, 1966, Sutherland, 1988, Vieira et al., 2008).

From an ecological point of view, goats are considered intermediate feeders that can behave either as browsers or as grazers, depending on the circumstances (Van Soest, 1994). For grazing ruminants, the occurrence of ruminal fiber stratification is relatively well described, whereas for browsing ruminants the stratification is not expected to occur (Clauss et al., 2010). Unfortunately, the literature does not clearly articulate which approach describing the dynamics of the rumen fiber pool in goats produces the best fit, although Tedeschi et al. (2012) have indicated that the k_p of goats consuming forage may be similar to those of cattle and sheep (i.e., grazers) when the goats are under confinement conditions.

Our hypothesis is that goats, despite being considered browsers in natural environments (where the stratification of digesta would not occur), exhibit the non-selective behavior typical of roughage eaters when fed forage-based diets in confined and intensive production systems and therefore experience fiber pool stratification. Therefore, the objectives of this paper were to investigate the stratification of digesta in the rumen of goats, to develop a mathematical approach to predict the mean retention time of forage and concentrates in goats and to perform a sensitivity analysis of the models developed.

MATERIALS AND METHODS

Dataset Descriptions

The criteria used to select the studies to construct the database were those outlined by Cannas et al. (2003), in which diets must have at least 20% forage (DM basis); intake must have been individually measured, not estimated; rumen contents must have been measured by complete evacuation; and feed and rumen contents must have been analyzed for DM, CP, NDF, physically effective NDF (**peNDF**) and lignin. In addition, the pulse dose and fecal concentrations of the forage and concentrate markers had to be from the same animals that were used to measure the rumen content. The data from three studies were used as follows and summarized in Table 1.

Experiment 1 (Lopes, 2009) consisted of a 3 x 3 completely randomized factorial design to evaluate the effect of the maturity of the forage, represented by age of cutting (35, 50 and 65 days), and the particle sizes of coast-cross hay (*Cynodon dactylon (L.) Pers., 1805*) on ruminal fiber mass in growing male goats. The data include 53 observations for rumen content collected 2 hours after feeding at slaughtering and 18 profiles of fecal fiber excretion from forage labeled with chromium (Cr_2O_3).

Experiment 2 (Felisberto, 2011) evaluated the effect of the combination of three particle sizes (2, 5 and 15 cm (arithmetic mean particle size)) and four levels of NDF from forage (**NDFf**) (340, 410, 490 and 570 $g \cdot kg^{-1}$ DM) on the milk production of goats (after 60 days of lactation) in a 3 x 4 completely randomized factorial design. The data include 48 observations for rumen content collected 2 hours after feeding at slaughtering and profiles of fecal fiber excretion from forage and concentrates labeled with ytterbium and lanthanum, respectively.

Experiment 3 (Matos, 2012) studied the effect of three fiber sources (Tifton hay, corn silage and alfalfa hay) and number of parities (primiparous and multiparous) on the milk yield of dairy goats (after 60 days of lactation). This experiment consisted of a pen study with 96 dairy goats in 12 pens in a 3 x 2 completely randomized factorial design with two replicates. For each pen, two goats were slaughtered (2 hours after feeding) and three goats were used to measure the profiles of fecal fiber excretion. Thus, the average nutrients intake parameters, rumen content and particle kinetics were used. The data consisted of 12 values for nutrient intake, rumen content and profiles of fiber excretion from forage and concentrates labeled with ytterbium and lanthanum, respectively.

Testing for the Existence of a Single Fiber Pool

The validity of the single rumen fiber pool approach for goats was evaluated using the test developed by Lucas et al. (1961) and adapted by Van Soest et al. (1992) to identify homogenous compartments in the rumen. This approach has been used to predict the turnover of dietary components (Cannas et al., 2003) and the homogenous fiber compartment in cattle and sheep (Vieira et al., 2008). The uniform pool of a determined feed or digesta component in the ruminoreticulum compartment can only be considered if the linear relation between the pool size ($\boldsymbol{Q_x}$) and intake rate ($\boldsymbol{F_x}$) yields acceptable goodness-of-fit estimates (Eq. 1) (Vieira et al., 2008):

$$Q_x = T_x \times F_x + Q_{xm} \quad (1)$$

in which Q_x and F_x are NDF or lignin rumen content and intake, expressed in g and g·day^{-1}, respectively; T_x represents the ruminal turnover; and Q_{xm} represents the metabolic portion of the component determined. The criteria adopted for the uniformity assumptions were the absence of a lack of fit for linear regression, a low value for the standard error of the linear regression parameters and an intercept that does not differ from zero for both NDF and lignin analysis, as fiber and lignin are not part of the endogenous matter (Van Soest, 1994). The uniformity assumption was tested by using all the data from Exp. 1 (n = 53), 2 (n = 48) and 3 (n = 12), because individual feed intake data were available and rumen contents were assessed by slaughtering all the animals.

Additionally, the Q_x and F_x were scaled to body weight using the allometric function $y = \alpha \times BM^{\beta}$. When β differed from unity (95% confidence interval), the scaled variables $Q_x^c = Q_x/BM^{\beta 1}$ and $F_x^c = F_x/BM^{\beta 2}$ were used to fit the linear regression (Eq. 1) and to evaluate the uniformity assumptions cited previously, yielding the variables T_x^c and Q_{xm}^c. If $\beta_1 = \beta_2$, $T_x^c = T_x$; otherwise, $T_x^c = T_x \times BM^{\beta_2 - \beta_1}$, which contradicts the assumptions of fiber uniformity and indicates that the ruminal turnover is a function of body weight, which is an additional assumption for applying the Lucas' test (Vieira et al., 2008).

The allometric equations were fitted according to the nonlinear mixed model methodology using the SAS macro %NLINMIX (SAS Inst. Inc., Cary, NC. version 9.3). The between-study variability was modeled by introducing a parameter u to the β allometric equation. The residual variance was modeled using the power-of-the-mean variance function when heteroscedasticity of the residuals was observed. The restricted maximum likelihood was used as the method of estimation, and the zero-order expansion method was used to fit the model.

The Lucas' test was performed using the following statistical model (Sauvant et al., 2008) via the MIXED procedure in the SAS software (SAS Inst. Inc., Cary, NC. version 9.3):

$$Y_{ij} = \beta_0 + S_i + \beta_1 X_{ij} + b_i X_{ij} + e_{ij} \quad (2)$$

where Y_{ij} = the dependent variable (Q_x or Q_x^c), expressed in g; β_0 = the overall (inter-study) intercept; S_i = the random effect of the i^{th} study, assumed to be independent and identically and normally distributed, or iid ~ N (0, σ_s^2); β_1 = the overall fixed regression coefficient of Y on X; X_{ij} = the value of the continuous predictor variable (F_x or F_x^c), expressed in g·day^{-1}; b_i = random effects of the regression coefficient of Y on X, assumed to be iid ~ N (0, σ_b^2); and e_{ij} = residual errors, assumed to be iid ~ N (0, σ_e^2). When the SAS software produced the error message that the "estimated G matrix is not positive definite," the model was re-parameterized without the respective variance component with a value of zero. Graphical analysis (conditional Pearson residuals vs. predicted values) indicated that there was within-subject (study) residual heteroscedasticity; thus, the power of the mean was used to account for this heteroscedasticity (Littell et al., 2006).

Rate Passage Analysis

The marker excretion profiles of the forage (Exp. 1 (n = 18), 2 (n = 48) and 3 (n = 12)) and concentrates (Exp. 2 (n = 48) and 3 (n = 12)) were kinetically interpreted with the model known as G*N*G1 (Eq. 3) (Matis, 1972, Matis et al., 1989, Pond et al., 1988, Vieira et al., 2008):

$$C_{(t)} = e, \quad 0 \le t < \tau$$

$$C_{(t)} = C_{(0)} k_e \left\{ \delta^N \exp[-k_e(t-\tau)] - \exp[-\lambda_r(t-\tau)] \times \sum_{i=1}^{N} \delta^i [\lambda_r(t-\tau)]^{N-i}/(N-i)! \right\} + e, \quad t \ge \tau \quad (3)$$

in which $\delta = \lambda_r/(\lambda_r - k_e)$; $C_{(t)}$ is the marker concentration at time t_i; $C_{(0)}$ is the mass ratio between the marker dose and the particulate NDF mass of the raft pool; λ_r is the age-dependent fractional rate for particle transference from the raft to the escapable pool (h^{-1}); k_e is the fractional rate of escape of particles from the escapable pool in the ruminoreticulum to the remaining parts of the stomach (h^{-1}); τ is the particle transit time from the ruminoreticular orifice to the first appearance in the feces; and N is the order of time-dependency. The GNG1 model allows us to estimate the compartmental mean residence time (**CMRT**) independently for each ruminal pool (raft and escapable) and the mean retention time (**MRT**) can be expressed as $MRT = N/\lambda_r + 1/k_e$, and the mean passage rate (k_p) can be expressed as $k_p = 1/MRT$.

The GNG1 functions were fitted to each profile for $0 < N \leq 6$ using the Marquardt algorithm of the NLIN procedure in SAS (SAS Inst. Inc., Cary, NC. version 9.3). The criteria adopted to choose the order of time-dependency were the following: 1) the convergence criterion; 2) estimates of λ_r that did not tend towards k_e; 3) the simplest model (smallest order of N), chosen using the approach described by Burnham and Anderson (2002) and Vieira et al. (2012) that used Akaike's information criterion (**AIC**), differences among AIC values, the Akaike weights or likelihood probabilities and the evidence ratio or relative likelihood; and 4) graphical analysis.

Modeling Empirical Equations to Predict Ruminal Fractional Passage

After the GNG1 fitting was completed and the most reasonable order of time-dependency was chosen, the λ_r and k_e parameters were modeled independently with the goal of understanding which factors influence each parameter most. As mentioned previously, the MRT is calculated as $N/\lambda_r + 1/k_e$. The first term was modeled to account for the order of time-dependency directly on the estimated coefficient of the parameter. In

other words, $N/\lambda_r = f(\beta_i, X_i) + e$. The second term was modeled directly in terms of the fractional rate $k_e = f(\beta_i, X_i) + e$.

The independent variable candidates that were common to all experiments were regressed against these parameters based on methods described by Tedeschi et al. (2012). Two groups of variables were used (Cannas et al., 2003): 1) predictors associated with diet composition, such as forage content (**For_{diet}**), concentrate content (**$Conc_{diet}$**), neutral detergent fiber (**NDF_{diet}**), crude protein (**CP_{diet}**) and lignin content (**Lig_{diet}**), which are expressed in g·kg^{-1} DM; and 2) predictors associated with intake level, such as DMI, neutral detergent fiber intake (**NDFI**), intake of physically effective NDF (**peNDFI**, particles > 1.18 mm) and lignin intake (**LIGI**), which are expressed in g·day^{-1}. The variables in group 2 were also expressed in g·kg^{-1} BW (relative variables); however, when these parameters were determined to have body weight effects, the variables were scaled to body weight (g·kg$^{-\beta}$ BW). In addition, the logarithms of all of the independent variables in group 2 were added. The logarithms of λ_r and k_e were also evaluated using the independent variables mentioned above (transformed variables). Because it has been shown that a relationship exists between the passage rates of concentrates and forage, the λ_r and k_e of forage and its logarithmic values were added to the λ_r and k_e of the concentrates as independent variables (Cannas and Van Soest, 2000).

The stepwise regression procedure PROC REG in SAS (SAS Inst. Inc., Cary, NC. version 9.3) was used to select the predictors (α = 0.10). To avoid problems with multicollinearity, the variance inflation factor (VIF < 10) and the condition index root (CI < 30) were considered. After this first step, a meta-analysis was performed using the extension of Eq. 2 to accommodate other independent variables. The N/λ_r and k_e values, adjusted for study effects, were computed, and the approximate coefficient of determination was calculated for the regression of y_{adj} on the fixed-effects variables

(Tedeschi et al., 2012) to determine the combination of N/λ_r and k_e equations that best fit the dataset. Conditional Pearson residuals were used to evaluate the statistical assumptions and values outside the range of -3.0 to 3.0 were removed.

Sensitivity Analysis

A sensitivity analysis was performed using the Monte Carlo simulation technique to evaluate the impact of the independent variables on the dependent variable through the standardized regression coefficients (**SRC**s). The SRC reflects the change in the standard deviation of a dependent (output) variable associated with a unit change in the standard deviation of an independent (input) variable with all other variables held constant (Helton and Davis, 2002). The Monte Carlo simulation was performed with the @Risk v. 5.5.1 (Palisade Corporation, Ithaca, NY) software using 10,000 iterations and the default Latin hypercube sampling as the selection method. Certain biological aspects were considered by restricting the lower and upper limits of the independent variables in the probability distribution fitting. The predictors associated with intake (i.e., DMI, NDFI, etc.) were restricted as follows: $0 \leq X_{(g\ or\ g \cdot kg^{-1}\ BM)} < a\ bounded\ unknown.$ The predictors associated with diet composition (NDF_{diet}, LIG_{diet}, etc.) were restricted as follows: $0 \leq X_{(g \cdot kg^{-1} DM)} \leq 1000$. The unknown bound specific to the distribution had a finite bound (that is, it does not extend to plus or minus infinity). Spearman correlations were assigned to input variables to maintain the expected correlation between independent variables during the simulation. The Chi-squared statistic was used to choose the probability distribution for the independent variables.

RESULTS

None of the β parameters of the allometric equations except for Q_{LIG} differed from unity (Table 2). Thus, both non-scaled and scaled variables were used to test the

assumption of the uniformity of the fiber pool. A value of β = 1 was used for the NDF intake and NDF rumen mass because neither parameter differed from unity. Consequently, the scaled variables were: $F_{NDF}^{c} = F_{NDF}/BM$ and $Q_{NDF}^{c} = Q_{NDF}/BM$. For the lignin intake and lignin rumen mass, the following scaled variables were used: $F_{LIG}^{c} = F_{LIG}/BM^{1.24}$ and $Q_{LIG}^{c} = F_{LIG}/BM^{1.59}$.

The values of Q_{NDF_m} and $Q_{NDF_m}^{c}$ did not differ from zero, which corroborates the homogeneous pool assumption. Furthermore, T_{NDF} and T_{NDF}^{c} were not different from each other (P = 0.84) (Table 3). Despite the lack of evidence of a difference between the power (P = 0.80) of the lignin intake and that of the lignin rumen mass (which would result in $T_{LIG}^{c} = T_{LIG}$ and support the homogenous pool assumption), the intercept differed from zero, indicating the presence of a potential metabolic component to the lignin, which is not possible (Table 3).

When the non-scaled variables were analyzed, there was clear heteroscedasticity of the residuals, indicated by θ values of 3.86 and 1.67 (P<0.001) for Q_{LIG} and Q_{NDF}, respectively, which suggests that MRT does not exhibit homogeneous behavior over the body weight range evaluated. The estimated variance components of the intercept and slope of the equations were not significant (P > 0.10) (Table 3).

The transformed variables in the forage model and untransformed variables in the concentrates model were chosen based on the previously mentioned criteria. For the transformed N/λ_r of forage, peNDFI, NDF_{diet} and $DMI/BW^{0.75}$ were selected (R^2 = 0.40; RMSE = 7.36 h). For the transformed k_e of forage, peNDFI/BW and lignin intake were selected (R^2 = 0.13; RMSE = 0.0240 h^{-1}) (Table 4). For the N/λ_r of concentrates, the natural logarithms of DMI and N/λ_r of forage were selected (R^2 = 0.34; RMSE = 5.48 h). For the k_e of concentrates, the natural logarithms of LIGI and the k_e of forage were selected (R^2 = 0.21; RMSE = 0.026 h^{-1}) (Table 4).

Despite the low values of the approximate coefficients of determination of the above-mentioned equations, when the equations were formulated to predict the mean retention times, the R^2 and RMSE were 0.53 and 9.78 h, respectively, for forage and 0.49 and 5.86 h, respectively, for concentrates.

Table 5 shows the values of the Spearman correlation coefficients used to preserve the structure of the original dataset in the Monte Carlo simulation. The correlation coefficient values were maintained in the simulation even when they were not significant. It is interesting to note that the correlation coefficient value between $N/\lambda_{r\ For}$ and $k_{e\ For}$ was not significant (P = 0.20). However, a low but significant value for the correlation between $N/\lambda_{r\ Conc}$ and $k_{e\ Conc}$ (not shown in Table 5) was found (r = 0.39; P = 0.012).

Figure 2 shows the SRCs for the N/λ_r and k_e of forage (panels A1 and A2) and concentrates (panels B1 and B2) from the simulated predictions using the equations in Table 4. For the forage value of N/λ_r, the NDF_{diet} and peNDFI are similar in intensity, but their effects have opposite signs; increasing the NDF_{diet} content also increases the time of residence in the raft pool, whereas increasing the peNDFI, decreases the residence time. For concentrates, increasing the DMI results in decrease in the residence time in the first fiber pool. Lignin intake has significant but opposite effects on the k_e of forage and concentrates. It is interesting to note that LIGI has a strong influence on $k_{e\ For.}$. An increase of one standard deviation in the LIGI value increases $k_{e\ For.}$ by 118%. Both concentrate pools were influenced by their respective forage pools. Figures 2-A3 and 2-B3 showed the effects of both fiber compartments on the rate of passage of fiber, calculated as $k_p = 1/(N/\lambda_r + 1/k_e)$. Both raft and escapable pools presented similar intensity and importance on the k_p. However, it is worth noting that the opposite effects are the result of the modeling approach, given that the first pool was modeled in terms of compartmental

mean residence time (h), whereas the second pool was modeled in terms of the fractional rate (h^{-1}).

Figure 3-A1 shows an overlap of the k_p probability distributions for forage and concentrates. A pronounced overlap was observed between these probability distributions. In the simulated dataset, the mean with 99% confidence interval of the k_p value for the forage was 0.0322 h^{-1} (0.0158 to 0.0556 h^{-1}). For the concentrates, the mean k_p value was 0.0334 h^{-1} (0.0146 to 0.0570 h^{-1}), very close to the forage k_p. Figure 3-B shows scatter plots of the simulated fractional rates of passage of forage and concentrates. A Pearson correlation coefficient of 0.66 ($P< 0.001$) was obtained.

DISCUSSION

Assessment of Heterogeneous Fiber Pools

The gut capacity of herbivores is scaled to the power of unity, whereas feed intake is scaled to the power of 0.75 (Demment and Van Soest, 1985). Nevertheless, feed intake can be considered to be scaled to 0.75 if and only if feed intake is under the control of metabolic factors rather than fill factors (Mertens, 1987). This assumption is based on basal metabolic energetic requirements. Animals raised in intensive production systems normally experience short periods of time in the basal energetic requirement range. The dataset used to access the fiber pool was obtained from studies that used both growing and producing animals fed diets with considerable fiber contents, which could have led to the restriction of feed intake due to physical constraints. This possibility is corroborated by the isometric relationship between NDF rumen content and body weight. Rumen lignin content scaled to the power of 1.59, most likely because the NDF in the rumen is under effect of degradation and passage rates, whereas lignin is only under effect of passage rate, and so the amounts that would accumulate in the rumen could vary, causing this allometry.

As discussed by Van Soest et al. (1992), evaluations of the presence of heterogeneous pools in the rumen and of fiber kinetic behavior are of particular importance. The Lucas' test allows the identification of heterogeneous pools and metabolic portions through simple linear regression analysis and to make inferences about the rumen turnover of these compartments. The turnover of NDF determined from non-scaled (0.97 d) and scaled (0.86 d) variables did not differ, which suggests that the presence of homogeneous pools of fiber in the rumen rather than the body weight affected NDF turnover. This result contradicts the findings of Illius and Gordon (1992), who suggested that only body weight affected rumen turnover. However, because the range of body weights encountered in the present study was low, no solid inferences could be made on the effect of body weight on NDF turnover.

Although Lucas' test using the NDF analysis corroborates the presence of a homogeneous fiber pool, the scaled and non-scaled analysis for lignin indicate the presence of a positive metabolic component, which violates the assumption of a homogeneous ruminal fiber pool, as lignin is a genuine cell wall component and has no endogenous component. In other words, homogeneous fiber pools cannot be assumed when goats are fed diets with considerable fiber contents. Vieira et al. (2008) obtained similar results for cattle and sheep; however, they used robust linear regression to fit the Lucas' test, which resulted in higher standard errors than those obtained in the present study. In addition, correlated errors may have occurred, leading to higher parameter standard errors, as meta-regression tools were not used.

Comparing the NDF results with the lignin results reveals an interesting phenomenon. For the intercept (the metabolic portion) of the Lucas' test of lignin, the standard errors of both the scaled and non-scaled variables are smaller than those of NDF; in contrast, for the slope (turnover), the standard errors are larger than those of NDF. This

phenomenon is illustrated in Fig. 1. It is unclear what causes this effect, but it is probable that noise in the lignin analysis (due to the lower values in the data) is a factor. Moreover, both non-scaled lignin and NDF presented heteroscedasticity, which highlights the necessity of scaling the variables to control for the effect of body weight. The assumption of heteroscedasticity could be used as an additional criterion in future analyses assessing homogeneous rumen compartments.

GNG1 Fit and Modeling the Two Fiber Pools Approach

The limitations of the evaluated dataset need to be considered. Experiment 1 used Cr_2O_3 as the forage fiber external marker, whereas Exp. 2 and 3 used Yb and La as the forage and concentrate fiber markers, respectively. Both rare earth and chromium markers present problems. Rare earth can migrate from the solid phase to the liquid phase, consequently reducing the estimated mean retention time in the rumen, whereas chromic oxide increases fiber density, most likely affecting the true mean retention time (Van Soest, 1994).

In contrast to Tedeschi et al. (2012), who found consistently lower sums of squared errors and greater relative likelihood probabilities with the G2G1 model using data from goats, a greater frequency of G1G1 was observed in our dataset (45.33%). This difference is probably due to the fact that our dataset contained data from lactating animals, which usually have faster rates of passage and a lower degree of time-dependency than the animals in the dataset analyzed by Tedeschi et al. (2012). When our dataset was split into non-lactating (Exp. 1) and lactating animals (Exp. 2 and 3), the most frequent time dependency numbers were G2G1 (33.33%) and G1G1 (56.14%), respectively, in agreement with the work by Tedeschi et al. (2012).

To avoid tedious approaches such as frequency analysis or a Bayesian approach to modeling the order of time-dependency (a factor that controls the length of time during

which the fiber remains in the raft pool in the rumen), we decided to model *N* directly as a component of the independent parameter (N/λ_r). The result is the CMRT expressed in hours in this first fiber pool. This method seems to be appropriate because this result is added to the CMRT of the fiber pool dispersed in the fluid phase (given as $1/k_e$) to calculate the total mean retention time. However, it is worth remembering that the first pool model fitted here gives us the value in terms of hours, whereas the second pool model gives us the value in terms of reciprocal time.

The nonlinearity indicated by the choice of the logarithms of the dependent (forage) and independent (concentrates) variables is consistent with the findings of Cannas et al. (2003) and Tedeschi et al. (2012). Despite the lower approximate coefficient of determination achieved in the fit, when the equations for N/λ_r and $1/k_e$ were combined to predict the mean retention time, the coefficient of determination and passage rate values were reasonable and consistent with those reported in other studies (Seo et al., 2006, Tedeschi et al., 2012).

Interestingly, the independent variables related to the concentrate dependent variables were the forage variables ($N/\lambda_{r\,For}$ and $1/k_{e\,For}$), indicating that these factors are related. Even though concentrates theoretically do not undergo fiber stratification in the rumen, concentrates are high in density (low fiber content). In diets in which high forage contents are used, the raft effect occasioned by forage fiber could delay the transfer of the concentrate fiber from the dorsal to the ventral region, especially if total mixed diets are used. Consequently, a heterogeneous fiber approach becomes valid for concentrates.

Sensitivity Analysis

The sensitivity analysis allowed us to identify important factors that influence the dependent variables. The SRC can be used to provide a measure of the importance of the independent variables. However, interrelationships and nonlinearity between independent

variables can lead to misinterpretation of the results, producing low values for SRC. This fact arises mainly if non-monotonic behavior occurs between independent and dependent variables (Cariboni et al., 2007), which did not seem to be the case in the data analyzed in this study despite the nonlinearity observed among the variables. Plots of the data (not shown) indicate a monotonic behavior in all nonlinear parameters.

The buoyancy of the first ruminal fiber pool is mainly caused by the gas entrapment; consequently, fermentability (rate and extension of digestion) plays an important role on the compartmental mean residence time. Therefore, the greater structures of gas retention and more potentially digestible tissues would yield greater contribution of the buoyancy to the total mean retention time. The NDF_{diet} is an important factor that affects the rate of passage in ruminants. The buoyancy of the NDF_{diet} caused by the low density of the cell walls increases the residence time in the raft pool (as indicated by positive SRC values), which negatively affects the rate of passage. This result is consistent with the findings of Tedeschi et al. (2012). However, while NDF_{diet} has a negative influence on the forage fiber passage rate, factors associated with intake levels such as peNDFI and DMI reduce the raft pool residence times for forage and concentrates, respectively. It is possible that the effect of the pressure caused by high feed ingestion pushes the fiber pool to the ventral region, enabling its escape from the gastro intestinal tract (**GIT**). Moreover, it has been demonstrated that increasing the peNDF dietary content stimulates chewing activity (Zhao et al., 2011). Because particle size influences the rate of passage of fiber out of the rumen, it makes sense that this factor is found to have a negative value in the sensitivity analysis.

The relationship between $N/\lambda_{r\,For}$ and $N/\lambda_{r\,Conc}$ and between $k_{e\,For}$ and $k_{e\,Conc}$ clearly demonstrates the interdependence of forage and concentrate passage rates. In the case of the raft pool residence time, this interdependence illustrates the delay effect

previously mentioned; in the case of the escapable pool, both forage and concentrate are influenced by the same predictor (lignin intake), and it appears that these fiber pools (forage and concentrates) are similarly affected in this pool.

The similar importance of N/λ_r and k_e to both forage and concentrates (Fig. 2-A3 and B3) is a significant finding because it demonstrates the important effect of the ascendant rate on the total mean retention time. Alternative interpretations using an age-independent model for this parameter are discussed by Grovum and Williams (1973) who assumes that, for bi-compartmental functions, an ascending rate describes the rumen outflow and a descending rate describes the lower tract turnover. The MRT obtained using age-dependent models such as G*N*G1 assumes two compartments in the rumen and two rates (λ_r and k_e). It is possible that higher values for MRT are estimated and that lower passage rates are predicted than with functions used to describe the kinetics of particles.

The overlap between forage and concentrate k_p (Fig. 3) in the simulated dataset suggests a considerable effect of forage on the concentrate k_p when significant fiber contents are used in the diets of goats. The maximum and minimum values for forage and concentrate k_p are in agreement with the results of the sensitivity analysis performed by Tedeschi et al. (2012). However, our upper limit was greater, likely because our dataset included lactating animals that usually have more rapid turnovers.

CONCLUSIONS

Heterogeneous rumen fiber pools should be assumed in goats fed diets with considerable fiber contents. Both λ_r and k_e are of similar importance to the passage rate in goats. The rates of passage of forage and concentrates in goats present a high degree of overlap and are closely related. The equations that account for heterogeneous fiber pools are an alternative approach to predict the mean retention time of forage and concentrate

fiber and are expected to provide more reasonable predictions for the digestibility of feed ingredients used in goat diets.

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Table 1. Summary of the dataset evaluated.

	Studies		
Variables[1]	(Lopes, 2009)	(Felisberto, 2011)	(Matos, 2012)
n	18	48	12
BW, kg	16.2 - 29.3[3]	37.3 - 72.0	42.1 - 60.5
DMI, g·day^{-1}	208.8 - 877.4[3]	665.5 - 2242.0	1647.0 - 2188.0
NDFI, g·day^{-1}	114.4 - 476.9[3]	330.4 - 1041.0	576 - 767.2
LIGI, g·day^{-1}	5.6 - 42.3[3]	20.3 - 66.5	28.1 - 106.8
peNDFI, g·day^{-1}	11.6 - 273.1	187.1 - 1067.0	424.1 - 614.7
For_{diet}, g·kg^{-1} DM	769.2 - 769.2	557.4 - 934.4	467.3 - 697.1
$Conc_{diet}$, g·kg^{-1} DM	230.8 - 230.8	65.6 - 442.6	302.9 - 532.7
NDF_{diet}, g·kg^{-1} DM	560.2 - 587.2	406.5 - 587.1	366.8 - 439.8
CP_{diet}, g·kg^{-1} DM	134.1 - 148.2	148.5 - 155.8	157.8 - 177.4
Lig_{diet}, g·kg^{-1} DM	33.2 - 47.4	25.3 - 39.1	21.2 - 58.6
$N/\lambda_{r\ For}$[2], h	2.96 – 38.61	1.14 – 24.12	2.11 – 14.40
$k_{e\ For}$[2], h^{-1}	0.016 – 0.055	0.018 – 0.219	0.042 – 0.105
$N/\lambda_{r\ Conc}$[2], h	-	0.344 – 31.22	3.46 – 17.50
$k_{e\ Conc}$[2], h^{-1}	-	0.025 – 0.185	0.055 – 0.105
MRT forage[2], h	26.9 - 92.6	18.2 - 57.3	22.4 - 33.1
MRT concentrate[2], h	-	9.2 - 50.8	21.0 - 28.3
$Q_{DM,}$ g	170.1 - 1158.0[4]	278.8 - 1830.0	1061.0 - 1842.0
Q_{NDF}, g	73.0 - 614.3[4]	186.9 - 1129.0	568.3 - 1126.0
Q_{LIG}, g	8.04 - 72.97[4]	30.9 - 266.7	72.6 - 251.8

[1] BW, body weight; DMI, dry matter intake; NDFI, neutral detergent fiber intake; LIGI, lignin intake; peNDFI, neutral detergent fiber physically effective intake; For_{diet}, diet forage content; $Conc_{diet}$, diet concentrate content; NDF_{diet}, diet NDF content; CP_{diet}, diet crude protein content; Lig_{diet}, diet lignin content; $N/\lambda_{r\ For}$, compartmental mean residence time of the first ruminal pool for forage; $k_{e\ For}$, fractional rate of escape of particles from the escapable pool for forage; $N/\lambda_{r\ Conc}$, compartmental mean residence time of the first ruminal pool for concentrate; $k_{e\ Conc}$, fractional rate of escape of particles from the escapable pool for concentrate; MRT forage, mean retention time of forage; MRT concentrate, mean retention time of concentrate; Q_{DM}, dry matter content in the rumen; Q_{NDF}, NDF content in the rumen; Q_{LIG}, lignin content in the rumen.

[2] Obtained from pulse dose studies and interpreted kinetically using the G*N*G1 model; $MRT = N/\lambda_r + 1/k_e$.

[3] When testing the single-pool approach, all data from experiment 1 were used (n = 53). Thus, BW: 16.2 – 31.3; DMI: 123.9 – 952.6; NDFI: 59.8 – 514.4; LIGI: 3.2 – 42.3.

[4] n = 53

Table 2. Parameters of the allometric equations[1] and 95% asymptotic confidence intervals.

Variable[2]	α (S.E)	95% CI		β (S.E)	95% CI		θ^4
		Lr[3]	Up[3]		Lr[3]	Up[3]	
F_{DM}	15.40 (8.50)	-1.45	32.25	1.16 (0.16)	0.86	1.47	1.5
F_{NDF}	8.07 (2.02)	4.07	12.08	1.12 (0.07)	0.98	1.25	1.5
F_{LIG}	0.38 (0.25)	-0.12	0.88	1.24 (0.18)	0.87	1.61	1.5
Q_{DM}	15.49 (8.32)	-1.01	31.99	1.11 (0.15)	0.81	1.40	1.5
Q_{NDF}	6.89 (3.39)	0.17	13.62	1.16 (0.14)	0.90	1.44	1.5
Q_{LIG}	0.24 (0.15)	-0.06	0.53	1.59 (0.18)	1.24	1.94	2.5

[1] $y = \alpha \times BM^{\beta}$

[2] Intake rate (F_x, g·day^{-1}) and rumen content (Q_x, g) of dry matter (DM), neutral detergent fiber (NDF) and lignin (LIG).

[3] Lr and Up are the lower and upper limits of the 95% confidence intervals (95% CI), respectively.

[4] Power-of-the-mean variance.

Table 3. Linear regression estimates for rumen mass (Q_x) and intake of NDF and lignin (scaled and non-scaled) with lower (Lr) and upper (Up) limits of the asymptotic 95% confidence intervals (95% CI) and P-Values for the variance components estimated.

Variable[1]	Q_{xm} or Q_{xm}^{c} [2] $(S.E)$	95% CI		T_x or T_x^c [2] $(S.E)$	95% CI		θ^3	P-Value	
		Lr	Up		Lr	Up		σ_Q^2	σ_T^2
Q_{LIG}	9.39 (2.47)	4.48	14.29	2.16 (0.49)	0.07	4.25	3.86	-[4]	0.17
Q_{NDF}	22.66 (16.27)	-9.58	54.91	0.97 (0.06)	0.71	1.23	1.67	-[4]	0.28
Q_{LIG}^{c}	0.11 (0.02)	0.07	0.15	0.32 (0.06)	0.04	0.60	-	-[4]	0.22
Q_{NDF}^{c}	1.97 (1.09)	-0.19	4.14	0.86 (0.09)	0.48	1.24	-	0.43	0.37

[1] Rumen content (Q_x, g) of neutral detergent fiber (NDF) and lignin (LIG).

[2] Intercept (Q_{xm} or Q_{xm}^{c}) and slope (T_x or T_x^c) of the linear regression. The subscript *m* for the intercept means metabolic component and the superscript *c* for the intercept and slope means scaled variable.

[3] Power-of-the-mean variance.

[4] SAS returned the error message "Estimated G matrix is not positive definite"; therefore, the model was re-parameterized without the respective variance component.

Table 4. Regression equations used to predict the mean retention time of dietary forage and concentrates.

Variable[1]	Equations[2]	R^2	RMSE
$N/\lambda_{r\ For}$	$0.358\ (3.313) \times exp\left(\begin{matrix}-0.0025\ (0.001)\times peNDFI_{(g\cdot day^{-1})}+0.0064\ (0.002)\\ \times NDF_{diet(g\cdot kg^{-1})}+0.0133\ (0.008)\times DMI_{(g\cdot day^{-1})}/BM_{(kg)}^{0.75}\end{matrix}\right)$	0.40	7.36
$k_{e\ For}$	$0.010\ (1.398) \times exp^{\left(-0.024\ (0.016)\times peNDFI_{(g\cdot day^{-1})}/BM\right)} \times LIGI_{(g\cdot day^{-1})}^{0.474\ (0.116)}$	0.13	0.024
$N/\lambda_{r\ Conc}$	$81.43\ (18.19) - 9.98 \times \ln\left(DMI_{(kg.day^{-1})}\right) + 2.68\ (1.54) \times \ln(N/\lambda_{r\ For.})$	0.34	5.48
$k_{e\ Conc}$	$0.274\ (0.07) - 0.0173\ (0.011) \times \ln\left(LIGI_{(g\cdot day^{-1})}\right) + 0.0448\ (0.014) \times \ln\left(k_{e\ For.}\right)$	0.21	0.026

[1] $N/\lambda_{r\ For}$, compartmental mean residence time of the first ruminal pool for forage, h; $k_{e\ For}$, fractional rate of escape of particles from the escapable pool for forage, h^{-1}; $N/\lambda_{r\ Conc}$, compartmental mean residence time of the first ruminal pool for concentrate, h; $k_{e\ Conc}$, fractional rate of escape of particles from the escapable pool for concentrate, h^{-1}.

[2] peNDFI, intake of physically effective NDF, $g\cdot day^{-1}$; NDF_{diet}, concentration of neutral detergent fiber in the diet, $g\cdot kg^{-1}$; DMI, dry matter intake, $g\cdot day^{-1}$; BW, body weight, kg; LIGI, lignin intake, $g\cdot day^{-1}$.

Table 5. Spearman correlation coefficients and *P*-values of the variables from the original dataset used in the sensitivity analysis simulation.

Spearman Correlation Coefficients[1]								
	peNDFI	NDF_{diet}	$DMI/BW^{0.75}$	NDFI/BW	DMI	$N/\lambda_{r\ For}$	LIGI	$k_{e\ For}$
peNDFI	1	-0.21 0.0602	0.63 <.0001	0.88 <.0001	0.74 <.0001	-0.54 <.0001	0.83 <.0001	0.26 0.0236
NDF_{diet}		1	-0.71 <.0001	-0.08 0.4984	-0.73 <.0001	0.32 0.0053	-0.43 <.0001	-0.37 0.0009
$DMI/BW^{0.75}$			1	0.59 <.0001	0.94 <.0001	-0.39 0.0006	0.78 <.0001	0.44 <.0001
NDFI/BW				1	0.57 <.0001	-0.43 0.0001	0.70 <.0001	0.13 0.2523
DMI					1	-0.49 <.0001	0.84 <.0001	0.44 <.0001
$N/\lambda_{r\ For}$						1	-0.42 0.0002	0.15 0.1982
LIGI							1	0.42 0.0002
$k_{e\ For}$								1

[1] peNDFI, intake of physically effective, g·day^{-1}; NDF_{diet}, concentration of neutral detergent fiber in the diet, g·kg^{-1}; $DMI/BW^{0.75}$, relative metabolic dry matter intake, g·kg$^{-0.75}$; NDFI/BW, relative neutral detergent fiber intake, g·kg^{-1}; DMI, dry matter intake, g·day^{-1}; $N/\lambda_{r\ For}$, compartmental mean residence time for forage, h; LIGI, lignin intake, g·day^{-1}; $k_{e\ For}$, fractional rate of escape of particles from the escapable pool for forage, h^{-1}.

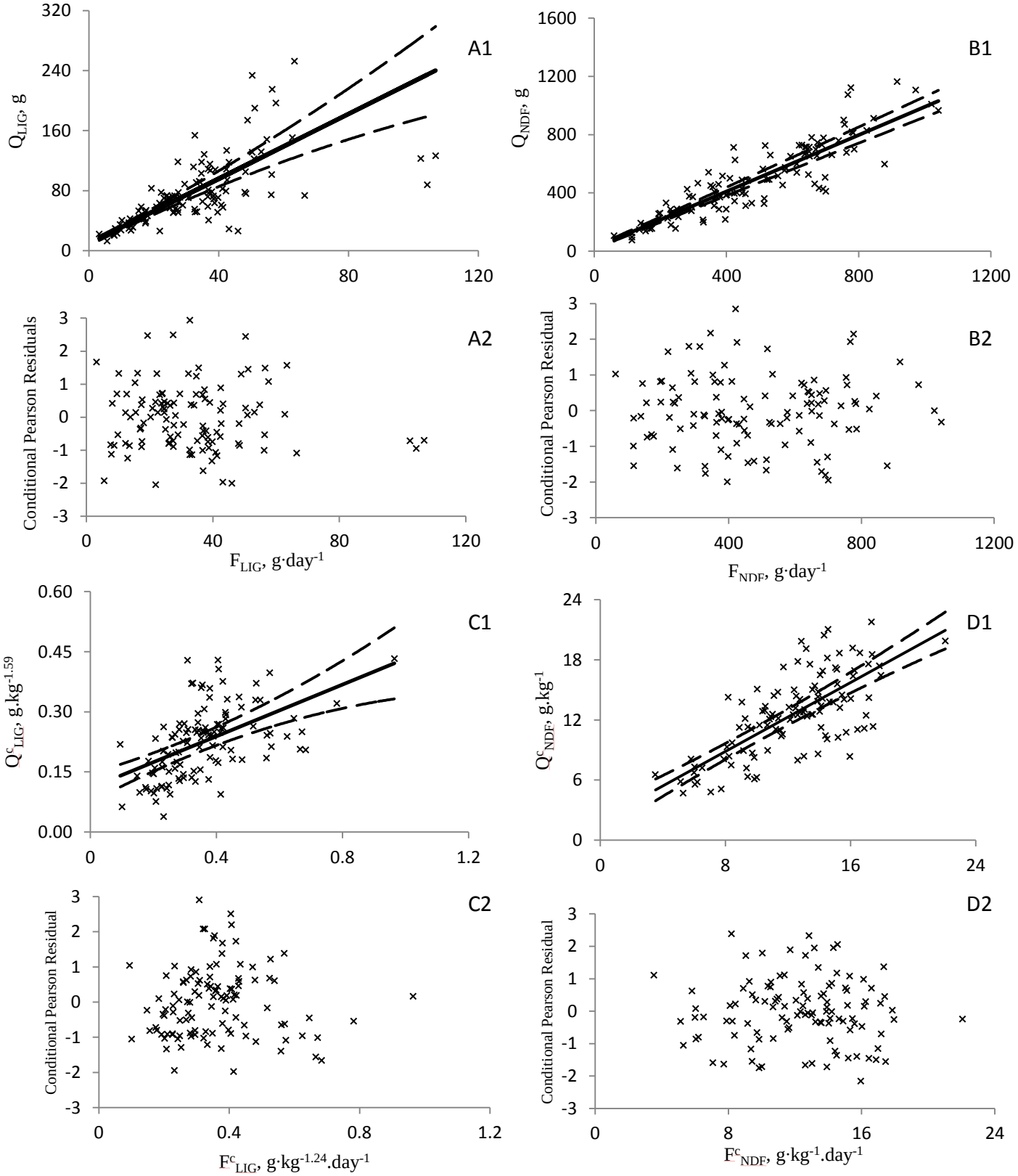

Figure 1. NDF and lignin ruminoreticular content regressed against NDF and lignin intake (panels A1 and B1 are non-scaled variables; panels C1 and D1 are scaled variables) and conditional Pearson residuals (panels A2, B2, C2 and D2).

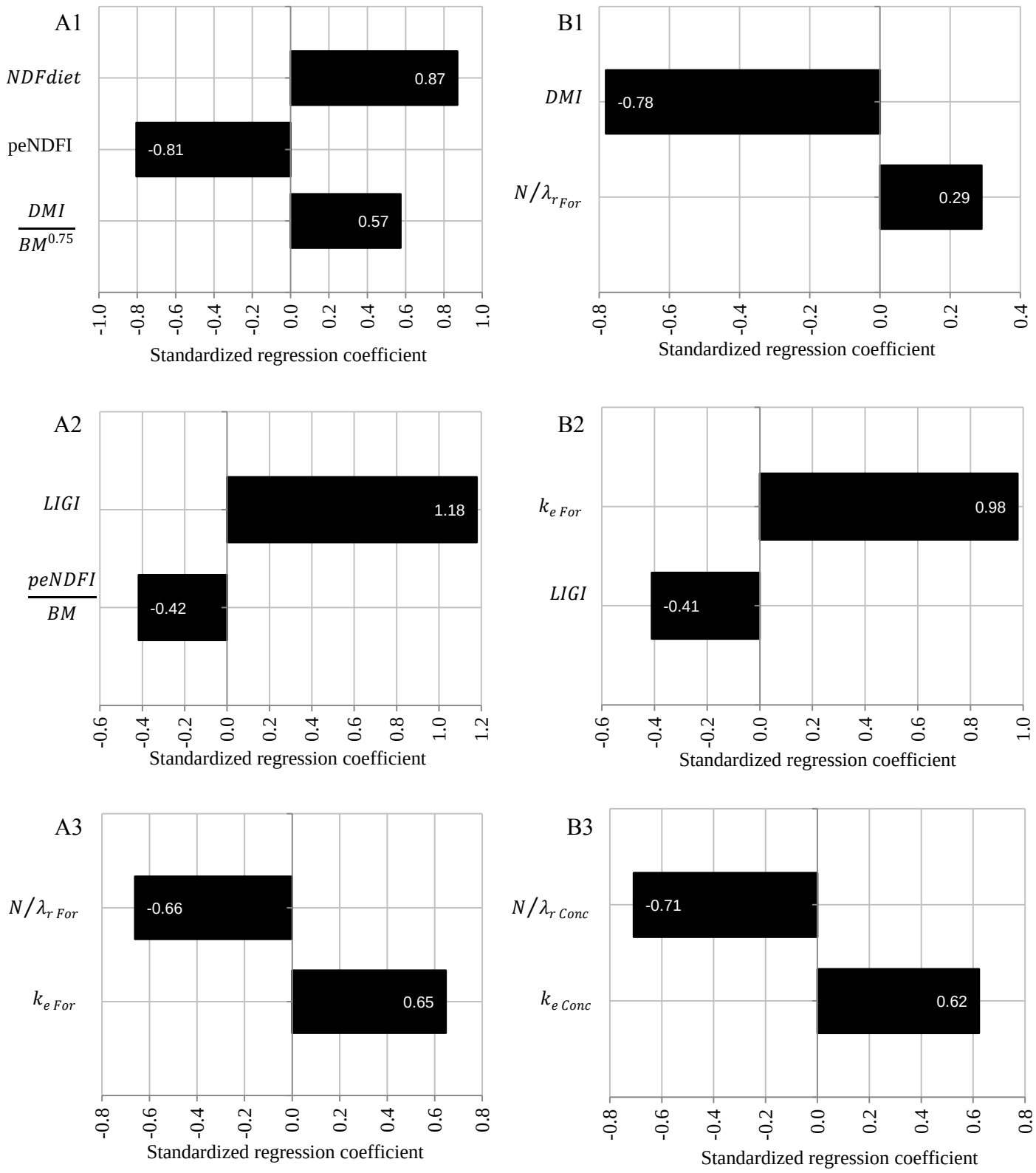

Figure 2. Standardized regression coefficients obtained from Monte Carlo simulation measuring the effects of the independent variables on the dependent variables. Panels A and B correspond to forage and concentrates, respectively. Panels 1, 2 and 3 correspond to n/λ_r, k_e and k_p, respectively.

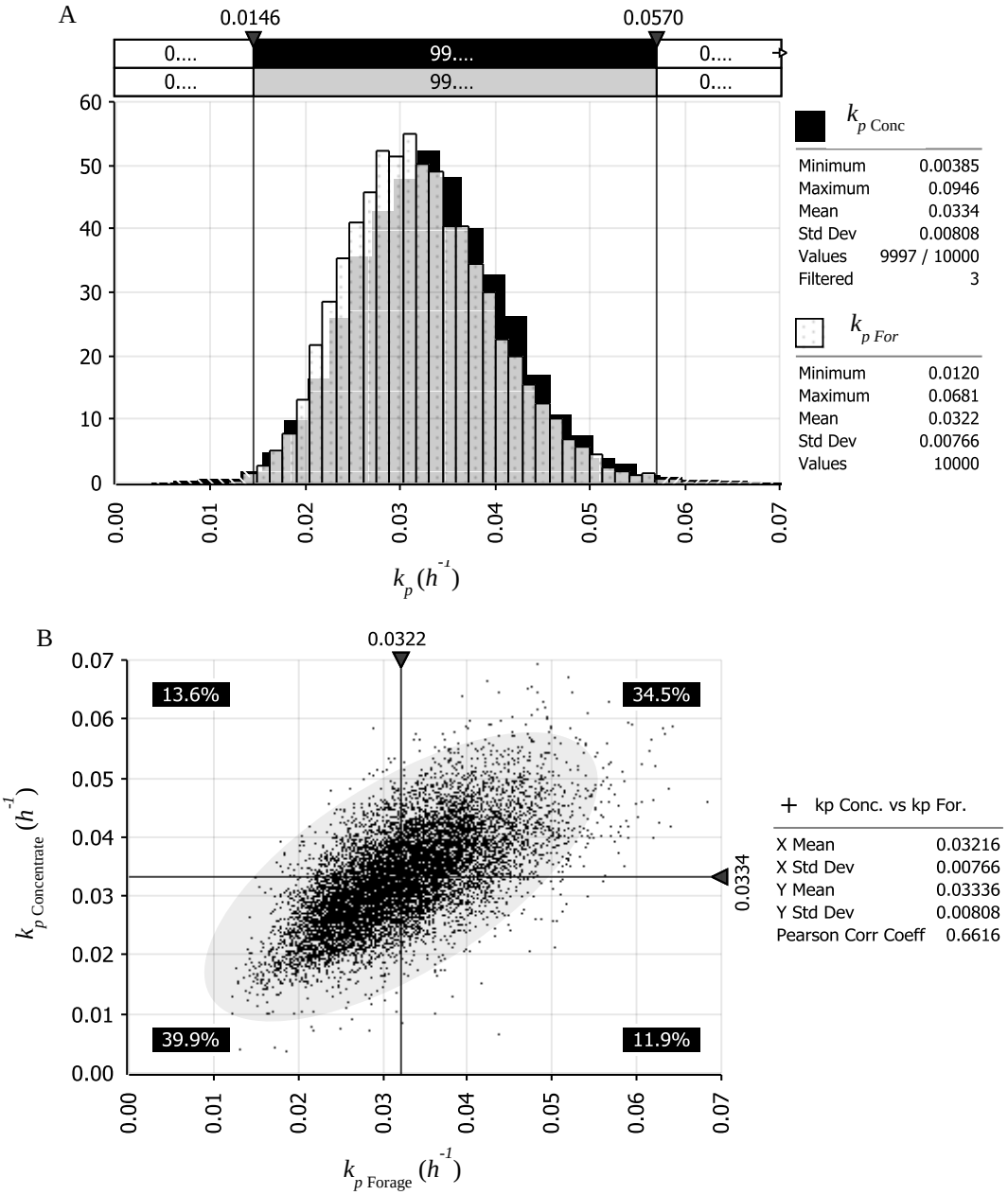

Figure 3. Histogram of the distributions (A) and scatter plot (B) of the simulated rates of passage (h^{-1}) of forage and concentrate fibers.

SRNS model: Considering the ruminal fiber stratification and evaluating an alternative approach to estimate the dry matter intake for goats[1]

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ABSTRACT

The objectives of this paper were (1) to assess the ability of a mechanistic model named Small Ruminant Nutrition System (**SRNS**) to predict the metabolizable energy intake (**MEI**) and milk yield (**MY**) by using a heterogeneous ruminal fiber pool scenario (**GnG1**) in comparison to a traditional homogeneous scenario (**G1**), and (2) to evaluate an alternative approach to estimate the dry matter intake (**DMI**) of goats. The GnG1 scenario considers that the first ruminal fiber pool (raft) follows an age-dependent fractional rate for particle transference from a raft to an escapable pool (λ_r) and that the second ruminal fiber pool (escapable) follows an age-independent fractional rate of particle escape from the escapable pool (k_e). For G1, a scenario with only one fiber pool, a single fractional rate passage (k_p) is adopted. All parameters were estimated individually by using equations published in the literature; however, for the G1 scenario, two rate passage equations were used, with one from cattle data (**G1-C**) and another one from goat data (**G1-G**). The alternative approach to estimating the DMI is based on an optimization process. The MEI, MY and DMI estimated by using these scenarios were compared with the results of an independent dataset (n = 327) that contained information regarding the DMI, MEI, MY and milk and dietary compositions. The evaluation of the scenarios was performed using a coefficient of determination (**r^2**) between the observed and predicted values; mean bias (**MB**); bias correction factor (**C_b**); concordance correlation coefficient (**CCC**). The MEI estimated by the GnG1 scenario yielded precision and accuracy (r^2 = 0.82; MB = 0.21 Mcal·day^{-1}; C_b = 0.98) statistics similar to that for the G1-C (r^2 = 0.83; MB = 0.11 Mcal·day^{-1}; C_b = 0.99) and G1-G (r^2 = 0.85; MB = 0.17 Mcal·day^{-1}; C_b = 0.99) scenarios. The results were also similar for MY; however, a significant MB was found: GnG1 (r^2 = 0.74; MB = 0.63 kg·day^{-1}; C_b = 0.79), G1-C (r^2 = 0.72; MB = 0.63 kg·day^{-1}; C_b = 0.82), and G1-G (r^2 = 0.65; MB = 0.65 kg·day^{-1}; C_b = 0.82). The alternative approach for DMI

estimation yielded better results with the G1-G scenario (r^2 = 0.89; MB = -74.87 g·day^{-1}; C_b = 0.98). The GnG1 scenario can be assumed to maintain the theoretical basis of mechanistic models, but it is necessary to develop a sub-model that allows tissue mobilization to be inserted into the SRNS model to avoid MY underprediction for goats in negative energy balance. The alternative approach for estimating the DMI is reasonable and can be used in diet formulations.

Key words: heterogeneous fiber pool, optimization, rumen capacity

INTRODUCTION

The nutritional values of ruminant feedstuffs are not constant; they depend on the retention time in the gastrointestinal tract (**GIT**), animal species, body mass, dry matter intake (**DMI**) and many other factors that can affect their availability and digestibility (Van Soest, 1994). The interactions between these variables can only be evaluated using non-specific and comprehensive methods. On the basis of this limitation, nutritional models focused on mechanistic approaches have been developed and are used today to predict aspects of ruminant nutrition. The Small Ruminant Nutrition System (**SRNS**) (Tedeschi et al., 2010) is the most recent nutritional model for sheep and goats; its set of equations explaining the dietary supply of energy and nutrients is based on the Cornell Net Carbohydrate and Protein System (**CNCPS**) for cattle (Fox et al., 2004) and sheep (Cannas et al., 2004). However, because this model is still being developed, it has not been completely evaluated for goats under diverse production scenarios. Only one paper that evaluated lactating goats with the SRNS exists in the literature (Cannas et al., 2010). Therefore, additional evaluations with a larger database are needed to study the predictive ability of this model.

Furthermore, all nutritional models used currently include a sub-model referring to DMI estimation, but the DMI is a result of a range of factors that operate and interact with one another. Theoretical and practical models that predict the DMI based on the physical intake control theory have been proposed and tested (Forbes, 2007, Illius and Gordon, 1991, Poppi et al., 1994), but none of them have been evaluated in mechanistic models with the same ruminal approach as the SRNS.

Vieira et al. (2008) proposed that DMI can be predicted by using an optimization process with typical constraints in ruminant diet formulation. If valid, this hypothesis may generate an interesting tool for diet formulations and ruminant DMI predictions. This model would provide the DMI, which is more biologically appropriate than using this parameter as a model input. Usually, the constraints adopted by software that are used to formulate ruminant diets encompass the nutritional requirements and nutritional bounds for avoiding dietetic issues (e.g., an excess of non-fibrous carbohydrates and fats). Extra constraints can be added to the optimization process to achieve physical fiber restriction.

Another factor that can influence the physical aspects of ruminant intake control is ruminal fiber stratification, which may occur when ruminants are fed a considerable amount of fiber. The equations referring to the passage rate and fiber digestion in the SRNS model do not account for ruminal fiber stratification; consequently, an evaluation of how to insert this approach into the model is necessary.

The objectives of this paper were (1) to evaluate the SRNS model with modifications in predicting goat performance; (2) to evaluate the insertion of a heterogeneous fiber pool approach into the SRNS model; and (3) to assess an alternative approach for estimating DMI for dairy goats.

MATERIALS AND METHODS

Model descriptions and general assumptions

The SRNS[1] with modifications was used to evaluate a heterogeneous rumen fiber approach and mass fiber restrictions for goats. The SRNS uses the equations described by Tedeschi et al. (2010) to predict goat nutritional requirements, and the equations described by Fox et al. (2004) are used to predict the dietary supply of energy and nutrients. However, updates have been made in the model, and some of them were considered in our evaluations, including modified predictions for ruminal pH and microbial growth (Tylutki et al., 2008). The sub-model of fatty acid absorption and new carbohydrate fractionation were not evaluated.

The following modifications and assumptions were considered: 1) four protein fractions instead of the five in the original model; this adaptation is based on the assumption that digestion of the original PB1 and PB2 fractions is uniform, so the fractions assumed here are PA (NPN), PB1 (soluble protein), PB2 (NDIP-ADIP) and PC (ADIP); 2) four carbohydrate fractions were used, namely CA (organic acids and sugars), CB1 (soluble fiber and starch), CB2 (available insoluble fiber) and CC (unavailable fiber fraction); 3) the fecal crude protein calculation was modified (Cannas et al., 2004, Tedeschi et al., 2010); 4) the heterogeneous pool evaluation was considered only for the fiber fraction (B2 + C). For the other feed fractions (carbohydrate and protein), ruminal digestibility was calculated using the original approach: $RD = k_d/(k_d + k_p)$. However, the rate passage used to estimate digestibility was calculated as the reciprocal of the total mean retention time (**TMRT**); and 5) the calcium and phosphorous requirements described in the AFRC (1993) were adopted.

[1] Available at: http://nutritionmodels.tamu.edu/srns.html (verified 15 July 2013).

Sub-model for estimating the passage rate and ruminal fiber digestibility

The sub-model described here is based on the occurrence of a heterogeneous fiber ruminal pool in goats receiving high-fiber diets, and it considers general assumptions regarding particle flow as described by Matis (1972) and Vieira et al. (2008). These general assumptions are that the first ruminal fiber pool (raft) is ruled by an age-dependent fractional rate for particle transference from the raft to the escapable pool (λ_r) and that the second ruminal fiber pool (escapable) is governed by an age-independent fractional passage rate (k_e). The compartmental mean residence time (**CMRT**) is calculated by using $CMRT_1 = n/\lambda_r$ and $\mathrm{CMRT_2} = 1/\mathrm{k_e}$ for the first and second pools, respectively. The *n* represents the order of time dependency, which is an integer from the gamma function that rules the CMRT in the first ruminal pool. The total mean retention time is calculated as the sum of both compartments and the passage rate (k_p) is the reciprocal of the TMRT.

Based on these assumptions, Vieira et al. (2008) extended the concept deduced by Waldo et al. (1972) to the digestion and fill effects to account for a heterogeneous fiber pool in the rumen. The equations described below were used to predict the ruminal digestibility of the B2 carbohydrate fraction and the filling effect of the fiber fraction:

$$\mathrm{RDCB2_j} = \mathrm{DMI_J} \times \mathrm{CB2_J} \times \left\{ \mathrm{k_{d_j}} \times \left[\left[\sum_{i=1}^{n} \lambda_{r_j}^{i-1} / \left(\lambda_{r_j} + \mathrm{k_{d_j}} \right)^{i} \right] + \lambda_{r_j}^{n} / \left[\left(\lambda_{r_j} + \mathrm{k_{d_j}} \right)^{n} \left(\mathrm{k_{e_i}} + \mathrm{k_{d_i}} \right) \right] \right] \right\} \quad [1]$$

$$\mathrm{Q_{fiber_j}} = \mathrm{FCI_j} \left\{ \frac{\mathrm{CB2_j}}{\mathrm{FCI_j}} \times \left\{ \sum_{i=1}^{n} \left[\lambda_{r_j}^{i-1} / \left(\lambda_{r_j} + \mathrm{k_d} \right)^{i} \right] + \lambda_{r_j}^{n} / \left[\left(\lambda_{r_j} + \mathrm{k_{d_j}} \right)^{n} \left(\mathrm{k_{e_j}} + \mathrm{k_{d_j}} \right) \right] \right\} + \frac{\mathrm{CC_j}}{\mathrm{FCI_j}} \left[\mathrm{n}/\lambda_{r_j} + 1/\mathrm{k_{e_j}} \right] \right\} \quad [2]$$

where RDCB2 is the ruminal degraded B2 carbohydrate fraction (g); DMI is the dry matter intake (g·day^{-1}); CB2 is the concentration of the B2 carbohydrate fraction in DM (g·kg^{-1}); k_d is the degradation rate for the B2 fraction; Q_{fiber} is the rumen fiber mass per

unit of intake (g); FCI is the fiber carbohydrate intake (g·day^{-1}); CC is the concentration of the C carbohydrate fraction in DM (g·kg^{-1}); and subscript *j* indicates each feed in the diet.

Age-dependent and independent rate parameters were estimated using the equations described by Regadas Filho (2013) for forage and concentrate.

$$n/\lambda_{r\ For} = 0.358 \times exp^{\left(-0.0025 \times peNDFI + 0.0064 \times NDF_{diet} + 0.0133 \times \left(DMI/BM^{0.75}\right)\right)} \quad [3]$$

$$k_{e\ For} = 0.010\ \times exp^{(-0.024\ \times peNDFI/BM)} \times LIGI^{0.476} \quad [4]$$

$$n/\lambda_{r\ Conc} = 81.43 - 9.98 \times \ln(DMI) + 2.68 \times \ln(N/\lambda_{r\ For}) \quad [5]$$

$$k_{e\ Conc} = 0.274 - 0.0173\ \times \ln(LIGI) + 0.0448 \times \ln\left(k_{e\ For}\right) \quad [6]$$

where $n/\lambda_{r\ For}$ is the mean compartmental residence time of the first ruminal pool for forage (h); $k_{e\ For}$ is the fractional rate of particle escape from the escapable forage pool (h^{-1}); $n/\lambda_{r\ Conc}$ is the mean compartmental residence time of the first ruminal concentrate pool (h); $k_{e\ Conc}$ is the fractional rate of particle escape from the escapable pool for concentrate (h^{-1}); peNDFI is the intake of physically effective NDF (g·day^{-1}); NDF_{diet} is the concentration of NDF in the diet (g·kg^{-1}); DMI is the dry matter intake (g·day^{-1}); BM is the body mass (kg); and LIGI is lignin intake (g·day^{-1}).

An indirect approach was used to access the λ_r parameters once the equations developed in the cited paper had predicted the compartmental mean residence time (n/λ_r); and the equations used to predict the RDCB2 and Q_{fiber} required the parameter *per se*. It was assumed that the order of age dependency decreases as the rate of fiber carbohydrate intake ($FCI^c = FCI/BM$) increases, and the following classes were used to access the estimated order of age-dependency (n`): $\forall\ FCI^c < 5, n' = 3;\ 5 \leq \forall\ FCI^c \leq 12, n' = 2$; and $\forall\ FCI^c > 12, n' = 1$ (Vieira et al., 2008).

The model used to address the heterogeneous (GnG1) fiber pool was compared with the traditional homogeneous (G1) fiber pool model to predict the metabolizable energy intake (**MEI**) and the milk yield (**MY**) from dairy goats. The passage rate in the

homogeneous fiber pool model was calculated by using forage and concentrate equations developed by Seo et al. (2006) for cattle (these equations are in current use) (G1-C). However, Tedeschi et al. (2012) established a specific equation for forage rate passage in goats, and this equation was also evaluated here (G1-G). Because Tedeschi et al. (2012) did not develop an equation for concentrate, the equation developed by Seo et al. (2006) was used. The rumen filling effects in homogeneous fiber pools scenarios were calculated using the following expression (Waldo et al., 1972):

$$Q_{\mathrm{fiber_j}} = \mathrm{FCI_j} \times \left[\left(\mathrm{CB2_j}/\mathrm{FCI_j}\right)/\left(\mathrm{k_{p_j}} + \mathrm{k_{d_j}}\right) + \left(\mathrm{CC_j}/\mathrm{FCI_j}\right)/\mathrm{k_{p_j}}\right] \quad [7]$$

Rumen fiber restrictions and predictions of dry matter intake

It is possible to use the Q_{fiber} estimated by both approaches (homogeneous and heterogeneous ruminal fiber pools) to evaluate the hypothesis raised by Vieira et al. (2008) that DMI can be predicted by using inequality constraints during ruminant diet optimization. Therefore, the DMI would not be a model input, but would rather act as an output. For this purpose, the SRNS model with modifications was inserted into an Excel spreadsheet, and dietary constraints based in previous nutritional knowledge were considered and are described in Equations (8-17). The DMI was calculated as the sum of all optimized diet ingredients for each animal (n = 327) in the three evaluated scenarios (G1-C, G1-G and GnG1). This DMI prediction was compared with the equation described in the AFRC (1993) for goats.

The objective function specified in Eq. (8) minimizes the cost function, where c_j is the price of j feed ingredients times their use (denoted X_j; g) in the optimal ration. Eq. (8) was subject to a series of constraints described in Eq. (9-17). The constraints represented in Eq. (9-12) refer to the nutritional requirements of MEI, metabolizable protein intake (**MPI**), calcium intake (Ca) and phosphorous intake (P). Subscript *req* indicates the requirement for a specific nutritional component. To allow more flexibility in the

optimization process, the *p* constant (range from 0 to 1) that represents the precision of the model was assumed to be 0.1.

$$DMI = \begin{cases} Minimize\ Cost = \sum_{j=1}^{n} c_j \times X_j, & [8] \\ \qquad\qquad subject\ to: & \\ (1-p) \times MEI_{req} \leq \sum_{j=1}^{n} MEI_j \leq (1+p) \times MEI_{req} & [9] \\ (1-p) \times MPI_{req} \leq \sum_{j=1}^{n} MPI_j \leq (1+p) \times MPI_{req} & [10] \\ (1-p) \times Ca_{req} \leq \sum_{j=1}^{n} Ca_j \leq (1+p) \times Ca_{req} & [11] \\ (1-p) \times P_{req} \leq \sum_{j=1}^{n} P_j \leq (1+p) \times P_{req} & [12] \\ [NFC_{diet}] = \frac{\sum_{j=1}^{n}[NFC_j] \times X_j}{\sum_{j=1}^{n} X_j} \leq 0.42 & [13] \\ [Fat_{diet}] = \frac{\sum_{j=1}^{n}[Fat_j] \times X_j}{\sum_{j=1}^{n} X_j} \leq 0.06 & [14] \\ [peNDF_{diet}] = \frac{\sum_{j=1}^{n}[peNDF_j] \times X_j}{\sum_{j=1}^{n} X_j} \geq 0.20 & [15] \\ 0 \leq BactNBalance \leq 10 & [16] \\ \sum_{j=1}^{n} Q_{fiber_j} \leq RC & [17] \end{cases}$$

The constraints represented in Eq. (13-15) refer to the no fiber carbohydrate (≤ 42%), fat (≤ 6%) and neutral detergent fiber physically effective (≥ 20%) concentrations in dietary dry matter. The *BactNBalance* (g.day^{-1}) in the constraint represented in Eq. 16 is an estimate of the rumen nitrogen balance, and it was used to avoid nitrogen limitations or excesses in the diet that would cause increases in the maintenance energy requirement in relation to urea excretion or limitations to microorganism growth. Bounds from 0 to 10 g·day^{-1} were arbitrarily assumed.

Eq. 17 shows the constraint relating to ruminal fiber. The addition of Q_{fiber} for each dietary component yields the average amount of fiber present in the rumen. The limitation of this component is that the value has to be less than or equal to the ruminal capacity (RC, g). Although the rumen capacity has been reported to be scaled to unity (Demment and Van Soest, 1985), we used the equation described in Regadas Filho (2013) to estimate the ruminal capacity of goats as: $\mathrm{RC} = 6.89 \times \mathrm{BM}^{1.16}$ (g of NDF).

The nonlinear generalized reduced gradient method (Lasdon et al., 1974) in Excel was used to solve this problem. A precision of 0.001 was adopted, and the central derivatives method was used to estimate the partial derivatives of the objectives and constraint functions.

Model evaluation

The dataset used to evaluate these scenarios was composed of 10 studies with 45 treatments for a total of 327 individual measures in lactating dairy goats (Alpine and Saanen) in the Universidade Federal de Viçosa (MG-Brazil) where the DMI, MEI, MY, milk composition and dietary composition were measured. The MEI ($Mcal \cdot day^{-1}$) and MY ($kg \cdot day^{-1}$) were used to evaluate these scenarios. The MY was calculated from the first limiting nutrient (ME or MP). Accurate information regarding the concentrations of different fractions (carbohydrates and proteins) in the diet and their respective degradation rates were not available; however, some of them could be calculated from feed chemical analyses. Fraction C of the carbohydrates was calculated as $\mathrm{Lig} \times 2.4$; and B2 was calculated as $\mathrm{NDF_j}$-$\mathrm{CC_j}$; protein fractions B2 and C were obtained from the NDFIP and ADFIP analysis available in all experiments. The concentration of fractions A1 and B1 in carbohydrates and proteins and the degradation rates of all fractions were obtained from the Tropical Library in the original SRNS model. Table 1 shows a summary of this dataset.

The model evaluation was conducted using assumptions described by Tedeschi (2006). The precision of the models was assessed using the coefficient of determination ($\mathbf{r^2}$) between the predicted and observed values in addition to the simultaneous *F*-test of the intercept and slope (H_0: intercept = 0 and slope = 1; $\alpha = 0.05$), whereas the accuracy was calculated based on the bias correction factor ($\mathbf{C_b}$), which indicates how far the regression line deviates from the slope of unity (45°); the concordance correlation coefficient (**CCC**), the root of mean square error prediction (**RMSEP**) and its decomposition into mean bias, systematic bias and random errors were also calculated. Evaluations were performed by Model Evaluation System software (available at http://nutritionmodels.tamu.edu/mes.html, verified 04 April 2013).

RESULTS

Metabolizable energy intake and milk yield estimation

Table 2 presents the parameters for MEI evaluation, and panels 1-A, 1-B and 1-C in Figure 1 show the linear regression between the observed and predicted values for the G1-C, G1-G and GnG1 scenarios, respectively. Despite the simultaneous F-test rejecting a hypothesis in which the intercept = 0 and slope = 1 for the three assessed scenarios, all of them had high precision, as indicated by the high r^2 = 0.83, 0.85 and 0.82 for the G1-C, G1-G and GnG1 approaches, respectively. A slight underprediction of the MEI was found; the G1-C scenario presented a lower mean bias (MB = 0.1144 Mcal·day^{-1}), but the MBs were not significant in all scenarios (P = 0.2995, 0.1244 and 0.0611 for the G1-C, G1.T and GnG1 approaches, respectively). All scenarios presented high accuracy (Cb = 0.99, 0.99 and 0.98 for the G1-C, G1-G and GnG1 approaches, respectively). The CCC and RMSEP estimates were also similar between them (values in Table 1). The MSEP

decomposition showed that random errors were the main component of the error for all scenarios.

Table 3 presents the parameters of the MY evaluation, and panels 2-A, 2-B and 2-C in Figure 1 show the linear regression between the observed and predicted values for the G1-C, G1-G and GnG1 scenarios, respectively. The milk production evaluation yielded poorer results than the MEI. The simultaneous F-test for the intercept and slope rejected H_0 for the study scenarios, and the precision of the model was similar, with a slight advantage for the GnG1 scenario ($r^2 = 0.74$). The models underestimated the MY. The mean biases were 0.63, 0.65 and 0.63 $kg \cdot day^{-1}$ for the G1-C, G1-G and GnG1 scenarios, and all of them were significant ($P<0.0001$). These mean biases had important effects on the RMSEP partition, contributing approximately 50% of the error decomposition. The remaining 50% was mostly explained by random error. The CCC is a measure of model accuracy and precision and supports the idea that the MY estimation was less than the MEI estimate.

Evaluation of the dry matter intake estimation

The optimization technique is an interesting tool for addressing DMI in ruminants; however, the complexity of the model and the interdependence of some variables did not allow all runs to reach a solution. Approximately 45% of the individual runs in each scenario reached a solution.

Table 4 shows the DMI evaluation parameters, and Figure 2 shows a linear regression between the observed and predicted values for the G1-C, G1-G, GnG1 and AFRC scenarios. The scenarios based on this optimization technique presented higher precision (G1-C, $r^2 = 0.84$; G1-G, $r^2 = 0.89$; and GnG1, $r^2 = 0.84$) than the empirical equation (AFRC equation, $r^2 = 0.78$). In all evaluated scenarios, there was a slight DMI overprediction, as indicated by the negative mean bias; the AFRC model presented lower

values (G1-C, MB = -68.4 g·day^{-1}, P = 0.1542; G1-G, MB = -74.9 g·day^{-1}, P = 0.0923; GnG1, MB = -87.3 g·day^{-1}, P = 0.0668; and AFRC, MB = -46.2 g·day^{-1}, P = 0.2013), but none of them were significant. The accuracy of the models was high (Cb ≈ 0.96-0.98). The G1-G scenario presented higher CCC (0.92) and lower RMSEP (224.22 g·day^{-1}). There was a different pattern here than that observed for the MEI and MY evaluations, which produced the same MSEP decomposition. For the G1-C and GnG1 scenarios, the systematic bias (48.3 and 44.2%, respectively) and random errors (46.6 and 47.7%, respectively) were of similar importance, whereas in the G1-G and AFRC scenarios, the random error (62.0 and 88.8%, respectively) was the main component.

DISCUSSION

Metabolizable energy intake and milk yield estimation

The scenarios evaluated here had essentially the same ability to predict goat MEIs; the SRNS model is able to predict the MEI independently of the three sets of equations used to estimate the passage rate. An identical result was found by Cannas et al. (2010) with kids and goats using the original rate passage equations. The higher participation of the random error component in RMSEP decomposition can be interpreted despite the presentation of good estimates by the MEI estimation models, further studies should be performed to identify possible effects that were not controlled and may be influencing the results. Another factor that could be influencing the results is that the A1 and B1 fractions and the degradation rates of all carbohydrate and protein food fractions were not measured; rather, they were adopted from a tabular dataset, which could affect the prediction values. It is extremely important to perform evaluations on these types of models by using real values for feed fractions and degradation rates.

Similar to the MEI evaluation, the three scenarios evaluated showed the same ability of prediction for the MY. In contrast, the MY prediction had a significant mean bias (approximately 0.64 kg·day^{-1}). The underprediction of MY found here is most likely explained by a lack of information regarding changes in the goat body mass in the dataset. It is likely that a significant portion of the animals was in negative energy balance, and their milk production should be partly based on mobilized body reserves. The current SRNS model does not consider this sub-model, which is already considered for cattle. In terms of evidence, we can demonstrate the effect of not addressing the energy and protein body mobilization in the model. We can assume a nominal body mass weight loss of 1 kg·week^{-1} during the first four weeks of lactation; considering the 5.71 Mcal·kg^{-1} for goat body mass (AFRC, 1993), this parameter would yield 0.82 Mcal·day^{-1} of body energy loss, and if we assume a 0.84 efficiency for mobilized tissue energy use (NRC, 2007), the energy allowable for milk production would be 0.68 Mcal·day^{-1}. The average milk concentration of fat and protein reported in the dataset was 3.17 and 2.96% (Table 1), respectively; which would yield an energy content of approximately 0.66 Mcal·kg^{-1} of milk. The mean bias found in the analysis was 0.64 kg·day^{-1} of milk, so 0.42 Mcal·day^{-1}, which is not expressively different from the 0.68 Mcal·day^{-1} allowable for milk production. The slight difference of 0.26 Mcal·day^{-1} is most likely caused by differences in body mass loss. The 1 kg·week^{-1} assumed here is for the initial four weeks of lactation; we have six experiments starting at 60 days after calving, one starting at 45 days after calving and one starting at 28 after calving, whereas two did not report this information in the dataset; hence, the animals were most likely losing less than 1 kg·week^{-1}. Moreover, we only considered the energy allowable for milk production; however, the metabolizable protein available for milk production is also an important factor. Tylutki et al. (2008) found that the MP was the first limiting nutrient for all diets under study. Tedeschi et al.

(2013) developed a dynamic model to simulate the DMI and the fluxes of fat and protein associated with body condition scores for cattle; however, that model has not yet been evaluated for goats and a specific model for goats may be needed. The relationship between the factors that affect the allowable ME or MP for milk production in goats can be different. Therefore, it is essential to develop and evaluate a sub-model of tissue mobilization specific to goats to be used by the SRNS model.

These results indicate that both homogeneous (G1-C and G1-G) and heterogeneous (GnG1) fiber pool approaches have similar robustness for predicting the MEI and MY of goats. If we consider only the principle of parsimony, we would adopt the simplest approach (homogeneous pool); however, mechanistic models are built on known or assumed biological, chemical or physical theories (Mertens, 2005) and not only on statistical analysis. The heterogeneous ruminal fiber pool approach has a robust mechanistic base, and its theory and the identification of influential factors have been studied and tested by numerous investigators (Clauss and Lechner-Doll, 2001, Tschuor and Clauss, 2008, Vieira et al., 2008); thus, this approach can be used.

Evaluation of the dry matter intake estimation

One important consideration must be made regarding the limitation of this evaluation. The DMI observed in these animals is a response to the diet obtained from a specific combination of ingredients; however, the DMI estimated using an optimization technique changes the composition of the diet. Therefore, animal performance would be expected to change when using the optimal diet formulated for these specific animals. For a more appropriate assessment of this theory, it is necessary to evaluate this approach from an *a priori* diet formulation, and then feed the animals and observe their intake. Nevertheless, the results found here were interesting for a first investigative analysis.

The DMI optimization theory seems to be an appropriate technique. The DMI as an output of the model is biologically appropriate. Predictions are based on nutritional concepts of degradation and passage rates, and they agree in part with the theories of the first limiting factor and optimization models for estimating the DMI as discussed by Forbes (2007). The constraints used here encompassed the most important nutritional requirements, and some possible dietetic issues were addressed. The ruminal fiber constraint prevented food intake from exceeding expectations. In addition, information on the degradation and passage rates are already available in mechanistic models, such as the CNCPC and SRNS, and can be easily used in the process of diet optimization. Extra constraints can be added depending of the goal of the users.

The high precision (high r^2) of the optimization process scenarios is indicative of high repeatability for the DMI estimation. The empirical equation adopted by AFRC (1993) does not account for dietary estimation effects; only animal information are considered. This empirical approach may cause bias in the estimation when evaluating animals with the same size and level of production that are fed with different food qualities. This bias is clearly indicated by the higher participation of the random errors component in the RMESP.

Apparently, the main problem in using this methodology for formulating ruminant diets is the development of an appropriate mathematical algorithm to address the hard nonlinearity and interdependence between variables in the model. As discussed previously, approximately 45% of the individual runs in each scenario reached a solution; hence, reliable DMI estimates are expected when using an algorithm capable of optimizing a broader range of scenarios.

CONCLUSIONS

The SRNS model can accurately and precisely predict the MEI for lactating goats, and if necessary, the heterogeneous ruminal fiber approach can be assumed to maintain the theoretical basis of mechanistic models. It is necessary to develop a sub-model that accounts for tissue mobilization to be inserted into the SRNS model to avoid MY underprediction for goats in negative energy balance. The alternative approach for estimating the DMI based on the optimization method is reasonable and can be used in dietary formulations.

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Table 1 – Dataset summary

Animal information	Mean	S.D.	Min.	Max.
BM (kg)	54.27	8.70	36.10	78.70
DMI ($g \cdot day^{-1}$)	1939.05	523.48	666.47	3451.00
Milk yield ($kg \cdot day^{-1}$)	2.45	1.18	0.09	6.81
Milk fat content (%)	3.17	0.59	1.85	5.04
Milk protein content (%)	2.96	0.36	2.29	4.95
MEI ($Mcal \cdot day^{-1}$)	5.02	1.32	1.53	8.21
Dietary information				
Forage ($g \cdot kg^{-1}$ DM)	415.10	19.06	205.22	941.46
Concentrate ($g \cdot kg^{-1}$ DM)	584.90	19.06	58.54	794.78
Ash ($g \cdot kg^{-1}$ DM)	63.83	1.52	38.37	104.76
CP ($g \cdot kg^{-1}$ DM)	172.78	2.01	116.92	225.19
Fat ($g \cdot kg^{-1}$ DM)	26.50	1.16	14.20	66.11
CHT ($g \cdot kg^{-1}$ DM)	741.82	2.39	690.52	814.44
NFC ($g \cdot kg^{-1}$ DM)	382.19	11.20	77.51	654.71
FC ($g \cdot kg^{-1}$ DM)	359.63	10.37	235.43	654.71

Table 2 – Parameters for evaluating the metabolizable energy intake of different scenarios

Parameters	MEI ($Mcal \cdot day^{-1}$)		
	G1-C (Seo et al., 2006)	G1-G (Tedeschi et al., 2011)	GnG1 (Regadas Filho, 2013)
r^2	0.83	0.85	0.82
P-Value $\alpha = 0$ & $\beta = 1$	<0.0001	<0.0001	<0.0001
Mean bias	0.1144	0.1744	0.2088
(P-Value)	(P = 0.2995)	(P = 0.1244)	(P = 0.0611)
Cb	0.99	0.99	0.98
CCC	0.90	0.91	0.89
RMSEP	0.6174	0.6172	0.6734
MB (%)†	3.43	7.98	9.62
SB (%)†	20.3	20.5	21.76
RE (%)†	76.27	71.52	68.62

†Decomposition of MSEP; MB = mean bias, SB = systematic bias, RE = random error.

Table 3 - Parameters for evaluating the milk yield from different scenarios

Parameters	Milk yield ($kg \cdot day^{-1}$)		
	G1-C (Seo et al., 2006)	G1-G (Tedeschi et al., 2011)	GnG1 (Regadas Filho, 2013)
r^2	0.72	0.71	0.74
P-Value $\alpha = 0$ & $\beta = 1$	<0.0001	<0.0001	<0.0001
Mean bias	0.63	0.65	0.63
(P-Value)	(P< 0.0001)	(P< 0.0001)	(P< 0.0001)
Cb	0.82	0.82	0.79
CCC	0.70	0.69	0.68
RMSEP	0.8885	0.9112	0.9278
MB (%)†	49.87	50.51	56.56
SB (%)†	1.13	0.37	1.15
RE (%)†	49.00	49.12	49.00

†Decomposition of MSEP; MB = mean bias, SB = systematic bias, RE = random error.

Table 4 – Parameters for evaluating the DMI of different scenarios

Parameters	DMI ($g \cdot day^{-1}$)			
	G1-C (Seo et al., 2006)	G1-G (Tedeschi et al., 2011)	GnG1 (Regadas Filho, 2013)	AFRC (AFRC, 1993)
r^2	0.84	0.89	0.84	0.78
P-value $\alpha = 0$ & $\beta = 1$	<0.0001	<0.0001	<0.0001	<0.0001
Mean bias	-68.39	-74.87	-87.36	-46.19
(P-value)	(P = 0.1542)	(P = 0.0923)	(P = 0.0668)	(P = 0.2013)
Cb	0.96	0.98	0.96	0.95
CCC	0.88	0.92	0.87	0.84
RMSEP	301.97	224.22	306.5	258.29
MB (%)†	5.13	11.15	8.12	3.2
SB (%)†	48.29	26.84	44.2	7.96
RE (%)†	46.58	62.01	47.68	88.84

†Decomposition of MSEP; MB = mean bias, SB = systematic bias, RE = random error.

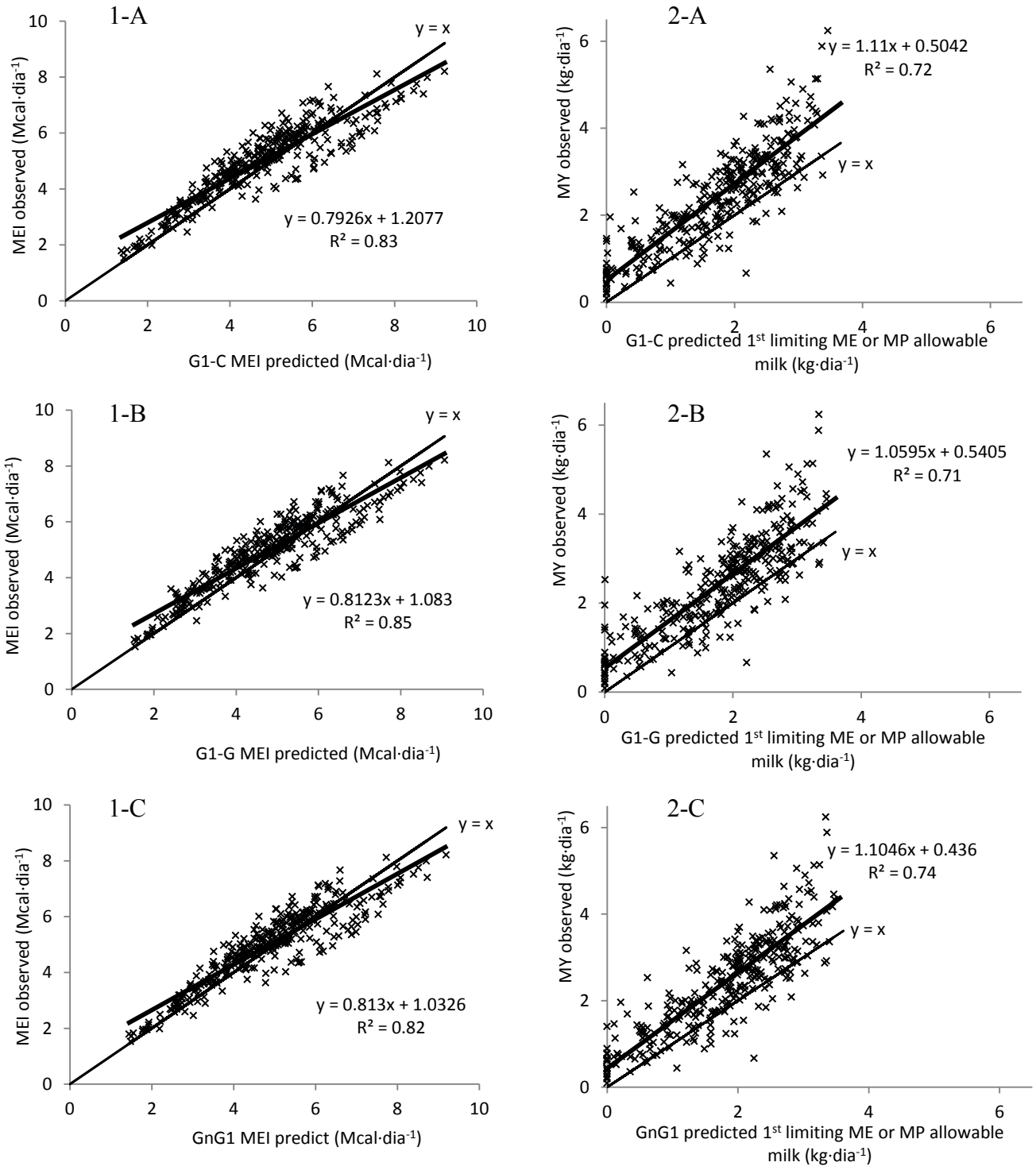

Figure 1 – Linear regression between the metabolizable energy intake (MEI, Mcal·day^{-1}) (Panels 1) observed and predicted, and between the milk yields (MY, kg·day^{-1}) (Panels 2) observed and predicted by the G1-C (Panels A), G1-G (Panels B) and GnG1 (Panels C) scenarios.

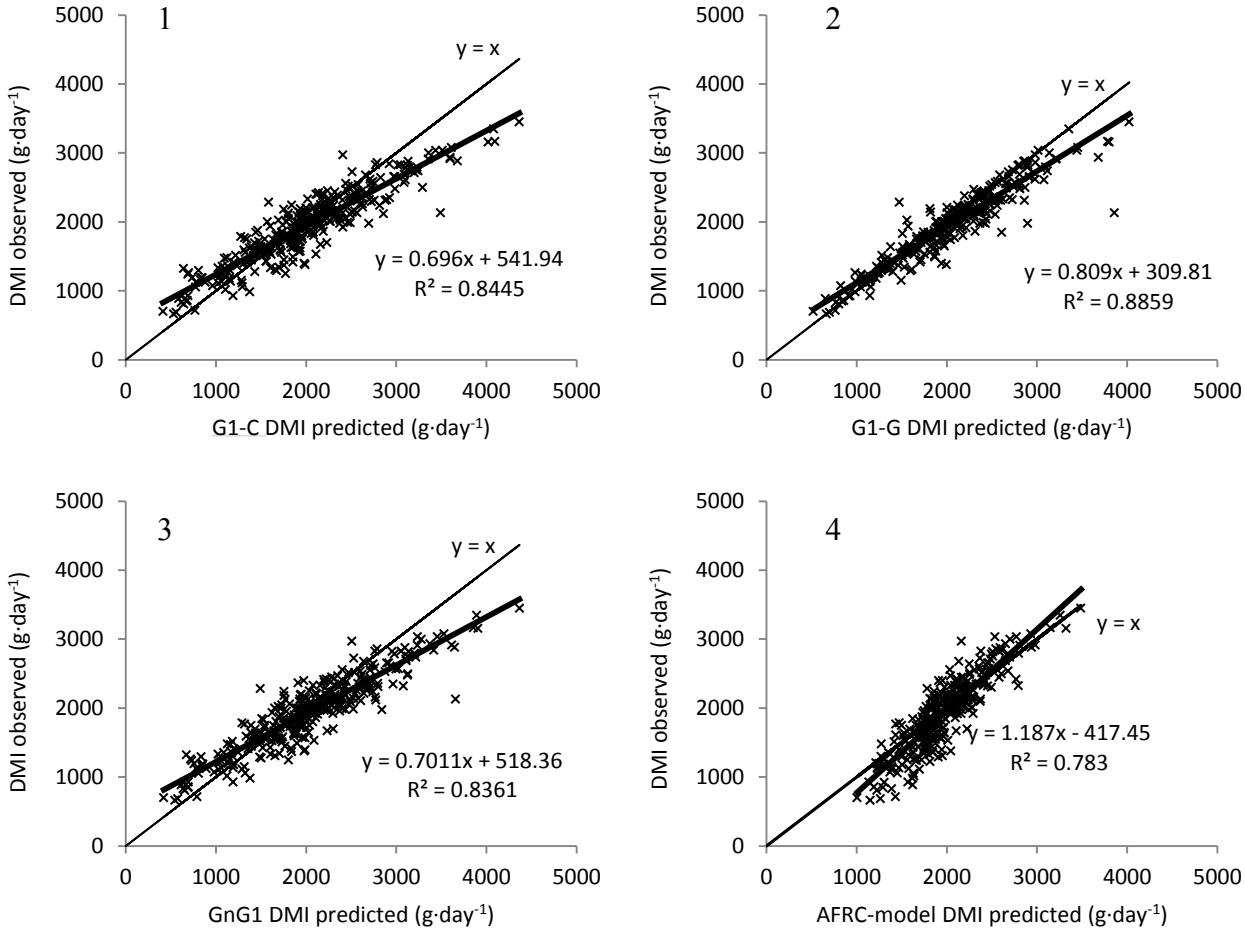

Figure 2 – Linear regression between the dry matter intake (DMI, g·day^{-1}) observed and predicted by the G1-C (Panel 1), G1-G (Panel 2), GnG1 (Panel 3) and AFRC (Panel 4) scenarios.

Technical Note: Comparison of fermentation kinetics of four feedstuffs using two in vitro gas production systems

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ABSTRACT

The objective of this study was to compare two computerized systems that are used to determine the fermentation kinetics of feedstuffs fed to ruminant animals. These systems use pressure sensors to measure the pressure generated by gas released during the in vitro anaerobic incubation. Both systems are based on the in vitro gas production technique (**IVGP**). The evaluated systems were the wireless ANKOM^{RF} Gas Production Systems® (**aIVGP**) and a wired generic in vitro anaerobic fermentation system (**tIVGP**). Four different samples of ground corn, alfalfa hay, dried distillers grain, and dried grass forage were used with four replicates (n = 16). In order to preserve the same fermentation conditions, all components (sample, rumen fluid, and incubation media) were maintained constant between the IVGP systems. After 48-h, the concentrations of hydrogen and methane in the bottle's headspace were collected with a syringe and analyzed using a gas chromatographer. The solution pH was measured after the gas sample was collected and bottles were unsealed and opened. The profiles of the alfalfa, DDG, and grass were kinetically interpreted using an exponential nonlinear model while the profiles of the ground corn were interpreted using the Gompertz nonlinear model. The total gas production (ml·100 mg^{-1} of DM), fractional production rate of gas (h^{-1}), pH of the solution, and hydrogen and methane concentrations in the bottle's headspace were used to compare the systems. The agreement was determined using the coefficient of correlation (**r**), simultaneous *F*-test of the intercept and slope (H_0: intercept = 0 and slope = 1; $\alpha = 0.01$), bias correction factor ($\mathbf{C_b}$), concordance correlation coefficient (**CCC**), mean bias (**MB**), and mean square error prediction (**MSEP**) and its decomposition. No significant concentration of hydrogen in the headspace of the tIVGP was found. The IVGP systems had similar values for total gas production (r = 0.90; C_b = 0.85; CCC = 0.77; MB = 3.58; *P* = 0.2165), methane concentration (r = 0.94; C_b = 0.83; CCC = 0.79; MB = 0.51; *P* =

0.0787), and solution pH (r = 0.95; C_b = 0.98; CCC = 0.93; MB = -0.04; *P* = 0.6480), but the estimated values of fractional production rate of gas differed (r = 0.66; C_b = 0.79; CCC = 0.52; MB = -0.022; *P* = 0.0032). The results suggested that both IVGP systems had similar fermentations patterns, but the difference in the fractional production rate of gas between these IVGP systems may be due to difference in the headspace gas composition.

Key words: gas production, headspace, *in vitro* systems, ruminants.

INTRODUCTION

The use of in vitro gas production systems (**IVGP**) has rapidly spreading during the last decades because of its ease of use, affordable cost, good accuracy and precision, and reliability when compared with in situ techniques (Williams, 2000). The gas production measures can be used in ruminant nutrition to describe the kinetic of degradation of feedstuffs, and from the kinetic degradation information, one can obtain parameters (degradation rate and total gas production) that may be related with nutritional value of the feed (Tedeschi et al., 2009).

There is a range of possible approaches described in the literature that are used for measuring gas production. Including systems based on manual or automatic recordings in which the measures are made using mobile or fixed sensors (McBee, 1953, Pell and Schofield, 1993); vented or closed systems in which gas may or may not be released into pre-determined time/pressure or at each measure (Pell and Schofield, 1993, Theodorou et al., 1994); and wired or wireless systems in which sensors are or are not connected to computers via cables (Tagliapietra et al., 2010, Tedeschi et al., 2008a). However, to be reliable, the parameters of interest (total gas pressure and fractional pressure rate, which is used as a proxy for fractional degradation rates) need to be obtained from consistent systems and universally accepted apparatus and methods. In addition, IVGP systems

cannot influence any characteristic of the fermentation process; that means that variables such as pH, H_2, volatile fatty acid (**VFA**) and methane (CH_4) concentration, for example, cannot vary among IVGP systems if the same proportion of headspace and solution are used.

The ANKOM Technology (2052 O'Neil Road, Macedon, NY, 14502[2]) has developed a commercial apparatus, automatic optional vented or closed wireless system called ANKOM^{RF} Gas Production System, to measure gas production. Because this system is still being developed, it needs to be thoroughly evaluated with different types of feeds and a comparison with a reliable method is required.

Therefore, the objective of the present study was to perform a comparison between the latest ANKOM^{RF} Gas Production Systems® (**aIVGP**) and a generic in vitro anaerobic fermentation system (**tIVGP**) as described by Tedeschi et al. (2008a).

MATERIAL AND METHODS

Feeds and Chemical Analysis

Four different feed samples of coarsely ground corn, alfalfa hay, dried distillers' grain (**DDG**), and dried mixed grass forage hay were used, totalizing 16 feed samples, to perform the proposed comparison. The ground samples of corn and alfalfa hay were collected from different facilities surrounding College Station, TX. The DDG samples (Dakota Gold BPX DDGS) were collected from different batches from Poet Bio-refinery of Dakota Gold Manufacturing, Sioux Falls, SD. The BPX DDG samples are a DDGS resulting from a low-heat processing method before fermentation, which is assumed to have less heat-damaged protein. The forage hay samples were collected from June, July, August, and September of 2012 at the King Ranch, TX (lat 27.52°; long -97.89°). All feed

[2] http://www.ankom.com/

samples were stored in a freezer at -10 ^{o}C until processing. The pasture was mainly composed of a mix of different range plants such as Kleberg Bluestem and Coastal Bermuda grass as the most dominant species. Before grinding, the forage hay samples were dried at 65°C in a forced-air oven.

The feed samples were ground to pass a 2-mm screen using a Model 4 Wiley® Mill (n^{o} 3375-E25, Thomas Scientific, Swedesboro, NJ 08085) and analyzed following the AOAC (2000) methods for DM (method 930.15), ash (method 942.05), fat (method 2003.05), CP (method 990.09), neutral detergent fiber (NDF) (1-mm) with thermostable α-amylase and without sodium sulfite (Van Soest et al., 1991), and lignin (method 973.18). The summary of the chemical analysis is described in Table 1.

In Vitro Fermentation Procedures

In order to preserve the same fermentation conditions, the same proportion among all components (sample, rumen fluid, medium and H_2O) was maintained constant between the IVGP systems. In order to accomplish this similarity, an initial evaluation of the bottle volumes for both systems were performed to maintain the same ratios (headspace to liquid phase ratio). Table 2 has the summary of the characteristics of the bottles used in the fermentations.

The tIVGP, as described by Tedeschi et al. (2008b), was used to develop the protocol used in the aIVGP. Briefly, the ruminal fluid inoculum was a mix of inocula obtained from two Angus steers that had access to total mixed rations (DM = 945.11 g·kg^{-1}; CP = 164.44 g·kg^{-1}; NDF = 268.33 g·kg^{-1}; ash = 56.33 g·kg^{-1}; fat = 40.11 g·kg^{-1}) constituted of forage grass, alfalfa hay, and concentrate (corn, molasses, DDG, and macro and micro minerals). The collected ruminal contents were put into in a sealed plastic container (Thermos) that was previously filled with warm water to maintain the ideal temperature about 39 ^{o}C. Then, the Thermos was transported to the Ruminant Nutrition

Laboratory at Texas A&M University. Immediately upon arrival, the Thermos content was filtered through four layers of cheesecloth and then through glass wool into a side-arm Erlenmeyer flask with an O_2-free headspace. The system was saturated with CO_2 whilst filtrating to minimize changes in microbial populations and to avoid O_2 contamination. The rumen fluid was maintained as close as possible to 39°C during the whole procedure.

The in vitro medium used was the phosphate-bicarbonate medium and reducing solution of Goering and Van Soest (1970), except for the addition of trypticase. The medium flask was ventilated with CO_2 at all times while it was heated to just below boiling temperature. Then, the medium was cooled to room temperature and cysteine hydrochloride was added. The medium pH and CO_2 saturation were controlled by color change of resazurin indicator from purple to pink/colorless; the optimum pH reference utilized was between 6.8 and 6.9. Feed samples were previously weighed and transferred to the bottles, containing a 5 cm magnetic stirring Teflon-covered bar. The feed samples were hydrated with boiled, double-distilled water that had been previously cooled to room temperature; the water was used to avoid particle dispersion, and discounted by the media. Each bottle was filled with media as described in Table 2.

For the tIVGP system, the 125-ml bottles were closed with previously unused, lightly greased with petroleum grease base (Lubriseal; stopcock grease, Thomas Scientific, Swedesboro, NJ 08085) butyl rubber stoppers under CO_2 ventilation, and crimp sealed with Aluminum caps. The filtered ruminal fluid, containing the rumen inoculum, was injected in the tIVGP systems directly through the rubber stoppers using a syringe with a 20G needle. For the aIVGP system, the filtered rumen fluid was injected while the bottles were opened and subsequently sealed with its respectively cover under CO_2 ventilation. The purging (elimination of oxygen) was performed through the built-in electromechanical valve by injecting CO_2. Blank bottles (bottles with rumen fluid and

media, but without feed samples) were used to correct for fermentation of remaining substrate contents in the filtered rumen inoculum.

Both IVGP systems were placed in sealed, temperature-controlled fermentation chambers. When fermentation chambers reached 39°C, the pressure inside the bottles was released (i.e., vented) using a syringe for the tIVGP system and using the electromechanical valve for the aIVGP. The respective data acquisition software (tIVGP: Pico Technology, Eaton Socon, Cambridgeshire, UK; and aIVGP: Gas Pressure Monitor, 2052 O'Neil Road, Macedon, NY, 14502) were set to collect pressure signals every 5 minutes for 48 h. After 48 h of fermentation, the final concentration of H_2 and CH_4 gases in the bottle's headspace was determined using a gas chromatographer (Allison et al., 1992). Then, the bottles were opened and the pH solution was measured.

Calculations, Model Fitting, and Statistical Analysis

The experiment was planned as a randomized complete block design with four blocks (samples from different facilities (corn ground and alfalfa), months (hay grass) or batches (DDGs)) with at least two laboratory replicates (fermentation bottle). The mean value of the laboratory replicates were used as the experimental unit, thus, 16 experimental units (n = 16) were analyzed.

Neither software measures the gas production volume directly: the sensors in the tIVGP measure volts whereas the sensors in the aIVGP measures psi or mbar. Therefore, it was necessary to develop standardized relationships between pressure and volume for these systems under the conditions they will be operated. For the tIVGP, we used the approach discussed by Tedeschi et al. (2008a) in which voltage readings (**V**) were converted to gas volume (ml) using individual conversion ratios between voltage and volume obtained during the calibration process of the pressure sensors. For the aIVGP, we

used the approach indicated by the manufacturer in the Operator's Manual[3] (page 44) (ANKOM-Technology, 2012) in which the gas pressure can be converted to moles of gas produced using the ideal gas law (Eq. [1]) and then converted to mL of gas produced using Avogadro's law (Eq. [2]).

$$n = (P \times V)/(R \times T) \quad [1]$$

$$mL\ of\ gas\ produced = n \times 22.4 \times 1000 \quad [2]$$

where n is gas produced in moles (mol), P is the pressure in kilopascal (kPa), V is the headspace volume in the bottles (L), R is the gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$) and T symbolizes the temperature in Kelvin (K). In the Equation 2, when atmospheric pressure is measured as psi (1 psi = 6.89478 kilopascal), 1 mole will occupy 22.4 L at 0°C and 101.323 kPa (standard conditions). Therefore, gas measured as moles can be converted to gas measured as ml.

The gas profiles were adjusted for the blank flasks to correct for the impact of residual substrate in the rumen fluid as discussed above. Afterwards, in order to have comparable parameters between the systems, a common nonlinear function that was able to fit both IVGP systems and had the same parameters' biological interpretation has to be chosen. Hence, unicompartimental models with fixed degradation rates (k_d not variable) were utilized. For that, an exploratory analysis using the Gas Production Fitting System (Gasfit) software (available at http://nutritionmodels.tamu.edu/gasfit.html, verified 04 April 2013) was performed to fit all unicompartimental models present in the software and discussed by Tedeschi et al. (2008b). The most frequent function that achieved the convergence for each feed was chosen. Some biological interpretation was lost with the unicompartimental models for the sake of simplicity when compared to the interpretation of parameters of bicompartimental models (i.e. two fractional degradation rates) that was

[3] Available at http://www.ankom.com/media/documents/RF_Manual_RevF_112912.pdf, verified 09 July 2013.

found for the DDG. Nonetheless, it was possible to compare the fractional degradation rates and total gas production between the systems for each feed using unicompartimental models. The profiles of the alfalfa, DDG, and forage hay samples were kinetically interpreted using an exponential model (shown in Eq. [3]) while the profiles of the ground corn were interpreted using the Gompertz model (shown in Eq. [4]).

$$y = \begin{cases} a \times \left(1 - \exp\left(-k_d \times (t - L)\right)\right);\ \forall\ t \geq L \\ 0;\ \forall\ t < L \end{cases} \quad [3]$$

$$y = a \times \exp\left(-\exp\left(1 + k_d \times (L - t)\right)\right) \quad [4]$$

where a represent the asymptotic gas production (mL); k_d represent the fractional degradation rate (h^{-1}); and L represent the lag time (h).

The parameters of the curves (a and k_d), pH of the solution, and H_2 and CH_4 concentrations in the headspace of the bottles were the variables of interest used to compare the IVGP systems. The level of agreement between the aIVGP and tIVGP systems was executed based on Tedeschi (2006). The precision was assessed by the coefficient of correlation (**r**) between both predictions and the simultaneous *F*-test of the intercept and slope (H_0: intercept = 0 and slope = 1; $\alpha = 0.01$). The accuracy was assessed based on the bias correction factor ($\mathbf{C_b}$; range from 0 to 1) that indicates how far the regression line deviates from slope of unity. Additionally, the concordance correlation coefficient (**CCC**; range from 0 to 1), mean bias (**MB**) and the mean square error prediction (**MSEP**) and its decomposition into mean bias, systematic bias and random errors were also calculated. The Model Evaluation System software was used to perform the evaluation (available at http://nutritionmodels.tamu.edu/mes.html, verified 04 April 2013).

RESULTS AND DISCUSSION

The profiles from the first block of the aIVGP and tIVGP are shown in Figures 1. Both systems were able to distinguish profiles with two pools such as that observed for the DDG samples (Panel 2). Additionally, for grass forage samples (Panel 4), the systems kept the lag time values constant indicating small variation among the fermentations.

The H_2 gas was not detected in the bottles' headspace in the tIVGP system; consequently, was not compared among them. Possible, the slight higher values of CH_4 concentration in the tIVGP system (MB = 0.51 μmole·ml^{-1} of gas; P = 0.0787) (Figure 2, Panel 2) may explain this difference. An increased use of H_2 for methanogenesis would not have allowed its release to the headspace, consequently, resulting in the absence of detectable H_2 peaks in the gas chromatography analysis in the tIVGP system. However, the CH_4 concentrations in the headspace of the systems had acceptable level of agreement. The level of precision between them (r = 0.94) (Table 3) was high, but the test for the intercept and slope rejected the hypothesis H_0 (P< 0.001). Still the accuracy had satisfactory values (C_b = 0.83); the CCC = 0.79 was also reasonable. The MSEP decomposition presented high effect of the mean bias (67.54%).

On the other hand, the assertion that the tIVGP presented higher methanogenesis is also supported by the slight advantage in the total gas production. However, there was no significant mean bias between the systems (MB = 3.53 ml·100 mg^{-1} of DM; *P* = 0.2165) (Table 3), but a strong tendency to reject the null hypothesis for the simultaneous test of intercept and slope was found (*P* = 0.0109) (Figure 2, Panel 3). When tested individually, neither the intercept (*P* = 0.8661) nor the slope (*P* = 0.2001) differed from zero and one, respectively. The total gas production estimated by aIVGP and tIVGP were highly correlated (r = 0.90) indicating high level of precision between the systems with reasonable accuracy (C_b = 0.85), yielding a moderate concordance correlation of 0.77. The

MSEP decomposition demonstrated similar contribution for mean bias (46.03%) and random errors (47.08%), suggesting that about half of the differences between these IVGP systems is due to uncontrolled errors.

The total gas production is directly related to the extension of degradation and it depends on the concentration of nicotinamide adenine dinucleotide (NAD^+) as an oxidizing agent to convert hexose into acetate. Notwithstanding, when H_2 is not used to form CH_4 throughout the interspecies H transfer phenomenon (Russell, 2002), it will accumulate and consequently the fermentation process would be reduced due to a high partial concentration of H_2. Therefore, an inhibition of NADH-linked hydrogenases would eventually reduce the total gas production. We initially hypothesized that changes in the fermentation conditions could be influencing the results, once it has been demonstrated the effect of pH on CH_4 production (Lana et al., 1998) and then on hydrogen concentration (Ellis et al., 2008). However, the simultaneous test for the pH of the solution between the systems did not reject the null hypothesis (P = 0.1913) and had practically the same values (MB = -0.04; P = 0.6480), suggesting the pH concentration tended to be similar, i.e., parallel (Y = X). Furthermore, the correlation between the IVGP systems was 0.95 (Figure 2, Panel 1), the accuracy (Cb) was 0.98, and the CCC was 0.93. The MSEP decomposition indicated that most of the errors associated with the IVGP systems were random (77.54%) (Table 3). These results suggested the pH remained constant between the IVGP systems. An alternative hypothesis is that a greater acetogenesis in the tIVGP system could have removed H_2 to produce acetate, however, we lack the information about the VFA profile (mainly ratio acetate/propionate) and H_2 concentration in the solution that would be necessary to confirm this hypothesis.

Another factor that may have affected the H_2 concentration is that in the tIVGP there is the requirement to penetrate the rubber stoppers to connect the pressure sensors.

The rubber stoppers may suffer a deformation due to the requirement of needle penetrations in the beginning, during the fermentation, and at the end of the process, which could possibly lead to a gas escape. However, this hypothesis only would be confirmed if this rubber stoppers had allowed only hydrogen escaping, once just this specific gas presented different result between the evaluated systems.

The Ankom^{RF} Gas Production Systems® uses the assumption of the ideal gas law as mean to convert psi or mbar to moles of gas produced, but this assumption does not account for the diffusion of gases from the headspace (mainly CO_2) into the liquid phase (Mauricio et al., 1999), and this could lead to overprediction of the gas being produced. It is encouraged to users perform individual calibrations for the aIVGP bottles in order to have specific linear regressions (pressure x gas volume) for each bottle, thus, correcting possible variations in the sensor of the bottles and in the bottle volumes after all.

The fractional degradation rate (k_d) had the most conflicting results. The simultaneous test of the intercept and slope rejected the null hypothesis (P = 0.0005) and the coefficient of correlation between aIVGP and tIVGP was 0.66. Despite the intermediate accuracy value (C_b = 0.79), the CCC was low (0.53) and a significant (i.e., different from zero) mean bias (MB = -0.022 h^{-1}; P = 0.0032) was observed. The MSEP decomposition had higher contribution for the systematic bias component (49.85%). Although the fractional degradation rate is an important parameter to be estimated, it is very sensible to a range of possible influential factors that were not accounted in our study. Additionally, the fractional degradation rate presents great natural variability; Tedeschi et al. (2008b) found average values of coefficient of variation (CV) for this parameter of 36% while Palmer et al. (2005) found CV of 93.5%.

Furthermore, it was recently demonstrated by Patra and Yu (2013) that the headspace gas composition, especially CO_2 concentration, may affect the pattern of

fermentation in IVGP systems. They indicated that the CH_4 concentration in headspace gas after fermentations was greater for bottles with higher initial CO_2 concentration, probably, due to the more availability of CO_2 in the culture media as the electron acceptor for the primary hydrogenotrophic methanogenesis pathway. The IVGP systems we evaluated had different ways to seal and purge (eliminate oxygen) the bottles, which may have resulted in different initial CO_2 concentration in the bottle headspace and considerably affected the degradation rates.

IMPLICATIONS

Both IVGP systems investigated in this study were able to replicate the same fermentations conditions. Both IVGP systems satisfactorily estimated the total gas production and can be used for in vitro gas production analysis interchangeably. Despite of divergences in the fractional degradation rate between the IVGP systems, this parameter is usually surrounded by great variability and sensitive to many factors including the initial CO_2 concentration in the bottle headspace. Although some limitations in the protocol are intrinsic to the IVGP systems, they are beyond the control of the user and these limitations have to be taken into account and acknowledged when comparing results between different IVGP systems.

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Table 1. Chemical composition (mean ± SE) of the analyzed feed samples.

Items	Corn	Alfalfa	DDG	Grass hay
DM, $g \cdot kg^{-1}$ as fed	897.3 ± 18.3	930.3 ± 2.40	893.7 ± 8.60	959.3 ± 0.20
Ash, $g \cdot kg^{-1}$ of DM	19.0 ± 1.20	96.0 ± 1.80	13.0 ± 1.30	86.5 ± 3.30
Fat, $g \cdot kg^{-1}$ of DM	35.5 ± 2.30	23.0 ± 2.10	40.6 ± 1.30	5.00 ± 0.40
CP, $g \cdot kg^{-1}$ of DM	94.0 ± 2.50	177.0 ± 11.5	468.5 ± 1.70	44.8 ± 5.50
NDF, $g \cdot kg^{-1}$ of DM	97.0 ± 2.40	412.5 ± 20.2	356.0 ± 47.0	758.3 ± 4.90
Lignin, $g \cdot kg^{-1}$ of DM	12.8 ± 1.90	77.9 ± 3.80	54.3 ± 7.80	87.1 ± 3.90

Table 2. Summary of the physical characteristics of the bottles and the fermentation protocols for two in vitro gas production (IVGP) systems

Parameters	tIVGP[1]	aIVGP[1]
Bottle volume, ml	158 (S.E ±0.12)	307 (S.E ±0.40)
Sample, g	0.20	0.39
Rumen fluid, ml	4.0	7.8
Medium, ml	14.0	27.3
Double-distiller water, ml	2.0	3.9
Total solution, ml	20	39
Headspace, ml	138	268
Ratio of bottle volume:total solution	7.9	7.9

[1] aIVGP, ANKOM^{RF} Gas Production Systems; tIVGP, generic in vitro anaerobic fermentation system.

Table 3. Evaluation of the parameters between aIVGP and tIVGP systems[1]

Parameters	Total gas production (ml·100 mg^{-1} of DM)	k_d (h^{-1})	pH	CH_4 (μmole·ml^{-1} of gas)
r	0.90	0.66	0.95	0.94
P-Value $\alpha = 0$ & $\beta = 1$	0.0109	<0.001	0.1914	<0.001
C_b	0.85	0.79	0.98	0.83
CCC	0.766	0.526	0.931	0.788
MB	3.53	-0.0225	-0.04	0.51
(P-Value)	(P = 0.2165)	(P = 0.0032)	(P = 0.6480)	(P = 0.0787)
MSEP	27.08	0.00226	0.00725	0.38
Mean bias	12.47 (46.03%)	0.00050 (22.33%)	0.0015 (21.35%)	0.26 (67.54%)
Systematic bias	1.86 (6.88%)	0.00113 (49.85%)	0.00008 (1.11%)	0.024 (6.15%)
Random errors	12.75 (47.08%)	0.00063 (27.82%)	0.0056 (77.54%)	0.101 (26.31%)

[1] aIVGP, ANKOM^{RF} Gas Production Systems; tIVGP, generic in vitro anaerobic fermentation system; k_d, fractional degradation rate; r, coefficient of correlation; C_b, bias correction factor; CCC, concordance correlation coefficient; MB, mean bias; MSEP, mean square error prediction.

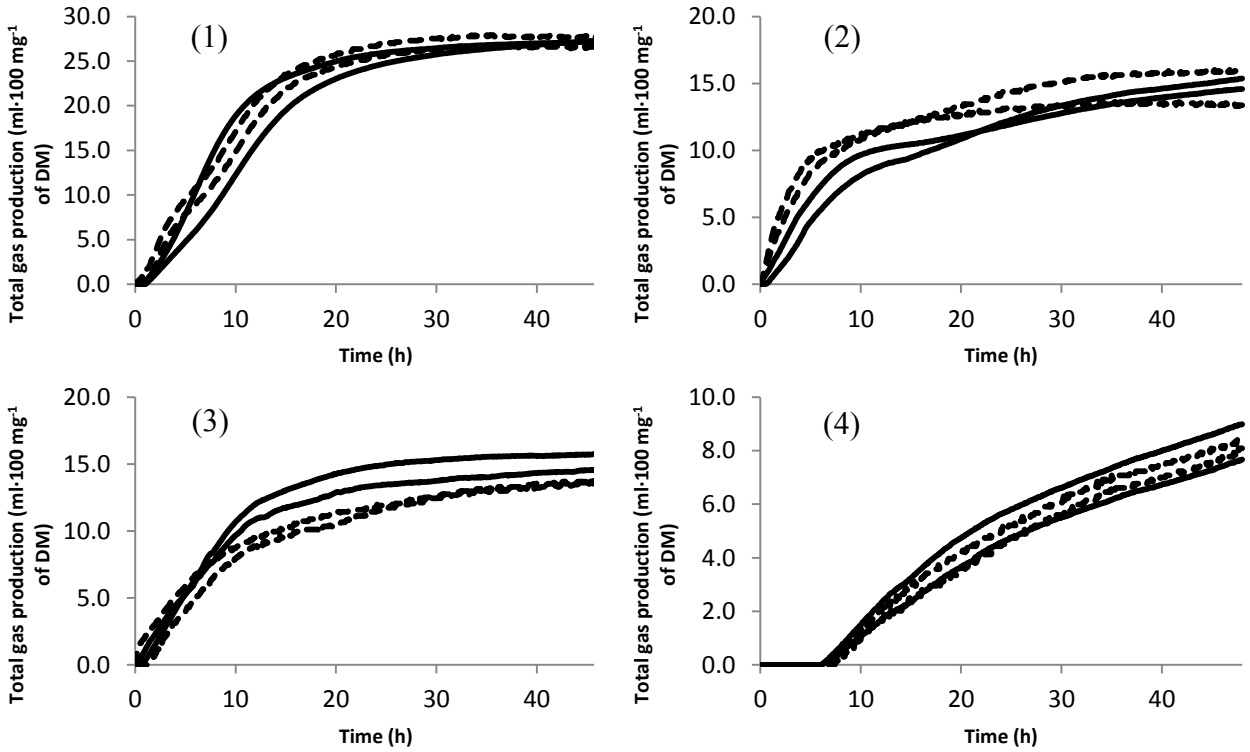

Figure 1 – Profiles of the aIVGP (dotted lines) and tIVGP (continuous line) corrected for blank for corn ground (Panel 1), DDG (Panel 2), alfalfa (Panel 3) and hay grass (Panel 4) obtained from the first fermentations block of the evaluated feedstuffs.

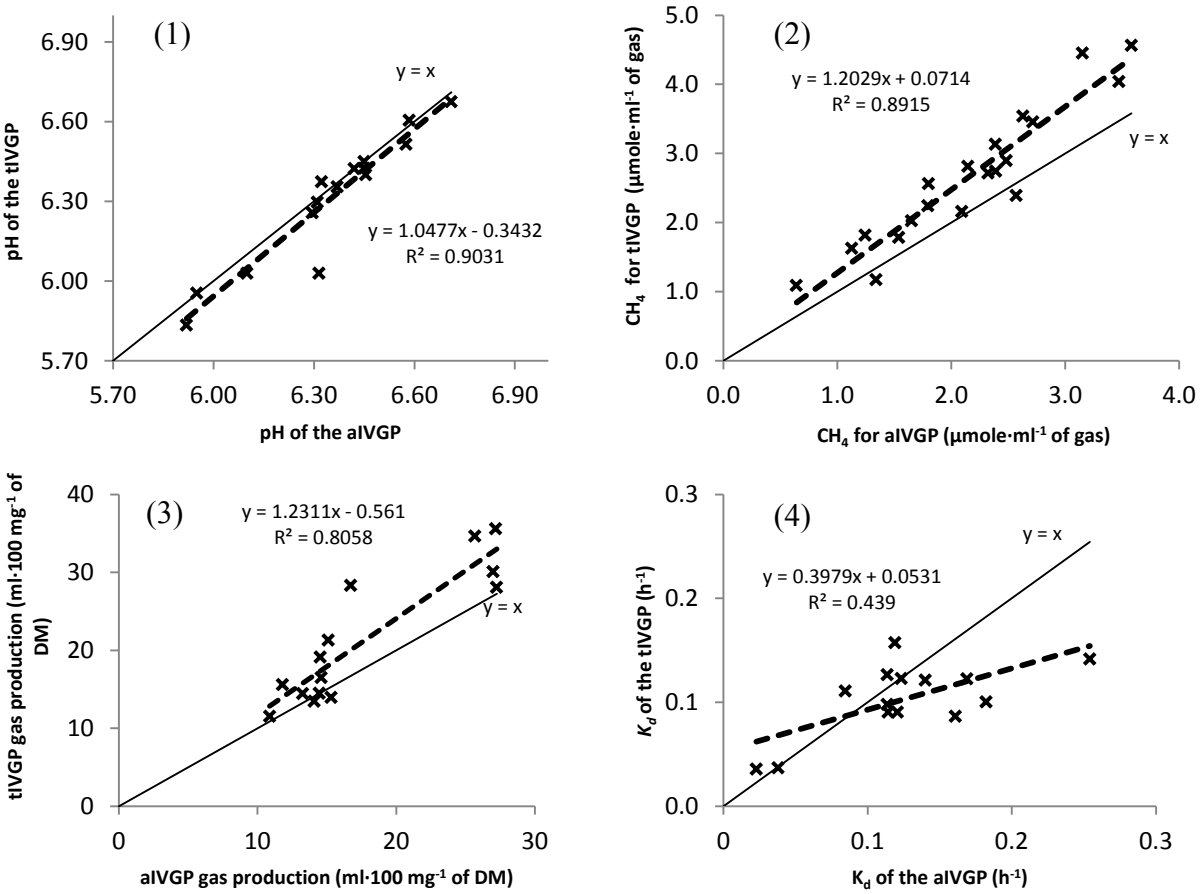

Figure 2 – Linear regression of the pH (Panel 1), methane concentration (Panel 2), total gas production (Panel 3) and asymptotically gas production rate (Panel 4) between aIVGP and tIVGP systems.

APPENDIX 1

Chapter 1 – SAS inputs

Fixed model:

```
PROC NLMIXED; /*Gaussian quadrature algorithm*/
/* Initial parameter estimates from PROC NLIN to start iterations;*/
parms b01=56 b11=59
      b02=.9 b12=.97
      b03=1.2 b13=1.5
      b04=.7 b14=.8
      s2e=20;
/*coding dummy variables for the goat genotypes*/
b1=b01*z1+b11*z2;
b2=b02*z1+b12*z2;
b3=b03*z1+b13*z2;
b4=b04*z1+b14*z2;
/*nonlinear Richards model*/
pred= b1*(1-b2*exp((-b3/1000)*x))**b4;
model y ~ normal (pred,s2e);
run;
```

Random model:

```
PROC NLMIXED method=firo; /*FIRO algorithm*/
parms b01=52 b11=53
      b02=.9 b12=.97
      b03=1.9 b13=2
      b04=.7 b14=.8
      s2u1=30 c13=.99 s2u3=1.5 s2e=10;
b1=b01*z1+b11*z2+u1; /*variance components u1 and u3 added*/
b2=b02*z1+b12*z2;
b3=b03*z1+b13*z2+u3;
b4=b04*z1+b14*z2;
pred= b1*(1-b2*exp((-b3/1000)*x))**b4;
model y ~ normal (pred,s2e) ;
/*Specifying random parameters with normal distribution */
random u1 u3 ~ normal ([0,0],[s2u1,c13,s2u3]) subject=animal ;
run;
```

Random model + Variance Power + Error Structure model:

```
%nlinmix(data=cresc,          /*Specifies the SAS data set*/
/*Coding dummy variables for the goat genotypes and request the
nonlinear Richards model*/
model=%str(
        b1=b01*z1+b11*z2+u1;
        b2=b02*z1+b12*z2;
        b3=b03*z1+b13*z2+u3;
        predv= b1*(1-b2*exp((-b3/1000)*x))**b4;
),
/*Initial parameters estimated*/
parms=%str(b01=53 b11=57 b02=.9 b12=.97 b03=2.7 b13=2.2 b4=.9),
/*Modeling the variance power statement*/
derivs=%str(
wt = 1/predv**(2.91*0.5);
),
/*Specifies the MIXED procedure to be executed for each iteration*/
stmts=%str(
class animal;
```

```
model pseudo_y = d_b01 d_b11 d_b02 d_b12 d_b03 d_b13 d_b4 / noint notest
solution cl residual outp=pred_matern;
/*Specifies the random parameters and matrix G*/
random d_u1 d_u3 / subject = animal type = un solution;
/*Specifies the matrix R*/
repeated / subject=animal type=sp(matern)(x);
weight wt;
),
/*Request the expansion method*/
expand = zero,
/*Request the REML method and test the variance components parameters*/
procopt = %str(method = reml) covtest
)
run;
```

APPENDIX 2

Chapter 2 – Original dataset

Table 1 – Literature dataset used in the Chapter 2 to assess the homogeneous fiber pool compartment in the rumen.

Experiment	Experimental unit	diet	BM kg	DMI $g \cdot day^{-1}$	NDF intake $g \cdot day^{-1}$	Lig intake $g \cdot day^{-1}$	Q_{DM} g	Q_{NDF} g	Q_{LIG} g
(Lopes, 2009)	183	B	26.93	647.08	346.17	19.32	970.00	532.10	66.26
	205	C	25.26	278.09	152.61	8.14	338.70	176.10	22.30
	206	B	21.06	349.46	195.13	10.19	485.20	250.60	31.59
	207	A	20.96	499.20	294.23	16.97	512.90	268.20	27.30
	209	C	29.25	835.44	448.15	24.98	761.90	386.00	35.15
	210	A	31.26	952.57	514.36	28.95	638.80	346.30	35.50
	211	A	28.00	515.81	281.22	16.14	788.20	416.90	44.36
	212	C	19.76	460.46	238.48	12.32	495.40	259.90	26.42
	213	C	26.94	707.73	380.27	20.89	764.20	359.70	40.22
	214	B	21.55	432.40	233.30	12.69	478.00	256.80	31.57
	217	B	28.94	694.75	358.83	17.64	843.00	431.10	38.32
	218	B	27.36	758.97	400.89	21.81	732.80	376.50	44.46
	219	C	25.63	281.29	155.27	8.73	296.50	134.30	15.53
	220	B	24.78	429.12	231.87	13.02	349.40	173.00	15.64
	221	A	28.74	851.41	464.55	27.61	867.50	471.10	53.69
	223	C	16.16	208.75	114.37	5.60	170.10	73.00	8.04
	224	A	25.44	747.33	410.42	23.71	938.10	488.20	46.94
	225	A	26.28	859.24	458.61	26.00	632.90	299.40	28.15
	227	F	16.93	123.85	59.76	3.17	198.20	103.80	19.16
	228	F	19.03	208.48	114.16	7.61	217.60	122.30	14.70
	229	E	28.49	679.81	363.70	25.65	767.90	397.80	50.31
	231	E	20.90	264.96	136.24	9.90	309.90	144.20	18.63
	232	F	26.80	600.01	325.64	22.58	665.60	319.30	40.78
	233	D	22.23	710.03	377.77	26.22	536.00	278.10	28.85
	234	D	21.93	436.80	230.96	15.55	546.50	279.70	30.96
	235	E	29.06	732.70	379.75	25.30	781.40	376.10	40.91
	236	F	24.23	257.68	140.96	9.62	372.90	187.80	26.39
	243	E	21.48	210.01	113.93	7.86	189.50	93.20	13.30
	244	D	24.58	446.76	246.21	17.40	369.90	149.30	21.56
	246	D	24.28	658.76	352.61	24.64	802.60	440.40	49.27
	248	E	24.03	414.23	217.71	15.37	608.90	325.40	39.70
	250	E	23.30	362.84	196.52	13.90	413.70	220.40	27.36
	251	D	26.75	572.63	313.64	22.28	915.40	458.70	58.31
	252	F	21.88	467.24	254.94	18.05	435.40	228.30	30.68
	253	D	20.85	315.88	174.63	12.33	292.40	150.90	18.94

(continued)

(Lopes, 2009)	254	F	25.88	460.34	249.95	17.37	538.60	282.00	36.17
	256	I	26.08	600.21	329.19	27.49	425.50	205.20	27.14
	257	G	28.73	538.36	298.10	24.17	672.40	364.30	45.89
	258	I	29.25	598.48	328.63	27.66	587.90	319.30	40.22
	259	G	28.88	821.17	446.48	36.93	779.70	418.10	44.78
	260	H	28.25	877.36	476.85	42.29	660.90	316.60	42.64
	261	H	27.70	794.13	431.76	38.39	773.30	389.30	39.68
	262	G	28.10	401.01	221.99	17.57	399.90	220.10	23.56
	263	G	27.15	304.09	168.56	13.60	331.40	148.30	19.31
	264	G	26.65	846.51	457.87	37.87	734.30	380.00	45.46
	265	G	29.25	777.97	426.02	34.65	1158.00	614.30	72.97
	266	H	24.83	729.31	396.22	36.70	489.00	207.40	27.21
	267	I	17.95	374.89	199.30	17.20	520.00	256.10	27.92
	268	H	27.53	729.73	399.45	35.03	557.30	276.40	34.92
	272	H	28.93	709.90	387.77	34.00	894.60	505.50	62.94
	273	I	26.93	669.76	364.09	30.69	594.30	335.90	43.89
	275	I	20.80	542.03	295.67	24.53	546.10	293.00	45.39
	278	I	25.28	527.16	287.39	23.85	684.80	369.70	51.93
(Felisberto, 2011)	3776	5	47.15	1163.38	613.01	39.65	903.90	575.33	59.78
	3286	5	48.00	1751.22	780.17	48.39	1323.88	790.67	86.50
	3530	15	53.10	1247.87	739.38	48.03	1119.45	731.30	88.50
	3016	15	47.20	1768.26	1041.26	66.52	1401.45	926.37	88.07
	3389	2	60.85	1413.80	650.41	41.29	1038.79	658.95	66.84
	3672	15	51.00	1445.50	636.99	38.74	1036.47	641.86	72.09
	3225	2	42.45	1603.67	667.23	43.16	770.99	436.37	38.47
	2710	2	66.50	1054.55	628.16	41.21	1044.17	705.22	91.65
	3788	2	40.40	1187.69	570.34	36.88	701.88	432.49	48.77
	3779	5	46.70	849.05	510.02	33.32	810.33	545.40	59.24
	3532	5	58.25	1124.49	593.86	38.44	1010.55	629.59	77.47
	2858	2	71.05	1322.45	700.39	45.96	567.94	384.46	36.36
	3442	15	52.20	1788.83	680.03	39.84	749.47	407.58	79.52
	3563	5	57.75	2241.68	878.51	54.81	1049.12	563.95	160.19
	3842	2	40.95	860.25	517.66	35.50	1056.85	706.49	136.03
	3478	5	52.85	1748.40	916.49	57.62	1830.04	1128.68	209.45
	3894	2	49.40	1697.22	688.51	43.22	1239.18	704.32	127.45
	3521	5	37.30	702.61	362.89	23.14	485.03	299.03	75.34
	3573	2	46.25	1191.80	657.76	42.41	1132.01	756.51	117.87
	3649	5	37.30	930.07	555.64	36.68	733.09	494.78	80.09
	3178	15	52.90	1362.64	612.97	36.83	808.33	516.63	89.09
	3144	2	58.60	1591.93	972.82	63.39	1717.91	1069.23	266.70
	3846	5	46.55	1638.86	638.78	38.98	1137.39	691.88	116.58
	3347	15	48.35	688.01	398.75	24.62	562.41	368.90	70.53
	3134	5	43.15	1111.97	424.94	26.56	637.08	327.40	61.27
	2837	2	55.10	833.06	446.94	29.53	721.22	472.28	95.22
	3850	2	42.25	806.87	376.25	24.05	706.68	444.87	78.84
	3355	15	50.40	923.16	532.51	34.73	979.55	640.27	125.92

(continued)

(Felisberto, 2011)	3365	2	48.50	1798.90	698.82	42.60	798.12	480.27	103.62
	3349	2	57.15	2184.75	1020.22	62.72	1531.69	971.52	164.45
	3345	15	42.70	666.47	330.39	21.88	278.81	186.08	30.90
	3543	15	49.75	1556.31	675.06	37.44	1285.13	720.30	115.18
	3165	5	54.50	1356.52	513.92	27.41	580.60	307.43	60.49
	3303	2	72.00	886.32	370.29	20.28	587.56	359.84	57.67
	3645	5	51.50	714.14	421.58	27.44	1042.52	697.04	124.34
	3586	15	45.70	1482.92	783.78	48.28	1199.94	783.49	115.72
	3581	2	48.60	1579.53	845.28	53.11	1370.87	879.99	143.32
	3395	15	49.70	1807.66	692.34	40.83	646.23	400.82	69.80
	2992	15	49.05	1514.83	786.17	50.91	1091.18	669.88	133.83
	3391	5	45.50	1189.69	523.52	32.27	857.56	471.03	118.76
	3146	5	66.80	1878.00	824.14	50.34	1379.43	797.09	142.34
	3552	15	44.10	1331.37	620.03	37.35	1218.64	703.58	123.60
	3302	5	40.00	1209.36	529.47	33.09	818.91	471.68	98.00
	3679	2	50.35	1265.20	757.36	48.93	1314.26	841.51	184.72
	3321	15	52.90	1833.67	718.63	42.50	1246.52	634.49	143.11
	3785	15	46.25	1132.64	423.96	26.48	701.52	380.86	79.17
	3374	5	59.00	1295.38	776.91	50.35	1683.86	1093.55	244.84
	3889	15	41.55	1124.63	577.62	36.73	931.78	553.94	116.83
(Matos, 2012)	1	1	58.30	1844.55	642.41	32.59	1479.97	758.58	76.18
	2	1	42.96	1869.79	651.25	32.67	1198.07	694.77	175.00
	3	2	42.50	2188.43	762.92	56.36	1216.86	727.92	137.76
	4	3	41.48	1991.72	660.74	105.47	1782.94	964.22	276.91
	5	2	53.58	2147.87	767.16	56.46	1841.88	1125.67	251.77
	6	3	58.88	2022.61	670.80	104.29	1287.33	742.81	155.22
	7	1	45.62	1646.87	575.96	28.11	1135.02	592.97	89.36
	8	1	60.54	1844.30	648.00	32.73	1060.60	568.28	72.65
	9	3	52.74	2057.57	674.11	106.82	1274.01	700.20	195.73
	10	3	43.00	1907.21	650.54	102.30	1316.83	758.11	189.11
	11	2	49.36	2135.30	754.57	56.17	1512.32	950.34	110.90
	12	2	55.39	1941.61	693.94	51.12	1230.87	824.50	223.01

Table 2 – Parameters obtained from profiles of fecal fiber excretion and interpreted using the G*N*G1 model used in the Chapter 2 to develop the equations to predict the compartmental mean residence time of forage and concentrate.

Experiment	Experimental unit	Forage			Concentrate		
		N	k_e h^{-1}	λ_r h^{-1}	N	k_e h^{-1}	λ_r h^{-1}
(Lopes, 2009)	218	2	0.0385	0.0518	-	-	-
	219	3	0.0160	0.0997	-	-	-
	220	3	0.0431	0.0900	-	-	-
	221	2	0.0304	0.6750	-	-	-
	223	2	0.0189	0.1076	-	-	-
	224	4	0.0484	0.1056	-	-	-
	248	2	0.0315	0.1310	-	-	-
	250	1	0.0251	0.0804	-	-	-
	251	3	0.0426	0.0963	-	-	-
	252	4	0.0526	0.2650	-	-	-
	253	1	0.0550	0.1147	-	-	-
	254	2	0.0406	0.1147	-	-	-
	256	3	0.0542	0.1147	-	-	-
	257	4	0.0352	0.1253	-	-	-
	258	4	0.0276	0.1424	-	-	-
	259	4	0.0311	0.2811	-	-	-
	260	3	0.0529	0.0778	-	-	-
	262	2	0.0431	0.1056	-	-	-
(Felisberto, 2011)	1	1	0.0477	0.2884	3	0.0655	0.2029
	2	1	0.0322	0.8773	-	-	-
	3	1	0.0294	0.2601	1	0.0527	2.1003
	4	1	0.0467	0.4883	-	-	-
	5	1	0.0346	0.5155	1	0.0246	0.5645
	6	1	0.0312	0.4782	-	-	-
	7	5	0.1480	0.2073	-	-	-
	8	4	0.1575	0.2199	-	-	-
	9	1	0.0728	0.1658	-	-	-
	10	3	0.0485	0.4548	-	-	-
	11	2	0.1039	0.1610	-	-	-
	12	7	0.2188	0.4118	-	-	-
	13	2	0.0682	0.1418	-	-	-
	14	1	0.0585	0.5151	-	-	-
	15	1	0.0247	0.1293	-	-	-
	16	1	0.0293	0.3044	2	0.0539	0.1599
	17	2	0.0476	0.2166	4	0.0591	0.2604
	18	1	0.0308	0.1745	2	0.0824	0.1180
	19	2	0.0525	0.2679	2	0.0692	0.2061

(continued)

(Felisberto, 2011)	20	2	0.0302	0.1695	4	0.0641	0.2875
	21	1	0.0391	0.3736	2	0.076	0.1888
	22	1	0.0478	0.1635	3	0.0917	0.1867
	23	1	0.0363	0.1620	2	0.0591	0.1737
	24	1	0.0310	0.4804	3	0.0758	0.1249
	25	1	0.0374	0.3297	3	0.0861	0.1653
	26	1	0.0375	0.0873	3	0.0279	0.2132
	27	2	0.0626	0.1907	3	0.0844	0.1266
	28	1	0.0418	0.1528	2	0.0993	0.1057
	29	2	0.0598	7.0732	1	0.0344	0.2490
	31	1	0.0379	0.1152	4	0.0521	0.1281
	32	2	0.0422	0.4129	1	0.0476	0.1899
	33	2	0.0451	0.1864	3	0.0808	0.1679
	34	1	0.0258	0.3043	3	0.0336	0.1616
	35	1	0.0327	0.1033	2	0.0427	0.1989
	36	2	0.0720	0.4699	3	0.0566	0.4400
	38	1	0.0520	6.7593	2	0.1126	5.8159
	39	4	0.0348	0.5934	3	0.0848	0.2284
	40	1	0.0185	0.3092	2	0.0419	0.0742
	41	1	0.0476	0.1762	5	0.0725	0.2757
	42	2	0.0441	0.7556	6	0.1305	0.3246
	43	1	0.0435	0.2700	-	-	-
	45	2	0.1175	0.1670	-	-	-
	46	2	0.0853	0.1990	10	0.1855	0.3503
	47	2	0.0647	0.2337	4	0.0679	0.2303
	48	1	0.0442	0.4447	1	0.0307	0.1501
(Matos, 2012)	3270	1	0.0449	0.4096	1	0.0542	0.2970
	3353	2	0.0576	0.1426	3	0.0865	0.1866
	3356	1	0.0480	0.5268	1	0.0594	0.2901
	3387	1	0.0502	0.2496	2	0.0567	0.2774
	3392	1	0.0590	0.1105	2	0.1078	0.1652
	3410	2	0.0499	0.1368	2	0.0786	0.1253
	3421	2	0.0563	0.1922	2	0.0813	0.1948
	3467	1	0.0428	0.5609	1	0.0562	0.2790
	3533	1	0.0352	0.3403	1	0.0583	0.1399
	3545	1	0.0511	0.1447	2	0.0694	0.1735
	3729	1	0.0617	0.1964	3	0.1508	0.1510
	3746	3	0.0679	0.1856	3	0.0944	0.1982
	3852	1	0.0281	0.1937	1	0.0474	0.1352
	3861	1	0.0489	0.4463	1	0.061	0.2907
	3874	1	0.0544	0.1311	2	0.0701	0.2120
	3888	2	0.0500	0.1623	3	0.0675	0.1906
	3890	2	0.0466	0.2071	1	0.0678	0.1441
	3893	1	0.0394	0.5472	1	0.0602	0.2251
	3910	1	0.0513	0.1554	2	0.0855	0.1871

(continued)

(Matos, 2012)	3937	2	0.0738	0.2218	3	0.1034	0.2403
	4032	1	0.0495	0.0684	2	0.0534	0.1481
	4079	1	0.0546	0.1217	1	0.0527	0.1746
	4086	2	0.0640	0.1064	2	0.1029	0.1030
	4087	2	0.1007	0.3071	3	0.1287	0.3201
	4088	3	0.0675	0.1936	-	-	-
	4098	1	0.0409	0.1826	1	0.067	0.1268
	4112	2	0.0578	0.1642	2	0.0948	0.1415
	4113	2	0.0427	0.1885	2	0.0916	0.1285
	4116	1	0.0408	0.4723	1	0.0576	0.1989
	4117	1	0.0438	0.1749	2	0.0586	0.2014
	4120	1	0.0652	0.0809	2	0.049	0.2582
	4126	1	0.0492	0.2214	3	0.0691	0.2701
	4128	1	0.0528	0.2386	2	0.0677	0.3275
	4251	1	0.0638	0.0664	1	0.0597	0.1095
	4285	3	0.2080	0.3767	2	0.0599	0.3651

Table 3 – Animal and dietary information used as independent variables in the stepwise regression in Chapter 2 to develop the equations to predict the compartmental mean residence time of forage and concentrate.

Experiment	Animal or pen	Diet	BM kg	DMI g·day^{-1}	NDF intake g·day^{-1}	Lig intake g·day^{-1}	peNDF intake g·day^{-1}	Fdiet g·kg^{-1} DM	Cdiet g·kg^{-1} DM	NDFdiet g·kg^{-1} DM	Cpdiet g·kg^{-1} DM	Ligdiet g·kg^{-1} DM
(Lopes, 2009)	218	2	27.36	758.97	400.89	21.81	245.34	769.23	230.77	569.98	148.20	33.15
	219	3	25.63	281.29	155.27	8.73	26.83	769.23	230.77	569.98	148.20	33.15
	220	2	24.78	429.12	231.87	13.02	129.08	769.23	230.77	569.98	148.20	33.15
	221	1	28.74	851.41	464.55	27.61	273.14	769.23	230.77	569.98	148.20	33.15
	223	3	16.16	208.75	114.37	5.60	21.71	769.23	230.77	569.98	148.20	33.15
	224	1	25.44	747.33	410.42	23.71	232.27	769.23	230.77	569.98	148.20	33.15
	248	5	24.03	414.23	217.71	15.37	78.94	769.23	230.77	587.21	134.35	40.31
	250	5	23.30	362.84	196.52	13.90	73.61	769.23	230.77	587.21	134.35	40.31
	251	4	26.75	572.63	313.64	22.28	160.60	769.23	230.77	587.21	134.35	40.31
	252	6	21.88	467.24	254.94	18.05	13.70	769.23	230.77	587.21	134.35	40.31
	253	4	20.85	315.88	174.63	12.33	90.18	769.23	230.77	587.21	134.35	40.31
	254	6	25.88	460.34	249.95	17.37	11.61	769.23	230.77	587.21	134.35	40.31
	256	9	26.08	600.21	329.19	27.49	59.57	769.23	230.77	560.21	134.12	47.38
	257	7	28.73	538.36	298.10	24.17	186.07	769.23	230.77	560.21	134.12	47.38
	258	9	29.25	598.48	328.63	27.66	49.92	769.23	230.77	560.21	134.12	47.38
	259	7	28.88	821.17	446.48	36.93	271.60	769.23	230.77	560.21	134.12	47.38
	260	8	28.25	877.36	476.85	42.29	203.25	769.23	230.77	560.21	134.12	47.38
	262	7	28.10	401.01	221.99	17.57	138.65	769.23	230.77	560.21	134.12	47.38
(Felisberto, 2011)	1	49	47.15	1163.38	613.01	39.65	457.97	803.20	196.80	529.06	155.81	34.31
	2	41	48.00	1751.22	780.17	48.39	490.33	672.10	327.90	459.66	151.04	29.00
	3	57	53.10	1247.87	739.38	48.03	754.10	934.40	65.60	587.06	148.52	39.14
	4	57	47.20	1768.26	1041.26	66.52	1067.43	934.40	65.60	587.06	148.52	39.14

(continued)

5	41	60.85	1413.80	650.41	41.29	362.16	672.10	327.90	459.66	151.04	29.00
6	41	51.00	1445.50	636.99	38.74	457.19	672.10	327.90	459.66	151.04	29.00
7	34	42.45	1603.67	667.23	43.16	328.47	557.40	442.60	406.52	153.00	25.30
8	57	66.50	1054.55	628.16	41.21	483.35	934.40	65.60	587.06	148.52	39.14
9	41	40.40	1187.69	570.34	36.88	330.34	672.10	327.90	459.66	151.04	29.00
10	57	46.70	849.05	510.02	33.32	438.54	934.40	65.60	587.06	148.52	39.14
11	49	58.25	1124.49	593.86	38.44	440.11	803.20	196.80	529.06	155.81	34.31
12	49	71.05	1322.45	700.39	45.96	456.48	803.20	196.80	529.06	155.81	34.31
13	34	52.20	1788.83	680.03	39.84	388.14	557.40	442.60	406.52	153.00	25.30
14	34	57.75	2241.68	878.51	54.81	454.20	557.40	442.60	406.52	153.00	25.30
15	57	40.95	860.25	517.66	35.50	404.82	934.40	65.60	587.06	148.52	39.14
16	49	52.85	1748.40	916.49	57.62	675.01	803.20	196.80	529.06	155.81	34.31
17	34	49.40	1697.22	688.51	43.22	332.42	557.40	442.60	406.52	153.00	25.30
18	49	37.30	702.61	362.89	23.14	261.86	803.20	196.80	529.06	155.81	34.31
19	49	46.25	1191.80	657.76	42.41	437.95	803.20	196.80	529.06	155.81	34.31
20	57	37.30	930.07	555.64	36.68	490.21	934.40	65.60	587.06	148.52	39.14
21	41	52.90	1362.64	612.97	36.83	457.57	672.10	327.90	459.66	151.04	29.00
22	57	58.60	1591.93	972.82	63.39	731.43	934.40	65.60	587.06	148.52	39.14
23	34	46.55	1638.86	638.78	38.98	328.34	557.40	442.60	406.52	153.00	25.30
24	57	48.35	688.01	398.75	24.62	414.65	934.40	65.60	587.06	148.52	39.14
25	34	43.15	1111.97	424.94	26.56	220.73	557.40	442.60	406.52	153.00	25.30
26	49	55.10	833.06	446.94	29.53	298.28	803.20	196.80	529.06	155.81	34.31
27	41	42.25	806.87	376.25	24.05	219.91	672.10	327.90	459.66	151.04	29.00
28	57	50.40	923.16	532.51	34.73	554.99	934.40	65.60	587.06	148.52	39.14
29	34	48.50	1798.90	698.82	42.60	318.88	557.40	442.60	406.52	153.00	25.30
30	41	57.15	2184.75	1020.22	62.72	575.04	672.10	327.90	459.66	151.04	29.00
31	49	42.70	666.47	330.39	21.88	280.02	803.20	196.80	529.06	155.81	34.31
32	41	49.75	1556.31	675.06	37.44	476.97	672.10	327.90	459.66	151.04	29.00
33	34	54.50	1356.52	513.92	27.41	254.10	557.40	442.60	406.52	153.00	25.30

(Felisberto, 2011)

(continued)

(Felisberto, 2011)	34	34	72.00	886.32	370.29	20.28	187.05	557.40	442.60	406.52	153.00	25.30
	35	57	51.50	714.14	421.58	27.44	377.39	934.40	65.60	587.06	148.52	39.14
	36	49	45.70	1482.92	783.78	48.28	697.45	803.20	196.80	529.06	155.81	34.31
	37	49	48.60	1579.53	845.28	53.11	558.29	803.20	196.80	529.06	155.81	34.31
	38	34	49.70	1807.66	692.34	40.83	413.03	557.40	442.60	406.52	153.00	25.30
	39	49	49.05	1514.83	786.17	50.91	684.65	803.20	196.80	529.06	155.81	34.31
	40	41	45.50	1189.69	523.52	32.27	326.67	672.10	327.90	459.66	151.04	29.00
	41	41	66.80	1878.00	824.14	50.34	499.57	672.10	327.90	459.66	151.04	29.00
	42	41	44.10	1331.37	620.03	37.35	473.93	672.10	327.90	459.66	151.04	29.00
	43	41	40.00	1209.36	529.47	33.09	311.19	672.10	327.90	459.66	151.04	29.00
	44	57	50.35	1265.20	757.36	48.93	569.34	934.40	65.60	587.06	148.52	39.14
	45	34	52.90	1833.67	718.63	42.50	441.37	557.40	442.60	406.52	153.00	25.30
	46	34	46.25	1132.64	423.96	26.48	244.63	557.40	442.60	406.52	153.00	25.30
	47	57	59.00	1295.38	776.91	50.35	666.02	934.40	65.60	587.06	148.52	39.14
	48	49	41.55	1124.63	577.62	36.73	501.73	803.20	196.80	529.06	155.81	34.31
(Matos, 2012)	1	1	58.53	1844.55	642.41	32.59	469.22	467.30	532.70	439.83	160.00	21.19
	2	1	44.80	1869.79	651.25	32.67	474.36	467.30	532.70	439.83	160.00	21.19
	7	1	45.76	1646.87	575.96	28.11	424.07	467.30	532.70	439.83	160.00	21.19
	8	1	60.47	1844.30	648.00	32.73	479.61	467.30	532.70	439.83	160.00	21.19
	3	2	45.68	2188.43	762.92	56.36	606.46	646.30	353.70	408.67	157.77	29.24
	5	2	54.09	2147.87	767.16	56.46	614.69	646.30	353.70	408.67	157.77	29.24
	11	2	53.08	2135.30	754.57	56.17	600.73	646.30	353.70	408.67	157.77	29.24
	12	2	54.97	1941.61	693.94	51.12	557.36	646.30	353.70	408.67	157.77	29.24
	4	3	42.09	1991.72	660.74	105.47	496.14	697.10	302.90	366.79	177.38	58.56
	6	3	57.41	2022.61	670.80	104.29	489.64	697.10	302.90	366.79	177.38	58.56
	9	3	56.00	2057.57	674.11	106.82	490.22	697.10	302.90	366.79	177.38	58.56
	10	3	43.14	1907.21	650.54	102.30	484.17	697.10	302.90	366.79	177.38	58.56

APPENDIX 3

Chapter 3 – Original dataset

Table 1 – Summary of the literature dataset used to evaluate the SRNS model with modifications in the Chapter 3.

Experiment*	Diet	Body mass kg	DMI $g \cdot day^{-1}$	Milk production $kg \cdot day^{-1}$	%fat	%CP	MEI $Mcal \cdot day^{-1}$
1	1	54.67	3169.00	6.24	3.20	2.96	7.99
1	1	67.75	2725.00	2.96	3.44	2.96	6.87
1	1	64.05	2796.00	4.57	2.79	2.96	7.05
1	1	52.80	2934.00	4.72	2.79	2.96	7.39
1	1	75.04	2287.00	0.67	3.55	2.96	5.76
1	1	56.90	2658.00	3.88	3.44	2.96	6.70
1	1	61.69	3078.00	5.14	3.50	2.96	7.76
1	1	45.26	2612.00	4.18	2.90	2.96	6.58
1	1	60.19	2515.00	3.01	2.86	2.96	6.34
1	2	57.24	2827.00	4.22	3.13	2.96	6.73
1	2	58.98	2998.00	4.54	3.48	2.96	7.13
1	2	51.84	3037.00	4.39	3.58	2.96	7.23
1	2	56.49	3159.00	6.71	2.52	2.96	7.52
1	2	65.02	2822.00	3.83	3.61	2.96	6.72
1	2	58.81	2910.00	4.90	2.77	2.96	6.93
1	2	52.49	3039.00	5.13	3.62	2.96	7.23
1	2	43.57	2194.00	3.28	2.59	2.96	5.22
1	2	64.37	3451.00	6.81	3.26	2.96	8.21
1	2	69.37	2302.00	3.30	2.83	2.96	5.48
1	3	64.05	2741.00	4.24	3.39	2.96	6.06
1	3	49.16	2302.00	2.71	2.91	2.96	5.09
1	3	67.92	3348.00	5.88	3.21	2.96	7.40
1	3	52.90	2556.00	3.58	2.99	2.96	5.65
1	3	50.24	2658.00	4.21	2.93	2.96	5.87
1	3	55.38	1113.00	1.44	3.75	2.96	2.46
1	3	50.10	2786.00	4.35	3.20	2.96	6.16
1	3	66.21	2915.00	5.06	3.05	2.96	6.44
1	3	44.64	2712.00	4.64	3.28	2.96	5.99
1	3	51.44	2130.00	2.10	3.41	2.96	4.71
1	3	59.46	2880.00	4.15	3.03	2.96	5.90
1	4	39.96	1994.00	3.38	3.14	2.96	4.09
1	4	61.98	2415.00	2.47	3.27	2.96	4.95
1	4	64.08	2691.00	4.09	2.98	2.96	5.52
1	4	63.73	2791.00	4.21	4.01	2.96	5.72
1	4	54.12	2752.00	4.17	3.90	2.96	5.64
1	4	55.15	2130.00	2.79	3.28	2.96	4.37

1	4	53.06	2573.00	4.10	3.38	2.96	5.27
1	4	67.88	2588.00	3.46	3.28	2.96	5.31
1	4	54.24	2186.00	2.72	3.70	2.96	4.48
1	5	58.25	2884.00	5.35	3.12	2.96	5.45
1	5	52.70	2317.00	2.98	3.31	2.96	4.38
1	5	46.39	2312.00	3.69	3.36	2.96	4.37
1	5	60.49	2828.00	3.39	3.17	2.96	5.35
1	5	64.43	2619.00	3.38	3.27	2.96	4.95
1	5	48.07	2295.00	3.60	3.33	2.96	4.34
1	5	66.78	2517.00	3.09	2.80	2.96	4.76
1	5	65.98	1920.00	2.25	3.51	2.96	3.63
1	5	65.80	2128.00	1.79	3.79	2.96	4.02
1	5	57.95	2497.00	4.27	3.43	2.96	4.72
2	1	38.05	1628.13	2.66	3.39	2.93	4.91
2	2	37.33	1072.92	1.42	2.79	2.41	3.18
2	3	52.03	1262.48	1.42	3.62	2.89	3.53
2	4	47.43	1016.40	1.69	4.52	2.50	2.98
2	5	40.87	2071.32	3.25	3.57	2.76	5.68
2	1	40.47	1843.85	3.26	2.79	2.49	5.53
2	2	44.20	1858.09	2.75	2.36	2.81	5.38
2	3	47.50	2379.05	3.22	3.10	2.71	6.72
2	4	55.08	1638.55	1.76	2.69	2.84	4.53
2	5	52.13	2131.92	2.53	3.37	2.98	5.50
2	1	57.68	1557.19	1.75	2.63	2.73	4.46
2	2	53.88	1555.53	2.02	2.74	2.64	4.44
2	3	41.62	1719.83	3.11	2.81	2.29	4.75
2	4	44.33	1545.95	2.30	2.34	2.54	4.19
2	5	49.63	2433.17	4.07	2.35	2.42	6.64
2	1	49.22	2330.74	4.12	2.49	2.82	6.16
2	2	60.95	1786.10	1.78	2.90	2.75	5.02
2	3	57.80	2193.84	2.29	2.97	2.57	6.27
2	4	38.72	1448.96	2.69	2.61	2.64	3.79
2	5	45.92	1526.69	2.47	2.51	2.61	3.91
2	1	58.55	2025.38	1.51	3.43	3.15	5.82
2	2	41.35	1861.59	2.70	2.91	2.59	4.77
2	3	45.18	1853.19	2.42	2.33	2.83	5.21
2	4	47.87	2209.75	3.26	2.45	2.62	6.06
2	5	63.15	1938.00	1.14	3.45	3.33	5.37
3	1	36.1	1061.10	1.77	2.36	2.33	3.28
3	2	40.62	1999.89	2.89	3.55	2.83	6.10
3	3	58.38	2446.53	4.17	2.60	2.60	7.66
3	4	41.5	1787.39	2.71	3.57	2.82	5.20
3	5	48.03	2007.31	3.77	2.94	2.76	5.71
3	1	43.73	1792.74	2.57	2.76	2.91	5.29
3	2	43.4	984.43	1.87	2.50	2.39	2.98
3	3	43.67	2367.07	2.87	2.28	2.63	6.98

3	4	44.68	2236.79	3.70	3.03	2.86	6.15
3	5	61.22	2539.65	4.34	2.66	2.68	7.27
3	1	48.22	1571.57	1.95	2.84	2.49	4.62
3	2	44.22	1957.41	2.66	2.23	2.50	5.80
3	3	50.05	2349.32	3.80	2.52	2.67	7.07
3	4	64.47	2420.24	4.46	1.97	2.55	7.12
3	5	46.38	2156.00	3.01	2.58	2.86	6.00
3	1	51.52	2041.95	2.99	2.79	2.67	5.77
3	2	65.15	2326.14	4.50	2.28	2.72	7.08
3	3	47.73	2150.46	3.28	2.43	2.90	6.24
3	4	55.08	2330.98	3.37	2.38	2.82	7.13
3	5	43.53	1811.19	2.71	2.86	3.10	5.25
3	1	61.2	1923.87	3.08	2.87	2.86	5.81
3	2	49.1	2423.02	3.14	2.57	3.02	7.18
3	3	57.35	2388.19	3.73	2.57	2.91	6.47
3	4	44.18	2000.47	2.49	2.82	2.98	5.83
3	5	46.95	1911.64	3.05	2.31	2.83	5.35
4	4	61.13	2208.34	2.68	2.09	2.46	6.50
4	3	72.66	1798.79	1.36	2.63	2.93	4.92
4	2	55.30	2057.07	2.81	2.68	2.81	5.77
4	1	48.31	2092.27	2.35	3.09	2.87	6.27
4	4	55.86	1532.45	2.45	2.42	2.55	4.73
4	3	53.56	2140.64	2.97	2.65	2.95	6.12
4	2	55.53	2446.46	2.95	2.94	2.79	6.72
4	1	58.89	1568.24	1.71	2.47	2.80	4.55
4	1	64.43	2046.16	2.57	2.34	2.50	4.79
4	4	75.94	1598.36	1.20	2.73	4.44	4.16
4	3	57.94	2157.38	3.20	2.76	2.77	5.91
4	2	54.68	2270.28	3.04	3.13	2.96	5.79
4	1	59.93	1639.36	2.09	2.42	2.54	4.49
4	4	54.39	2116.28	3.28	2.78	3.10	5.81
4	3	57.04	2361.16	2.99	2.82	2.78	6.10
4	2	59.06	1623.25	1.88	3.71	3.19	4.48
4	2	65.78	2082.97	2.53	2.44	2.57	5.32
4	1	76.75	1355.61	0.78	3.36	3.39	3.60
4	4	62.01	2322.74	3.55	2.85	2.85	6.27
4	3	56.31	2351.29	3.49	2.91	2.98	5.62
4	2	61.11	1554.15	1.91	2.78	2.69	3.90
4	1	54.24	1986.14	3.00	3.10	3.11	4.92
4	4	57.79	2478.85	3.72	2.97	2.76	6.58
4	3	61.89	1718.23	1.97	3.68	3.19	4.70
4	3	67.55	2072.50	2.29	2.63	2.75	5.10
4	2	75.15	1226.36	0.76	3.86	3.47	2.96
4	1	58.63	2028.05	2.73	2.98	2.92	5.17
4	4	56.28	2313.94	3.21	2.87	3.12	6.35
4	3	63.53	2004.33	2.14	2.89	2.87	5.06

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4	2	55.23	2238.96	3.11	3.20	3.24	5.67
4	1	57.00	2215.96	3.01	3.23	2.87	6.35
4	4	61.00	1568.06	1.90	3.46	3.40	4.66
5	1	51.85	2130.00	3.37	2.67	2.60	6.17
5	2	59.93	2260.00	3.16	3.12	2.84	5.97
5	3	54.80	1980.00	2.70	2.81	3.74	5.30
5	4	48.75	930.00	1.40	3.37	3.43	1.99
5	5	56.25	1380.00	2.53	1.85	2.76	3.48
5	3	51.58	1980.00	2.96	3.12	2.63	3.96
5	5	55.33	1930.00	2.02	3.36	3.28	4.70
5	1	49.80	1740.00	1.73	3.91	3.35	4.87
5	2	59.85	2570.00	3.37	2.46	2.70	6.90
5	1	63.35	2420.00	3.30	2.94	2.70	6.79
5	2	56.95	2130.00	2.57	2.87	3.21	6.52
5	3	52.55	1430.00	1.37	4.02	3.59	4.39
5	4	62.25	2110.00	3.00	2.46	2.80	5.46
5	2	54.90	2390.00	3.22	2.94	2.64	6.72
5	3	61.10	1700.00	2.86	3.46	2.82	4.85
5	4	57.38	1800.00	1.98	2.92	3.63	4.62
5	5	56.23	1400.00	0.65	4.69	4.95	3.24
5	1	66.93	2820.00	3.48	2.88	2.88	6.08
5	4	52.65	1490.00	2.17	3.61	2.84	3.78
5	5	61.13	1310.00	1.96	4.24	2.83	3.60
5	1	56.20	1730.00	1.89	3.09	3.16	4.48
5	2	61.65	1750.00	0.44	3.97	3.01	4.16
5	3	64.50	2130.00	3.16	4.55	4.54	5.63
6	1	52.25	2200.00	2.66	2.10	2.60	6.21
6	2	70.45	1960.00	2.03	3.03	2.84	5.57
6	3	54.23	1850.00	2.45	2.91	2.47	4.91
6	4	67.55	1290.00	1.46	2.85	2.95	3.31
6	5	41.05	1150.00	1.27	2.67	2.79	2.58
6	3	54.88	2470.00	2.6	3.05	2.72	6.25
6	4	70.1	1640.00	1.25	3.68	3.04	4.12
6	5	54.3	1560.00	1.47	3.73	2.63	3.54
6	1	73.85	2040.00	2.05	3.36	2.82	6.11
6	2	40.53	1610.00	1.44	2.38	2.71	4.53
6	1	71.8	2000.00	1.24	3.16	2.95	5.63
6	2	52.28	1900.00	2.3	3.10	2.70	5.43
6	3	73.43	1980.00	1.72	3.48	2.86	5.09
6	4	41.55	1300.00	0.84	3.14	3.23	3.41
6	2	54.8	2220.00	2.28	3.24	2.72	5.80
6	3	76.78	1720.00	0.57	3.79	3.43	4.62
6	4	54.4	1510.00	1.62	3.66	2.74	3.67
6	5	74.68	1470.00	0.7	4.57	3.21	2.89
6	1	41.1	1140.00	0.73	4.79	4.11	3.88
6	4	56.88	1770.00	1.95	2.67	2.83	4.40

6	5	78.7	1240.00	0.09	3.31	3.56	2.59
6	1	46.98	1250.00	2	3.91	2.93	3.74
6	2	77.9	1950.00	1.25	4.26	3.09	5.15
6	3	41.93	1200.00	0.74	2.90	3.54	2.93
7	1	48.87	1750.76	2.20	3.51	3.05	5.22
7	1	52.14	1343.67	1.79	4.28	3.04	4.75
7	1	58.04	2143.76	2.81	3.55	3.09	6.42
7	1	49.92	2042.85	2.34	4.46	3.34	5.90
7	1	56.38	1760.15	2.05	4.39	3.36	5.20
7	2	61.03	1572.50	1.90	5.05	3.45	5.01
7	2	61.84	1921.82	2.60	3.72	3.06	5.16
7	2	48.70	1417.31	2.06	4.71	3.35	4.27
7	2	68.23	1409.75	1.28	4.22	3.32	4.23
7	2	65.53	1634.69	1.56	3.49	3.29	5.33
7	2	56.77	1278.46	1.24	3.45	4.00	4.15
7	3	63.38	1866.07	1.69	3.80	3.74	5.67
7	3	53.07	1914.00	2.36	3.48	3.22	6.01
7	3	51.12	1716.22	2.18	4.19	3.19	4.87
7	3	48.45	1962.79	2.01	4.55	3.52	5.53
7	3	66.99	1519.55	0.99	4.65	3.07	4.98
7	4	55.41	1332.12	1.78	3.72	3.17	4.23
7	4	52.02	1513.44	1.12	3.83	3.69	4.43
7	4	59.78	1582.80	1.62	4.45	3.55	4.58
7	4	56.04	1427.57	1.68	3.07	3.07	4.04
7	4	66.43	1544.97	1.42	3.17	3.44	5.02
8	M	52.35	2360.00	3.77	2.60	2.81	6.11
8	N	48.43	1180.00	1.20	2.88	2.57	3.12
8	P	49.07	2020.00	3.40	2.75	2.43	5.41
8	C	49.75	2450.00	3.36	2.33	2.59	6.29
8	P	58.98	2090.00	2.51	2.80	2.67	5.38
8	N	49.35	1840.00	2.13	1.95	2.67	5.01
8	C	50.55	1830.00	1.86	2.30	2.42	4.83
8	M	64.43	2100.00	1.55	3.34	2.93	5.82
8	C	44.07	2060.00	2.65	3.20	2.70	5.53
8	M	43.48	2140.00	2.51	3.07	2.85	5.67
8	N	37.57	1750.00	2.92	2.56	2.44	5.05
8	P	48.27	1770.00	2.35	3.17	2.65	4.64
8	P	51.08	1670.00	3.02	3.16	2.74	4.15
8	C	54.63	1850.00	1.23	2.40	2.80	4.82
8	N	51.65	2160.00	3.34	2.76	2.57	6.20
8	M	50.32	2340.00	3.29	2.58	2.73	6.43
8	C	60.53	2130.00	2.52	2.68	2.75	5.41
8	M	50.52	1830.00	2.36	2.64	2.59	4.92
8	N	52.15	1700.00	1.75	2.23	2.67	4.80
8	P	65.63	1510.00	1.22	3.54	2.92	4.22
8	M	46.12	1930.00	2.86	3.05	2.53	5.32

8	N	45.82	2090.00	2.60	2.93	3.02	5.96
8	P	39.63	1830.00	3.09	3.42	2.69	4.92
8	C	52.58	2250.00	3.04	2.48	2.62	5.89
8	C	56.90	2530.00	3.87	2.93	3.13	6.39
8	P	55.97	1620.00	1.06	3.48	3.24	4.26
8	M	52.62	2050.00	2.98	2.91	2.68	5.13
8	N	51.47	2120.00	3.28	2.79	3.06	5.95
8	M	60.10	1960.00	2.33	3.20	3.44	5.22
8	C	56.43	2400.00	2.08	3.40	3.05	6.13
8	P	54.45	1750.00	1.74	4.64	3.20	4.65
8	N	67.32	2000.00	1.28	3.13	3.58	5.82
8	P	46.23	1740.00	2.70	2.81	2.52	4.78
8	C	49.18	2400.00	2.63	3.17	2.85	5.73
8	M	41.68	2030.00	2.88	3.06	2.60	5.32
8	N	54.07	2050.00	2.72	2.48	2.72	5.89
8	N	61.28	2360.00	3.43	2.78	2.94	6.02
8	M	59.48	1640.00	1.04	3.01	2.63	4.16
8	C	54.00	2240.00	3.15	2.80	2.90	5.49
8	P	51.78	1970.00	2.66	2.40	3.02	4.95
8	N	64.07	2330.00	2.25	3.21	3.20	6.25
8	P	57.50	1910.00	2.01	2.68	2.88	4.75
8	M	54.23	1370.00	1.64	3.64	3.03	3.61
8	C	70.83	2000.00	1.00	2.81	2.83	5.07
8	N	47.08	2020.00	2.97	2.70	2.68	5.90
8	P	47.78	1680.00	2.23	3.18	2.74	4.10
8	C	42.17	2200.00	2.89	2.85	2.70	5.82
8	M	56.25	1970.00	2.74	2.43	2.68	4.99
9	N	42.98	2330.00	3.29	3.29	2.90	6.43
9	P	53.12	2180.00	2.73	3.38	2.57	5.74
9	T	46.58	2310.00	3.59	3.16	2.66	5.86
9	A	45.93	2110.00	2.80	3.41	2.85	5.46
9	T	63.22	2160.00	2.76	3.07	3.10	5.84
9	A	65.17	2160.00	2.88	3.93	2.94	5.97
9	P	42.55	2080.00	3.10	3.16	2.50	5.21
9	N	43.33	2490.00	3.96	2.99	2.55	6.85
9	P	41.20	1620.00	2.65	3.28	2.78	4.12
9	T	52.02	2050.00	2.04	2.61	2.52	5.36
9	A	48.90	2040.00	3.42	3.57	2.69	5.18
9	N	47.70	2640.00	3.69	2.29	2.89	6.47
9	A	64.52	1980.00	2.73	3.41	3.09	5.03
9	N	70.45	2840.00	3.63	2.53	2.85	8.12
9	T	44.25	2560.00	3.18	3.21	2.79	6.46
9	P	43.32	1860.00	3.44	3.06	2.44	4.77
9	A	42.45	1900.00	2.43	3.58	2.79	4.89
9	N	56.12	2970.00	2.92	2.89	2.59	7.79
9	P	45.08	1650.00	2.93	3.41	2.50	4.33

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9	T	49.80	2680.00	3.19	2.16	3.11	6.45
9	P	63.90	2010.00	2.89	2.85	3.06	5.24
9	T	71.40	2860.00	3.36	2.50	2.88	7.35
9	N	42.77	2250.00	3.38	2.78	2.66	6.07
9	A	46.60	1400.00	2.75	3.10	2.71	3.33
9	T	43.22	1670.00	1.97	2.48	2.91	3.75
9	A	53.75	2000.00	2.22	2.90	2.59	5.07
9	N	45.80	1980.00	2.99	3.29	2.49	5.97
9	P	48.10	1970.00	2.86	2.82	2.71	4.59
9	N	64.62	2170.00	2.84	2.90	3.11	5.93
9	P	68.45	2100.00	2.94	2.59	2.81	5.20
9	A	44.95	2050.00	3.00	3.08	2.62	5.04
9	T	46.00	2200.00	2.98	2.42	2.53	5.20
10	49	47.15	1163.38	0.74	3.30	3.30	2.73
10	41	48.00	1751.22	1.74	2.95	3.00	4.52
10	57	53.10	1247.87	1.13	3.60	2.98	2.74
10	57	47.20	1768.26	1.05	3.79	3.25	3.66
10	41	60.85	1413.80	1.31	3.73	2.95	3.62
10	41	51.00	1445.50	1.64	3.35	2.86	3.69
10	34	42.45	1603.67	1.60	3.50	3.10	4.33
10	57	66.50	1054.55	0.61	3.06	3.36	2.24
10	41	40.40	1187.69	0.79	4.09	3.30	3.10
10	57	46.70	849.05	0.61	3.41	3.24	1.90
10	49	58.25	1124.49	0.89	3.39	2.91	2.65
10	49	71.05	1322.45	0.19	2.92	3.62	3.09
10	34	52.20	1788.83	0.88	2.14	3.57	4.93
10	34	57.75	2241.68	1.84	2.70	2.98	6.00
10	57	40.95	860.25	0.62	4.24	3.38	1.96
10	49	52.85	1748.40	1.93	3.76	2.48	4.19
10	34	49.40	1697.22	1.43	4.25	3.51	4.66
10	49	37.30	702.61	0.23	3.62	2.81	1.83
10	49	46.25	1191.80	0.52	3.54	3.57	2.87
10	57	37.30	930.07	0.66	3.67	3.02	2.12
10	41	52.90	1362.64	1.37	4.55	3.31	3.44
10	57	58.60	1591.93	1.36	3.89	3.39	3.61
10	34	46.55	1638.86	1.38	4.18	3.10	4.59
10	57	48.35	688.01	0.42	4.63	3.50	1.79
10	34	43.15	1111.97	1.14	4.16	3.31	3.19
10	49	55.10	833.06	0.45	2.94	3.39	1.95
10	41	42.25	806.87	0.65	2.69	2.90	1.95
10	57	50.40	923.16	0.44	3.86	3.44	2.21
10	34	48.50	1798.90	2.41	3.66	3.35	4.60
10	41	57.15	2184.75	1.88	3.85	2.97	5.48
10	49	42.70	666.47	0.38	4.18	3.75	1.53
10	41	49.75	1556.31	1.20	3.89	3.41	4.10
10	34	54.50	1356.52	0.36	2.88	3.90	3.64

(continued)

10	34	72.00	886.32	0.30	3.80	3.36	2.49
10	57	51.50	714.14	0.79	4.53	3.47	1.72
10	49	45.70	1482.92	0.61	3.83	4.10	3.54
10	49	48.60	1579.53	2.01	2.71	2.80	3.92
10	34	49.70	1807.66	1.93	3.45	2.95	4.90
10	49	49.05	1514.83	0.88	3.22	3.32	3.59
10	41	45.50	1189.69	0.70	3.99	3.54	3.06
10	41	66.80	1878.00	0.91	2.95	3.78	4.69
10	41	44.10	1331.37	1.03	3.69	3.00	3.45
10	41	40.00	1209.36	0.75	3.88	3.44	3.16
10	57	50.35	1265.20	0.45	2.80	3.20	2.69
10	34	52.90	1833.67	1.55	3.98	3.30	5.00
10	34	46.25	1132.64	1.22	2.58	2.73	2.91
10	57	59.00	1295.38	0.60	2.84	3.27	2.85
10	49	41.55	1124.63	0.71	3.93	2.99	2.69

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