Universidade Federal de Minas Gerais Escola de Veterinária Colegiado de Pós-Graduação em Zootecnia

EXIGÊNCIA EM PROTEÍNA PARA CORDEIRAS DESLANADAS EM CRESCIMENTO

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Exigência em proteína para cordeiras deslanadas em crescimento

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Epígrafe

O Rei dos Animais

Saiu o leão a fazer sua pesquisa estatística, para verificar se ainda era o Rei das Selvas. Os tempos tinham mudado muito, as condições do progresso alterado a psicologia e os métodos de combate das feras, as relações de respeito entre os animais já não eram as mesmas, de modo que seria bom indagar. Não que restasse ao Leão qualquer dúvida quanto à sua realeza. Mas assegurar-se é uma das constantes do espírito humano, e, por extensão, do espírito animal. Ouvir da boca dos outros a consagração do nosso valor, saber o sabido, quando ele nos é favorável, eis um prazer dos deuses. Assim o Leão encontrou o Macaco e perguntou: "Hei, você aí, macaco - quem é o rei dos animais?" O Macaco, surpreendido pelo rugir indagatório, deu um salto de pavor e, quando respondeu, já estava no mais alto galho da mais alta árvore da floresta: "Claro que é você, Leão, claro que é você!".

Satisfeito, o Leão continuou pela floresta e perguntou ao papagaio: "Currupaco, papagaio. Quem é, segundo seu conceito, o Senhor da Floresta, não é o Leão?" E como aos papagaios não é dado o dom de improvisar, mas apenas o de repetir, lá repetiu o papagaio: "Currupaco... não é o Leão? Não é o Leão? Currupaco, não é o Leão?".

Cheio de si, prosseguiu o Leão pela floresta em busca de novas afirmações de sua personalidade. Encontrou a coruja e perguntou: "Coruja, não sou eu o maioral da mata?" "Sim, és tu", disse a coruja. Mas disse de sábia, não de crente. E lá se foi o Leão, mais firme no passo, mais alto de cabeça. Encontrou o tigre. "Tigre, - disse em voz de estentor -eu sou o rei da floresta. Certo?" O tigre rugiu, hesitou, tentou não responder, mas sentiu o barulho do olhar do Leão fixo em si, e disse, rugindo contrafeito: "Sim". E rugiu ainda mais mal humorado e já arrependido, quando o leão se afastou.

Três quilômetros adiante, numa grande clareira, o Leão encontrou o elefante. Perguntou: "Elefante, quem manda na floresta, quem é Rei, Imperador, Presidente da República, dono e senhor de árvores e de seres, dentro da mata?" O elefante pegou-o pela tromba, deu três voltas com ele pelo ar, atirou-o contra o tronco de uma árvore e desapareceu floresta adentro. O Leão caiu no chão, tonto e ensangüentado, levantou-se lambendo uma das patas, e murmurou: "Que diabo, só porque não sabia a resposta não era preciso ficar tão zangado".

MORAL: CADA UM TIRA DOS ACONTECIMENTOS A CONCLUSÃO QUE BEM ENTENDE.

Millôr Fernandes

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Resumo Geral

Essa tese é composta por dois estudos. O primeiro trabalho objetivou determinar as exigências líquidas de proteína (NPg) para cordeiras Santa Inês em crescimento, e secundariamente avaliar as predições de cinco sistemas nutricionais. Cinquenta e sete cordeiras foram abatidas seguindo os procedimentos comuns a técnica de abate comparativo, sendo 21 abatidas no início do experimento e as demais divididas em um delineamento inteiramente ao acaso, em arranjo fatorial 2 x 3 (dois regimes alimentares, *ad libitum* e restrito, e três pesos ao abate, 20, 28 ou 36 kg, seis animais por grupo). A composição corporal dos animais foi obtida, e o teor e massa dos nutrientes foram modeladas utilizando as funções matemáticas de Huxley e von Bertalanffy. Paralelamente, as predições para NPg dos sistemas AFRC (1993), CSIRO (2007), NRC (1985), NRC (2007) e SRNS (2010) foram avaliadas. As assíntotas estimadas pelas funções ajustadas de von Bertalanffy apresentaram valores razoáveis. A estimativa da NPg conforme a função de Huxley foi 12,5 g/100 g de peso de corpo vazio em animais com 30 kg. A avaliação dos sistemas revelou que os modelos nutricionais tendem a subestimar a NPg de cordeiras Santa Inês. O sistema SRNS apresentou a melhor acurácia para estimativa do NPg (CCC = 0.948, r = 0.985, Cb = 0.963, RMSEP = 1.80 g). O segundo trabalho objetivou avaliar os efeitos do peso ao abate e do manejo nutricional sobre a carcaça e desenvolvimento corporal de cordeiras Santa Inês. Foram utilizados os mesmos animais do estudo anterior. Modelos lineares foram ajustado para acessar o efeito nutricional e do peso ao abate sobre as variáveis quantitativas. Um estudo alométrico multivariado foi realizado para visualização da relação entre partes corporais associadas ao efeito nutricional durante o crescimento. Concomitante ao crescimento do peso ao abate, a condição corporal, gordura subcutânea e intracavitária, peso de carcaça fria e cortes também aumentaram. O plano nutricional influenciou o peso de carcaça quente e fria ($P \le 0,002$), assim como o peso da perna, paleta, costelas/flanco e pescoço, que apresentaram menores pesos para animais sob restrição (P < 0.05). O estudo alométrico revelou que os componentes corporais crescem em diferentes taxas e que o plano nutricional afeta alguma delas, como a costela/flanco. Ademais, o desenvolvimento dos depósitos adiposos no corpo não se dá de forma isométrica, e um plano nutricional alto pode direcionar a energia ingerida para gordura visceral ao invés da carcaça. Animais sob restrição apresentaram um melhor equilíbrio na distribuição da gordura corporal, o que indica que as exigências nutricionais recomendadas por sistemas nutricionais em voga podem superestimar as verdadeiras exigências de ovinos brasileiros, e possivelmente reduzem a eficiência de sistemas produtivos.

Palavras chave: alometria, modelagem, nutrição, produção, Santa Inês

Abstract

This thesis was composed of two studies. The first work was conducted to determine the net protein requirements for gain (NPg) of Santa Inês female lambs, and secondarily, evaluate five feed systems predictions for this characteristic. Fifty-seven female lambs were slaughtered following common procedures of comparative slaughter technique, being twenty-one slaughtered at the beginning of trial and the remaining animals were assigned in a completely randomized design with a 2 x 3 factorial arrangement (two nutritional planes, *ad libitum* or restricted, versus, three slaughter weights, 20, 28 or 36 kg, six animals per group). Animals' body composition was assessed, and nutrients percentage and amount were modelled by means of Huxley's and von Bertalanffy's mathematical functions. Besides, the predictions from AFRC (1993), CSIRO (2007), NRC (1985), NRC (2007) and SRNS (2010) were evaluated. The estimated asymptotes from fitted von Bertalanffy function were in a reasonable value for the evaluated animals. The net protein requirements derived from Huxley's function resulted in an average NPg of 12.5 g/100 g o EBW gain in animals with 30 kg of shrunk BW. The models evaluation showed that Santa Inês female lambs present a higher NPg compared to the feed systems predictions. Moreover, the SRNS (2010) presented the best accuracy for NPg estimative (CCC = 0.948, r = 0.985, Cb = 0.963, RMSEP = 1.80 g). The second study aimed to evaluate the effect of slaughter weight and feeding management on carcass and body development of Santa Inês female lambs. The same animals from first study were used. Linear models were fit to assess nutritional and slaughter weight effects on body traits, carcass yields and composition. Also, a multivariate allometric study was performed to visualize the relationship between body parts associated to nutritional regimen during growth. Concurrent with an increase of slaughter weight body condition score, fat thickness, visceral fat depots, cold carcass weight, cuts and carcass composition also increased. Nutritional plane influenced hot and cold carcass weights ($P \le 0.002$), as well as hindlimb, blade, rib/flank and neck, which presented lower weights for restricted animals compared to *ad libitum* ones (P < 0.05). The allometric study revealed that body parts grow in different rates and nutritional plane influences some parts such as ribs/flank. Moreover, fat distribution among depots is not isometric, and a higher nutritional regimen may drive the energy intake to visceral fat rather than to carcass. Restricted animals presented a better balance on fat distribution, what indicates that common nutritional systems may overestimate nutrient demands for Brazilian sheep and possibly reduce livestock system efficiency.

Keywords: allometry, modelling, nutrition, production, Santa Inês

Introdução Geral

A caprino-ovinocultura brasileira representa uma atividade pecuária de grande relevância por garantir segurança alimentar a pequenos produtores e ainda poder gerar lucro a empreendimentos agrários, principalmente em regiões pressionadas por desafios edafoclimáticos (*e.g.*, semi-árido, aclives) que impossibilitam o sucesso de outras atividades. Por outro lado, esse setor carece de desenvolvimento de tecnologia apropriada e também de treinamento de recursos humanos capazes de tornar tais sistemas o mais eficiente possível.

Neste cenário, a ciência animal brasileira tem se dedicado a definir critérios e técnicas que possam ser aplicadas de forma mais acurada aos sistemas de criação de pequenos ruminantes em voga no país, bem como avaliar se estes sistemas são de fato os mais adequados. Ainda que a aplicação de tecnologias estrangeiras, oriundas de países com mais tradição na criação de pequenos ruminantes, possam trazer vantagens, a adaptação de tais técnicas bem como o desenvolvimento de ferramentas customizadas devem ser o foco da pesquisa brasileira.

Essa tese apresenta dois trabalhos que buscam entender melhor como se expressam as exigências proteicas de cordeiras Santa Inês, e ainda frente ao atendimento desses requisitos, como se dá o desempenho desses animais.

O primeiro capítulo intitulado "Body composition and net protein requirement for weight gain of Brazilian hair ewe lambs and evaluation of international nutritional models" apresenta o resultado da exigência proteica para ganho em cordeiras deslanadas estimado a partir do abate de 57 animais do genótipo Santa Inês, com peso de abate entre 20 e 37 kg. Ademais, o trabalho avalia a aplicabilidade de modelos de crescimento para modelagem da participação de nutrientes no peso de corpo vazio desses animais, e ainda, avalia a acurácia e precisão de cinco modelos nutricionais para predição da exigência proteica para ganho baseado nos dados obtidos pelo trabalho.

O segundo capítulo dessa tese apresenta os resultados relativos ao desempenho dos animais que foram submetidos a dois planos nutricionais, *ad libitum* ou restrito, em função do experimento de exigência nutricional. Neste trabalho foram avaliados os rendimentos cárneos bem como a distribuição de depósitos adiposos. Para esta segunda hipótese, foi realizado um estudo multivariado de alometria ontogênica, onde componentes principais foram estimados a partir da matriz de covariância dos resultados obtidos para as partes em estudo. O estudo multivariado permite de forma concisa avaliar-se não só a relação de partes com o todo (*e.g.*, pernil *versus* carcaça), mas também a comparação pareada de todas as partes. Para se avaliar o

efeito dos planos nutricionais sobre os parâmetros alométricos, uma abordagem *bootstrapp* foi desenvolvida a fim de criarem-se intervalos de confiança para os coeficientes estimados, e dessa forma testa-se a hipótese de igualdade do grupo de animais alimentados à vontade e restritos.

Literature review

The first studies on protein requirements for cattle were must likely conducted in the first decades of the 20th century. Those experiments were based on feed trials, therefore any protein recommendations were supported by cattle productive response when fed with feeds with known quantity of nitrogen, (i.e., protein) (Tedeschi et al., 2013). Later, with additional studies, the Subcommitte on Animal Nutrition, chaired by Dr. Mitchell in 1926 provided a detailed report with enough evidence that the protein composition, what means, different amino acids proportions, would influence protein digestibility and use (Mitchell, 1926). Therein, in 1929, the first guidelines for minimum protein requirements for cattle, based on a factorial approach, were outlined (Mitchell, 1929).

These first evidences of different protein demands and feed composition started a long research field on protein requirements and use by ruminants. This history was described in details by Tedeschi et al. (2013). Moreover, it is interesting to understand that researchers all around the globe started to investigate this theme, and however they presented some discrepancies between protein requirements for different species, production stages and regions, they all tended to share the same factorial approach. An effort to represent the intricate relationship between research centers investigating nutrient requirements is depicted in Figure 1 (Tedeschi et al., 2014).

From figure 1 is possible to understand that some studies had a major impact over nutrition models development, such as the classical papers of Blaxter (1962), NRC (1945a, 1945b), and Baldwin et al. (1977), which provided the fundamental basis for the development of British, and North American models, respectively. It is also possible to realize that, in the beginning of 21st century, the presence of horizontal lines become more frequent, what indicates a more intense exchange of information between models, and systems like LRNS (*i.e.*, Large Ruminant Nutrition System) arrived. This exchange of information may be improve model's prediction quality, since most likely will expand its use in different conditions. Such hypothesis was tested by Tedeschi et al. (2014) that evaluated different models using an independent dataset with information regarding milk production all around the world. In this occasion, the LRNS, level 2, presented the second best prediction.



Figure 1. Chronological evolution of mathematical nutrition models (red boxes) and key references (blue boxes). Year of publication or release is shown on the left. The green boxes represent models not yet released to the public. The solid line represents a direct relationship of influence, and the dashed line represents that at least one other version or edition was released in between the marks. References are: (A1) NRC(1945*a*, 1945*b*), (A2) Leroy (1954), (B1) Blaxter (1962), (B2) Van Soest (1963*a*, 1963*b*), (C1) Nehring *et al.* (1966), (C2) Lofgreen and Garrett (1968), (C3) Moe *et al.* (1970), (D1) Schiemann *et al.* (1971), (D2) Waldo *et al.* (1972), (D3) Hoffmann *et al.* (1974), (D4) Ministry of Agriculture, Fisheries and Food (1975), (D5) Van Es (1975), (E1) Baldwin *et al.* (1977), (E2) Baldwin *et al.* (1980), (F1) France *et al.* (1982), (F2) Gill *et al.* (1984), (F3) Fox and Black (1984), (F4) Conrad *et al.* (1984), (G1) Danfær (1990), (H1) Illius and Gordon (1991), (H2) France *et al.* (1992), (H3) Russell *et al.* (1992), Sniffen *et al.* (1992), and Fox *et al.* (1992), (H4) Dijkstra *et al.* (1992), Neal *et al.* (1992), and Dijkstra (1993), (H5) Tamminga *et al.* (1994), (J1) Nagorcka *et al.* (2000), (J2) Mills *et al.* (2001), (J3) Fox *et al.* (2004), (J4) Cannas *et al.* (2004), (K1) Bannink *et al.* (2006), (K2) Bannink *et al.* (2008), and (L1) Gregorini *et al.* (2013). RNS is the Ruminant Nutrition System. Adapted from (Tedeschi et al., 2014).

With small ruminants, this scenario is quite similar, and most of traditional nutritional systems adopted the same approach used by cattle researchers. One clear example of this fact is the evolution of Small Ruminant Nutrition System - SRNS (Tedeschi et al., 2010), which was first denominated as "The Cornell Net Carbohydrate and Protein System for Sheep, CNCPS-S" (Cannas et al., 2004), obviously, an allusion to the cattle nutritional system developed by Dr. Danny Fox and colleagues, CNCPS (Fox et al., 2004). In the same way, the Australian nutritional system, developed by the Commonwealth Scientific and Industrial

Research Organisation, CSIRO (2007), presented only different values for equations' coefficients used for all species in their compendium.

Nevertheless, this scientific field is far from an ending. Not just because some gaps still exist about protein metabolism, but because nowadays a new concern about protein usage has arrived, being the dietary nitrogen use efficiency the main subject, since its excretion in the environment contributes to the greenhouse effect (Koenig and Beauchemin, 2013; Waldrip et al., 2013) by increasing nitrous oxide production (Eckard et al., 2010).

In Brazil, there are few researchers in the field of nutrient requirements for ruminants. For cattle, the first nutritional Brazilian system was recently released (Valadares Filho et al., 2006), and was denominated Br-CORTE, with a large database, mainly dedicated to Zebu cattle. The second revised edition, with more data and crossings, was released four years later (Valadares Filho et al., 2010). On the other hand, for small ruminants, there is not a Brazilian system well defined, even though an increasing number of studies were conducted in the last decade (Resende et al., 2010) and some were published (Galvani et al., 2008; Regadas Filho et al., 2011a; Regadas Filho et al., 2011b; Regadas Filho et al., 2013).

Brazilian lamb production still incipient, what can be concluded in face of the low production (84 thousand tons/year) combined to a low per capita consumption (700 g/year), but in opposition, with a steady importation of meat from neighbors countries, such as Uruguay and Argentina. Notwithstanding, the Brazilian herd is not as small as its production and consumption (16.81 million heads; IBGE, 2010), what may indicate a low efficiency of conversion of animals in products. This condition is partially explained by the low level of technology applied by producers associated to the type of animals with natural low production, such as hair sheep.

One of the most common breeds in Brazil is the Santa Inês. This sheep is characterized by ewes with small to medium frame size, weighing around 50 kg when mature and in medium body condition score. Most likely, this animal is the result of crossings between Italian Bergamacia ewes and Brazilian northeastern native sheep, and present a good maternal ability, rusticity and adaptation to tropical conditions. Moreover, Santa Inês females are less sensitive to photoperiod, therefore allowing a more flexible window for reproduction. Consequently, this breed have been explored by Brazilian producers as dams in crossings with meat breeds, such as Dorper and Texel, where both male and female offspring are directed to slaughter. However due to the scarcity of information regarding Santa Inês nutrient requirements, technicians tend to associate their empirical experience to international nutritional recommendations, such as those from North American, Australian, British, and French committees. (e.g., Institute National de la Recherche Agronomique (1988); Agricultural and Food Research Council (1993); Commonwealth Scientific and Industrial Research Organisation (2007); National Research Council (2007)). Because of region discrepancies and probably because of animal type, those models, although present good accuracy have been pointed out as imprecise when evaluated with Brazilian sheep (Galvani et al., 2008; Regadas Filho et al., 2011a). This result is even worse when dealing with hair sheep, such as Santa Inês, due to its vast heterogeneity of phenotypes.

The knowledge of growth behavior of livestock animals is a key information for technicians that intend to produce meat. In a simple view, the growth is depicted as the increase in size, but this increase is not similar among all body parts (Widdowson, 1980). Classically, the body growth is divided as a function of tissues growth, where the skeleton is the first to develop, followed by muscle and adipose tissue (Fowler, 1980). Moreover, not all members develop together, so understand this phenomenon is a key step to achieve desired cuts and traits yields.

The study of allometry is classically used by biologists to better understand evolutionary shape and morphology of species by interpreting their relative growth of body parts (Stevens, 2009). This technique was basically classified into three categories: (i) Static or size allometry, (ii) ontogenetic or growth allometry, and (iii) evolutionary allometry (Klingenberg, 1996). It is obvious that when the interest is on growth pattern, the second technique is the most appropriate, by using longitudinal data, and/or cross-sectional data with different specimens in several known stages. Due to possible changes on rate of growth of different body parts for different experimental units at dissimilar stages of life, the linear bivariate approach proposed by Huxley (1932), often sufficient for explaining the relationship between body parts (Stevens, 2009), may not hold. Therefore, Klingenberg (1996) presented a multivariate technique using principal components which is supported by the frequent find that the first eigenvector (β_1) estimated by a principal component analysis (PCA), often contains the largest proportion of the total variance. This approach allows both comparisons between groups using β_1 estimated from absolute weights of parts and also the overall isometry between parts tested together. This way, this technique can be used to compare the growth of all parts in a one-step approach, and simultaneously investigate possible differences due to different treatments, such as nutritional managements or breeding selection.

Chapter 1 - Body composition and net protein requirement for weight gain of Brazilian hair ewe lambs and evaluation of international nutritional models

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ABSTRACT: This experiment was conducted to determine the net protein requirements for gain (NPg) of Santa Inês female lambs, and secondarily, evaluate international feed systems predictions for this characteristic. Fifty-seven weaned lambs were acquired from local farms. Twenty-one were slaughtered at the beginning of trial to give information about initial empty body weight and composition for the remaining animals, following comparative slaughter methodology. The latter lambs were assigned in a completely randomized design with a 2 x 3 factorial arrangement (two nutritional planes, ad libitum or restricted, versus, three slaughter weights, 20, 28 or 36 kg, six animals per group). Animals' body composition was assessed, and nutrients percentage and amount were modelled by means of Huxley's and von Bertalanffy's mathematical functions. The net protein requirements for gain was obtained from the first derivative of Huxley's function. Besides, the predictions from AFRC (1993), CSIRO (2007), NRC (1985), NRC (2007) and SRNS (2010) were evaluated. Lambs from restricted group presented lower intake compared to ad libitum (~30% less, P < 0.001), also lower weight at slaughter and smaller average daily gain (P < 0.001). The von Bertalanffy's growth function was successfully fitted to nutrient percentage on empty body weight, and provided valuable information regarding body composition changes. The estimated asymptotes were in a reasonable value for the evaluated animals. The net protein requirements derived from Huxley's function resulted in an average NPg of 12.5 g/100 g o EBW gain in animals with 30 kg of shrunk BW. The models evaluation showed that Santa Inês female lambs present a higher NPg compared to the international feed systems predictions. Moreover, the SRNS (2010) presented the best accuracy for NPg estimative (CCC = 0.948, r = 0.985, Cb = 0.963, RMSEP = 1.80 g).

Keywords: digestibility, modeling, nutrition, production, Santa Inês

1. Introduction

Historically, the production of small ruminants is highly associated to developing countries all around the world. In such places, raising sheep and goat, due to their multifunctional characteristic, represent a livestock activity that may support both financial and food security, mainly in smallholder systems (Hilali et al., 2011; Devendra and Liang, 2012; Oluwatayo and Oluwatayo, 2012). Nonetheless, the low production efficiency in this scenario, becomes a challenge in times of climate change and global policies to promote sustainable intensification (Thornton et al., 2009; Herrero et al., 2010; Garnett et al., 2013; Herrero et al., 2014; Vervoort et al., 2014). In this sense, information about feed quality and nutrient requirements of farm animals are essential to improve feed-use efficiency.

This situation is even worst in either developing countries or semiarid regions (Herrero et al., 2013). The caatinga is the predominant biome in the northeast of Brazil, characterized by a semiarid climate condition, with irregular rainfall distribution and low stocking rates (Santos et al., 2010). In this region prevails the biggest percentage of Brazilian sheep herd, where hair native breeds with small to medium mature size are typical, such as Santa Inês. It is believed that this genotype was originated from crossings between Italian Bergamacia ewes and Brazilian northeastern native breeds, and it is depicted as adult ewes with medium mature size, around 50 kg of live weight (LW) at body condition score of 3.0 (*i.e.* scale from 0 to 5). They are also known for its rusticity, good maternal ability and, adaptation to tropical conditions, being usually used in pure breed systems or on crossings with specialized meat breeds (Sousa et al., 2003). Besides, for this sheep, the photoperiod dependency for reproduction is less pronounced, which gives a big advantage and flexibility to farmers when use them as dams, justifying its popularity all over the country. However, information about nutritional requirements of this hair sheep, and others native breeds, are scarce in the literature(Regadas Filho et al., 2013).

Protein is a key nutrient in livestock systems since it respond to a large cost in farm input resources and at the same time, explain the value aggregation in the final products (*e.g.*, milk, meat, wool). Moreover, the waste of these compounds may cause in both economical and energy loss, also environmental impact (Montes et al., 2013). Rearing female lambs for either ewe replacement or to slaughtering, demands good knowledge of protein requirements, mainly for maintenance and tissue gain. For the last, its net requirement is highly dependent on body composition, thus on characteristics such as breed, gender, and life stage (Cannas et al., 2004). Although, due to the lack of information for Brazilian hair lambs requirements and body composition, diets formulation for these animals are mostly based on recommendations from

international committees (NRC, 1985a; AFRC, 1993; CSIRO, 2007; NRC, 2007). There are several differences among these feeding systems, such as feeds used, animals evaluated, and modeling approach as well (Tedeschi et al., 2013), which may interfere on requirements accuracy and prediction (Tedeschi et al., 2014).

The main objective of this work was to use body composition data from Santa Inês female lambs to estimate their net protein requirement for live weight gain. Secondarily, the feed systems from North-America (NRC, 1985a; NRC, 2007; SRNS;Tedeschi et al., 2010), United Kingdom (AFRC, 1993) and Australia (CSIRO, 2007) were evaluated regarding their precision and accuracy in predicting protein requirements for Santa Inês lambs weight gain.

2. Material and Methods

Animal procedures were approved by the Animal Experimentation Committee of Universidade Federal de Minas Gerais, Belo Horizonte, Brazil (Protocol 197/2010, Appendix B).

2.1. Location, animals and experimental design

The experiment was conducted, between December 2010 and March 2011, in the Laboratory of Metabolism and Calorimetry at Veterinary School of Universidade Federal de Minas Gerais, Brazil. Fifty-seven Santa Inês weaned female lambs, about three months age, were acquired from local farms. On arrival (d -15 ± 7), the lambs were brought to the working facility, where they were 1) weighed individually, 2) identified with a uniquely numbered ear tag, 3) vaccinated against *clostridium spp.* and 4) treated for parasites with closantel. Animals were assigned in three groups, lighter, intermediate and heavier, based on their initial live weight and body condition score (16.5 ± 2.4 , 21.3 ± 2.5 , 28.2 ± 1.87 kg, respectively). Within each group, 12 animals were randomly selected and divided in two groups of six lambs each, where the first (AL) had ad libitum access to diet (allowance of 15 % of refusal), whereas for the second (R) it was imposed an intake restriction initially targeted at 30 % of the computed daily intake of animals with unrestricted access to feed (*i.e.*, $[DMI_R = \overline{DMI_{AL}} \times 0.7, g/kg^{0.75}]$). The animals from lighter, intermediate and heavier groups were fattened to achieve the following slaughter weights: 20, 28 or 36 kg of LW. Every time an animal from AL group achieved its target weight, it and a previously selected animal from restricted group were simultaneously slaughtered. The animals were housed in a large room with controlled environment. They were allocated into individual metabolism cages provided with food, fresh water and salt containers. These cages also had a slated floor to allow excrete collection. Every morning, last day orts were collected and weighed, and first meal was offered, therefore daily intake could be computed. The remaining 21 lambs were slaughtered on day 0 of the trial. These animals' body composition were used to estimate initial empty body weight and composition for the animals that continued in the experiment.

2.2. Feeds, diet chemical composition and digestibility trial

The experimental diet (Table 1) consisted of corn meal, soybean meal, chopped Tifton hay (*Cynodon spp.*, 2 cm length) and minerals. Nutrient requirements were obtained from Small Ruminant Nutritional System (Tedeschi et al., 2010), simulating a condition of live weight gain around 200 g/d for animals weighing 30 kg BW. The roughage was cut in a stationary forage chopper before being fed. Concentrate and hay were offered simultaneously, and diet was split into two equal meals fed at 8:00 am and 4:00 pm. The amount fed was weekly adjusted, after lambs weighing.

Chemical Compostion, %Dry matter	Concentrate	Roughage	Diet
Amount (% as fed)	55.45	45.55	100.00
Dry matter	89.46	95.75	93.22
Ash	10.47	5.70	8.40
Crude protein	28.98	7.88	19.66
Neutral detergent fiber	16.75	66.96	39.79
Acid detergent fiber	5.82	38.53	20.78
Lignin	1.64	7.07	4.13
Fat	1.22	0.97	1.12
Non-fiber carbohydrate	44.20	19.84	33.55
Total digestible nutrients ²	72.55	52.04	63.93

Table 1 – Chemical composition of experimental diet

1 – Diet composition, % Dry matter: Corn meal = 26.06, soybean meal = 27.39, dicalcium phosphate = 0.08, limestone = 1.03, sodium-bicarbonate = 0.89, cynodon hay = 44.56. 2 – Calculated based on NRC (2001).

In the last week prior to slaughter, during five days, feed, refusals (whenever existed), feces and urine were collected, weighed and sampled (10% by day), being stored at -17°C. A solution of 6M HCl was daily added to the urine bucket in a quantity of 100 mL, to avoid nitrogen loss by volatilization. At the end of the collection period, each material was homogenized to form a composite by animal representing the five days of collection. All composites with exception of urine were air dried for 72h at 55°C, subsequently were ground

in a Wiley mill to pass a 1-mm screen. Urine density was measured using a refractometer. All samples were analyzed for gross energy (GE) using a bomb calorimeter, and also for nitrogen content (Kjeldahl method), following AOAC (1990). The diet metabolisable energy (ME, Mcal/kg) was computed by subtracting from feed GE, the gross energy presented on urine, feces and methane emission. The last one was estimated following recommendation of Blaxter and Clapperton (1965). A linear model was fitted to evaluate the relation between ME content and metabolic body weight (kg^{0.75}).

2.3. Slaughter procedure and body composition analyses

The slaughter day for each pair of animals (*i.e.*, one from AL and one from R group) was defined based on the expected day when the animal from AL group would reach its respective target weight (20, 28 or 36 kg LW). This prediction was possible based on the animal weekly weighing history. Feed was withdraw for 16 h previous to slaughter, when the shrunk BW was obtained, as well the BCS at slaughter accordingly to NRC (2007) scale. Lambs were stunned by percussion, hooked and exsanguinated by cutting main vessels of the neck, following common humane slaughtering procedures. Blood was collected in a bucket, weighed and sampled. All body components (carcass, head, fore and hind feet, hide, tong, traquea and esophagus, lungs, heart, rumen, reticulum, omasum, abomasum, small and large intestInês, liver, bladder, gallbladder, pancreas, kidneys, spleen, diaphragm, uterus, mammary gland, and visceral fat) were weighed separately and stored in cooling chamber (-17 °C). The digestive tract, also bladder and gallbladder were weighed before and after emptying, in order to obtain digestive content weight, thus, by subtracting its weight from SBW was obtained the empty body weight (EBW).

Lambs carcasses were split at the midline using a band saw. The right side half was passed three times through an industrial meat grinder (plate with 0.32 cm holes), homogenized and sampled. This same procedure was done with head, fore and hind feet and hide. Organs and viscera were cut in small pieces and sampled following proportional weight in relation to EBW. They were mixed and passed three times through meat grinder, homogenized and sampled. All components were air dried in oven (55 °C, 72 h), following immersion in petroleum ether for 48 h to be obtained the pre-defatted dry weight by weight difference. This material was ground to pass a 1-mm screen. Samples were analyzed for fat (final ether extraction was obtained in soxhlet apparatus), nitrogen content (Dumas combustion using LECO FP-528), and ash by complete combustion in a muffle furnace at 600°C for 6 h (AOAC, 1990).

2.4. Calculations

Total nutrients amount in EBW was calculated following equation 1.

$$NA_{i} = \sum_{j=1}^{n} BP_{ij} NP_{ij} / 100$$
 [1]

Where *NA* is the nutrient amount in the EBW of the i^{th} animal (g), *BP* is the weight of the j^{th} body part (g) in the i^{th} animal, and *NP* is the nutrient percentage on the j^{th} body part of the i^{th} animal (%), and *n* is the number of body parts.

The initial EBW of animals that continued in the experiment was estimated based on a linear model regression of all animals EBW against their SBW, as depicted on equation 2.

$$EBW_i = \alpha + \beta \times SBW_i + \varepsilon_i$$
[2]

Where *EBW* is the estimative of empty body weight for the *i*th animal (kg), *SBW* is the shrunk body weight of the *i*th animal (kg), whereas α is the intercept (kg), β is the slope (dimensionless) and ε_i corresponds to the random error associated to the *i*th observation.

Due to the asymptotic nature of body components growth, the von Bertalanffy's nonlinear function was fitted to data in order to predict water, ash, protein and fat percentages relative to EBW, following equation 3. Models' parameters were estimated by least square method, using Gauss-Newton algorithm.

$$NP_{ij} = \alpha_j \times (1 - \beta_j \times exp^{(-\kappa_j \times EBW_i)})^3 + \varepsilon_{ij}$$
[3]

Where, *NP* is the *j*th nutrient percentage in a specific *EBW* (kg) of the *i*th animal, α is the asymptote (%), β is a constant related to the intercept and its sign defines whether NP will increase or decrease with the change in the values of EBW (%), and κ is the deposition rate (1/%).

Net protein requirements for growth were estimated according to ARC (1980) by establishing the allometric relationship between protein amount (g) and EBW (kg), by means of Huxley (1932) mathematical function, as follows (Equation 4). Allometric coefficients were estimated by least square method, using Gauss-Newton algorithm. To calculate protein accretion on EBW, thus net protein requirements for gain (NPg), the first derivative of Huxley's function was used, as presented on equation 5.

$$PA_i = \alpha \times (EBW_i)^{\beta} + \varepsilon_i$$
[4]

$$NPg = \hat{\alpha} \times \hat{\beta} \times (EBW)^{\hat{\beta}-1} \times ADG \qquad [5]$$

Where *PA* is protein amount (g) in the *EBW* (kg) of the *i*th animal, α and β are allometric coefficients, while ε represent the random error associated to the *i*th observation. *NPg* is protein (g) accreted into *EBW* (kg) accordingly to an average daily gain (ADG, kg), based on parameters estimated by equation 4.

2.5. Models evaluation

The estimated net protein requirements for gain computed for each animal in the present study, by means of equation 5, was used to evaluate five feed systems: AFRC-1993, CSIRO-2007, NRC-1985, NRC-2007 and SRNS-2010. The equations used to compute the requirements by each committee are presented in equations 6 to 11.

AFRC (1993)
$$NPg_{AFRC} = ADG(156.1 - 1.94BW + 0.0173BW^2)$$
 [6]

$$L = \frac{MEI}{(0.062SBW^{.75}e^{-0.03})/.644}$$
[7]

CSIRO (2007) and
$$P = BW/50$$
 [8]
SRNS (2010)

$$NPg_{CSIRO/SRNS} = ADG\left(212 - 8(L-1) - \frac{A - 8(L-1)}{1 + e^{-6(P-0.4)}}\right)$$
[9]

NRC (1985)
$$NPg_{NRC85} = ADG \left(268 - 29.5 \frac{317BW^{.75}ADG}{(ADG \times 1000)} \right)$$
[10]

NRC (2007)
$$NPg_{NRC07} = 0.92ADG\left(0.256 - 0.0670\left(27/(1 + e^{-6(P - 0.4)})\right)\right)$$
 [11]

Where NPg is the net protein requirement for gain (g), ADG is the average daily gain of full body weight (kg) and, BW is the body weight (BW). The L factor, necessary for CSIRO-2007, SRNS-2010 and NRC-2007 systems, stand for the intake of metabolisable energy above maintenance requirement. The latter was estimated following NRC (2007) recommendations, while the former was obtained by calculating the daily intake of metabolisable energy during the digestibility trial. The P parameter stands for the maturity index, and is calculated by the

rate between current BW and BW at mature size, which was assumed as 50 kg in the present study. The *A* parameter in Equation 9 assumes the value of 120 in SRNS model and 140 in CSIRO.

Models evaluations were performed by plotting and regressing the observed values for net protein accretion on the EBW of each animal over the predicted by each system (Equation 12).

$$Y_i = \beta_{0_i} + \beta_{1_i} \times f(x)_{ij} + \varepsilon_i$$
[12]

Where, *Y* is the *i*th observed value, β_0 and β_1 are the intercept and slope, respectively, f(x) represents the *i*th output from the *j*th model, while the ε coefficient stands for the random error (*i.e.*, residue) associated to the ith paired data point, which is independent and identically distributed ~ N (0, σ^2).

The fitted linear regression coefficients β_0 and β_1 were tested for the null hypothesis of equality to 0 and 1, respectively. Additionally, were computed the following statistics as suggested by (Tedeschi, 2006): Mean square error of prediction (MSEP), Root mean square error (RMSE), Mean bias (MB), and, Concordance correlation coefficient (CCC) decomposed in correlation coefficient (r) and bias correction factor (C_b), in order to evaluate model precision and accuracy, respectively. These analyses were performed by means of the Model Evaluation System (http://nutritionmodels. tamu.edu/mes.htm, verified November 2014).

2.6. Statistical analyses

Variables obtained through digestibility trial and for body composition were analyzed as a completely randomized design with a 2 x 3 factorial arrangement. The statistical model used is shown below:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$
[13]

Where y is the measured variable for the *i*th nutritional plane in the *j*th slaughter weight for the *k*th repetition, μ is the overall mean, α_i is the fixed effect, β_j is the fixed effect for, $(\alpha\beta)_{ij}$ is the interactive effect, and ε_{ij} represents the error term. The degree of freedom (df) for this model includes 5 df for nutritional planes plus slaughter weight (*i.e.*, 1 df for diet, 2 df for live weight at slaughter, and 2 df for interaction). Linear and quadratic contrasts were used for the effect of slaughter weight (20, 28 or 36 kg LW) within each nutritional plane.

All analyzes were performed in R environment (R Core Team, 2014).

3. Results and discussion

3.1. Animals performance and diet digestibility

Results of animal performance and digestibility trial are presented in Table 2. Even though animals from different regimens started the trial with the same weight (P > 0.05), nutritional restriction did reduce average daily gain (ADG), thus reducing shrunk body weight at slaughter weight (P < 0.05). Body condition score, however, was not affected by regimen, but was linearly increased as lambs got heavier. The average daily gain was slightly lower than the predicted by SRNS at the moment of diet calculation (*i.e.*, 200 g/d). However not evaluated in this paper, similar result was found by Regadas Filho et al. (2011a) who worked with Santa Inês male lambs, and conclude that in average, SRNS tended to overestimate average daily gain for this sheep. On the other hand, Galvani et al. (2008) working with Texel crossbred lambs in Brazilian conditions found that CNCPS-S ((Cannas et al., 2004), latter revised and renamed to SRNS by Tedeschi et al. (2010)) under predicted the ADG of these lambs. These authors agreed that such differences might be the result of both animal and environmental discrepancies between Brazilian scenery and that one used throughout SRNS development. Moreover, these results indicate that even a mechanistic approach such that used by SRNS may result in inaccuracy and imprecision when evaluated using independent data sets, therefore it is fundamental to evaluate such models constantly so they can evolve.

As expected, dry matter intake was reduced by influence of nutritional plane, in an average ratio of 73% of intake of animals from the AL group (g/kg^{0.75}), close to designed restriction (*i.e.*, 30%). Nevertheless, all registered intake were in the range of DMI observed in the data bank collected by Vieira et al. (2013) (*i.e.*, 2.3 to 5.4% LW) that conducted a meta-analysis to study the intake of Santa Inês male lambs reared in Brazilian feedlot condition. In addition, DMI was also quadratically reduced in animals with unrestricted access to feed (P < 0.001) as slaughter weight increased. This result is in agreement with CSIRO (2007) approach regarding voluntary intake.

Item	Ad libitum				Restricted						P-Values			
	LW-20	LW-28	LW-36	LW-20	LW-28	LW-36	Pooled SEM	Regimen	LW	Interaction	Ad libitum		Restricted	
											L	Q	L	Q
IBW (kg)	18.60	21.47	27.58	16.87	23.20	28.88	0.724	0.469	< 0.001	0.046	< 0.001	0.077	< 0.001	0.716
SBW (kg)	19.92	27.80	35.78	18.17	26.49	33.62	0.897	0.024	< 0.001	0.893	< 0.001	0.964	< 0.001	0.590
ADG (g/d)	56.99	139.53	162.13	54.21	95.04	70.28	11.320	< 0.001	< 0.001	0.002	< 0.001	0.039	0.3234	0.025
BCS (1-5)	2.08	3.00	4.25	2.08	3.17	3.83	0.139	0.467	< 0.001	0.113	< 0.001	0.334	< 0.001	0.229
DMI (g/d)	970.62	934.01	1127.08	653.04	661.65	744.74	31.766	< 0.001	< 0.001	0.227	0.002	0.006	0.047	0.339
DMI (g/kg ^{0.75} /d)	101.88	75.57	73.59	76.58	55.13	52.96	2.065	< 0.001	< 0.001	0.435	< 0.001	< 0.001	< 0.001	0.001
MEI (kcal/d)	2946.23	2826.13	3581.13	1829.43	1974.06	2362.86	145.246	< 0.001	< 0.001	0.429	0.006	0.02	0.013	0.491
ME (Mcal/kg)	2.76	3.03	3.18	2.81	2.99	3.00	0.070	0.155	0.001	0.324	< 0.001	0.508	0.063	0.326
N Intake (g/d)	35.36	31.96	40.30	20.68	20.91	23.44	1.335	< 0.001	0.001	0.1	0.017	0.001	0.148	0.481
N feces (g/d)	22.88	13.48	19.16	9.67	6.41	10.31	1.545	< 0.001	0.002	0.174	0.132	< 0.001	0.754	0.082
N feces (% of NI)	60.77	41.95	47.17	46.14	31.99	44.76	3.777	0.062	0.002	0.282	0.028	0.013	0.784	0.01
N urine (g/d)	7.35	8.1	11.01	5.65	8.67	9.79	0.938	0.266	0.001	0.454	0.01	0.337	0.005	0.427
N urine (% of NI)	21.15	25.58	27.69	27.17	40.53	40.68	2.827	< 0.001	0.003	0.28	0.109	0.728	0.003	0.075
N balance (g/d)	14.28	10.37	10.13	6.3	9.41	6.69	2.235	0.038	0.71	0.305	0.213	0.508	0.902	0.288
N bal. (% of NI)	42.39	32.47	25.14	31.22	44.9	28.81	8.127	0.745	0.321	0.362	0.157	0.897	0.833	0.139

 Table 2 – Animal performance and digestibility trial results

IBW = Initial body weight, SBW = Shrunk body weight, ADG = Average daily gain, BCS = Body condition score, DMI = Dry matter intake, MEI = Metabolisable energy intake, ME = Metabolisable energy content, N = Nitrogen, NI = Nitrogen Intake, N bal. = N balance = N Intake - (N feces + N urine). L = Linear, Q = Quadratic.

This committee assumes that potential intake increases in a quadratic fashion as animals become mature, achieving the maximum when they reach 85% of mature weight, in a scale denominated relative size. It is believed that Santa Inês ewes have mature weight around 50 kg, what corroborates the last inference, since the heavier animals in the present study would be close to 85% for relative size.

Since the same diet was given to all animals, nutrients absolute intake accompanied the DMI, as may be seen from results for metabolisable energy intake and nitrogen intake. However, ME content was linearly affected by slaughter weight (P < 0.1), getting higher as animals become heavier. This relationship was modelled by a linear first order regression between ME content and body metabolic weight, that presented the following results:

$$ME_{(Mcal_{kg})} = 2,3142 \ (\pm 0.142) + 0.0543 \ (\pm 0.012) BW^{0.75}; \ r^2 = 0.43, RMSE = 0.15_{Mcal_{kg}}, P < 0.001.$$
[14]

This increase in diet metabolisability may be related to a more developed gastro intestinal tract, as reported by Cavalcanti et al. (2014), thus improving feed-use efficiency. Otherwise, it can also be a result of diet selection, since heavier animals received a larger portion of feed, they tend to eat more concentrate and refuse roughage, this way increasing the metabolisable energy of diet by increasing concentrate portion on actual intake. Indeed, the effect of age over diet metabolisability is well documented (Vermorel and Bickel, 1980), and energy digestibility tends to be lower in growing animals compared to adults, mainly in lambs. However, none feed system allows any correction in this sense.

Nitrogen excretion, feces plus urine, did not follow the same pattern of N intake, but in general were affected by nutritional plane, where N on feces was lower in an absolute scale for animals from the R group (P < 0.001) and also tended to be lower in a relative manner (P = 0.062), whereas for urinary N, only the relative excretion was influenced, being higher for animal under restriction. Nitrogen in urine was also increased as slaughter weight increased.

Item	Ad libitum				Restricted		_	P-Values						
	LW-20 LW-28 LW-36		LW-36	LW-20	Pooled LW-28 LW-36 SEM Regimen LW Interaction <u>Ad libitum</u>		LW-36 Pooled EW-36 SEM		Ad libitum		Restricted			
											L	Q	L	Q
Water	65.37	52.1	50.49	67.37	52.53	49.2	1.543	0.766	< 0.001	0.573	< 0.001	0.004	< 0.001	0.005
Protein	17.26	15.8	14.7	16.88	16.09	16.33	0.544	0.254	0.022	0.19	0.002	0.793	0.474	0.449
Fat	12.76	28.15	30.96	10.91	27.29	30.06	1.398	0.301	< 0.001	0.922	< 0.001	0.001	< 0.001	< 0.001
Ash	4.61	3.95	3.86	4.84	4.09	4.41	0.200	0.071	0.003	0.554	0.012	0.263	0.141	0.037

Table 3 – Body composition in percentage of empty body weight

LW = Live Weight, SEM = Standard error of the mean, L = Linear, Q = Quadratic.

Regimen also influenced on nitrogen balance, being lower for animals in restricted group (g/d, P < 0.05). However, in relation to N intake, balance was not affected by neither slaughter weight nor by regimen. Ruminants have a notorious ability to retain nitrogen in levels necessary to maintain both rumen microbial activity and also support host maintenance requirements (Obitsu and Taniguchi, 2009). Starke et al. (2012) showed that goats' kidneys responds to a lower nitrogen income by upregulating urea transporters in renal cortex, thus increasing urea reabsorption. In the present study, lambs under restriction presented a bigger excretion of nitrogen in urine compared to AL animals, therefore, differing from last authors finds. Notwithstanding, even restricted animals from this work received a large amount of nitrogen since diet had a high crude protein concentration (> 19% on DM basis). Therefore, it is possible that the imposed restriction was more effective in reducing energy availability than crude protein to rumen microbes, thus causing an asynchrony between nitrogen wastage (Hristov et al., 2005), probably overcoming kidneys capacity of reabsorption in restricted animals.

3.2. Empty body weight composition

The equation for empty body weight estimative is presented below.

$$EBW = -2.1093(\pm 0.5055) + 0.8899(\pm 0.02) \times SBW; r^2 = 0.97, RMSE = 1kg, P < 0.001$$
 [15]

Following this equation, EBW of animals with 20 and 40 kg of SBW would be 15.7 and 33.49 kg, respectively, thus between 78 and 83% of SBW. This result is slightly lower than the value adopted by SRNS model, where the EBW is computed as a fixed rate of 85.1% of SBW (Cannas et al., 2004). On the other hand, this values are close to the one found by Regadas Filho et al. (2011b) (*i.e.*, 80.36% of BW) who worked with Santa Inês male lambs in a similar slaughter weight range.

The body composition is presented in Table 3. It is possible to notice that none of body components were affected by regimen when evaluated as a percentage of empty body weight. However, in relation to live weight, all components, with exception of fat tended to decrease as body weight got heavier.

The results for von Bertalanffy's parameters fitted to water, protein, fat and ash percentage on empty body weight are presented in Table 4, and depicted in Figure 1. All models presented a satisfactory adjustment, with lower errors of prediction, as shown by the lower values of RMSE. Moreover, the high values for correlations between predicted and observed values denote a high precision. These results indicate that the growth model used is sufficient to explain body composition changes of growing lambs. Additionally, the estimated values for β parameter in each model shows that only for fat, this coefficient had a positive value, what corroborates the fact that as animals become heavier, fat percentage tends to get higher whereas the others, although being deposited on tissue gain, are accreted in lower rates, thus having their proportion reduced as EBW increases. In addition, the significance of parameter α indicates that all nutrients were reaching a plateau as animals got heavier. This asymptotic-like pattern is typical for animals getting close to maturity, when all tissues weight tends to became steady. In Figure 1, the x axis (*i.e.*, empty body weight, kg) was extended to values not found in the present data bank. This approach was used to evidence the asymptote in each fitted model. Also, the dashed lines shows the exactly value of α . It is possible to notice that, with exception of protein, the heavier animals in data set were close to the asymptote. Furthermore, it is also possible to realize that all lambs would have all nutrients percentage steady when they reach an EBW around 50 kg, what agrees with the common find of Santa Inês ewes with this body weight when adults. Obviously, a more complete data bank, with animals slaughtered in more advanced ages would be necessary to confirm this technique, since extrapolations out of explored x-space are somewhat dangerous (Draper and Smith, 1981). However, these results indicate that this approach may be used with success to define a mature weight of a population based on their body composition. Moreover, this modeling process can be useful to predict body composition as function of body weight, and probably, adding covariables to this model, such as body condition score, would improve its accuracy and precision.

Nutrient	Von Be	ertalanffy's para	imeters	DMCE (0/)		DCE
	α	β	κ	- KMSE (%)	Г	KSE
Water	46.948 ± 2.964	$\textbf{-0.513} \pm 0.171$	0.114 ± 0.039	4.67	0.81	4.793
Protein	24.865 ± 3.960	$\textbf{-0.818} \pm 0.118$	0.085 ± 0.027	4.44	0.88	4.558
Fat	63.952 ± 2.843	1.640 ± 0.652	0.164 ± 0.033	5.90	0.89	6.062
Ash	7.206 ± 0.872	-1.447 ± 0.401	0.128 ± 0.033	1.82	0.85	1.867

Table 4 – Von Bertalanffy's function fitted to water, protein, fat and ash percentage on empty bodyweight.

RMSE = Square root of mean square error, r = correlation between observed and predicted values, RSE = Model's residual standard error.

The fitted Huxley's function to protein amount on EBW and estimative of protein net requirements, following equation 4 and 5 resulted in the equations below:

$$PA = 290.04(\pm 34.49) \times EBW^{0.813(\pm 0.038)};$$

$$r^{2} = 0.897, RMSE = 289.51 g, P < 0.001$$
[16]

$$NPg = 290.04 \times 0.813 \, EBW^{-0.187} \times ADG$$
 [17]

Equation 16 presented a good adjustment, with a high coefficient of determination, and relative low square root of mean square error. From these equations is possible to estimate net protein requirements for gain, as presented in Table 5. The values for protein requirements are quite close to the ones found by Regadas Filho et al. (2011b) that worked with Santa Inês lambs in similar condition. In fact, these authors presented slightly higher values for protein accretion on empty body weight gain, what is appropriate since they worked with males. From Table 5 is also possible to observe that protein content on EBW gain tends to decrease as animals get heavier. This result corroborates the ones obtained through modelling body composition using von Bertalanffy's growth function. Such pattern was also found by Silva et al. (2010) and Gonzaga Neto et al. (2005) who worked with Santa Inês castrated males lambs in Caatinga condition and with Morada Nova lambs (*i.e.*, other hair lamb genotype), respectively. As well as Regadas Filho et al. (2011b), these authors found a slightly higher net protein requirements for gain than the ones showed in the present study, what can be partially explained by gender effect.



Figure 1 – Von bertalanffy's function (continuous line) fitted to percentage of fat (red dots), protein (black dots) and ash (blue dots) on empty body weight free of water. The dashed lInês represent the estimate asymptote of each model.

SDW (Irg)		CP on	CP on NPg as function of ADG (kg) and EBW (kg)							
SD W (Kg)	EDW (Kg)	EBW (g/kg)	0.050	0.100	0.150	0.200	0.250			
20	15.691	165.74	6.739	13.478	20.217	26.956	33.695			
25	20.140	158.97	6.464	12.928	19.392	25.856	32.319			
30	24.590	153.65	6.247	12.495	18.742	24.990	31.237			
35	29.040	149.29	6.070	12.140	18.210	24.280	30.351			
40	33.490	145.61	5.921	11.841	17.762	23.682	29.603			

Table 5 – Protein on empty body weight and net protein requirements for weight gain of Santa Inês ewe lambs

 \overline{SBW} = Shrunk body weight, \overline{EBW} = Empty body weight, \overline{CP} = Crude protein, \overline{NPg} = Net protein requirements for gain, \overline{ADG} = Average daily gain.

3.3. Models evaluation

As pointed out by Tedeschi et al. (2014) most intercomparisons of the adequacy of livestock mathematical models' predictions are made only as needed, and often are conducted in order to promote a single model rather than highlight important gaps and models' application in different scenarios. In this sense, such comparisons tend to overvalue the use of a specific model, mostly when its accuracy and precision is evaluated using datasets similar to those ones used throughout model development. However, recently, nutritional models development has been based on mechanistic and stochastic approach, which may provide a more generalist application (Baldwin, 1995). Although, most models evolved their mechanistic approach more on the nutrient supply side than in nutrient requirements or efficiency of use of protein, and old values of reference (ARC, 1980; NRC, 1985b; CSIRO, 1990). still being adopted (Tedeschi et al., 2013). Such imbalance was mainly caused because there was a greater advance in the field of feed analysis and nutrient supply with the advent of carbohydrate and protein fraction systems (Russell et al., 1992; Fox et al., 2004), that was not followed by research on protein requirements. With the global pressure for nitrogen wastage mitigation in livestock systems, most likely, more researchers will engage on protein metabolism studies and new data will surge in the upcoming years (Eckard et al., 2010).

Regarding protein requirements for sheep live weight gain, the evaluated models in this study use a common approach based on the protein content in empty body weight. Moreover, the most recent models (*i.e.*, NRC (2007), CSIRO (2007) and SRNS (2010)) added a correction factor for relative size, thus animals with different frame sizes can be better compared, and the last two, included a factor for interaction between protein and energy metabolism. As depicted on Figure 2, the NRC (1985) and SRNS (2010) seems to be the most accurate models, since the quite homogenous scattering around Y=X line. In fact, the first one was the sole model that

presented equality to 0 and 1 for the estimated values of α and β , respectively, in the linear regression analysis (P > 0.05). This result indicates that this model may have good accuracy in predicting protein requirements for animals in the live weight range studied. This inference is corroborated by the value of Cb, which evaluate accuracy, and for this model was the bigger among the ones evaluated (Table 6). In this statistic, NRC (1985) was followed by SRNS (2010), that also presented the lowest value for MSEP, RMSEP, MB (*i.e.*, absolute value) and the biggest for CCC and r. These results indicate that, although SRNS (2010) presented the intercept and slope different from 0 and 1, respectively, in the linear regression analysis, in a more holistic view, it tends to be more accurate and also more precise, the last one due to bigger correlation, and consequently, a coefficient of determination. For precision, however, AFRC (1993) showed to be the more constant, which is a result of the simplest approach adopted by this system, where the protein requirement is only determined by a direct relation to body weight (equation 6). In spite of that, this type of error can be easily corrected by employing a correction factor.

 Table 6 – Model's adequacy comparison.

Model	Mean	SD	Median	MCED	DMCED	MD	Ch	_	000
Observed	12.6386	6.0427	12.0261	MSEP	KMSEP	MB	CD	r	uu
AFRC (1993)	10.9576	5.2361	10.3878	3.4788	1.8652	1.681	0.948	0.9996	0.948
CSIRO (2007)	10.2323	4.3819	9.7137	11.0273	3.3207	2.4063	0.861	0.9504	0.818
NRC (1985)	14.1621	6.2726	13.2002	4.1942	2.0480	-1.5235	0.97	0.9752	0.946
NRC (2007)	10.6213	4.5194	10.2134	10.4496	3.2326	2.0173	0.895	0.9223	0.826
SRNS (2010)	11.4532	5.0658	10.8524	3.2516	1.8032	1.1855	0.963	0.9846	0.948

 $[\]overline{SD}$ = Standard deviation (g), MSEP = Mean square error of prediction (g², smaller is better), RMSEP = Square root of MSEP (g, smaller is better), MB = Mean bias (g, closer to zero is better), Cb = Bias correction factor (closer to one is better), r = Correlation (closer to one is better), CCC = Concordance correlation coefficient (*Cb* × *r*, closer to one is better).

Nevertheless, all models predicted protein requirement quite close to the observed in the present study, what is partially revealed by the low values found for RMSEP, never bigger than four grams per day. However, it is possible to notice that with exception of NRC (1985), all models presented a positive MB and lower mean and median values of prediction compared to observations, which meant that, it is almost unanimous that observed protein requirements for hair sheep are bigger than international systems' predictions. These results are in agreement with a review of nutrient requirements for hair sheep, where Resende et al. (2005) suggested that protein requirements for the latter are higher than for wool sheep. This higher protein deposition is probably related to the finding that carcasses of hair sheep tend to be leaner

compared to wool sheep (Garcia et al., 2000; Gutiérrez et al., 2005), thus, proportionally, the protein amount is greater in body weight gain.



Figure 2 – Models evaluation by regression of observed values on predicted by each system. Symbols after coefficients denote difference from 0 and 1 for α and β , respectively. '*'= P < 0.1, '**'= P < 0.1, '**'= P < 0.01.

4. Conclusion

Santa Inês female lambs present a sigmoid asymptotic pattern for nutrients deposition on empty body weight. Such phenomena can be modelled by using growth function, such von Bertalanffy's model. Moreover, this Brazilian hair lamb have a higher protein requirement when compared with international feed systems recommendations. However, all models evaluated presented close recommendations for net protein requirements, but a mechanistic approach such the one presented by SRNS (2010) resulted in greater accuracy. Further research is needed to investigate the efficiency of dietary protein use and to assign dietary recommendations for this breed.

5. Conflict of interest

The authors declare that there is no conflict of interest.

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Chapter 2 - Evaluation of nutritional plane and slaughter weight effect on carcass characteristics and multivariate allometric growth of Brazilian hair ewe lambs

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ABSTRACT: Studies on growth of sheep are necessary to understand their nutritional requirements as well as their production. Therefore, this research aims to evaluate the effect of slaughter weight and feeding management on carcass and body development of Santa Inês female lambs under two nutritional planes. A 2x3 factorial arrangement was used to randomly allocate 36 Brazilian hair ewe lambs in two nutritional regimens (restricted or ad libitum access to food) and at three slaughter weight (20, 28 and 36 kg). Linear models were fit to assess nutritional and slaughter weight effects on body traits, carcass yields and composition. Also, a multivariate allometric study was performed to visualize the relationship between body parts associated to nutritional regimen during growth. Concurrent with an increase of slaughter weight body condition score, fat thickness, visceral fat depots, cold carcass weight, cuts and carcass composition also increased. Nutritional plane influenced hot and cold carcass weights $(P \le 0.002)$, as well as hindlimb, blade, rib/flank and neck, which presented lower weights for restricted animals compared to *ad libitum* ones (P < 0.05). Cooler shrinkage, dressing percentage and Longissuus Dorsi area were not affected by regimen. All fat depots linearly increased as slaughter weights raised, but were concomitantly influenced by nutritional plane (P < 0.01), revealing a significant interaction effect (P < 0.01). The allometric study revealed that body parts grow in different rates and nutritional plane influences some parts such as ribs/flank. Moreover, fat distribution among depots is not isometric, and a higher nutritional regimen may drive the energy intake to visceral fat rather than to carcass. Even though the nutritional treatment influenced average daily gain, the imposed restriction in this study caused minor effects on carcass traits yields and allometry, but, restricted animals presented a better balance on fat distribution, what indicates that international nutritional systems may overestimate nutrient demands for Brazilian sheep and possibly reduce livestock system efficiency.

Keywords: allometry, nutrition, production, Santa Inês

1. Introduction

Brazilian lamb meat production is about 84.4 ton a year, but its consumption is less than 700 grams per person (IBGE, 2007; FAO, 2011; MAPA, 2014). Eating lamb is not a common habit in Brazil mainly because of Brazilian cultural differences, offer irregularity, bad meat quality and poor commercial presentation, and therefore its salability is questionable. However, there is still space to increase production and market opportunities to attract consumers, which is confirmed by the constant import flow of sheep from traditional neighbor producers such as Uruguay and Argentina. Thus, there is a high demand to produce high quality meat, with leaner carcasses, thereby stimulating studies on carcass yield and meat quality (Díaz et al., 2006). It is known that females have greater proportion of fat, in the carcass and in internal depots, and lower proportion of bone and muscle when compared to males (Al-Owaimer et al., 2013). Slaughter weight and breed genotype (i.e., milk production breed or meat production breed) also affect fat distribution on carcass, being greater in females, justifying a lower slaughter weight for ewe lambs in order to avoid too much carcass fat (Al-Owaimer et al., 2013; Díaz et al., 2006; Hammell and Laforest 1999). Nevertheless, this type of evaluation is rare in hair sheep.

When there is herd stabilization (*i.e.* number of animals) or an increase in market demand, slaughtering female lambs may be both necessary and economically viable, although, there is a paucity of information on carcass yield and meat production when dealing with female lambs. Furthermore, feedlots with female lambs should have a different strategy for slaughter age because, among other differences, they tend to mature earlier (Hopkins et al., 2007). However, such decision has to be supported by a good knowledge of carcass growth and body composition (Tedeschi et al., 2004). this information are quite obscure when working with hair sheep. For this type of lamb it is believed that fat deposition is primarily accreted on internal depots rather than on carcass, as a physiological strategy for energy storage, as occurs with new world camelids and fat-tailed sheep. These types of animals can use subcutaneous and intramuscular fat for energy supply, and also mobilize rapidly the fat accumulated in the tail when facing feeding scarcity (Ben Salem et al., 2011). This metabolic pathway, despite representing an evolutionary step for survival of these species, may drive the uptake of metabolisable energy (ME) to these non-marketable tissues (*i.e.* visceral fat depots), reducing meat production efficiency. This hypothesis has been tested in other genotypes (Abouheif et al., 2013; Rios-Rincon et al., 2014) and it has been shown that growth and body composition are largely influenced by nutrition, and also that fat depots are metabolically independent (Kenéz et al., 2013; Samadi et al., 2013). However, specific information regarding Santa Inês female lambs is scarce.

The study of allometry is classically used by biologists to better understand evolutionary shape and morphology of species by interpreting their relative growth of body parts (Stevens, 2009). This technique was basically classified into three categories: (i) Static or size allometry, (ii) ontogenetic or growth allometry, and (iii) evolutionary allometry (Klingenberg, 1996). It is obvious that when the interest is on growth pattern, the second technique is the most appropriate, by using longitudinal data, and/or cross-sectional data with different specimens in several known stages. Due to possible changes on rate of growth of different body parts for different experimental units at dissimilar stages of life, the linear bivariate approach proposed by Huxley (1932), often sufficient for explaining the relationship between body parts (Stevens, 2009), may not hold. Therefore, Klingenberg (1996) presented a multivariate technique using principal components which is supported by the frequent find that the first eigenvector (β_1) estimated by a principal component analysis (PCA), often contains the largest proportion of the total variance. This approach allows both comparisons between groups using β_1 estimated from absolute weights of parts and also the overall isometry between parts tested together. This way, this technique can be used to compare the growth of all parts in a one-step approach, and simultaneously investigate possible differences due to different treatments, such as nutritional managements or breeding selection.

This study aims to evaluate the effect of slaughter weight and feeding management on carcass and body development of Santa Inês female ewe lambs under two nutritional planes.

2. Material and Methods

Animal procedures were approved by the Animal Experimentation Committee of Universidade Federal de Minas Gerais, Belo Horizonte, Brazil (Protocol 197/2010, Appendix B).

2.1. Location, animals and experimental design

The experiment was conducted, between December 2010 and March 2011, in the Laboratory of Metabolism and Calorimetry at the Veterinary School of Universidade Federal de Minas Gerais, Brazil. Thirty-six Santa Inês female lambs were used in this study. This genotype is a prevalent Brazilian hair sheep, and originated from crossings between Italian Bergamacia ewes and Brazilian northeastern native breeds, with a medium mature size, around

60 kg of live weight (LW) for adult ewes with medium body condition score (*i.e.*, 3.0). Known for its rusticity and adaptation to tropical conditions, it is usually used as a pure breed or in crossings with specialized meat breeds (Sousa et al., 2003). Besides, for this breed, the photoperiod dependency for reproduction is less pronounced, which gives a big advantage and flexibility to farmers when using them as dams. The latter justifies this breed being scattered throughout the country.

On arrival (d -15 \pm 7), the lambs were taken to the working facility, where they were 1) weighed individually, 2) identified with a uniquely numbered ear tag, 3) vaccinated against *clostridium spp.* and 4) treated for parasites with closantel. The ewe lambs were stratified by LW in three categories (initial LW of 17.7 \pm 2.1, 22.3 \pm 1.7 and 28.2 \pm 1.9 kg), and every two animals from each category were assigned randomly to one of dietary treatments, *ad libitum* or restricted. The animals were housed in a large room with controlled environment that guaranteed continuous climate condition. They were allocated into individual metabolism cages provided with food, fresh water and salt containers. These cages also had a slated floor to allow excrete collection. Every morning, last day orts were collected and weighed and first meal was offered, therefore daily intake could be computed.

2.2. Feeds and diet chemical composition

Concentrate and roughage were offered simultaneously, split into two equal meals offered at 8:00 am and 4:00 pm. The diet composition is presented in Table 1 and it was mainly composed by corn meal, soybean meal and Tifton hay (*Cynodon spp.*, chopped, 2 cm length). The estimated nutrient requirements for the animal category used in this experiment was calculated by means of the SRNS model (Tedeschi et al., 2010) which has predicted an average daily gain of 200 g for the animals with *ad libitum* access to the diet evaluated. The amount of feed to be offered was determined by *ad libitum* animals, to which was provided enough food to allow at least 15% of refusal. On the other hand, 30% less food than the computed intake (g/kg^{0.75}) by animals fed in *ad libitum* regimen was provided to restrict animals. The amount offered was corrected on a weekly basis due to weighing intervals. In this way, a quantitative restriction based on feed intake was created.

Chemical Compostion, %Dry matter	Concentrate	Roughage	Diet
Amount (%)	55.45	45.55	100.00
Dry matter (%)	89.46	95.75	93.22
Ash (%)	10.47	5.70	8.40
Crude protein (%)	28.98	7.88	19.66
Neutral detergent fiber (%)	16.75	66.96	39.79
Acid detergent fiber (%)	5.82	38.53	20.78
Lignin (%)	1.64	7.07	4.13
Fat (%)	1.22	0.97	1.12
Non-fiber carbohydrate (%)	44.20	19.84	33.55
Total digestible nutrients ² (%)	72.55	52.04	63.93

 Table 1 – Chemical composition of experimental diet

1 – Diet composition, % Dry matter: Corn meal = 26.06, soybean meal = 27.39, dicalcium phosphate = 0.08, limestone = 1.03, sodium-bicarbonate = 0.89, cynodon hay = 44.56. 2 – Calculated based on NRC (2001).

2.3. Carcass data collection

The day of slaughter was decided based on the expected day when each animal fed in ad libitum regimen would reach its target weight (i.e., 20, 28 or 36 kg LW) according to its own previous weighing records. Consequently, its formerly assigned pair from restricted group was killed simultaneously. On the previous day of slaughtering, feed was withheld overnight with free access to water. The animals were weighed the next morning to get the shrunk body weight (SBW) and body condition score (BCS), following NRC (2007). Immediately, the lambs were stunned by percussion, hooked and exsanguinated by cutting main blood vessels of the neck. After hide removal, they were eviscerated and the carcass was obtained by separation of the head, at atlanto-occipital joint, and fore and hind feet (removed at the carpal and tarsal joints, respectively). All visceral fat, namely, omental fat, mesenteric fat, pericardial fat and perirenal fat were also removed and weighed separately. Afterward, hot carcass weight (HCW) was recorded and used to determine dressing percentage $(100 \times HCW/LW)$, thereafter carcasses were chilled for 24h at 4°C. After the cooling period, carcasses were reweighed to record cold carcass weight (CCW), and the relative difference between hot and chilled carcasses weights was used to calculate cooler shrinkage (CS, $((HCW - CCW)/HCW) \times 100$). Subsequently, the carcasses were split at the midline using a band saw and the right half was ribbed between 12th and 13th ribs, thus fat thickness could be measured opposite the *longissimus* muscle with a caliper. Later, the longissimus muscle area (LDA) was traced upon acetate paper and digitalized through a table scanner. Images were processed by means of UTHSCSA Image tool software (http://compdent.uthscsa.edu/dig/itdesc.html). The left half carcass was cut with a band saw in

the following traits: loin, short ribs, ribs/flank, blade, hindlimb, neck, posterior forelimb, and anterior forelimb, accordingly to Furusho-Garcia et al. (2006) (Figure 1). The whole left half of the carcass was grind and homogenized and 350 g were sampled for analysis of fat and crude protein content.



Figure 1 – Location of cuts

2.4. Calculations and statistical analyses

To estimate the average daily gain (ADG), a linear, first order model, was fit for each animal, regressing the live weight on experimental days, and the estimated slope was considered the daily gain in kg per animal.

Linear models were used to analyze all quantitative variables by the complete randomized design with a 2 x 3 factorial arrangement. The statistical model used is shown below:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$
[1]

Where y is the measured variable for the *i*th nutritional plane in the *j*th slaughter weight in the *k*th lamb, μ is the overall mean, α_i is the fixed effect, β_j is the fixed effect for, $(\alpha\beta)_{ij}$ is the interactive effect, and ε_{ij} represents the error term. The degree of freedom (df) for this model includes 5 df for nutritional planes plus slaughter weight (*i.e.*, 1 df for diet, 2 df for live weight at slaughter, and 2 df for interaction). Linear and quadratic contrasts were used for the effect of slaughter weight (20, 28 or 36 kg LW) within each nutritional plane.

Two allometric studies were conducted by performing a separate multivariate analysis for each nutritional plane, using in the first one the data regarding carcass traits and in the second the visceral fats plus the absolute mass of fat in the carcass. A principal component analysis (PCA) based on the covariance matrix was computed using the natural logarithm of each variable, and parametric standard errors for the eigenvectors coefficient estimates (equation 2) and for the eigenvalues (equation 3) were calculated.

$$s(\boldsymbol{\beta}_{m}) = \left[\frac{1}{n}l_{h}\sum_{\substack{j=1\\j\neq h}}^{p}\frac{l_{j}}{(l_{j}-l_{h})^{2}}\boldsymbol{\beta}_{mj}^{2}\right]^{1/2}$$
[2]

$$s(l_j) = \sqrt{2/nl_j}$$
 [3]

Where, *s* stands for the standard error, *n* is the number of subjects, *l* is the eigenvalue vector, and $\boldsymbol{\beta}$ is the eigenvector with *p* coefficients and counted by *m*. Whereas, *h* and *j* are counters for the eigenvalue vector and must be different between each other. In the present study, only the first eigenvector ($\boldsymbol{\beta}_1$) will be explored, therefore *h* can be fixed as one.

To evaluate the accuracy of PCA estimative, a bootstrap approach with 5,000 random iterations, with replacement, was performed allowing PCA calculations for each resampling and also the calculation of standard errors for those, following recommendations of Efron and Tibshirani (1993).

In order to evaluate the hypothesis of overall isometry, which means that all parts have similar allometric coefficients, a chi-square test with degree of freedom equal the number of parts being tested (*p*) minus one (*i.e.*, df = p - 1) was performed by regimen, comparing the first eigenvector obtained in each study (β_1) to the perfect isometric vector (β_1^0), which is

assumed as a vector with p elements equal $p^{-0.5}$. This test calculation is presented on equation 4, following recommendation of Flury (1988).

$$\chi_q^2 = n \left(l_1 \beta_1^{0'} \mathbf{S}^{-1} \beta_1^0 + l_1^{-1} \beta_1^{0'} \mathbf{S} \beta_1^0 - 2 \right)$$
 [4]

Where, *q* is the degree of freedom for the analysis, *n* is the number of observations, l_1 is the first eigenvalue, β_1^0 is the vector with isometric condition and **S** is the covariance matrix for observation of parts being analyzed.

With the intention to evaluate whether animals from different nutritional planes share a common allometric pattern, a 90% confidence interval, as evaluated by Timmerman et al. (2007), was build based on the difference between the bootstrapped estimated coefficients of β_1 for each regimen. The presence of zero within this interval, for each variable coefficient, would confirm similarity among regimens.

All analyses were conducted on R environment (R Core Team, 2014). The R script used through multivariate analysis is shown in the Appendix section.

3. Results

3.1. Body weight, carcass traits

All animals remained healthy during the whole experimental period and presented a satisfactory daily intake, obviously with a smaller consumption for animals from the restricted group (Dry matter intake (DMI) equal 83.68 *versus* 61.55 g/LW^{0.75} for *ad libitum* and restricted animals, respectively). It is clear from Table 2, that slaughter weight was the main significant effect for changes over all variables, linearly increasing all traits when evaluated as absolute weight, regardless of nutritional plane (P < 0.001). This result is corroborated by the fact that actual lamb live weight at slaughter (*i.e.*, SBW) increased from lighter groups to heavier, what was reflected on carcasses (*e.g.*, HCW) and consequently over all traits. However, for animals on restricted plane, a reduction on SBW was observed (P = 0.024). These results agree with those found for ADG, which were affected by the nutritional regimen, whereas animals from the *ad libitum* group presented a higher daily gain (P < 0.001). An interaction was also observed between slaughter weight and regimen for ADG, since animals with unrestricted access to food presented a linear increase of slaughter weight and so did FT, although only the latter showed a tendency to be affected by nutritional plane (P < 0.1).

Item	Ad libitum				Restricted		P-Values							
	SW-20	SW-28	SW-36	SW-20	SW-28	SW-36	Pooled SEM	Regimen	SW	Interaction	Ad lii	bitum	Restr	icted
											L	Q	L	Q
IBW (kg)	18.60	21.47	27.58	16.87	23.20	28.88	0.724	0.469	< 0.001	0.046	< 0.001	0.077	< 0.001	0.716
SBW (kg)	19.92	27.80	35.78	18.17	26.49	33.62	0.897	0.024	< 0.001	0.893	< 0.001	0.964	< 0.001	0.590
ADG (g/d)	56.99	139.53	162.13	54.21	95.04	70.28	11.32	< 0.001	< 0.001	0.002	< 0.001	0.039	0.3234	0.025
BCS (1-5)	2.08	3.00	4.25	2.08	3.17	3.83	0.139	0.467	< 0.001	0.113	< 0.001	0.334	< 0.001	0.229
HCW (kg)	8.09	13.70	18.10	7.25	12.54	16.30	0.442	0.001	< 0.001	0.550	< 0.001	0.269	< 0.001	0.167
CCW (kg)	7.69	13.05	17.58	6.82	11.89	15.75	0.470	0.002	< 0.001	0.572	< 0.001	0.489	< 0.001	0.315
CS (%)	4.99	4.66	2.88	5.92	4.93	3.41	0.529	0.179	< 0.001	0.824	0.007	0.287	0.002	0.690
Dressing (%)	40.37	49.32	51.00	39.81	47.50	48.49	1.524	0.200	< 0.001	0.811	< 0.001	0.061	< 0.001	0.083
FT (mm)	1.05	2.37	4.18	0.72	1.91	3.21	0.420	0.099	< 0.001	0.724	< 0.001	0.643	< 0.001	0.917
LDA (cm ²)	6.27	10.09	11.88	6.71	8.71	10.85	0.523	0.191	< 0.001	0.190	< 0.001	0.153	< 0.001	0.911
Absolute (g)														
Neck	589.13	964.03	961.57	509.47	776.90	1028.63	37.746	0.039	< 0.001	0.008	< 0.001	< 0.001	< 0.001	0.866
Blade	555.97	889.58	1169.67	489.00	809.33	1080.90	42.307	0.030	< 0.001	0.967	< 0.001	0.609	< 0.001	0.641
Loin	258.70	534.67	672.40	249.93	510.98	658.63	30.556	0.542	< 0.001	0.970	< 0.001	0.075	< 0.001	0.140
Short Ribs	507.65	1096.30	1104.00	464.48	1010.38	1266.63	88.766	0.878	< 0.001	0.339	< 0.001	0.012	< 0.001	0.193
Ribs/Flank	691.10	1513.67	2151.90	554.48	1266.95	1672.82	70.067	< 0.001	< 0.001	0.059	< 0.001	0.291	< 0.001	0.084
Hindlimb	1129.05	1811.47	2348.15	1036.20	1655.45	2265.90	61.959	0.037	< 0.001	0.814	< 0.001	0.345	< 0.001	0.954
Yield (g/kg CCW)	77 4	71.02	5177	74.00	65 75	65.92	2 870	0 608	0.001	0.081	< 0.001	0.242	0.005	0.240
Neck	77.4	/1.92	54.77	74.99	05.75	65.82	3.870	0.698	0.001	0.081	< 0.001	0.242	0.095	0.349
Diaue	72.71	66.16	66.55	/1./5	67.16	68.50	2.153	0.716	0.031	0.780	0.046	0.213	0.279	0.284
	34.15	40.51	38.26	36.21	40.95	42.37	2.456	0.258	0.054	0.762	0.233	0.177	0.078	0.598
Short ribs	67.14	89.05	62.71	68.48	87.45	79.46	5.782	0.219	0.004	0.245	0.580	0.003	0.177	0.076
Ribs/Flank	88.55	117.05	122.41	80.83	106.01	105.94	3.428	< 0.001	< 0.001	0.425	< 0.001	0.012	< 0.001	0.007
Hindlimb	147.03	139.84	133.71	152.44	137.43	144.77	3.781	0.109	0.009	0.232	0.016	0.913	0.150	0.027

Table 2 - Body weight, carcass traits mass and yield of hair ewe lambs submitted to two regimens

S2W = Slaughter weight, IBW = Initial body Weight, SBW = Shrunk body weight, ADG = Average daily gain, BCS = Body condition score, HCW = Hot carcass weight, CCW = Cold carcass weight, CS = Cooler shrinkage, FT = Fat thickness, LDA = Longissimus dorsi area, L = Linear, Q = Quadratic.

The hot and cold carcass weights were influenced by nutritional plane ($P \le 0.002$), and this behavior was also observed for the following traits: hindlimb, blade, rib/flank and neck which presented lower weights for restricted animals compared to *ad libitum* ones (P < 0.05). On the other hand, loin and short ribs were not affected by the level of feeding (P > 0.1). Interestingly, it was observed that only for the neck the interaction effect between slaughter weight and regimen was significant (P = 0.008), which was evidenced by the highly significant quadratic effect for this trait (P < 0.001), however only in animals from the *ad libitum* group. Looking at the perspective of traits masses in relation to CCW mass, there was a strong effect of slaughter weight among all cuts; however, the effect direction was not common for all. For instance, neck, hindlimb and blade did decrease their relative masses with increase on slaughter weight, whereas ribs/flank and short ribs took a higher participation on CCW weight (P < 0.05). Only ribs/flank relative mass was affected by regimen, being larger in animals on *ad libitum* regimen (P < 0.001).

3.2. Carcass composition and fat depots

Table 3 presents the results regarding carcass composition (*i.e.*, protein and fat content) and weights of visceral fat depots. These variables were linearly influenced by slaughter weight (P < 0.05). The bigger the body weights at slaughter, the heavier the fat and protein depots. However, when carcass components were evaluated in a relative perspective (*i.e.* percentage of carcass weight), both, protein and fat, were affected by slaughter weight, but in opposite directions, where the fat increased with the increase of body weight (P < 0.001) while the protein percentage tended to decrease (P = 0.063), independently of nutritional plane. Moreover, differently from protein, fat percentage was quadratically increased in response to the increase of slaughter weight. Observing the numerical results for this variable, this behavior seems reasonable since the fat percentage remained steady after 28 kg of body weight. However, for all fat depots, and including their totality, slaughter weight linearly increased fat weights, which were concomitantly influenced by nutritional plane (P < 0.01), revealing a significant interaction effect (P < 0.01). The sum of both effects can be observed in Figure 2,

where it becomes clear that regardless of nutritional plane, fat depots were increased by rising lamb weight at slaughter, but the rate of fat accretion for animals on *ad libitum* regimen was bigger, mainly after 30 kg of live weight. This interaction effect was constantly significant for all visceral fat depots (P < 0.05), except for pericardial fat depot (P > 0.1). On the contrary, carcass composition was not influenced by nutrition (P > 0.05).



Figure 2 – Amount of fat and protein on carcass and visceral fat depots. The color of elements differentiates the nutritional plane. The line type denotes different locals (carcass *versus* visceral fat depots) while the shape of points stands for type of nutrient (carcass fat, carcass protein or visceral fat). The error bars in both directions represents the standard errors of the mean for each group of six animals, where horizontal bars stands for slaughter weight error and vertical bar for the error of weight of each component.

Item	Ad libitum				Restricted			P-Values						
	SW-20 SW-28 SW-36		SW-20) SW-28 SW-36		Pooled SEM	Regimen	SW	Interaction	Ad lil	oitum	Restricted		
											L	Q	L	Q
Carcass Fat (kg)	1.22	4.27	5.47	0.89	3.8	5.37	0.331	0.275	< 0.001	0.855	< 0.001	0.030	< 0.001	0.108
Carcass Fat (%)	14.68	31.25	30.16	12.17	30.37	33.02	2.014	0.916	< 0.001	0.403	< 0.001	0.001	< 0.001	0.004
Carcass CP (kg)	1.44	2.28	2.78	1.25	2.05	2.74	0.099	0.066	< 0.001	0.616	< 0.001	0.178	< 0.001	0.629
Carcass CP (%)	18.02	16.62	15.38	17.23	16.33	16.85	0.638	0.806	0.063	0.194	0.007	0.921	0.679	0.372
Visceral Fat (g)	364.43	1653.32	3374.88	257.23	1397.00	2027.68	163.575	< 0.001	< 0.001	0.001	< 0.001	0.289	< 0.001	0.214
Mesenteric (g)	135.93	465.50	797.82	114.00	439.13	558.88	38.696	0.005	< 0.001	0.012	< 0.001	0.977	< 0.001	0.038
Omental (g)	106.88	729.25	1436.72	57.47	609.37	869.17	71.62	< 0.001	< 0.001	0.002	< 0.001	0.631	< 0.001	0.106
Perirenal (g)	53.10	347.48	852.55	35.38	244.83	442.73	57.473	0.001	< 0.001	0.005	< 0.001	0.145	< 0.001	0.935
Pericardial (g)	68.52	111.08	287.8	50.38	103.67	156.9	32.395	0.058	< 0.001	0.125	< 0.001	0.101	0.027	1.000

 Table 3 – Influence of nutritional planes on carcass composition and visceral adipose tissues

 $S_2V = Slaughter weight, LW= Live Weight, L=Linear, Q=Quadratic.$

3.3. Allometric growth

Results from the multivariate allometric study based on principal components analysis is presented in Table 4. Carcass traits presented a non-isometric pattern in spite of nutritional plane (P < 0.001), compared to the perfect isometry vector which was in this case: $\beta_1^0 =$ (0.408, 0.408, 0.408, 0.408, 0.408, 0.408)'. Estimated coefficients for β_1 were larger for ribs/flank and smaller for neck in both regimens. The first principal component accounted for 91.76% of total variance in the sample of cuts from animals at *ad libitum* regimen and for 92.80% of animals in restricted condition. Only ribs/flank bootstrapped coefficients were affected by regimen, showing significant difference between animals of distinct nutritional planes, with high values for animals with free access to food (P < 0.10).

Table 4 – Principal component analysis for multivariate ontogenetic allometry test of carcass traits and fat depots in growing Santa Inês female lambs.

	Ad libitum			Restricted			Boostrapped 90%
-	$\widehat{oldsymbol{eta}}_1$	Parametric s.e.	Bootstrapped s.e.	$\widehat{oldsymbol{eta}}_1$	Parametric s.e.	Bootstrapped s.e.	CI for difference
Carcass Traits							
Neck	0.2597	0.027	0.028	0.2968	0.033	0.028	(-0.103;+0.025)
Blade	0.3495	0.028	0.023	0.3523	0.018	0.020	(-0.051;+0.046)
Loin	0.4553	0.021	0.026	0.4532	0.033	0.033	(-0.063;+0.076)
Short Ribs	0.4039	0.052	0.043	0.4627	0.039	0.046	(-0.160; +0.050)
Ribs/Flank	0.5613	0.021	0.019	0.5028	0.018	0.016	(+0.016; +0.098)
Hindlimb	0.3534	0.016	0.014	0.3399	0.016	0.013	(-0.020;+0.042)
Eigenvalue (Inertia)	0.8684 (91.76%)	0.2895	0.1872	0.9786 (92.80%)	0.3262	0.2049	
Isometry test	$H_0: \mathbf{\beta}_1 = \mathbf{\beta}_1^0; \ \chi_5^2 = 157,78, P < 0.001$			$H_0: \mathbf{\beta}_1 = \mathbf{\beta}_1^0; \ \chi_5^2 = 62.59, P < 0.001$			
Fat depots							
Carcass	0.3346	0.023	0.024	0.3954	0.019	0.020	(-0.114 ; -0.013)
Omental	0.5737	0.020	0.023	0.6072	0.013	0.011	(-0.079;+0.002)
Mesenteric	0.3747	0.017	0.014	0.3535	0.013	0.012	(-0.011;+0.049)
Perirenal	0.5917	0.033	0.023	0.5422	0.016	0.015	(+0.009; +0.095)
Pericardial	0.2615	0.040	0.047	0.2367	0.026	0.027	(-0.058;+0.119)
Eigenvalue (Inertia)	4.5118 (93.81%)	1.5039	0.9882	4.2321 (97.18%)	1.4107	0.7794	
Isometry test	$H_0: \boldsymbol{\beta}_1 = \boldsymbol{\beta}_1^0; \ \chi_4^2 = 550.67, P < 0.001$			$H_0: \boldsymbol{\beta}_1 = \boldsymbol{\beta}_1^0; \ \chi_4^2 = 314.54, P < 0.001$			

s.e. = standard error

Similarly to carcass traits, fat depots presented a allometry between places studied, $\beta_{1}^{0} =$ comparing the first eigenvector obtained in each regimen to (0.447, 0.447, 0.447, 0.447, 0.447)'. Variable coefficients that were most distanced from the isometric value (*i.e.*, $p^{-0.5} = 0.447$) were, negatively, pericardial fat in both nutritional planes (0.26 and 0.24, ad libitum and restricted, respectively), and, positively, perirenal fat in animals from the *ad libitum* group (0.59) and, omental fat in restricted animals (0.61). Perirenal and carcass fat depot bootstrapped coefficients were the only ones that presented a significant difference of zero between animals of different nutritional planes, being the first bigger for *ad libitum* animals and the second for the restricted group (P < 0.10).

The standard errors calculated by both approaches and in both studies were small and quite similar among them.

4. Discussion

Study of carcass cuts and traits is the first step to evaluate the yield of a feedlot, because they represent the final product of the livestock value chain.

The choice by SRNS model (Tedeschi et al., 2010) to predict lamb nutritional requirements was made because there is no Brazilian nutrient requirement table for this type of sheep and also because this system is based on a mechanistic approach, therefore, it would probably generate a better approximation of real nutrient demands compared to other nutritional systems. Its predictability was not evaluated in this work, but some papers have already shown that the system is accurate when it was evaluated in Brazilian conditions using Santa Inês sheep (Regadas Filho et al., 2011). Nevertheless, the main hypothesis of the present paper about the effect of nutritional plane on growth pattern is independent of this, since the nutritional restriction imposed on animals from the restricted group was guaranteed by the intake limitation of the same diet offered to ad libitum animals. The actual intake of animals with free access to the diet regardless of slaughter weight was around 83.68 g/LW^{0.75}. This value is in the range of results summarized by Vieira et al. (2013) for dry matter intake of Santa Inês male lambs raised in Brazilian feedlot conditions. Moreover, even for animals under restriction, the observed intake was above the minimum values found by those authors, and represented a reduction around 26% when compared to unrestricted animals. Yet, this limited ingestion was not sufficient to cause a loss of weight. Indeed, a positive ADG was observed for all animals, but, naturally it was bigger for animals on *ad libitum* regimen. ADG was slightly lower than SRNS general prediction (*i.e.* 200 g/d), what was also observed by Regadas Filho et al. (2011), who found an average overestimation on ADG of 5.18% when evaluating this system with Santa Inês sheep data. This discrepancy is most likely a consequence of differences between feeds and also animal type used in those papers when compared to the ones used in the development of this model (Galvani et al., 2008).

The effect of growth, here caused by increasing slaughter weight, became evident due to the absolute increase of all body parts. Interestingly, the dressing percentage was also augmented, what reveals that the carcass did become more representative on total live weight with age. This increase in carcass yield due to elevation on slaughter weight is frequently observed for lambs in feedlot conditions (Kremer et al., 2004; Majdoub-Mathlouthi et al., 2013), and when compared by gender, it tends to be equivalent (Peña et al., 2005; Soares et al., 2012). However, in spite of scarce information, there is some evidence that, for Santa Inês female lambs, dressing percentage is bigger than for males (Garcia et al., 2000). According to these authors, the higher dressing percentage observed for females may be due to earlier fat accretion on carcass when compared to males, mainly when close to maturity. In the present study, there was a significant increase on fat tissue absolute mass within the carcass as well on fat cover and visceral fat depots. The quadratic pattern observed for relative weight of fat on carcass in both nutritional planes indicate that this tissue reached a plateau, what is expected for mammals that are getting close to mature weight.

Following the same pattern, body condition score went up with the increase on slaughter weight. This relationship between BCS and fat is well documented for bovInês (NRC, 2000; 2001) and also for adult ewes after first lambing (Cannas et al., 2004), where BCS and live weight were used to calculate changes on protein and energy body reserves. This intense relation between these variables was also observed in this study (r = 0.9158). However, it may be overestimated since all animals used in this work were at growth stage, therefore all tissues (e.g. protein and fat) were in an anabolic directed metabolism, what is evidenced by their absolute positive gain. It is not known if during catabolism this high correlation would be conserved, mainly because of the type of energy storage in hair sheep, which is highly related to visceral fat depots (Ríos et al., 2011; Rios-Rincon et al., 2014). Nevertheless, there was an increase on the depth of fat layer on carcass with the elevation on slaughter weight, with average fat thickness of 0.89 and 3.7 mm for lighter and heavier animals, respectively. The observed FT for animals with medium and heavier slaughter weights were higher than the values observed by Santos et al. (2013) (*i.e.*, 1.1 mm) and Lage et al. (2014) (*i.e.*, 0.9 mm), who worked with Santa Inês male lambs at the same slaughter weight range. This difference may be related to both or either diet and/or gender. The latter effect was evaluated by Peña et al. (2005) who found on average a positive difference of 0.4 mm for females. Moreover, in an extensive meta-analysis study developed by Sales (2014), it was proved that the effect of castration in male lambs improves both backfat thickness and dressing percentage. The author justified this phenomena as a consequence of absence of testosterone, which may partially explain the higher values found in this study when compared to the ones found in literature.

The negative correlation between FT and cooler shrinkage (r = -0.6623) may be explained by the protective effect engendered by the lipid layer, reducing the moisture vapor transmission rate from the exterior of muscle to the surrounding air currents during cooling period. This result is in agreement with Smith and Carpenter (1973) who presented a reduction of 0.5 to 0.8 percent on weight loss when comparing animals with lower fat thickness to animals with deeper fat layers, and suggested that a fat covering over 2.5 mm at the 12th rib, would be sufficient to mitigate shrinkage in sheep carcasses during cooling. In the present study, the increase on FT might have prevented a loss of 2.11 % of carcass weight after chilling.

There was an expressive contrast between fat deposition pattern among carcass and visceral depots. Actually, for both locations, slaughter weight increase did increase their masses, but regimen, on the other hand, influenced only the visceral depots, which were greater for animals on *ad libitum* nutritional plane, mainly after 28 kg of slaughter weight (Figure 1). From an energetic point of view, it is more efficient, in growing sheep, to convert metabolisable energy into fat than into protein for either calculation basis, kcal of ME/kcal of tissue (i.e. protein or fat), or kcal of ME/g of tissue (Rattray et al., 1974). However, since adipose tissue contains less moisture than muscle (Smith and Carpenter, 1973), it is easier to increase live weight (*i.e.*, tissue + water) based on a corporal gain composed mostly by protein (*i.e.*, muscle). This result is quite important from the market perspective, since the visceral fat tissues are not marketable, or represent a lower income compared to carcass cuts, and also because its increase is associated with lower average daily gain, which is not interesting for farmers. Moreover, the results from this paper show that a higher nutritional plane, does not, obligatory, incur in profit, because, although the live weight and the ADG were greater for animals in the ad libitum group, a large proportion of energy intake was transformed in visceral fat, and was therefore not converted in product. This production inefficiency goes in the opposite direction of current policies for livestock production such as sustainable intensification (Garnett et al., 2013). Other authors who have tested the effect of different nutritional planes over body gain composition of hair sheep support these results (Abouheif et al., 2013; Rios-Rincon et al., 2014), but they worked with male lambs from other hair breeds than Santa Inês. Notwithstanding, Alves et al. (2003) working with Santa Inês male lambs, presented a solid linear increase of visceral fat with the rise on metabolisable energy intake. Those results corroborate with the inference above, even though, in this study, the effect of nutritional plane was evaluated by a qualitative diet change design, increasing energy intake by raising corn proportion on total diet, differently to the quantitative restriction used in the present trial. These authors also showed a linear increase of blade and ribs mass with the increase of metabolisable energy intake. This same pattern was observed in the present study, however the higher nutritional plane has also augmented the mass of the neck and hindlimb cuts.

All traits become greater with the increase on slaughter weight. The average mass for these cuts is in the same range of the ones found for other authors who worked with Santa Inês lambs in similar slaughter conditions (Alves et al., 2003; Soares et al., 2012; Lage et al., 2014). All cuts, with exception of loin and short ribs were influenced by nutritional plane. Alves et al. (2003) working with different levels of metabolisable energy on diet (2.42 to 2.83 Mcal/kg DM) found a nutritional effect only over the blade and ribs. This different result may indicate that the nutritional effect observed in the present trial can be related more to a total intake restriction than to a qualitative restriction as tested by these authors, and/or that this restriction may be more intense over females, since in males hormone stimulus may improve their efficiency for mass gain.

Changes on proportion of cuts mass within carcass with the increase on slaughter weight are evidence that there is a heterogonic growth between them. The multivariate allometric study made it clear that the growth of parts was not similar in the live weight range studied, since the coefficients from β_1 are quite different among themselves. Figure 3 summarizes the density of distributions of bootstrapped coefficients in each regimen for each carcass trait.



Figure 3 – Density of boostrapped coefficients for each carcass trait. The color denotes different nutritional plane. The dashed vertical line represents the isometric value for reference, p = 0.408.

Visually, it seems that all traits have similar distributions in spite of regimen, but it is possible to observe that some of them are on the right side of the isometric value of reference, whereas others are on the opposite side. This antagonism may be interpreted as higher or lower intensity of trait growth in each sample. Following this logic, one can understand that for carcass traits in female lambs with slaughter weight between 20 and 36 kg, loin and ribs/flank are the parts with more intense proportional growth, being around two times bigger than traits

such as neck (e.g., 0.5613/0.2597 = 2,16). This direct type of comparison among parts is one of the advantages of working with multivariate analysis rather than the classic bivariate approach (Klingenberg, 1996), allowing multiple pairwise comparisons by simply calculating the ratio between parts coefficients. Moreover, due to normalization of coefficients to have unity length, it is possible to calculate the bivariate allometric coefficient for each of the variables (*i.e.*, traits) against the measure of overall size (*e.g.*, carcass weight) by multiplying each coefficient by the root of p, which stands for the number of variables in the multivariate allometric study. For instance, if one would like to calculate the allometric coefficient of the hindlimb of animals from ad libitum regimen against total carcass weight, this may be achieved by the following operation: $0.3534 \times \sqrt{6} = 0.8656$. This same calculation would render the value of 1.1152 for the loin allometric coefficient. These results are in agreement with the coefficients estimated for the same parts by Mora et al. (2014) for sheep in similar conditions. These authors estimated the allometric coefficients using the classic Huxley's bivariate approach, and found that compared to the whole carcass, these coefficients are different from one, and therefore, considered heterogonic and, positive for the loin and negative for the leg. According to these authors, the higher coefficient for the loin indicates that this part has a late development compared to others. As observed, this type of conclusion can be derived from the multivariate approach as well. Hence, ribs/flank presented the highest allometric coefficient compared to the whole carcass (i.e., 1.3749), which shows its late development as well as an intense growth in the animals evaluated, mainly in lambs fed on *ad libitum* regimen. The last assertive is corroborated by the significant difference observed between the boostrapped coefficients for the ribs/flank estimated for each nutritional plane. Although not measured, a considerable increase was noticed on fat cover over ribs and flank of animals with ad libitum access to food, mainly for heavier animals, which may partially explain its greater weight compared to restricted lambs, and justifies its bigger allometric coefficient. This fat accelerated accretion on ribs is possibly related to the gender of animals used in this trial.

In the allometry study of fat, the highly significant difference between β_1 and β_1^0 shows how different the dynamic of fat depots are. Figure 4 clearly shows the opposite direction of bootstrapped coefficients, where the perirenal and omental are placed on the right side of the isometry reference value, and the others on the opposite side. This result enforces those presented in Table 4 and clearly shows a more intense growth of the main visceral fat depots in detriment of carcass fat deposition. It is also possible to observe that the perirenal and carcass fat allometric coefficients were different among animals from different nutritional planes. The distribution of bootstrapped coefficients for these two parts in animals from the restricted group was closer to the isometric value (*i.e.*, p = 0.447), which corresponds to a more balanced distribution of fat among these depots, which is highly desirable from the farmer's perspective.



Figure 4 – Density of boostrapped coefficients for each fat depot. The colors denotes different nutritional regimen. The dashed vertical line represents the isometric value for reference, p = 0.447.

All inferences about principal components analyses are just possible due to the high inertia observed for the first element of eigenvalue vector (*e.g.*, bigger than 91% in all studies), and also because there is a fair stability of coefficients of the first eigenvector, which can be concluded by the lower values of standard errors. For the latter ones, the high similarity between the parametric and bootstrapped estimated standard errors reveals that the coefficients distribution is close to a multivariate normal condition, which is essential for the first technique.

5. Conclusion

This paper brings valuable information regarding growth and slaughter characteristics of hair female sheep. Moreover, it is shown that slaughter weight massively influences most variables evaluated, including carcass traits, which is important to better understand when it would be more interesting to end fattening periods in order to achieve both higher profits and production efficiency. In this sense, for Santa Inês lambs, higher slaughter weight here evaluated presented higher dressing percentage. This result was accompanied by increase on visceral fat deposition, which is not desirable. Therefore, an intermediary slaughter weight would be more interesting from this point of view. Furthermore, the imposed restriction in this study caused minor effects on carcass yield and allometry, which indicates that international nutritional systems may overestimate nutrient demands for Brazilian sheep. However, restriction influence on average daily gain, thus more days in feedlot would be necessary to acquire the same slaughter weight of non-restricted animals, must likely would raise costs. Besides, the multivariate allometric approach is a concise way to deeply look into growth patterns and allows a rapid interpretation of physical dynamics, such as the fat distribution over the body.

6. Conflict of interest

The authors declare that there is no conflict of interest.

7. Acknowledgements

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Appendix A

PRC.inf – Principal components's inferences

Description

Returns inferences about eigenvector's and eigenvalue's stability for principal components analysis. Also tests equality between first principal component eigenvector and a specified vector, which can be an isometry vector.

Algorithm developed by Luigi F. L. Cavalcanti and Fabio L. B. Toral.

R programmed by Luigi F. L. Cavalcanti.

Reference for statistic inferences on principal components analysis in:

FLURY, B. 1988. Common principal components and related multivariate models. Wiley, New York.

Usage

PRC.inf(data,...)

Default

PRC.inf(data,cov=FALSE,dec.places=4,n=NA,iso=T)

Arguments

data: an R object of either class data.frame or matrix.

cov: if TRUE data object is a data.frame with brute data, if FALSE data is a matrix object with a covariance table, default=FALSE.

dec.places: rounds the output values to the specified number of decimal places, default=4

n: number of observations. Only necessary if COV=TRUE, otherwise, n=NA.

iso: if TRUE the function will perform a chi-square test to evaluate the null hypothesis of equality between first principal components eigenvector and isometry vector, which is calculated as a vector with replicated elements with length equal the number of variables (p), and value equal p raised to - 0.5. Otherwise, a vector with P elements may be passed to iso in order to test equality of this vector and first eigenvector.

Function

```
PRC.inf<-function(data,cov=FALSE,dec.places=4,n=NA,iso=T){
if(cov==FALSE){
    n<-nrow(data)
    names(data)->carac
    data<-cov(data)</pre>
```

```
}
  eigen(data)$values->values
  eigen(data)$vectors->vector
if(is.na(n)) stop("Sample size must be pass to n argument")
 result<-matrix(NA, ncol=ncol(vector), nrow=nrow(vector))</pre>
for (h in 1:length(values)) {
  for (m in 1:nrow(vector)) {
    p<-1:length(values)</pre>
    soma2<-c()</pre>
    soma<-c()</pre>
    for(j in p[-h]) {
      soma<-(values[j]/((values[j]-values[h])^2))*vector[m,j]^2</pre>
      soma2<-cbind(soma2,soma)</pre>
    }
    result[m,h]<-sqrt(1/n*values[h]*sum(as.vector(soma2)))</pre>
  }
 }
 result<-round(result,dec.places)</pre>
result<-as.data.frame(result)</pre>
 row.names(result)<- paste("Characteristic",row.names(result),sep="_")</pre>
 if(cov==F) {
  row.names(result)<-carac</pre>
 }
 names(result)<-paste("PC",1:ncol(result),sep="")</pre>
as.data.frame(vector)->vector
names(vector)<-names(result)</pre>
row.names(result)->row.names(vector)
 round(vector,dec.places)->vector
 round(values,dec.places)->values
 round(cumsum(values)/sum(values)*100,dec.places)->cumasum
 round(sqrt(2/n)*values,dec.places)->epvalues
 Principal components and eigenvalues\n\n")
 print(vector)
cat("\n
Eigenvalues\n\n")
 print(values)
cat("\n
Inertian^{n}
print(cumasum)
 Princiapal components standard errors\n\n")
print(result)
cat("\n
Eigenvalues standard errors\n\n")
 print(epvalues)
)
#### Isometry test or vector comparison
if(is.matrix(iso)){
```

```
b0<-iso
  n*((values[1]*(t(b0)%*%solve(data)%*%b0))+(values[1]^-1*(t(b0)%*%data%*%
b0))-2)->xcalc
  pchisq(xcalc,nrow(result)-1,lower.tail = F)->piso
  if (piso<0.05) {
    cat("\n According to Chi-square test, the first eigenvector is differe
nt from the vector passed to iso argument\n")
    cat("Chi square =",xcalc," on ", nrow(result)-1, "degrees of freedom \
n")
    print(paste("P=",piso,sep=""))
  } else {
    cat("\n According to chi-square test, the first eigenvector is equal f
rom the vector passed to iso argument\n")
    cat("Chi square =",xcalc," on ", nrow(result)-1, "degrees of freedom \
n")
    print(paste("P=",piso,sep=""))
  }
} else {
 matrix(rep(1/sqrt(nrow(result)), nrow(result)), ncol=1, nrow=nrow(result))-
>b0
  n*((values[1]*(t(b0)%*%solve(data)%*%b0))+(values[1]^-1*(t(b0)%*%data%*%
b0))-2)->xcalc
  pchisq(xcalc,nrow(result)-1,lower.tail = F)->piso
  if (piso<0.05) {
    cat("\n According to chi-square test, tested variables are not isometr
ic\n")
    cat("Chi square =",xcalc," on ", nrow(result)-1, "degrees of freedom \
n")
    print(paste("P=",piso,sep=""))
  } else {
    cat("\n According to chi-square teste, tested variables are isometric\
n")
    cat("Chi square =",xcalc," on ", nrow(result)-1, "degrees of freedom \
n")
    print(paste("P=",piso,sep=""))}
  }
 return(list(vector,values,result,epvalues,piso))
}
```

Appendix B



UNIVERSIDADE FEDERAL DE MINAS GERAIS COMITÊ DE ÉTICA EM EXPERIMENTAÇÃO ANIMAL - C E T E A -

CERTIFICADO

Certificamos que o **Protocolo nº 197/2010**, relativo ao projeto intitulado *Composição corporal e exigências nutricionais de fêmeas Santa Inês do desmame à puberdade*, que tem como responsável(is) **Iran Borges**, está(ão) de acordo com os Princípios Éticos da Experimentação Animal, adotados pelo *Comitê de Ética em Experimentação Animal* (CETEA/UFMG), tendo sido aprovado na reunião de 1/ 12/2010.

Este certificado expira-se em 1/ 12/ 2015.

CERTIFICATE

We hereby certify that the **Protocol nº 197/2010**, related to the project entitled "*Body composition na nutrient requirements of Santa Ines ewes from weaning to puberty*", under the supervisiors of **Iran Borges**, is in agreement with the Ethical Principles in Animal Experimentation, adopted by the *Ethics Committee in Animal Experimentation* (CETEA/UFMG), and was approved in December 1, 2010.

This certificate expires in December 1, 2015. Dezembro de 2010. Belo Horizonte, 3 Prof^a. Jacqueline Isaura Alvarez-Leite

Coordenadora do CETEA/UFMG

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(Mod.Cert. v1.0)