UNIVERSIDADE ESTADUAL PAULISTA “JULIO DE MESQUITA FILHO”
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

PROTEIN AND ENERGY REQUIREMENTS FOR MAINTENANCE AND GROWTH IN DAIRY GOATS: A META-ANALYSIS

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TÍTULO: PROTEIN AND ENERGY REQUIREMENTS FOR MAINTENANCE AND GROWTH IN DAIRY GOATS: A META-ANALYSIS

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BIOGRAPHY

ANAIANE PEREIRA SOUZA was born in September 22, 1988, in Miguel Calmon, Bahia, Brasil. From 2006 to 2010, she did her undergraduate study in Animal Science at Universidade Federal da Paraíba, Areia, Paraíba, Brasil. During her undergrad, she completed an internship at Universidade Técnica de Lisboa, Lisboa, Portugal, from September 2008 to February 2009. She became graduate student at Universidade Federal da Paraíba and concluded her Master’s degree in Animal Science in February 2013. She focused on nutritional requirements for indigenous goats under supervision of Dr. Ariosvaldo Nunes de Medeiros. In 2013, she began her doctoral degree at Universidade Estadual Paulista, Jaboticabal, São Paulo, Brasil, and she worked on a meta-analysis of nutritional requirements for dairy goats under supervision of Dr. Izabelle Auxiliadora Molina de Almeida Teixeira. She also completed a graduate internship at The Ohio State University, Columbus, Ohio, USA, from January 2015 to December 2015 under supervision of Dr. Normand St-Pierre.

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SUMMARY

PROTEIN AND ENERGY REQUIREMENTS FOR MAINTENANCE AND GROWTH IN DAIRY GOATS: A META-ANALYSIS

ABSTRACT - A database of seven comparative slaughter studies of Saanen goats was gathered to predict the protein and energy requirements for maintenance and growth of dairy goats. For the evaluation of energy utilization by dairy goats we used 238 Saanen goats subjected to three levels of intake. The experimental design provided different levels of metabolizable energy intake (MEI) and body weight (BW), allowing the development of regression equations to predict the net energy requirements for maintenance (NE_M). The nonlinear relationship between MEI and heat production was used to estimate the NE_M and the requirements of ME for maintenance (ME_M). The efficiency of energy utilization for maintenance (k_m) was calculated as the relationship between NE_M and ME_M. The slope between retained energy (RE) and metabolizable energy intake above maintenance (MEI_G) was adopted as the efficiency of utilization of ME for growth (k_g). The efficiency of utilization of energy for protein and fat deposition (k_p and k_f, respectively) were calculated using a multiple regression on MEI_G (model intercept equal to 0) on the RE as protein (RE_p) and RE as fat (RE_f). For the development of linear and non-linear equations we used MIXED and NLMIXED procedures in SAS considering sex (castrated male, intact male, and female, *n* = 80, 98, and 60, respectively) as fixed effect and block nested in study and sex as random effect. The NE_M was affected by sex where castrated males and intact males have similar requirements (75 kcal/kg$^{0.75}$ empty BW); on the other hand, females presented a lower value (64 kcal/kg$^{0.75}$ empty BW). The k_m did not differ between sexes (0.62). The k_g was different between sexes (0.32 for castrated males, 0.26 for intact males, and 0.31 for females) but the k_p (0.21) and k_f (0.80) were similar between sexes. For the evaluation of the net protein requirements for maintenance (NP_M) of dairy goats we used 185 Saanen goats subjected to three levels of intake. The equations were analyzed using MIXED procedure of SAS, sex was considered as fixed effect and block nested in study and sex as random effect. The NP_M was assumed to be the intercept of the linear regression of the N retained (g/kg$^{0.75}$ BW) on the total N intake (g/kg$^{0.75}$ BW) multiplied by 6.25. The NP_M was similar between sexes. Using the comparative slaughter technique, the daily estimated NP_M was 1.23 g/kg$^{0.75}$ BW; lower than the one using N balance method (3.18 g/kg$^{0.75}$ BW) for dairy growing goats and previous reports by the current feeding systems. For estimating the net requirements of protein (NP_G) and energy (NE_G) for growth we used only animals fed ad libitum (n = 238). The allometric equation included the fixed effects of sex (castrated male, male and female, *n* = 73, 94, and 71, respectively) and the random effect of study. The net requirements for growth were estimated as the first partial derivative of allometric equations in relation to empty BW. The estimated parameters were obtained using the MIXED procedure of SAS. Sex affects the NP_G, where female goats showed lower NP_G than male goats (castrated males and intact males). The NE_G of castrated males was greater than intact males, and lower than females.

Keywords: allometry, comparative slaughter, nutritional requirements, Saanen

EXIGÊNCIAS DE PROTEÍNA E ENERGIA PARA MANTENÇA E CRESCIMENTO DE CAPRINOS LEITEIROS: UMA METANÁLISE

RESUMO - Um banco de dados de sete estudos de abate comparativo utilizando caprinos Saanen foi construído para predizer as exigências de proteína e energia para mantença e crescimento de caprinos leiteiros. Para a avaliação da utilização de energia por caprinos leiteiros foram utilizados 238 caprinos Saanen submetidos a três níveis alimentação. O delineamento experimental proporcionou variação no consumo de energia metabolizável (CEM) e peso corporal (PC), permitindo o desenvolvimento de equações de regressão para predição das exigências líquidas de energia para mantença (EL_M). A relação não linear entre CEM e produção de calor foi utilizada para estimativa das exigências de EL_M e as exigências de energia metabolizável para mantença (EM_M), a eficiência de uso de energia para mantença (k_m) foi calculada como a relação entre EL_M e EM_M. O coeficiente de inclinação entre a energia retida (ER) em relação ao consumo de energia metabolizável acima da mantença (CEM_G) foi adotado como a eficiência de utilização de EM para crescimento (k_g). A eficiência de utilização de EM para retenção de proteína (k_p) e gordura (k_f) foram calculadas utilizando uma regressão múltipla do CEM_G (modelo com intercepto igual a 0) na ER como proteína e na ER como gordura. Para o desenvolvimento das equações lineares foi utilizado o PROC MIXED e para as não lineares o PROC NLINMIXED do software SAS considerando a classe sexual (macho castrado, macho inteiro e fêmea; 80, 98, e 60, respectivamente) como efeito fixo e bloco aninhado a estudo e a classe sexual como efeito aleatório. Classe sexual afetou a EL_M, de modo que machos castrados e machos inteiros não diferiram e apresentaram exigências superiores (75 kcal/kg^{0,75} PC vazio) aos valores obtidos para fêmeas (64 kcal/kg^{0,75} PC vazio) utilizando o método do abate comparativo. Os valores de k_m não diferiram entre classes sexuais (0,62). Os valores de k_g foram diferentes entre classes sexuais (0,32 para machos castrados, 0,26 para machos inteiros, e 0,31 para fêmeas) e k_p (0,21) e k_f (0,80) foram semelhantes entre classes sexuais. Para avaliação das exigências líquidas de proteína para mantença (PL_M) de caprinos leiteiros foram utilizados 185 caprinos Saanen submetidos a três níveis de alimentação. As equações foram analisadas usando o PROC MIXED do SAS, a classe sexual foi considerada como efeito fixo e bloco aninhado a estudo e classe sexual foi considerado efeito aleatório. A PL_M foi assumida como o intercepto da regressão linear entre o N retido (g/kg^{0,75} PC) em relação ao N ingerido (g/kg^{0,75} de PC) multiplicado por 6,25. A PL_M foi semelhante entre sexos. Usando a técnica de abate comparativo a PL_M foi 1,23 g/kg^{0,75} PC foi inferior à estimada usando balanço de N (3,18 g/kg^{0,75} PC) para caprinos leiteiros em crescimento e recomendações dos atuais sistemas de alimentação. Para as exigências líquidas de proteína e energia para crescimento (PL_G e EL_G respectivamente) foram utilizados apenas animais alimentados ad libitum (n = 238). Os parâmetros foram estimados usando o PROC MIXED do SAS. O modelo incluiu o efeito fixo de classe sexual (macho castrado, macho inteiro e fêmea; 73, 94 e 71, respectivamente) e o efeito aleatório de estudo. As exigências líquidas para crescimento foram estimadas como a primeira derivada parcial das equações alométricas em relação ao PC vazio. Houve efeito de classe sexual nas exigências líquidas de proteína (PL_G) e energia (EL_G) para crescimento, em que para PL_G, machos inteiros e machos castrados não diferiram, no entanto apresentaram valores superiores aos valores obtidos para fêmeas. Em relação aos valores de EL_G, machos castrados apresentaram valores superiores aos valores de machos inteiros e inferiores aos valores de fêmeas.

Palavras-chave: abate comparativo, alometria, exigências nutricionais, Saanen

LIST OF ABBREVIATIONS

ADG	Average daily gain
AL	Ad libitum
BW	Body weight
CP	Crude protein
DE	Digestible energy
DMI	Dry matter intake
EBW	Empty body weight
EWG	Empty weight gain
GE	Gross energy
GH	Growth hormone
HP	Heat production
k_f	Efficiency of energy retained as fat
k_g	Efficiency of energy utilization for growth
k_m	Efficiency of ME utilization for maintenance
k_p	Efficiency of energy retained as protein
LCI	Lower confidence limit
ME	Metabolizable energy
ME_G	Metabolizable energy requirement for growth
MEI	Metabolizable energy intake
MEI_G	Metabolizable energy intake above maintenance
ME_M	Metabolizable energy requirement for maintenance
ML	Maintenance level
MP_G	Metabolizable protein requirement for growth
MR	Moderate feed restrition
N	Nitrogen
NE_G	Net energy requirement for growth
NE_M	Net energy requirement for maintenance
NP_G	Net protein requirement for growth
NP_M	Net protein requirement for maintenance
Q	Metabolizability
RE	Retained energy
RE_f	Energy retained as fat
RE_p	Energy retained as protein
SD	Standard deviation
SEM	Standard error of mean
UCI	Upper confidence limit
σ^2_e	Estimated residual variance
σ^2_s	Estimated study variance
$\sigma^2_{b:s}$	Estimated block nested in study variance

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DISSERTATION STRUCTURE

Chapter 1 is a literature review, about protein and energy requirements for maintenance and growth, covering the main concepts, factors that influence, and the methods used to predict these requirements. It was written following the guidelines of the Graduate Program in Animal Science of Unesp, Jaboticabal Campus.

Chapter 2 describes the energy requirements and efficiency of energy utilization by dairy goats. This chapter was also written following the guidelines of the Journal of Dairy Science except by the letter style, spaces between lines, and position of tables. The paper authors are A. P. Souza, N. R. St-Pierre, M. H. R. M. Fernandes, A. K. Almeida, J. A. C. Vargas, K. T. Resende and I. A. M. A. Teixeira.

Chapter 3 is a technical note about the protein requirements for maintenance in growing dairy goats. This chapter was also written following the guidelines of the Journal of Dairy Science except by the letter style, spaces between lines, and position of tables. The authors are A. P. Souza, N. R. St-Pierre, M. H. R. M. Fernandes, A. K. Almeida, J. A. C. Vargas, K. T. Resende and I. A. M. A. Teixeira.

Chapter 4 describes the protein and energy requirements for growth of dairy goats. This chapter was modified from the paper published in the Journal of Dairy Science in 2017 (doi/10.3168/jds.2016-11895). The paper authors are A. P. Souza, N. R. St-Pierre, M. H. R. M. Fernandes, A. K. Almeida, J. A. C. Vargas, K. T. Resende and I. A. M. A. Teixeira.

Chapter 5 describes the main implications of this study, written following the guidelines of the Graduate Program in Animal Science of Unesp, Jaboticabal Campus.

CHAPTER 1. GENERAL CONSIDERATIONS

INTRODUCTION

Goats are rustic animals existing in distinct regions of the world, which have been historically used for many purposes such as milk, meat, fiber and skin production (DUBEUF et al., 2004). The goat's milk production in the world has risen by about 60% in the last 20 years (FAOSTAT, 2015). Making the correct choice of the goat's breed and providing a diet adequately formulated to supply the nutrients and energy for optimal production are essential steps for meeting the demand of goat dairy products, thereby improving the efficiency of the production system. In this sense, the Saanen breed is one of the best breeds used in dairy production, because, on average, they produce a greater amount of milk compared to other dairy breeds (RIBEIRO, 1997).

Knowledge about the protein and energy requirements of the dairy goats and about the composition of the feedstuffs used, are the basis for providing a balanced diet in the production system. The importance of an adequate supplying of protein lies in its function as it pertains to animal production and the high costs of the sources of protein for diets. Additionally, animals that are either underfed or overfed in energy may exhibit reproductive problems throughout their lives (RUKKWAMSUK et al., 1999; FENWICK et al., 2008).

The knowledge about body composition is important for estimating the nutritional requirements because they are associated (NRC, 2007). One of the factors that may affect the body composition is the sex of an animal (GEAY, 1984; HERRING et al., 2013). The effects of sexual hormones on the deposition of muscle and adipose tissue have been studied in cattle where differences in body composition between sexes, and, consequently, differences between protein and energy requirements for maintenance and growth were found (ARC, 1980; NRC, 2007). However, the effect of the sex of an individual on the requirements for maintenance and growth remains poorly quantified in goats.

This review will discuss the main factors related to protein and energy requirements for maintenance and growth in dairy goats. Furthermore, it will discuss the main methods used to predict those requirements.

PROTEIN AND ENERGY REQUIREMENTS FOR MAINTENANCE

Protein and energy requirements are frequently estimated by the factorial approach (NRC, 2007). The requirements for maintenance describe the nutrient quantities, or energy, for the basic functions in the body (AFRC, 1998). The losses of nitrogen in urine, feces, and skin are associated with the concept of nitrogen required for maintenance, since this considers the sum of the losses that occur in the body for the basal functions (AFRC, 1998). On the other hand, the energy requirements for maintenance have been defined as the amount of the feed energy intake that will not result in net loss or gain of energy from tissues of the animal body (NRC, 2007, NRC 2016).

The body composition also may affect the requirements for maintenance because the metabolic activity differs between tissues that constitute the body. The expenditure of energy by muscular tissues and organs will be different from the expenditure in adipose tissues, for example (GILL et. al, 1989).

Protein requirements for maintenance

Factors related to the animal as well as diets may affect the protein requirements for maintenance (CANNAS et al., 2008). The metabolic fecal crude protein includes crude protein (CP), such as enzymes and epithelial cells in the true endogenous losses (AFRC, 1998; NRC, 2007). Urinary CP includes costs associated with protein turnover, and those costs are usually lower than the fecal CP. Protein included in dermal losses, such as scurf and fiber, are also described as requirements for maintenance (AFRC, 1998; NRC, 2007).

The effect of sex is not reported in the protein requirements for maintenance by the current feeding systems (AFRC, 1998; CSIRO, 2007; NRC, 2007). Due to a lack of information about the protein requirements for maintenance, the current feeding systems still consider similar requirements to those reported for sheep and cattle. In a meta-analytical study, SALAH et al (2014) showed that the net protein required for maintenance (NP_M) in sheep was greater than the value found in goats (3.36 g/kg$^{0.75}$ BW vs 2.38 g/kg$^{0.75}$ BW). In the same study, they reported that the protein requirements for animals in warm climates are different from the values reported by the current feeding systems (AFRC, 1998; CSIRO, 2007; NRC, 2007).

Energy requirements for maintenance

The factors that affect the metabolizable energy requirement for maintenance (ME_M) are associated with animal characteristics, as well as the diets used in each situation (AFRC, 1993). Functions comprising the energetic costs for maintenance include body temperature regulation, essential metabolic processes, and physical activity (NRC, 2016). Among factors related to the animal, the effect of sex has been discussed and the current feeding systems for ruminants have reported that intact males have 15% greater requirements than females (ARC, 1980; CSIRO, 1990; NRC, 2000; NRC, 2007; BR-CORTE, 2016; NRC, 2016). The differences between sexes are associated with differences in body composition and the stage of maturity at a given BW (NRC, 2000).

As reported in the NE_G, it is preferred to express the requirements for maintenance in net terms than based on digestible energy (DE), total digestible nutrients (TDN), or metabolizable energy (ME). However, it requires predicting the efficiency of the utilization of energy.

In a meta-analysis using animals in warm climates, Salah et al. (2014) reported a value of ME_M of 105 kcal/kg$^{0.75}$ BW for goats. The reports by the current feeding systems are greater, when NRC (2007) and AFRC (1998) reported 128 and 117 kcal/kg$^{0.75}$ BW of ME_M, respectively. Independent studies conducted in goats have found that sex did not affect the energy requirements for maintenance (ASH & NORTON, 1987; BOMPADRE et al., 2014; ALMEIDA, et al., 2015a, FIGUEIREDO, et al., 2016b).

METHODS TO ESTIMATE THE PROTEIN AND ENERGY REQUIREMENTS

Different methods have been used to estimate the protein requirements in ruminant nutrition such as N balance and the comparative slaughter technique (ARC, 1980). In the N balance method, the maintenance requirements are estimated based on the losses of N in feces and urine, measuring them during a metabolism trial. The N balance is considered a better conceptual representation of the protein requirements for maintenance (ALMEIDA, et al., 2015b), however, this is based on a short period of experiment; consequently, this may be affected by any condition during this specific period (FORBES, 1973; HEGSTED, 1976). Due to limitations reported for the N balance method, the comparative slaughter technique, first developed for estimating energy requirements (LOFGREEN & GARRET, 1968), has been also adopted to determinate the protein requirements for maintenance (CHIZZOTTI et al., 2008.; ALMEIDA, et al., 2015b). In the comparative slaughter technique, the body composition of animals submitted to different levels of intake is evaluated; the procedures measure both protein intake and retained protein. In both methods, a regression of the retained N in the daily gain on N intake is used to calculate the net N requirement for maintenance. The intercept of the regression equation is assumed to be the endogenous and metabolic losses of N, which multiplied by the factor 6.25, is defined as the NP_M (ARC, 1980).

Basically, three methods have been used to measure the energy requirements: feeding trials, the comparative slaughter technique, and calorimetric methods. Using the feeding trials, it is the energy requirements for maintenance that are estimated by the quantity of feed needed to maintain BW that is determined. In the comparative slaughter technique, included in The California System, it is possible to obtain the energy requirements in net terms (LOFGREEN & GARRET, 1968), the differences in the body composition of animals fed in different levels of energy is obtained, and the heat production (HP) is calculated based on the retained energy (RE). The efficiency of energy utilization for maintenance (k_m) is obtained using the simple ratio between net energy for maintenance (NE_M) and the metabolizable energy for maintenance (ME_M), and the efficiency of energy utilization for growth (k_g) is estimated as the slope of the linear regression between RE and MEI above maintenance. The comparative slaughter technique presents the measurement of the body composition but lacks complexity and the costs of measurements (NRC, 2000); in this sense, the number of animals is limited. The calorimetric method has traditionally been used to estimate the energy requirements for maintenance, where the method is conducted in respiration chambers to measure gas exchange, fasting heat production and energy loss via urine and methane with animals fed at maintenance level (SALAH et al., 2014).

PROTEIN AND ENERGY REQUIREMENTS FOR GROWTH

The simplest definition of growth means getting bigger. In one individual animal we could refer to cell growth, tissue growth, or organ growth, but this discussion would be restricted to the physical aspects of growth (LAWRENCE, et al., 2012). The main interest when we study dairy animals lies in the growth of specific parts of the body such as bone, muscle, fat, or the development of the mammary gland, because these ones will be associated with the reproductive and productive lives of the animals (PETERS, 1983; LAWRENCE, et al., 2012). In this sense, we will develop the concept of growth related to body composition to evaluate, herein, in the field of nutritional requirements.

The net protein and net energy requirements for growth (NP_G and NE_G, respectively) can be more accurately defined based on the deposition of different tissues in the body (NRC, 2000). One of the factors that may affect the deposition of the different tissues, and consequently the nutritional requirements, is the genotype, since different breeds, for example, have different body composition mainly for presenting different weights at maturity (NRC, 2007; WEBSTER, 1986). Beyond the species or breeds, the body composition, and consequently the protein and energy requirements, may be affected by the physiological stage and sex of the animal in which the growth rate of bones, muscles, and adipose tissues are affected by different hormones during life (GEAY, 1984; LAWRENCE et al., 2012). Indeed differences in sexes also represent differences in maturity weight in dairy goats because of the distinct deposition of fat and protein in males and females, where females reach maturity earlier than males (ALMEIDA et al., 2016). Younger animals tend to present more protein and minerals in their bodies; on the other hand the deposition of fat increases with aging (OWENS et al., 1993; LAWRENCE et al., 2012).

Over the years, tissue growth and body composition have been studied in different species. BRODY (1945) detailed aspects related to bioenergetics and growth in ruminants. Different methods for determine body composition were also revised by BLAXTER (1989). Different researchers have verified that the animal grows to its adult weight following a sigmoid curve for cumulative growth (LAWRENCE, et al., 2012). In an attempt to model these variables, the model proposed that best describes the postnatal growth was the allometric model. Allometry, by definition, designates the changes in relative dimensions of parts of an organism that are correlated with changes in overall size (GAYON, 2000). The allometric equation usually takes the form of a two-parameter power function:

$$Y = a\ X^b$$

where Y is a biological variable of special interest, X is a measure of body size, and a and b are fitted parameters known as the allometric coefficient and allometric exponent, respectively (PETERS, 1983).

The concept of allometry has been adopted since 1968 in the comparative slaughter technique to predict NE_G (LOFGREEN & GARRET, 1968). This concept is adopted for understanding the requirements of an animal based on the specifics constituents (fat or protein).

Most investigators, however, work with logarithmic transformations of their data, so the mentioned equation is commonly expressed in the mathematical equivalent form as:

$$\log Y = \log a + b \log X$$

Traditional practice is to fit a straight line to logged values, usually by the method of ordinary least squares, and then to back-transform the resulting equation from logarithmic to arithmetic scale to obtain estimates for the parameters *a* and *b* (ZAR, 1968; SMITH, 1993). After that, the errors are multiplicative. The multiplicative error model assumes that the measures differ by equal proportion, and this is in line with the multiplicative nature of biological processes (KERKHOFF & ENQUIST, 2009). In this sense, the logarithmic transformation remains as an important and advantageous tool in allometry.

Protein requirements for growth

The current feeding systems for goats (NRC, 2007) and for cattle (NRC, 2000; NRC, 2016; BR-CORTE, 2016) have expressed the protein requirements in terms of metabolizable protein (MP) rather than crude protein (CP). The adoption of MP is basically explained by two reasons: there is now more information about the MP system which allows at more accurate prediction, and also because the CP system is based on an invalid assumption that the feedstuffs have an equal extent of protein degradation in the rumen (NRC, 2016). Net terms are also adopted to express the protein requirements for representing the absorption and incorporation of protein in the body (NRC, 2016). Net protein required is determined based on the retention of the protein in the body, and this will directly represent the amount of protein that an animal needs for archiving a specific average daily gain. The problems related to net proteins are the necessity of understanding the efficiency of utilization of the protein intake that will be affected either by the animals, or by the characteristics of each ingredient, or even by the combination of the ingredients in the diets (NRC, 2016).

The effect of sex on the body protein was verified in cattle, in which males present greater lean content than females at a similar body weight (BW; BERG & BUTTERFIELD, 1976; SEIDMAN et al., 1982; GEAY, 1984). On the other hand, studies with goats did not report differences in body protein between sexes (ALMEIDA et al., 2015b; FIGUEIREDO et al., 2016b), and consequently in the protein requirements. The current feeding systems for goats do not make a distinction in regards to the effect of sex on the protein requirements. The NRC (2007) reported that the MP estimated for growth (MP_G) in dairy goats is 290 g/kg BW gain, irrespective of sex. In the AFRC (1998) the NP_G ranged from 126 to 154 g/kg BW gain in goats weighing between 5 and 45 kg BW, evaluating mainly data from castrated goats.

Energy requirements for growth

The NE_G is defined based on the content of the tissue deposited using the comparative slaughter, which energy is a function of the proportion of fat and protein in the body (GARRET et al., 1980). The energy requirements are preferred expressed in net terms rather than based on digestible energy (DE), total digestible nutrients (TDN), or metabolizable energy (ME). However, this requires predicting the efficiency of energy use, and there are still few serial slaughter studies to allow this estimation (SAHLU et al., 2004; NRC, 2007).

Sex is a factor that determines the composition of growth, where hormonal regulations can establish biological limits for protein and fat deposition (BYERS, 1982). In response to changes in absorbed nutrients, the hormonal regulations in females results in a greater increase of fat in their body, and consequently, a greater amount of energy than in males (CHIZZOTTI et al., 2008; ALMEIDA et al., 2015a). This is possibly because of the earlier fat deposition in the abdominal tissues of females, which is an innate preparation of the female for future pregnancy (BERG & BUTTERFIELD, 1976). Sexual hormones are involved in the control of many mechanisms, and testosterone is one of the hormones that affect the secretion of growth hormone (GH); and it is also synergistic with estrogen for enhancing deposition of lean tissue (OWENS et al., 1993). The importance of GH in modulating lipid metabolism by decreasing glucose transportation and lipogenesis was detailed by LOUVEAU & GONDRET (2004).

The NE_G estimated by AFRC (1998) ranges from 2.2 to 4.1 Mcal/kg EBW gain, but there is no distinction between sexes in their estimation. The AFRC (1998) used studies mainly with castrated males, by the comparative slaughter technique. The NRC (2007) also does not incorporate a sex effect on the energy requirements for growth. In NRC (2007), the requirements are expressed in ME units (ME_G; 5.5 Mcal/kg BW gain). Variation in diet and body composition components has been reported to affect the partial efficiency of energy use for gain in lambs (k_g; GALVANI et al., 2014; ALMEIDA et al., 2015a).

META-ANALYSIS IN ANIMAL SCIENCE

The research in ruminant nutrition has markedly increased in the last years. In particular, there is a notable increase in the number of publications, which enhanced the number of experimental data available (ST-PIERRE, 2007). The aggregation of the information of several experiments may increase the possibility to get a better understanding of nutritional processes in the animal, allowing for the conclusion about animal responses in a broader application range than individual experiments (ST-PIERRE, 2001). Despite this, controlled and non-controlled factors, such as the basal plane of nutrition, vary from study to study, thus eventually requiring a quantitative summarization technique (SAUVANT et al., 2008). At this point, the meta-analysis can be proposed as a quantitatively summarization technique to attend this objective, which isolates the study effect.

In this context, the meta-analysis stands out as a statistical procedure to obtain reliable results regarding the values of protein and energy requirements in dairy goats. The development of a meta-analysis is done in several stages. The first defines the objectives and identifies the previous selection criteria for including variables in the database, the characterization of the variables as discrete or continuous, and definitions of the effects as fixed and random should also be taken into consideration (ST-PIERRE, 2007; ST-PIERRE, 2011). The objectives and reasons for using meta-analysis as a statistical procedure in animal studies were detailed by LOVATTO et al. (2007) and by SAUVANT et al. (2008). These researchers pointed out five important objectives of using meta-analysis: to obtain new results; to synthetize results; to improve the power of an analysis; to provide a better representation across studies; and even to generate new hypotheses.

Over the past few decades, multiple comparative slaughter studies were conducted at Universidade Estadual Paulista to quantify the effect of sex on protein and energy requirements for maintenance and growth in dairy goats (GOMES, 2011; BOMPADRE et al., 2014; MEDEIROS et al., 2014; ALMEIDA et al., 2015a,b; FERREIRA et al., 2015; FIGUEIREDO et al., 2016a,b). A meta-analysis of the individual records from these studies will be presented in the next chapters.

OBJECTIVE

The main objective of the research described in this dissertation is to predict protein and energy requirements for maintenance and growth in dairy goats of different sexes over a wide range of body weight.

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CHAPTER 2. Energy requirements and efficiency of energy utilization in growing dairy goats of different sexes

ABSTRACT: The aim of this study was to evaluate the effect of sex on the energy requirements and on the efficiencies of energy utilization in dairy goats. A database from seven comparative slaughter studies of 238 Saanen goats subjected to three levels of intake was gathered to provide information to develop equations to predict energy requirements and energy efficiencies of utilization. The experimental design provided different levels of metabolizable energy intake (MEI) and body weight (BW). The data were analyzed considering sex (intact males, castrated males, and females; *n* = 98, 80, and 60, respectively) as fixed effect, and blocks nested in studies and sex as random effects. For the development of linear and non-linear equations we used MIXED and NLMIXED procedures in SAS. Empty BW (EBW) was estimated as the difference between BW at slaughter and the contents of the gastrointestinal tract, bladder and biliary vesicle. Non-linear regression equations were developed to predict heat production (HP, kcal/kg$^{0.75}$ EBW; dependent variable) from MEI (kcal/kg$^{0.75}$ EBW; independent variable). Applying the comparative slaughter technique, the net energy requirement for maintenance (NE_M) was calculated as the value of HP at which MEI is zero. The metabolizable energy requirement for maintenance (ME_M) was calculated as the value at which HP is equal to MEI. The efficiency of ME utilization for maintenance (k_m) was calculated as the ratio between NE_M and ME_M. The efficiency of energy utilization for growth (k_g) was assumed to be the slope of the linear regression of RE on MEI above the maintenance (model intercept equal to 0). The k_p and k_f were calculated using the multiple linear regression of MEI above the maintenance (model intercept equal to 0) on RE_p and RE_f. Sex affected the NE_M (75.0 ± 1.76 kcal/kg$^{0.75}$ EBW for males and 63.6 ± 2.89 kcal/kg$^{0.75}$ EBW for females) but did not affect the k_m (0.63). The k_g was different between sexes (0.32 for castrated males; 0.26 for intact males; and 0.31 for females) but the k_p (0.21) and k_f (0.80) were similar between

sexes. This information may be useful for improving robustness of energy requirements recommendations for dairy goats.

Key words: metabolizable energy, net energy, partitioning of energy, Saanen goat

INTRODUCTION

The energy expenditure for accretion and maintenance is different between the adipose, muscular and other tissues in the body (Gill et al., 1989). In this sense, the body composition of an animal is directly associated with its energy utilization. Despite a relevancy of understanding the effects of body composition on the energy requirements and efficiency of energy utilization, the estimative of the energy requirements has been mainly obtained gathering results from calorimetry methods or feeding trials. These methods do not evaluate the direct effect of body composition on the energy requirements (Sahlu et al., 2004; NRC, 2007; Salah et al., 2014).

Animal characteristics as genotype, stage of maturity and sex may affect the pattern of tissue deposition and consequently the body energy. It has been reported that castrated males, intact males and females differ in their protein and fat amounts and proportions in the body (Geay, 1984; Herring et al., 2013). For this reason, we also expected the sex of one goat affects its energy requirements. The NRC (2007) stated that castrated males, females and intact males have similar ME_G, on the other hand, for maintenance, different feeding systems suggested that intact males require more ME_M than females (ARC, 1980; CSIRO, 1990; NRC, 2000; NRC, 2007) where the differences are mainly associated with body composition and stage of maturity at a given BW (NRC, 2007). However, iIndividual studies conducted with goats have generally concluded that sex affects the energy requirements for growth but does not affect the energy requirements for maintenance (Ash and Norton, 1987; Bompadre et al., 2014; Almeida et al., 2015; Figueiredo et al., 2016b).

To address this controversy in knowledge, we assembled a dataset with observations from individual animals from 7 comparative slaughter studies. Our objective for this study was to evaluate the effect of sex on the energy requirements and on the efficiencies of energy utilization in dairy goats.

MATERIALS AND METHODS

Data Collection

A dataset that included general information (e.g., author name), qualifying (e.g., sex, level of intake, and block), and necessary quantitative data was gathered for this study. Quantitative information included days on feed, initial and final BW, empty BW (**EBW**), DMI, ME intake (**MEI**), and body contents for each individual animal. Body samples were analyzed for fat content (AOAC, 1990, method 930.15), protein content by N analysis performed via Dumas combustion using LECO FP-528LC (Etheridge et al., 1998), and energy content using an adiabatic calorimetric bomb under protocols described in each of the published sources.

Data from individual animals were obtained from 7 comparative slaughter studies (Gomes, 2011; Bompadre et al., 2014; Medeiros et al., 2014; Almeida et al., 2015; Ferreira et al., 2015; Figueiredo et al., 2016a; Figueiredo et al., 2016b) resulting in 238 records (intact males, castrated males, and females; *n* = 98, 80, and 60, respectively). In all studies, each block was composed by 3 pair-fed goats within sex randomly allocated to 1 of 3 levels of intake (ad libitum; moderate restriction, 25 or 30% of feed restriction; and maintenance level, 50 or 60% of feed restriction). The daily intake of the restricted-fed goats within a block was determined by the DMI of the goat fed ad libitum within the same block on the previous day. The protein and energy contents of diets fed ranged from 137 to 204 g/kg CP and from 2.4 to 2.7 Mcal/kg ME. The summary statistics of the main variables of the dataset by sex is presented in Table 1.

Table 1. Summary of descriptive statistics of body composition in Saanen goats used in this study

Variables	n[1]	Mean	SD	Range
EBW[2] (kg)				
All animals	238	22.3	9.83	4.1 to 41.7
Castrated male	80	22.2	8.56	4.1 to 39.7
Intact male	98	21.3	10.6	5.1 to 41.7
Female	60	24.2	9.94	6.6 to 40.4
ADG (g/day)				
All animals	238	96.6	64.1	-18.8 to 264
Castrated male	80	95.0	68.4	-16.8 to 259
Intact male	98	111.4	64.5	-13.6 to 264
Female	60	74.4	50.2	-18.8 to 186
EWG[3] (g/day)				
All animals	238	82.5	54.2	-16.0 to 239
Castrated male	80	83.8	60.5	-8.7 to 239
Intact male	98	90.6	53.1	-16.0 to 219
Female	60	67.5	43.8	-10.3 to 171
DMI (g/day)				
All animals	238	600	332	104 to 1528
Castrated male	80	681	343	128 to 1440
Intact male	98	535	335	104 to 1528
Female	60	600	290	130 to 1287
MEI (kcal/day)				
All animals	238	1603	690	415 to 3272
Castrated male	80	1729	706	484 to 3272
Intact male	98	1503	700	415 to 3218
Female	60	1598	632	525 to 2895
RE[4] (kcal/day)				
All animals	238	261	212	-83 to 1061
Castrated male	80	291	237	-76 to 1061
Intact male	98	227	181	-83 to 930
Female	60	274	215	-83 to 853
RE_p[5] (kcal/day)				
All animals	238	84.5	62.6	-71.8 to 302
Castrated male	80	91.6	68.7	-21.6 to 289
Intact male	98	93.1	62.9	-71.8 to 302
Female	60	60.9	46.1	-38.7 to 164
RE_f[6] kcal/day)				
All animals	238	197	180	-133 to 802
Castrated male	80	220	194	-133 to 717
Intact male	98	148	148	-54.9 to 802
Female	60	245	193	-40.5 to 783

[1]Number of records in the study.
[2]Slaughter empty BW.
[3]EWG is empty weight gain.
[4]RE is retained energy. The measurements of energy were obtained using bomb calorimeter.
[5]RE_p is RE as protein.
[6]RE_f is RE as fat.

All procedures used in the individual studies were followed in accordance with the University's Animal Care Committee (Comissão de Ética e Bem-Estar Animal – CEBEA), under protocols described in each of the published sources. During the experiments, mean daily minimum and maximum temperatures were 16.3 and 35.7 °C respectively, and minimum and maximum relative humidity of the air were 21.5 and 88.6% respectively.

Data Calculation and Analyses

Prediction of MEI. The MEI was calculated based on the ME concentration of the diet (kcal/kg DM) estimated from the GE intake, total energy losses from feces, urine and gaseous products of digestion. Fecal and urinary excretion were obtained from total collection. Energy losses from gaseous products of digestion were predicted according to Blaxter and Clapperton (1965). We evaluated the ratios between the gross energy (**GE**), DE and ME. Values of predicted ME concentration for animals that were not part of the digestibility trials were derived from a linear model that predicted the ME concentration of the diet from DMI obtained during the experiment. The model included blocks and study as random effect, and sex as fixed effect.

Energy requirements. The procedures used to estimate the net energy requirement for maintenance (**NE_M**) using the comparative slaughter technique were similar to those described by Lofgreen and Garrett (1968). The initial body energy was calculated as follow: 1) initial EBW of the animals was predicted from initial BW, and 2) initial body energy was predicted from initial EBW by allometric equations across all trials, according to the equations reported by Souza et al. (2017).

Daily heat production (**HP**, kcal/kg$^{0.75}$ EBW) was calculated as the difference between daily MEI (kcal/kg$^{0.75}$ EBW) and daily RE (kcal/kg$^{0.75}$ EBW). The NE_M was estimated as the value of HP at which MEI was zero and ME_M was the value at which MEI was equal to HP. The relationship between HP and MEI was modeled as a nonlinear mixed model as follow:

$Y_{ijkl} = B_{0i} \times \exp(B_{1i} \times X_{ijkl}) + s_j + z_{k(j)} + e_{ijkl}$ [1]

where, Y_{ijkl} is the HP (kcal/kg$^{0.75}$ EBW) for the l^{th} animal of the i^{th} sex in the j^{th} study in the block k^{th},

X_{ijkl} is the daily MEI (kcal/kg$^{0.75}$ EBW) for the l^{th} animal of the i^{th} sex in the j^{th} study in the block k^{th},

B_{0i} and B_{1i} are parameters to be estimated for each of the i = 1, 2, 3 sexes,

s_j is the random effect of the j^{th} study ~ $N(0, \sigma_s^2)$,

$z_{j(k)}$ is the effect of block k^{th} nested in study j^{th},

e_{ijkl} is residual error ~ $N(0, \sigma_e^2)$.

The approach proposed by Luo et al. (2004) based on the feeding trial was also used to estimate ME_M; we used the same animals that were used in the comparative slaughter technique. The MEI (kcal/kg$^{0.75}$ BW) was regressed against ADG (g/kg$^{0.75}$ BW). This was also modified and done using the daily MEI (kcal/kg$^{0.75}$ EBW) regressed against empty BW gain (**EWG**; g/kg$^{0.75}$ EBW). In addition to the regression analyses that provide estimates of the ME_M, the requirement of ME for growth (**ME_G**) was also estimated as the slope of the linear regression.

Efficiencies of ME Utilization. Using the data from the comparative slaughter technique, the efficiency of ME utilization for maintenance (**k_m**) was estimated as the ratio between NE_M and ME_M.

The partial efficiency of ME utilization for growth (**k_g**) was assumed to be the slope of the linear regression of RE on MEI above maintenance (**MEI_G**) = MEI – ME_m, assuming that RE is null when MEI_G = 0 (i.e., model intercept = 0), according to Galvani et al. (2014).

The efficiencies of RE as protein (**k_p**) and as fat (**k_f**) were calculated using the multiple linear regression of MEI_G where RE as protein (**RE_p**) and the RE as fat (**RE_f**) were calculated as the difference between final and initial BW of the respective body protein or fat multiplied by the energetic values of protein and fat. The partitioning of MEI to RE_p and RE_f was computed using a multiple regression as follow:

$$Y_{ijkl} = b_{1i} \times RE_p + b_{2i} \times RE_f + s_j + z_{k(j)} + e_{ijkl} \qquad [2]$$

where,

Y_{ijkl} is the MEI_G (kcal/kg$^{0.75}$ EBW) for the l^{th} animal of the i^{th} sex in the j^{th} study in the block k^{th},

RE_p and RE_f are the RE (kcal/kg$^{0.75}$ EBW) as fat and as protein respectively,

b_{1i} and b_{2i} are parameters to be estimated for each of the i = 1, 2, 3 sexes,

s_j is the random effect of the j^{th} study ~ $N(0, \sigma_s^2)$,

$z_{k(j)}$ is the effect of block k^{th} nested in study j^{th},

e_{ijkl} is residual error ~ $N(0, \sigma_e^2)$.

The k_p and k_f were calculated as the inverse of the parameters estimates b_1 and b_2, respectively.

Statistical Analysis

Statistical analysis was performed using SAS (version 9.4). The linear regression analyses were computed with MIXED procedure. The statistical models included blocks and study as random effect, sex (castrated male, intact male and females) as fixed effect. When sex was found to be significant ($P < 0.10$), indicating a different intercept for at least 1 sex, 3 CONTRAST statements were used to conduct all 3 pairwise comparisons of sex. Likewise, 3 CONTRAST statements were used to conduct all 3 pairwise comparisons when the interaction between sex and regressor effects was found to be significant ($P < 0.10$), indicating that at least 1 sex had different slope. Outliers were removed when their normalized residuals were > |3|.

The NLMIXED procedure was used to fit nonlinear models. The statistical models included sex (castrated male, intact male and females) as fixed effect and block nested in study and sex as random effect. We used dummy variables approach to assess the effect of sex on the regression parameters. That is, 3 dummy variables (a1, a2, and a3) were created. For castrated males, a1 = 1, a2 = 0, and a3 = 0; for intact males, a1 = 0, a2 = 1, and a3 = 0; and for females, a1 = 0, a2 = 0, and a3 = 1. CONTRAST statements were used for testing whether a regression parameter differed across the 3 sexes.

A Monte Carlo based simulation was used to calculate numerical estimates of the variance and confidence intervals for efficiencies of energy utilization and energy requirements. Simulated values were generated using a multivariate normal distribution for parameter estimates using the algorithm of Fan et al. (2002).

RESULTS

Estimation of MEI.

Sex affected the ratio DE:GE (P = 0.048), where females presented slightly greater energy digestibility compared with intact males (0.74 vs. 0.71; P = 0.01) and castrated males (0.74 vs. 0.72; P = 0.09), whereas DE:GE was similar between intact males and castrated males (0.72; P = 0.48). On the other hand, sex did not affect the ratio ME:DE (P = 0.47) and the ratio ME:GE (***q***; P = 0.16) in the digestibility trial (Table 2).

The level of intake affected the ratio DE:GE (P < 0.001) and q (P = 0.011; Table 2). Animals fed at maintenance level (**ML**) presented greater energy digestibility than animals fed at moderate feed restriction (**MR**; 0.75 vs 0.71; P < 0.001) and animals fed ad libitum (**AL;** 0.75 vs 0.71; P < 0.001), whereas DE:GE was similar between animals fed at MR and animals fed AL (P = 0.80). Additionally, the q was also greater in animals fed at ML than in animals fed MR (0.61 vs 0.59; P = 0.005) and animals fed AL (0.61 vs 0.59; P = 0.01), whereas q was similar between animals fed at MR and animals fed AL (0.59; P = 0.68). On the other hand, the level of intake did not affect the ratio ME:DE (P = 0.21) with an average value of 0.83 for all animals.

Values of predicted ME concentration (kcal/kg DM) for animals that were not part of the digestibility experiment were derived from the model obtained in Eq. [3]. The model was obtained using data of ME concentration and the daily DMI (g/kg$^{0.75}$ BW). The parameters did not differ between sexes (P > 0.10) and the fitted equation was as follow, (n = 206, σ^2_s = 50612, σ^2_e = 82791):

$$\text{All sexes: ME} = 2919.54\ (\pm 102) - 5.14\ (\pm 1.33) \times \text{DMI} \quad [3]$$

Energy requirements

The nonlinear regression indicated that HP exponentially increased as MEI increased (Figure 1). Sex affected the parameters estimates of the equations presented in Table 3. The parameters *a* (P = 0.75) and *b* (P = 0.51) were similar between castrated and intact males. However, *a* (P = 0.003) and *b* (P = 0.01) differed between castrated males and females, as well as between intact males and females, *a* (P = 0.003) and *b* (P = 0.03). Therefore, we reported the applicable parameters to each sex and one general equation to all males because castrated and intact males were similar (Table 3). The value of HP when MEI is zero (NE_M) was estimated to be 75.0 ± 1.76 kcal/kg$^{0.75}$ EBW for males, this value is 16% greater than the NE_M obtained for females (63.6 ± 2.89 kcal/kg$^{0.75}$ EBW) as well as observed to ME_M (Table 3).

Table 2. Metabolism trial of castrated male, intact male, and female Saanen goats under different levels of intake

Variables	Level of intake[4]									SEM[5]	*P*-value[6]		
	Castrated males			Intact males			Female						
	AL	MR	ML	AL	MR	ML	AL	MR	ML		S	L	L*S
DE:GE[1]	0.711	0.706	0.746	0.704	0.705	0.733	0.726	0.735	0.758	0.0116	0.048	< 0.001	0.89
ME:DE[2]	0.831	0.827	0.82	0.831	0.827	0.819	0.840	0.841	0.837	0.0111	0.47	0.21	0.97
q[3]	0.592	0.579	0.600	0.585	0.579	0.598	0.597	0.604	0.641	0.0159	0.16	0.01	0.61

[1]DE:GE is the ratio of digestible energy (DE) and GE.

[2]ME:DE is the ratio of ME and DE.

[3]*q* is the metabolizability, the ratio of ME and GE.

[4]Ad libitum (AL), moderate restriction (25 or 30% of feed restriction based on AL feed intake), maintenance level (50 or 60% of feed restriction based on AL feed intake).

[5]The largest SEMs are reported.

[6]Significance of the main effects of level of intake (L), sex (S), and their interaction.

Table 3. Parameter estimates of the regression equations for heat production (HP, kcal/kg$^{0.75}$ of empty BW) according to ME intake (MEI, kcal/kg$^{0.75}$ of empty BW) to estimate energy requirements for maintenance by sex on dairy goats[1]

Sex	n[2]	*a*	*b*	ME_M			k_m[3]		
				Mean	LCI[4]	UCI[5]	Mean	LCI[4]	UCI[5]
Castrated male	80	75.5 ± 2.73	0.00388 ± 0.000161	120.5	113.0	127.6	0.63	0.61	0.64
Intact male	94	74.4 ± 2.20	0.00402 ± 0.000126	121.0	114.2	127.2	0.62	0.60	0.62
Female	60	63.6 ± 2.89	0.00459 ± 0.000221	101.2	92.3	108.8	0.63	0.62	0.64
All males[5]	174	75.0 ± 1.76	0.00395 ± 0.000104	120.9	115.7	125.5	0.62	0.61	0.63

[1]Model: HP = $a \times \exp(b \times \text{MEI})$. The parameters *a* and *b* did not differ between castrated males and intact males ($P > 0.10$). The NE_M was calculated as the value at the MEI is zero (value of parameter *a*). The ME_M was calculated by iteration assuming HP is equal to MEI at maintenance. The variances were $\sigma^2_{b:s} = 4.148$ and $\sigma^2_e = 144.1$.

[2]Total number of animals used to estimate parameters.

[3]The efficiency of use of ME for NE_M was calculated as NE_M/ME_M.

[4]Lower 90% confidence limit of requirement.

[5]Upper 90% confidence limit of requirement.

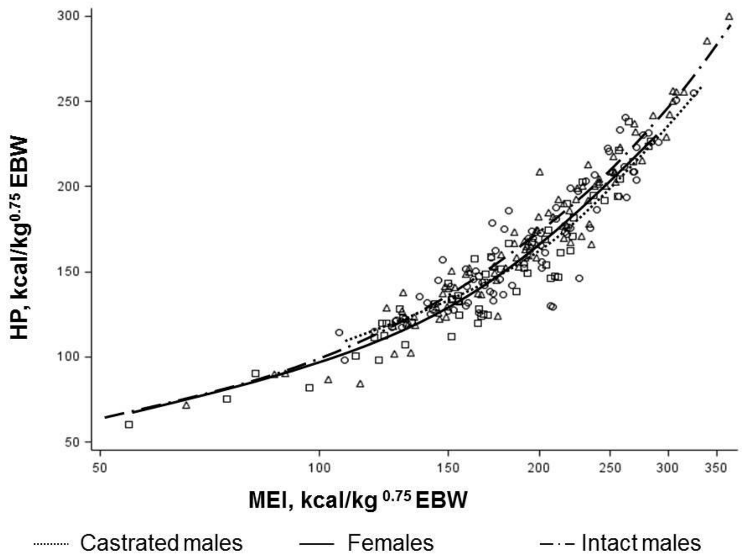

Figure 1. Relationship between heat production (HP) and MEI of Saanen goats of different sexes (○ castrated males, ∆ intact males, and □ females): for males HP = 75.0 (± 1.76) × exp (0.00395 (± 0.000104) × MEI); for females HP = 63.6 (± 2.89) × exp (0.00458 (± 0.000221) × MEI). The estimated study variances ($\sigma^2_{b:s}$) and the residual variances (σ^2_e) were 4.84 and 144.1 respectively. The parameters of the equation did not differ between castrated males and intact males (*P > 0.10*).

Using the approach suggested by Luo et al. (2004), we developed the relationship between ADG (g/kg$^{0.75}$ BW) and MEI (kcal/kg$^{0.75}$ BW) of Saanen goats (Eq. [6]; n = 235, $\sigma^2_{b:s}$ = 214.0, σ^2_e = 753.4). The ME_M did not differ between sexes (*P* = 0.28) and the overall value for ME_M was 111.6 (± 3.72) kcal/kg$^{0.75}$ BW, whereas the overall value for ME_G was 12.5 kcal/g ADG.

All sexes: MEI = 111.6 (± 3.72) + 12.5 (± 0.732) × ADG [6]

where, MEI is daily MEI (kcal/kg$^{0.75}$ BW) and ADG expressed in g/kg$^{0.75}$ BW.

When the same approach was scaled by EBW, the estimated ME_M was (126.2 ± 3.89) kcal/kg$^{0.75}$ EBW (Figure 2). There were no differences between the parameters estimates for different sexes (P = 0.56). In the same approach the ME_G were assumed to be the slope of the linear regression, so the estimated ME_G was 15.0 kcal/g EWG.

Efficiencies of ME Utilization

The k_m values were estimated as the ratio between NE_M and ME_M and did not differ between sexes, ranging from 0.62 to 0.64 for females and from 0.61 to 0.63 for males (Table 3).

The k_g was estimated using the regression equation of MEI_G on RE (Eq. [7], [8] and [9]; n = 230; $\sigma^2_{b:s}$ = 37.8, σ^2_e = 90.9). The slope is assumed to be the k_g for each sex and it did not differ between intact males and females (P = 0.14). However, the slope for castrated males was greater than for intact males (P = 0.055) and did not differ to the slope for females (P = 0.79). Therefore, we reported the applicable parameters to each sex, where the values of k_g for castrated males, intact males and females were 0.32, 0.26 and 0.31 respectively.

Castrated males: $RE = 0.320\ (\pm 0.0237) \times MEI_G$ [7]

Intact males: $RE = 0.260\ (\pm 0.0195) \times MEI_G$ [8]

Females: $RE = 0.310\ (\pm 0.0279) \times MEI_G$ [9]

where, RE is daily RE (kcal/kg$^{0.75}$ EBW) and MEI_G is daily MEI above maintenance (kcal/kg$^{0.75}$ EBW).

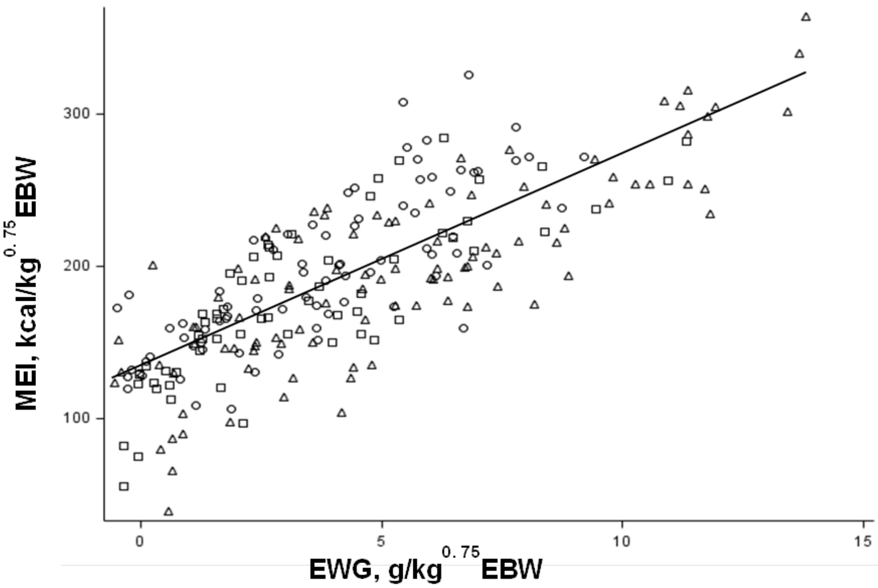

Figure 2. Relationship between empty weight gain (EWG) and MEI of Saanen goats (○ castrated males, ∆ intact males, and □ females). For all animals MEI = 126.2 (± 3.89) + 15.7 (± 0.801) × EWG. The estimated study variances ($\sigma^2_{b:s}$) and the residual variances (σ^2_e) were 112.7 and 959.2 respectively. The parameters of the equation did not differ between sexes (*P > 0.10*).

In search of understanding the effect of sex on the k_p and k_f, we developed a multiple regression of the partitioning of MEI to RE_p and RE_f (Table 4). Sex did not affect the k_p (*P* = 0.80) and k_f (*P* = 0.84). The overall value of k_p (0.21) was lower than the value for k_f (0.80; Table 4). The uncertainty in the estimates for k_f was noticeably greater than in the values for k_p.

Table 4. Regression of retained energy (RE) as fat and as protein on ME intake above maintenance (MEI_G) to estimate the partial efficiency of energy retention as fat and as protein on dairy goats[1]

Sex	n[2]	Coefficients		k_p			k_f		
		b_1	b_2	Mean	LCI[3]	UCI[4]	Mean	LCI	UCI
Castrated male	76	4.41 ± 0.606	1.17 ± 0.232	0.227	0.172	0.295	0.854	0.630	0.989
Intact male	93	4.88 ± 0.386	1.36 ± 0.259	0.205	0.177	0.237	0.735	0.520	0.945
Female	60	4.78 ± 0.652	1.20 ± 0.258	0.209	0.159	0.271	0.833	0.592	0.989
Overall	229	4.69 ± 0.323	1.24 ± 0.144	0.213	0.189	0.242	0.806	0.653	0.955

[1]Model: daily MEI_G (kcal/kg$^{0.75}$ EBW) = b_1 RE_p + b_2 RE_f, where RE_p and RE_f = daily RE as protein and as fat respectively (kcal/kg$^{0.75}$ EBW). The parameters b_1 and b_2 did not differ between sexes (P > 0.10).The efficiencies of RE as protein and fat (k_p and k_f) were calculated as 1/b_1 and 1/b_2, respectively.

[2]Total number of animals used to estimate parameters.

[3]Lower 90% confidence limit of requirement.

[4]Upper 90% confidence limit of requirement.

DISCUSSION

The effect of sex on the energy requirements and on the efficiency of energy utilization was evaluated in dairy goats. We found that sex affects the NE_M, as well as ME_M estimated by the comparative slaughter technique. Conversely, sex did not affect the ME_M and ME_G estimated by the feeding trial. Sex did not affect the k_m but affected k_g values, on the other hand the k_p and k_f were not different between castrated males, females and intact males.

The difference of NE_M between sexes is consistent with what has been reported by different feeding systems (ARC, 1980; CSIRO, 1990; NRC, 2000; NRC, 2007; NRC, 2016) that intact males have 15% greater requirements than females. The difference in our findings is that the castrated males presented similar results to intact males instead of females. This is possibly because of the differences in body composition and the stage of maturity at a given BW (NRC, 2000). This agree with what was reported by Souza et al. (2017) about the effect of sex on body protein and body fat in dairy goats, where the body protein of intact males and castrated males was greater than that found in females and the body fat of castrated males was greater than that of intact males and lower than that of females. Similar findings in the body protein and fat were reported in studies using other ruminants and pigs (Berg and Butterfield, 1976; Seidman et al., 1982; Geay, 1984). It is well known that the net protein deposition is much less efficient than the body fat, with estimates from 10 to 40% (Garret, 1980) and we also verified that with the data from the present study (Table 4) what can be strongly associated with the costs of maintenance of the adipose and muscular tissues. We verified in our study that castrated males were similar to intact males and not to females as reported to ME_M by NRC (2007), what may be more associated with similar body protein composition to intact males. Regarding the stage of maturity, Almeida et al. (2016) evaluated the maturity EBW in dairy goats and the authors pointed out that females are more precocious than castrated males and intact males. In this sense, animals of different sexes have different EBW at maturity, where the EBW at maturity was 34.1, 25.8 and 42.9 kg EBW for castrated males, females and intact males respectively (Almeida et al., 2016). Therefore, females have 60% of the mature

EBW reported for intact males at maturity and it agrees with the lower energy requirements for maintenance.

The NE_M for males (75 ± 1.76 kcal/kg$^{0.75}$ EBW) was similar to the values reported in cattle by Chizzotti et al. (2008) using also the comparative slaughter technique (75 kcal/ kg$^{0.75}$ EBW). The requirements scaled by EBW allow a more adequate index since only tissues are analyzed (Owens et al., 1995). In addition, we agree that the use of requirements in net terms is more accurate because avoids the effect of different diets and different efficiencies on the results, enabling a less biased comparison.

Regarding to the ME_M, this was calculated by two different approaches. The estimates obtained from the comparative slaughter technique differed between males (with confidence interval from 115.7 to 125.5 kcal/kg$^{0.75}$ EBW) and females (with confidence interval from 92.3 to 108.8 kcal/kg$^{0.75}$ EBW) and the estimates from the feeding trial were from 119.5 to 131.2 kcal/kg$^{0.75}$ EBW. Hence, the values from the feeding trial are slightly greater and closer to the estimates obtained to males by the comparative slaughter technique. The ME_M for males (in unit of BW) using the comparative slaughter technique was also lower than values reported by the current feeding systems, where our value (102 kcal/kg$^{0.75}$ BW) was similar to the value reported by Salah et al. (2014; 105 kcal/kg$^{0.75}$ BW) but lower than reports by NRC (2007) and AFRC (1998) of 128 and 117 kcal/kg$^{0.75}$ BW respectively. The values that we obtained herein using the feeding trial (111. 6 ± 3.72 kcal/kg$^{0.75}$ BW) were also lower than what was reported by the NRC (2007; 149 kcal/ kg$^{0.75}$ BW) for intact males. When we evaluated the variance of the errors between models we verified that the nonlinear model obtained using HP and MEI was more precise and thus this was able to confirm the differences between sexes than the linear model developed based on the MEI and EWG.

In the approach obtained by the feeding trial, we also reported the value for ME_G of 12.5 kcal/g ADG and 15.0 kcal/g EWG as the slopes of the linear regression between MEI and ADG, and between MEI and EWG respectively. Those values are greater than the values reported by NRC (2007; 5.52 kcal/g ADG) that also did not report differences between sexes. When we evaluated the k_g between sexes we found values for castrated males, intact males and females of 0.32, 0.26 and 0.31 respectively. This means that the net energy for gain (**NE_G**) could be predicted as 4.0, 3.25 and 3.87 kcal/g ADG or 4.8, 3.9 and 4.65 kcal/g EWG for castrated males, intact males and females, respectively. The values of NE_G were greater than the values reported by Souza et al. (2017), who evaluated the NE_G based on comparative slaughter technique and have reported values from 1.74 to 3.75 kcal/g EWG for castrated males, 1.73 to 2.89 kcal/g EWG for intact males and 1.86 to 4.77 kcal/g EWG for females. Both approaches agreed that intact males have lower NE_G in response of lower fat deposition than castrated males and females. The NE_G values in the present study were similar to the NE_G reported by AFRC (1998) from 2.2 to 4.1 kcal/g EWG.

The uncertainty verified for the k_m was lower than that found for k_g. The k_m is by definition related to fasting heat production (Garret, 1980) and the estimates in the present study (0.61 to 0.64) were consistent with findings reported by Nie et al. (2015) using lambs, 0.64 to 0.65, and findings reported to beef cattle by Chizzotti et al., (2008), 0.67, that also did not find effect of sex on the k_m. It was observed that the values for k_m to the animals in the dataset were reasonably constant.

It has been largely reported the effect of diet on the efficiencies as reported by the ARC (1980), which proposed an approach to calculate the k_m using the *q* of the diet where: $k_m = 0.30 \times q + 0.546$. Applying this approach and using the *q* obtained for animals fed at the maintenance (0.61) in the present study, then the estimated k_m was 0.73. Using this equation can hence underestimate ME_M. Differences in k_m related to diets are associated with extra heat and differences in time required to chew the feed for example (Susenbeth et al., 1998; Salah et al., 2014) but the results showed by Galvani et al. (2014) evaluating low-quality and medium-quality diets concluded that k_m was not related to the energy concentration of the diets used. Our results confirm that k_m may not be related to differences in body composition as well.

Hence, it is expected that the differences of body composition would be more evident in animals that are in process of growing than at the maintenance, it may be a possible reason for finding different k_g between sexes since represents the energy utilization to deposit protein and fat in the body. Although Ferrel (2003) has discussed the effect of body composition on k_g, the current feeding systems (AFRC, 1998; CSIRO, 2007; NRC 2007) have ignored the effects of gain composition on k_g, and it has been predicted based on the characteristics of the diet. In general, using these models overestimated the values of k_g in our study.

The results from the partitioning in k_p and k_f showed that these efficiencies are not affected by sex. Although the values of k_p and k_f are similar between sexes, the proportion of protein and fat deposited in the body are different between sexes, so the k_g is affected as a response of different proportions of protein and fat in the body. In our results using confidence intervals we found a large uncertainty of the k_f, which represents a great individual uncertainty on the fat deposition by each animal. The deposition of protein was more precise than deposition of fat, which reflects in a lower uncertainty. The reported k_p (0.20) and k_f (0.75) for cattle (Geay, 1984) were similar to the value reported in our study.

The k_g estimates in the present study were different between sexes (0.32 , 0.26 and 0.31 for castrated males, intact males and females respectively) where the lower efficiency was reported in males possibly because the proportion of fat and protein retained by each sex. In previous researches evaluating the effect of sex on the k_g in other ruminants, the authors did not report differences regarding to the effect of sex and showed it is, in average, 0.43 and 0.44 for beef cattle and sheep, respectively (Chizzotti et al., 2008; Nie et al. 2015) what could not maybe be verified due to the uncertainty in the data.

The estimates of energy requirements in net terms provide a more precise quantification of the values since we avoid an interference caused by different efficiencies. The body composition did not affect the efficiency of utilization of ME for maintenance but affected the partial efficiency of energy for gain, where castrated males and females are more efficient than males. To our knowledge, this is the first study aimed at quantifying the energy requirements related to the efficiency of utilization of energy by dairy goats over a wide range of BW. Thus, the information presented here may improve the knowledge about the partition of energy and the application in diets for dairy goats.

CONCLUSION

The sex affects the energy requirements and the efficiency of energy utilization in dairy goats. The NE_M, as well as ME_M estimated by the comparative slaughter technique, are greater for males than for females. Dairy goats of different sexes have different k_g, but similar k_p, k_f, and k_m.

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CHAPTER 3. Protein requirements for maintenance in growing dairy goats

ABSTRACT: The objective of this study was to estimate the net protein requirements for maintenance (NP_M) in growing dairy goats of different sexes using a meta-analytical approach. For this purpose, we used two datasets. Dataset 1 was used to evaluate the effect of sex on the NP_M using the comparative slaughter technique. This dataset was composed of 185 individual records from 6 studies. Dataset 2 was used to evaluate the effect of sex on NP_M using the N balance method. This dataset was composed of 136 individual records from 6 studies. The experimental design provided different levels of N intake and different body weight (BW), allowing the development of regression equations to predict the NP_M. The NP_M was assumed to be the intercept of the linear regression of the N retained (g/kg $^{0.75}$ BW) on the total N intake (g/kg $^{0.75}$ BW) multiplied by 6.25. The data were analyzed using MIXED procedure of SAS, sex was considered as fixed effect and block nested in study and sex was considered random effects. The NP_M was similar between sexes. Using the comparative slaughter technique, the daily estimated NP_M was 1.23 g/kg $BW^{0.75}$; lower than the one using N balance technique (3.18 g/kg $BW^{0.75}$) for dairy growing goats and previous reports by the current feeding systems.

Key words: comparative slaughter, nitrogen balance, Saanen goat, sex

INTRODUCTION

The productive performance of goats depends on the supplying of adequate diets, which requires accurate information about the nutrient requirement including factors that may influence the recommendation of nutrient demand. It has been recognized that sex affects the body composition however the approaches adopted by the current feeding systems (AFRC, 1998; NRC, 2007) did not account for changes in body composition and do not consider the effect of sex in protein requirements for maintenance of dairy goats.

By definition, protein requirement for maintenance includes endogenous urinary protein, endogenous fecal protein and dermal protein losses (AFRC, 1998). Based on it, N balance is a method frequently used by measuring those losses (Almeida et al., 2015). Nonetheless, N balance studies are based on few days of measurements and may be affected by the environmental condition of that period when it is conducted (Forbes, 1973) as well as can lead to biases of overestimation of N retention (Spanghero and Kowalski, 1997). Another technique used for estimating protein requirements for maintenance is the comparative slaughter technique, which is based on differences in body composition of animals slaughtered at different weights.

Although supplying adequately protein in diets is unquestionable important, there is no recent study that has focused on the protein requirements for maintenance of dairy goats of different sexes using a large dataset and considering the body composition. The objective of this study was to estimate protein requirements for maintenance of growing dairy goats of different sexes using a meta-analytical approach.

MATERIALS AND METHODS

Data Collection

A dataset that included general information (e.g., author name), qualifying (e.g., sex, level of intake, and block) and necessary quantitative data of body composition and intake was gathered for this study. Data from individual animals were obtained from 6 comparative slaughter studies (Medeiros et al., 2001; Boaventura Neto, 2011; Figueiredo, 2011; Gomes, 2011; Ferreira et al., 2015; and Almeida et al., 2015).). In all studies, each block was composed by 3 pair-fed goats within sex randomly allocated to 1 of 3 levels of intake (ad libitum; moderate restriction, 25 or 30% of feed restriction; and maintenance level, 50 or 60% of feed restriction). The daily intake of the restricted-fed goats within a block was determined by the DMI of the goat fed ad libitum within the same block on the previous day. The CP and ME contents of solid diets fed ranged from 137 to 175 g/kg DM and 2.4 to 2.7 Mcal/kg DM, CP of milk ranged from

283 to 285 g/kg DM. Body and diet protein contents were measured by N analysis performed via Dumas combustion using LECO FP-528LC (Etheridge et al., 1998). Diet energy content using an adiabatic calorimetric bomb under protocols described in each of the published sources.

All procedures used in the individual studies were followed in accordance with the University’s Animal Care Committee (Comissão de Ética e Bem-Estar Animal – CEBEA), under protocols descripted in each of the published sources.

The net protein requirement for maintenance (**NP_M**) was calculated using the comparative slaughter technique (Lofgreen and Garrett, 1968) and N balance as described below.

Comparative slaughter technique

The initial body protein was calculated as follow: 1) initial EBW of the animals was predicted from initial BW, and 2) initial body protein was predicted from initial EBW by allometric equations across all studies, as described by Souza et al. (2017). The summary statistics of the main variables of the dataset by sex is presented in Table 1.

For estimating NP_M using the comparative slaughter technique, we used data of 185 dairy goats (62 castrated males, 80 intact males, and 43 females). A linear regression of the N retained in the daily gain (g of N/kg$^{0.75}$ BW) on N intake (g of N/kg$^{0.75}$ BW) was used to calculate net N requirement for maintenance. The intercept of the regression was assumed to be the endogenous and metabolic losses of N, which when multiplied by 6.25 is assumed to be the NP_M.

Table 1. Summary of descriptive statistics of body composition and intake of Saanen goats used in the comparative slaughter technique

Variables	n[1]	Mean	SD	Range
BW (kg)				
All animals	185	27.4	12.5	6.2 to 51.0
Castrated male	62	27.6	11.0	6.2 to 47.4
Intact male	80	25.8	13.5	8.0 to 51.0
Female	43	30.2	12.5	8.4 to 46.0
EBW[2] (kg)				
All animals	185	22.6	10.9	4.1 to 41.7
Castrated male	62	22.5	9.5	4.1 to 39.7
Intact male	80	21.0	11.6	5.1 to 41.7
Female	43	25.4	11.3	6.6 to 40.4
ADG (g/day)				
All animals	185	93.7	66.8	-18.8 to 264
Castrated male	62	91.2	71.2	-16.8 to 259
Intact male	80	112	66.5	-13.6 to 264
Female	43	64.0	48.1	-18.8 to 162
DMI (g/day)				
All animals	185	604	364	104.7 to 1528
Castrated male	62	701	376	127.6 to 1440
Intact male	80	530	362	104.7 to 1528
Female	43	604	325	130.9 to 1287
CPI[3] (g/day)				
All animals	185	95.2	46.7	23.8 to 209
Castrated male	62	106	49.3	26.7 to 205
Intact male	80	87.6	45.5	23.8 to 209
Female	43	93.4	43.0	29.1 to 193
Protein retained in tissue (g/day)				
All animals	185	13.5	11.0	-12.6 to 53.1
Castrated male	62	14.5	11.7	-3.8 to 41.1
Intact male	80	15.4	11.3	-12.6 to 53.1
Female	43	8.3	7.2	-6.8 to 24.9

[1]Number of records.
[2]Empty BW at slaughter.
[3]CPI is crude protein intake.

N balance method

For estimating NP_M using the N balance method, we used data of 136 dairy goats obtained from digestibility trials (43 castrated males, 59 intact males, and 34 females). Their feed intake and feed refusals were recorded and feces and urine were collected for a minimum period of 5 days after an adaptation period as detailed in the published sources. A descriptive analysis is presented in Table 2. We adopted 0.018 g N/kg$^{0.75}$ BW to dermal losses (ARC, 1980). The N retained was obtained assuming the difference between N intake and N excreted (sum of fecal, urinary and dermal N). As described for the comparative slaughter technique, the intercept of the regression of N retained on N intake was assumed to be the endogenous and metabolic losses of N, which when multiplied by 6.25 is assumed to be the NP_M.

Table 2. Summary of descriptive statistics of N balance in Saanen goats used in this study

Variables	n[1]	Mean	SD	Range
BW[2] (kg)				
All animals	136	27.2	10.0	7.7 to 42.1
Castrated male	43	27.7	8.25	7.7 to 40.2
Intact male	59	24.6	11.5	7.9 to 42.1
Female	34	31.0	8.00	8.0 to 39.6
DMI (g/day)				
All animals	136	724	339	43.7 to 1672
Castrated male	43	730	339	79.8 to 1299
Intact male	59	710	358	64.7 to 1672
Female	34	738	310	43.7 to 1339
N intake (g/day)				
All animals	136	18.1	8.50	1.11 to 37.6
Castrated male	43	19.4	9.68	2.02 to 37.6
Intact male	59	17.6	8.27	1.64 to 36.8
Female	34	17.5	7.33	1.11 to 32.2
N feces (g/day)				
All animals	136	5.42	3.05	0.430 to 13.3
Castrated male	43	6.31	3.46	0.599 to 13.3
Intact male	59	4.71	2.88	0.430 to 12.2
Female	34	5.53	2.52	0.472 to 11.7
N urine (g/day)				
All animals	136	8.39	5.10	0.559 to 24.9
Castrated male	43	9.87	5.22	0.934 to 24.9
Intact male	59	7.63	5.39	0.740 to 21.5
Female	34	7.87	3.90	0.559 to 14.2

[1]Number of records.
[2]BW during the digestibility period.

Statistical Analyses

Statistical analysis was performed using SAS (9.4) with MIXED procedure. A mixed model was used assuming sex as fixed effect, and the effect of block nested in study and sex as random effect.

The general statistical model used was as follows:

$Y_{ijk} = a_{0i} + a_1X_{ijk} + s_j + z_{k(j)} + e_{ijkl}$

[1]

where,

Y_{ijk} = is the dependent variable (N retention, g/kg $BW^{0.75}$) for the *l^th^* animal of the *i^th^* sex in the *j^th^* study in the block *k^th^*,

X_{ijk} is the independent variable (N intake, g/kg $BW^{0.75}$) for the *l^th^* animal of the *i^th^* sex in the *j^th^* study in the block *k^th^,*

a_{0i} and a_{1i} are the parameters to be estimated for each of the *i* = 1, 2, 3 sexes,

s_j is the random effect of the j^{th} study ~ $N(0, \sigma_s^2)$,

$z_{k(j)}$ is the effect of block k^{th} nested in study j^{th},

e_{ijkl} is residual error ~ $N(0, \sigma_e^2)$.

When sex effect was significant on intercept, suggesting that at least two intercepts differed between sexes, three CONTRAST statements were applied to conduct pairwise comparisons of sex. Furthermore, three CONTRAST statements were applied to conduct pairwise comparisons when the interaction between sex and BW or EBW was found to be significant, indicating that at least two slopes differed between sexes (St-Pierre, 2001). Outliers were removed when their normalized residuals were > |3|. For the comparative slaughter technique, five data points were removed (2 castrated males, 2 intact males and 1 female), for N balance method also five data points were removed (2 castrated males, 1 intact males and 2 females).

RESULTS

The NP_M estimates using both approaches, the comparative slaughter technique (Figure 1) and the N balance method (Figure 2), were similar between sexes

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Comparative slaughter technique. Using the comparative slaughter technique, we evaluated a relationship between N intake (g/kg$^{0.75}$ BW) and N retained in tissues (g/kg$^{0.75}$ BW) in dairy goats (Eq. [2], Eq. [3], Eq. [4], Eq. [5]; *n* = 180, $\sigma^2_{b:s}$ = 0.0109, σ^2_e = 0.00655). The NP_M (i.e. the intercept of this regression), did not differ between sexes (*P* = 0.67) and the overall value was 197 mg of N/kg$^{0.75}$ BW (at N intake = 0), which corresponds to a NP_M of 1.23 g/kg$^{0.75}$ BW.

Castrated male: N retained = -0.233 (± 0.0487) + 0.293 (± 0.0264) × N intake [2]

Intact male: N retained = -0.182 (± 0.0431) + 0.334 (± 0.0241) × N intake [3]

Female: N retained = -0.176 (± 0.0622) + 0.249 (± 0.0401) × N intake [4]

All sexes: N retained = -0.197 (± 0.0300) + 0.292 (± 0.0179) × N intake [5]

When this equation was scaled by metabolic EBW, The NP_M (i.e. the intercept of this regression) also did not differ between sexes (*P* = 0.61). We presented the relationship between N intake (g/kg$^{0.75}$ EBW) and N retained (g/kg$^{0.75}$ EBW) in dairy goats (Eq. [6], Eq. [7], Eq. [8], Eq. [9]; n = 180, $\sigma^2_{b:s}$ = 0.0144, σ^2_e = 0.00916). The overall value of NP_M was 234 mg of N/kg$^{0.75}$ EBW (at N intake = 0), which corresponds to a NP_M of 1.46 g/kg$^{0.75}$ EBW.

Castrated male: N retained = -0.281 (± 0.0576) + 0.298 (± 0.0268) × N intake [6]

Intact male: N retained = -0.210 (± 0.0507) + 0.333 (± 0.0246) × N intake [7]

Female: N retained = -0.211 (± 0.0738) + 0.255 (± 0.0417) × N intake [8]

All sexes: N retained = -0.234 (±0.0355) + 0.295 (± 0.0184) × N intake [9]

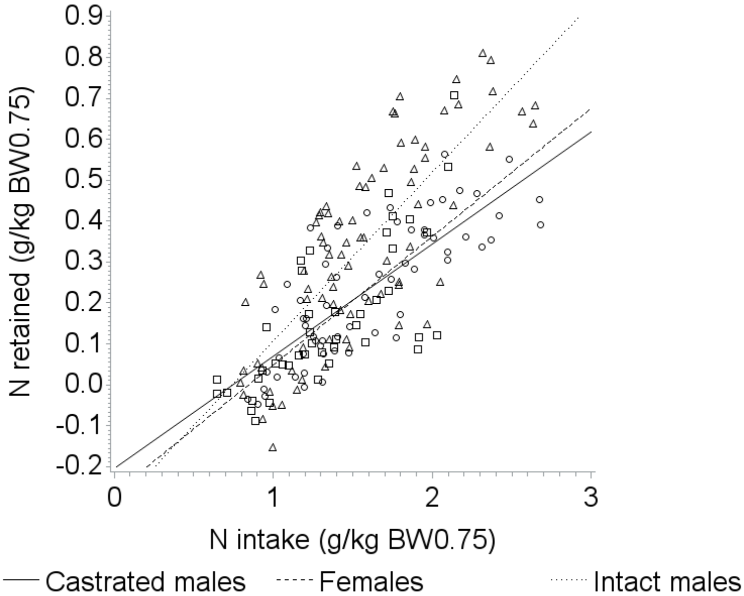

Figure 1. Relationship between N retained and daily N intake of Saanen goats of different sexes (○castrated males, ∆ intact males, and □ females) using the comparative slaughter technique. For all animals: N retained = - 0.197 (± 0.0300) + 0.292 (± 0.0179) × N intake. The estimated block nested to study variances ($\sigma^2_{b:s}$) and the residual variances (σ^2_e) were 0.0109 and 0.00655 respectively. The parameters of the equation did not differ between castrated males and intact males (*P > 0.10*).

N balance method. Using the N balance approach, we evaluated a relationship between N intake (g/kg$^{0.75}$ BW) and N retained in N Balance (g/kg$^{0.75}$ BW) in dairy goats (Eq. [10], Eq. [11], Eq. [12], Eq. [13]; n = 131, $\sigma^2_{b:s}$ = 0.0503, σ^2_e = 0.0482). The NP_M (i.e. the intercept of this regression) also did not differ between sexes (P = 0.38) and the overall value was 509 mg of N/kg$^{0.75}$ BW (at N intake = 0), which corresponds to a NP_M of 3.18 g/kg$^{0.75}$ BW.

Castrated male: N retained = - 0.653 (± 0.135) + 0.582 (±0.0773) × N intake [10]
Intact male: N retained = - 0.525 (± 0.172) + 0.662 (± 0.596) × N intake [11]

Female: N retained = - 0.348 (± 0.0817) + 0.508 (± 0.121) × N intake [12]

All sexes: N retained = - 0.509 (± 0.0817) + 0.584 (± 0.0519) × N intake [13]

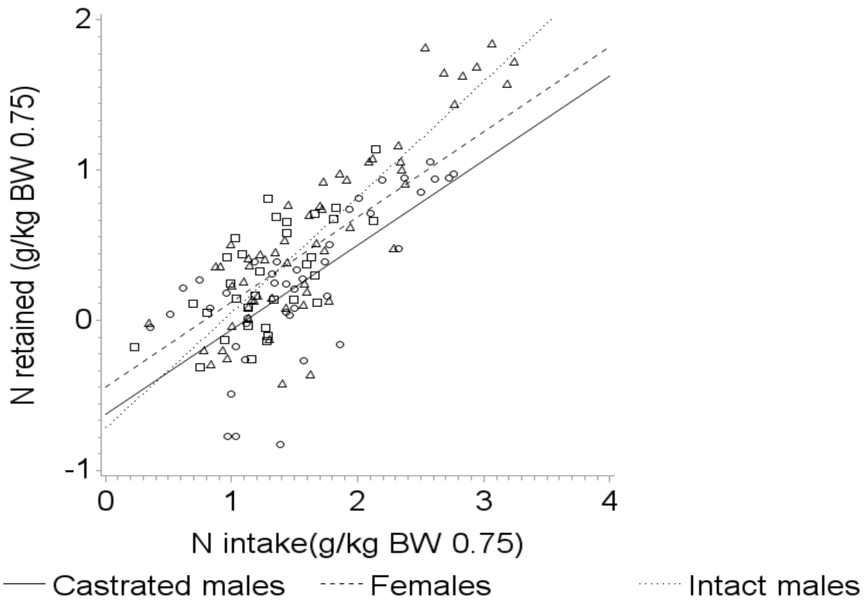

Figure 2. Relationship between N retained and daily N intake of Saanen goats of different sexes (○castrated males, Δ intact males, and □ females) using the N balance method. For all animals: N retained = - 0.509 (± 0.0817) + 0.584 (± 0.0519) × N intake. The estimated block nested to study variances ($\sigma^2_{b:s}$) and the residual variances (σ^2_e) were 0.0503 and 0.0482 respectively. The parameters of the equation did not differ between castrated males and intact males (*P > 0.10*).

DISCUSSION

Sex did not affect the NP_M for growing dairy goats. The estimate obtained using the comparative slaughter technique was less than 50% of that estimate obtained using N balance.

Results scaled by metabolic EBW provide an estimative more accurate of the requirements, since this is the most adequate index when just tissues are weighted and analyzed (Owens et al., 1995). However, the choice of presenting the results scaled by metabolic BW is relevant to compare with results reported in the literature where it is usually adopted the unit metabolic BW.

The similar values of NP_M between sexes is in accordance with reports by the current feeding systems (AFRC, 1998; NRC, 2007). Although females present less protein and more fat than males dairy goats in the body composition (Souza et al., 2017), those differences do not affect the protein requirements for maintenance. This can be explained because the protein content reach a point when this is constant in the de body, what is adopted as mature weight, and the remarkable changes in the body are consequence of the increasing fat deposition (Almeida et al., 2016).

The NP_M obtained using comparative slaughter technique herein (1.23 g/kg$^{0.75}$ BW) is similar to the values obtained by the independent studies used in this dataset, as expected. Medeiros (2001) evaluating Saanen kids from 5 to 20 kg of BW reported NP_M of 1.32 g/kg$^{0.75}$ BW, and Almeida et al. (2016) evaluating Saanen goats from 30 to 45 kg BW reported NP_M of 1.46 g/kg$^{0.75}$ BW. On the other hand, the value of 1.23 g/kg$^{0.75}$ BW is lower than the NP_M obtained for Boer goats by Fernandes et al. (2006) of 2.04 g/kg$^{0.75}$ of BW using also the comparative slaughter technique. The requirements obtained with meat goats and goats used for fiber production are clearly greater them values obtained for dairy goats herein. The greatest values of protein requirements for maintenance were estimated by Luo et al. (2004a, 2004b) for Angora (3.35 g/kg$^{0.75}$ BW) and for meat, dairy, and indigenous goats (3.07 g/kg$^{0.75}$ BW), in metabolizable terms. Besides the differences between body composition between genotypes that are associated with the body protein losses, we also have an important influence of methodologies adopted between researchers.

In the present study, our estimates using the comparative slaughter technique were 60 and 44% lower than that recommended by NRC (2007) and AFRC (1998), respectively (assuming an efficiency of protein utilization for maintenance of 1.0; NRC, 2007). Both feeding systems (AFRC, 1998; NRC, 2007) use N balance data for prediction. This fact raises the hypothesis that N balance may overestimate the NP_M according to the variation during the metabolism trial.

Although the N balance is a good representation of the concept of protein requirements for maintenance, the results of N balance can lead to biases of overestimation of N retention (Spanghero and Kowalski, 1997; Almeida et al., 2015). Estimates of NP_M by the comparative slaughter technique also yielded a more precise equation than those obtained from N balance data, which can be verified by the dispersion of the points in the graphs presented herein. As previously discussed by Waterlow (1999), little is known about how metabolic processes influences the N recycling in the body since short-term regulation as ureagenesis can be reflect in variation that are not clearly understood. Body proteins constantly undergo breakdown and resynthesis but these aspects remain not clearly understood (Waterlow, 1999).

In bovines, feeding low protein diets has been discussed in the last years, where no differences in animal performance among Nellore bulls fed diets containing 10, 12 or 14% CP were detected (BR-Corte, 2016). The main interest in feeding diets with less protein content is that it can reduces N input, improving N utilization efficiency and reducing the environmental impact caused by N losses from manure (Aschemann et al., 2012). Our results about requirements for maintenance of growing dairy goats also support the fact that dietary CP perhaps has been overestimated throughout the years. Mechanisms of recycling urea should be better evaluate in dairy goats.

CONCLUSION

The NP_M is similar between sexes in growing Saanen goats. The NP_M predicted by comparative slaughter technique is lower than that using N balance technique and previous reports by the current feeding systems.

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CHAPTER 4. Sex effects on net protein and energy requirements for growth of Saanen goats

ABSTRACT: Requirements for growth in the different sexes remain poorly quantified in goats. The objective of this study was to develop equations for estimating net protein (NP_G) and net energy (NE_G) for growth in Saanen goats of different sexes from 5 to 45 kg body weight (BW). A dataset from seven comparative slaughter studies (238 individual records) of Saanen goats was used. Allometric equations were developed to determine body protein and energy contents in the empty BW (EBW) as dependent variables and EBW as the allometric predictor. Parameter estimates were obtained using a linearized (log transformation) expression of the allometric equations using the MIXED procedure in the SAS software. The model included the random effect of the study, and the fixed effects of sex (intact male, castrated male, and female; 94, 79, and 71, respectively), EBW and their interactions. Net requirements for growth were estimated as the first partial derivative of the allometric equations with respect to EBW. Additionally, net requirements for growth were evaluated based on the degree of maturity. Monte Carlo methods were used to estimate the uncertainty of the calculated net requirement values. Sex affected allometric relationships for protein and energy in Saanen goats. The allometric equation for protein content in the EBW of intact and castrated males was $\log_{10}$ protein (g) = 2.221 (± 0.0224) + 1.015 (± 0.0165) × $\log_{10}$ EBW (kg). For females, the relationship was $\log_{10}$ protein (g) = 2.277 (± 0.0288) + 0.958 (± 0.0218) × $\log_{10}$ EBW (kg). Therefore, NP_G for males was greater than for females. The allometric equation for the energy content in the EBW of intact males was $\log_{10}$ energy (kcal) = 2.988 (± 0.0323) + 1.240 (± 0.0238) × $\log_{10}$ EBW (kg); of castrated males, $\log_{10}$ energy (kcal) = 2.873 (± 0.0377) + 1.359 (± 0.0283) × $\log_{10}$ EBW (kg); and of females, $\log_{10}$ energy (kcal) = 2.820 (± 0.0377) + 1.442 (± 0.0281) × $\log_{10}$ EBW (kg). The NE_G of castrated males was greater than that of intact males, and lower than that of females. Using the degree of maturity for estimating NP_G and NE_G, we can remove the differences between sexes. These results indicate that NP_G and NE_G differ among sexes in growing Saanen goats

and this should be accounted by feeding systems. Including the degree of maturity as predictor cancels out those differences across sexes in protein and energy requirements.

Key words: allometry, comparative slaughter, degree of maturity, mature weight, nutrient requirement

INTRODUCTION

Annual world production of fresh goat's milk has risen by about 60% in the last 20 years (FAOStat, 2015). To meet the demand for goat dairy products whereas improving the efficiency of the production system, goats must be fed a balanced diet to supply the nutrients and energy required for optimal production.

Sex is one among other variables which affect the rate of body protein and fat deposition in different species (Geay, 1984; Herring et al., 2013). The effects of sexual hormones on the development of muscle and adipose tissue have been extensively studied. Therefore, it is expected that the differences in body composition between sexes can consequently affect the nutritional requirements for growth (NRC, 2007). However, the effect of the sex of an individual on the requirements for growth remains poorly quantified in goats. The most recent recommendations for energy and protein requirements for goats (NRC, 2007) did not account for differences in the composition of gain between sexes. Our hypothesis is that the sex of an individual has an effect on the net protein and net energy requirements for growth in dairy goats.

Results from multiple comparative slaughter studies conducted at our institution were used to quantify the effect of sex on the net protein and energy requirements of growing goats. The objective of this study was to develop equations for estimating net protein (**NP_G**) and NE_G required for growth in Saanen goats of different sexes with BW ranging between 5 and 45 kg.

MATERIALS AND METHODS

Dataset

A dataset from 7 comparative slaughter studies including 238 individual records of Saanen goats was assembled (Gomes, 2011; Bompadre et al., 2014; Medeiros et al., 2014; Almeida et al., 2015a,b; Ferreira et al., 2015; Figueiredo et al., 2016a,b; Table 1). The studies were conducted at the Universidade Estadual Paulista (Jaboticabal – SP, Brazil) with intact males, castrated males, and female goats (94, 73, and 71, respectively). Empty BW (**EBW**) was calculated as the difference between BW at slaughter and the contents of the gastrointestinal tract, bladder and biliary vesicle. Total body protein and energy content of each animal in all experiments were calculated from the chemical composition of the body, where the amount of protein, expressed as g/kg, and energy, expressed as kcal/kg, were obtained from whole body composition multiplied by the EBW, expressed in kg. Body protein content was obtained by N analysis performed via Dumas combustion using LECO FP-528LC (Etheridge et al., 1998), and body energy content using an adiabatic calorimetric bomb under protocols described in each of the published sources. A summary of the body composition data used in this study is reported in Table 2.

Only animals fed ad libitum were included in the current study. The protein and energy composition of the diets fed to the study animals ranged from 137 to 204 g/kg CP and 2.4 to 2.7 Mcal/kg ME. In all trials, ME was estimated from digestible energy measured by total fecal collection, with energy lost to gaseous products of digestion calculated using the equation of Blaxter and Clapperton (1965). The ratio of roughage to concentrate ranged between 25:75 and 50:50. All procedures used across studies were reviewed by the University's Animal Care Committee (Comissão de Ética e Bem-Estar Animal – CEBEA).

Table 1. Summary characteristics of the seven studies used to assemble the dataset

Studies	n[1]	Sex	Phase[2]	BW (kg)	EBW[3] (kg)
Gomes (2011)	18	Intact Male	Weaned	30.0 to 51.0	19.4 to 41.7
Bompadre et al. (2014)	10	Castrated Male	Suckling	4.7 to 16.7	3.9 to 13.7
	19	Intact Male	Suckling	4.7 to 16.5	3.9 to 12.8
	18	Female	Suckling	4.6 to 16.3	3.5 to 13.4
Medeiros et al. (2014)	23	Intact Male	Suckling	5.1 to 21.6	4.9 to 17.5
Almeida et al. (2015a, 2015b)	16	Castrated Male	Weaned	27.8 to 47.4	21.7 to 39.7
	14	Intact Male	Weaned	27.6 to 46.6	21.3 to 39.7
	17	Female	Weaned	27.4 to 44.9	23.1 to 38.2
Ferreira et al. (2015)	27	Castrated Male	Weaned	20.6 to 35.5	15.6 to 30.3
Figueiredo et al. (2016a)	20	Female	Weaned	29.5 to 46.0	20.8 to 40.4
Figueiredo et al. (2016b)	20	Castrated Male	Weaned	15.3 to 32.5	13.1 to 26.4
	18	Intact Male	Weaned	15.7 to 34.0	12.8 to 28.3
	18	Female	Weaned	14.8 to 31.7	11.9 to 26.3

[1]Total number of records from the study.

[2]Suckling refers to goats that were fed milk and solid diet and weaned refers to goats that were fed just solid diet.

[3]EBW = Empty BW.

Table 2. Summary of statistics related to body composition of dairy goats used in this study

Variables	n[1]	Mean	SD	Range
Body Protein (g/kg EBW[2])				
All animals	238	173	18.4	114 to 264
Castrated male	73	173	16.6	114 to 206
Intact male	94	178	18.4	130 to 264
Female	71	166	18.1	123 to 214
Body Fat (g/kg EBW)				
All animals	238	144	78.7	21 to 390
Castrated male	73	145	69.8	22 to 345
Intact male	94	110	51.3	22 to 258
Female	71	188	95.1	21 to 390
Body Energy (kcal/kg EBW)				
All animals	238	2230	679	936 to 4,519
Castrated male	73	2213	648	936 to 4,143
Intact male	94	1975	470	936 to 3,330
Female	71	2586	841	1,086 to 4,519
Body Ash (g/kg EBW)				
All animals	220	43	11.1	25 to 80
Castrated male	55	40	7.60	25 to 56
Intact male	94	48	12.1	24 to 80
Female	71	38	9.50	25 to 60
Body Water (g/kg EBW)				
All animals	220	640	79.8	416 to 809
Castrated male	55	644	81.2	457 to 809
Intact male	94	663	59.9	478 to 809
Female	71	607	90.7	416 to 777

[1]Number of animals in the study.
[2]EBW = Empty BW.

Statistical Analysis and Parameter Estimation

Estimation of EBW. Regression equations were developed for estimating EBW from BW. The mixed model included the fixed effects of sex (female, castrated male, and intact male) and phase (suckling or weaned), and the study as a random effect.

Allometric Equations. The methods used to estimate NP_G and NE_G were similar to those described by Lofgreen and Garrett (1968). Allometric equations were developed for body protein and energy contents in the EBW. Statistically, the allometric equation can take two forms: untransformed (i.e., native scale) and log transformed to linearize the model. In our application, the native scale results in the following nonlinear mixed model [1]:

$$Y_{ijk} = \dot{B}_{0_i} W_{ijk}^{\dot{B}_{1_i}} + \dot{s}_j + \dot{e}_{ijk} \qquad [1]$$

where, Y_{ijk} is the value of the dependent variable (either total energy in the EBW expressed as kcal, or total protein expressed as g in the EBW) for the k^{th} animal of the i^{th} sex in the j^{th} study,

W_{ijk} is the EBW of the k^{th} animal of the i^{th} sex in the j^{th} study,

$\dot{B}_{0_i}$ and $\dot{B}_{1_i}$ are parameters to be estimated for each of the three sexes,

$\dot{s}_j$ is the random effect of the j^{th} study ~ $N(0,\sigma_s^2)$, and

$\dot{e}_{ijk}$ is the residual error ~ $N(0,\sigma_e^2)$.

The allometric portion of model [1] (i.e., the first term on the right-hand side in [1]) can be linearized through a log transformation, resulting in the following statistical model:

$$\log Y_{ijk} = B_{0_i} + B_{1_i} \log W_{ijk} + s_j + e_{ijk} \qquad [2]$$

where, Y_{ijk} and W_{ijk} are as previously defined,

B_{0_i} and B_{1_i} are parameters to be estimated for each of the sexes i,

s_j is the random effect of the j^{th} study ~ $N(0,\sigma_s^2)$, and

e_{ijk} is the residual error ~ $N(0,\sigma_e^2)$.

The 'dot' symbol was used in model [1] to make clear that although the *B* parameters are mathematically equivalent in both models, their estimates are not statistically the same. Both models include a random effect of the study to account for systematic differences between studies (St-Pierre, 2001). The NLINMIX macro of SAS software (SAS Inst. Inc., Cary, NC; version 9.4) was used for fitting model [1], whereas the MIXED procedure was used to fit model [2]. Model [1] assumes that the residual errors are additive in the native scale. That is, their variance is homogeneous with respect to *W*. This is in contrast to model [2] that assumes additive errors in the transformed (i.e., log) scale, which implies errors that are multiplicative in the native scale and variances that are proportional to *W* in the native scale. Therefore, choosing the model that is best for a given set of records should be determined by the pattern of the standardized conditional residuals. For all variables that we analyzed, the pattern of residuals was clearly more homogeneous with model [2] than model [1]. Therefore, model [2] was used to estimate parameters and to conduct significance tests. Outliers were removed when their normalized residuals were >|3|. For body protein three data points were removed, for body fat and body energy two data points were removed. The outliers were from three different studies (Bompadre et al., 2014; Almeida et al., 2015a; and Figueiredo et al., 2016a).

Whenever the effect of sex was found to approach significance ($P < 0.10$), indicating a different intercept for at least one sex, three contrast statements were used to conduct all three pairwise comparisons of sex. Likewise, three contrast statements were used to conduct all three pairwise comparisons when the interaction between sex and the regressor *W* was found to approach significance ($P < 0.10$), indicating that at least one sex had a different slope. In the study of Bompadre et al. (2014), intact male kids slaughtered at 5 kg BW were also considered for developing the equation for castrated male kids (for details check Bompadre et al., 2014), then a correction of the degrees of freedom was performed using the DDF option in the MODEL statement of MIXED procedure of SAS (Appendix).

Net Requirements for Growth. The coefficients to estimate net requirements for growth were obtained as the first partial derivative of the allometric equations with respect to EBW. That is, model [2] was first back-transformed to the native scale (done separately for each sex here to simplify the notation):

$$Y = 10^{B_0} W^{B_1} 10^{s}\, 10^{e} \qquad [3]$$

The first partial derivative of this is:

$$\frac{dY}{dW} = B_1\, 10^{B_0+s+e}\, W^{B_1-1} \qquad [4]$$

In [4], the expectations of *s* and *e* are both equal to zero. Therefore, the expectation (i.e., mean value) for the derivative is:

$$E\left(\frac{dY}{dW}\right) = B_1\, 10^{B_0}\, W^{B_1 - 1} \qquad [5]$$

That is, [5] is used to estimate the mean net requirement in g or kcal per kg EBW gained, with the explicit understanding that either B_0 or B_1, or both, can be different across sexes. Because of the complexity of [4] and the correlation between the estimated B_0 and B_1, the variance of dY/dW is not analytically tractable. A Monte Carlo based simulation was used to calculate numerical estimates of the variance and confidence intervals for dY/dW (i.e., net requirements). For each sex, and for each W for which a net requirement was being calculated, 10,000 simulated values of dY/dW were generated using a multivariate normal distribution for B_0, B_1, and e for the error of estimation, and B_0, B_1, s and e for the error of prediction using the algorithm of Fan et al. (2002). This was done on the conditional values of the study effect, where, the error of estimation for the requirement of a given animal among the seven studies used in the analyses is known (what we refer to as 'error of estimation'); this was also done for the general case, where, the value of the effect of the study is unknown, to reflect the uncertainty around the calculated requirements when applied to an animal not in the dataset (what we call the 'error for prediction').

Taking into account that degree of maturity affects body composition, the relationship between protein or energy (g or kcal/kg EBW gain) and degree of maturity was evaluated according to model [6]. The degree of maturity was calculated as a ratio between EBW and mature EBW of Saanen goats reported by Almeida et al. (2016) as 34.9, 42.6, and 26.0 kg EBW for castrated males, intact males and females, respectively.

$$Y_{ijk} = B_{0_i} + B_{1_i} W_{ijk} + s_J + e_{ijk} \quad [6]$$

where, Y_{ijk} is the value of the dependent variable (either energy in the EBW gain expressed as kcal/kg EBW gain in the ratio between EBW and mature EBW, or protein expressed as g/kg EBW gain in the ratio between EBW and mature EBW) for the *k*th animal of the *i*th sex in the *j*th study,

B_{0_i} and B_{1_i} are parameters to be estimated for each of the sexes i,

s_j is the random effect of the *j*th study ~ $N(0, \sigma_s^2)$, and

e_{ijk} is the residual error ~ $N(0, \sigma_e^2)$.

For this approach, we included just data from goats up to degree of maturity equal 1 because we just had data from goats with degree of maturity greater than 1 for females and castrated males. Then we used data of 94 intact males, 68 castrated males, and 47 females. Outliers were removed when their normalized residuals were >|3|. For protein seven data points were removed, for energy four data points were removed. The outliers were from four different studies (Gomes, 2011; Bompadre et al., 2014; Almeida et al., 2015a,b; and Figueiredo et al., 2016a). Monte Carlo simulation, as previously described, was also applied for this approach.

RESULTS

Estimation of EBW

The effect of sex did not approach significance for any of the parameter estimates ($P > 0.10$) in the regression equations developed for estimating EBW from BW. On the other hand, phase (suckling or weaned) affected both the intercept ($P = 0.006$) and the slope ($P < 0.001$). Hence, we used distinct equations (Eq. [7] and Eq. [8]) to estimate EBW from BW (n = 242, $P < 0.0001$, $\sigma^2_s = 0.568$, $\sigma^2_e = 0.856$):

Suckling: EBW = 0.76 (± 0.598) + 0.749 (± 0.0209) × BW [7]

Weaned: EBW = -2.38 (± 0.515) + 0.892 (± 0.0114) × BW [8]

Protein Requirements for Growth

The total protein in the EBW tended to be different between sexes (*P* < 0.10; Table 3). Intercepts (*P* = 0.59) and slopes (*P* = 0.68) were similar between castrated and intact males. However, intercepts (*P* = 0.03) and slopes (*P* = 0.01) differed between castrated males and females, as well as intercepts (*P* = 0.09) and slopes (*P* = 0.02) between intact males and females. Therefore, we are reporting the applicable parameters to each sex and one general equation to all males because castrated and intact males were similar (*P* > 0.10) (Table 3). Figure 1 illustrates the good fit of the allometric equations for body protein.

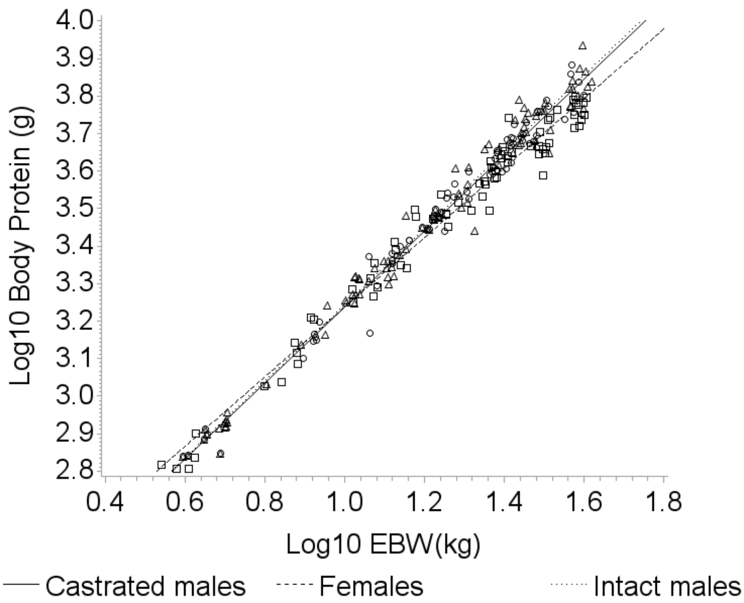

Figure 1. Relationship between Log_{10} Protein (g) and Log_{10} Empty BW (EBW) (kg) of growing dairy goats of different sexes (○ castrated males, □ females, and ∆ intact males): for males, $\log_{10}$ protein = 2.221 (± 0.0224) + 1.015 (± 0.0165) × $\log_{10}$ EBW; for females, $\log_{10}$ protein = 2.277 (± 0.0288) + 0.958 (± 0.0218) × $\log_{10}$ EBW. The estimated study variances (σ^2_s) and the residual variances (σ^2_e) were 0.000388 and 0.00140. Intercepts and slopes were different for males and for females (*P* = 0.028 and *P* = 0.006, respectively). The observations were adjusted for the study effect.

Table 3. Allometric equations of $\log_{10}$ of body protein (g), $\log_{10}$ of body fat (g) and body energy (kcal) on $\log_{10}$ EBW[1] (kg) of growing dairy goats

Item[2]	b_0	b_1	n[3]	*P*-value[4] b_0	*P*-value[4] b_1
Protein				0.084	0.024
Castrated male	2.213 ± 0.0289	1.020 ± 0.0220	77		
Intact male	2.228 ± 0.0244	1.010 ± 0.0184	93		
Female	2.277 ± 0.0288	0.958 ± 0.0218	71		
All males[5]	2.221 ± 0.0224	1.015 ± 0.0165	170		
Fat				<0.001	<0.001
Castrated male	1.210 ± 0.0714	1.708 ± 0.0518	78		
Intact male	1.384 ± 0.0618	1.506 ± 0.0437	93		
Female	1.053 ± 0.0703	1.873 ± 0.0509	71		
Energy				<0.001	<0.001
Castrated male	2.873 ± 0.0377	1.359 ± 0.0283	78		
Intact male	2.988 ± 0.0323	1.240 ± 0.0238	93		
Female	2.820 ± 0.0377	1.442 ± 0.0281	71		

[1]EBW = Empty BW.

[2]The estimated study variances (σ^2_s) and the residual variances (σ^2_e) were, respectively, 0.000388 and 0.00140 for protein, 0.00551 and 0.00699 for fat, 0.000956 and 0.00223 for energy respectively.

[3]Number of animals used to estimate parameters.

[4]*P*-value for fixed effect tests to check whether the intercept (b_0) and the slope (b_1) differ across the three sexes.

[5]An overall equation for males is reported because the differences in intercept and slope between castrated and intact males were not statistically significant ($P > 0.10$).

Our results show that females and males have different NP_G. Therefore, two different equations are necessary to calculate these requirements:

All males: $NP_G = 1.015 \times 10^{2.221} \times EBW^{0.0150}$ [9]

Females: $NP_G = 0.9586 \times 10^{2.277} \times EBW^{-0.0414}$ [10]

Monte Carlo methods were used to estimate the uncertainty of NP_G when equations [9] and [10] are used (i.e. SD of NP_G). For males and females, the greatest uncertainty for NP_G was at an EBW of 37.8 kg (corresponding to 45 kg BW). For males with BW between 5 and 45 kg, NP_G ranged from (mean ± SD) 173 ± 3.08 to 179 ± 5.64 g/kg EBW gain (Table 4). For females with similar BW, NP_G was lower and ranged from 171 ± 3.74 to 156 ± 6.84 g/kg EBW gain (Figure 2). When presented in unit of BW as opposed to EBW, the NP_G in males ranged from 156 to 150 g/kg BW gain, whereas in females NP_G ranged from 154 to 131 g/kg BW gain.

Table 4 compares estimated and predicted values obtained for NP_G. Although, the estimated and predicted means of NP_G (i.e., expectation) were the same, the SD and confidence limits are greatest when simulating external dataset.

Table 4. Effect of sex on estimated and predicted net protein requirements[1] of growing dairy goats from 5 to 45 kg BW

Sex	BW (kg)	EBW[2] (kg)	Estimation[3]				Prediction[4]			
			Mean	SD	LCI[5]	UCI[6]	Mean	SD	LCI	UCI
Males										
	5	4.5	173	3.09	171	174	173	8.44	169	177
	15	12.0	175	3.17	174	177	176	8.59	172	179
	25	19.8	177	4.07	174	179	177	9.03	172	182
	35	28.8	178	4.94	176	180	178	9.50	174	182
	45	37.8	179	5.64	176	181	179	9.91	174	183
Females										
	5	4.5	171	3.74	168	174	171	8.62	164	178
	15	12.0	164	4.06	162	166	164	8.50	159	169
	25	19.8	160	5.15	155	166	160	8.96	150	171
	35	28.8	158	6.11	153	163	158	9.47	151	166
	45	37.8	156	6.84	152	160	156	9.90	151	162

[1]Net protein requirements (g/kg EBW gain) were calculated as the first derivative of the allometric equation. For males $NP_G = 1.015 \times 10^{2.221} \times EBW^{0.015}$. For females: $NP_G = 0.958 \times 10^{2.277} \times EBW^{-0.0414}$.

[2]EBW = Empty BW. The values were calculated from the equation: EBW = 0.76 (± 0.597) + 0.75 (± 0.0209) × BW when 5 < BW < 15 kg, and from the equation EBW = -2.38 (± 0.515) + 0.892 (± 0.0114) × BW when BW ≥ 15 kg.

[3]Estimation refers to the statistics for an animal within the dataset used. It is conditional to the study effect. Hence, the uncertainty related to the study is not factored in.

[4]Prediction refers to statistics for an animal not within the dataset used, for example, a future observation. It is not conditional on a known study effect. Hence, the uncertainty related to the study is factored in.

[5]Lower 95% confidence limit of requirement.

[6]Upper 95% confidence limit of requirement.

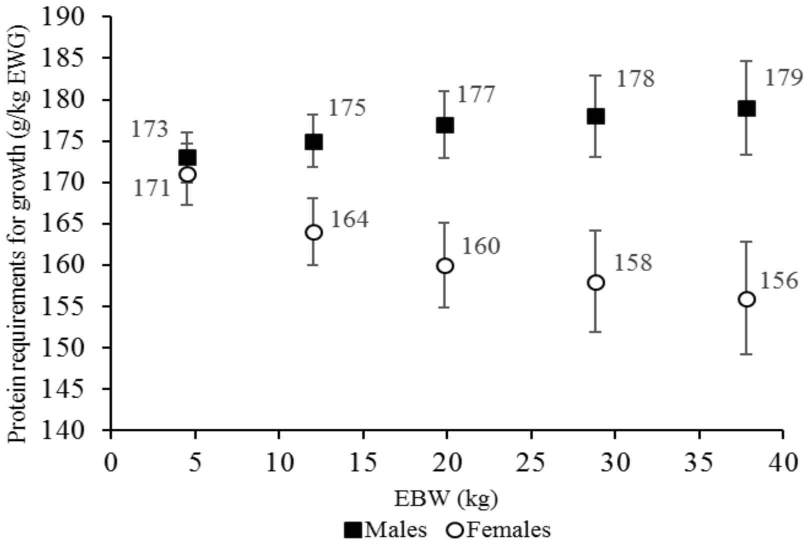

Figure 2. Net protein requirements of growing dairy goats from 5 to 45 kg BW

The NP_G based on degree of maturity was estimated using equation of protein in gain on ratio between EBW and mature EBW (Eq. [11], [12], and [13]; n = 202; σ^2_s = 156.2, σ^2_e = 2,237).

Castrated males: NP_G = 188.8 (± 17.4) – 11.2 (± 28.7) × EBW/mature EBW [11]

Intact males: NP_G = 185.6 (± 13.4) - 4.18 (± 24.0) × EBW/mature EBW [12]

Females: NP_G = 153.5 (± 21.5) + 25.2 (± 30.2) × EBW/mature EBW [13]

Sex does not affect the NP_G applying this approach (P = 0.26) consequently we obtained a general model (Figure 3). The slope of this relationship was not significant in the general model (P = 0.86) therefore we cannot describe this relationship based on the independent variable ratio between EBW and mature EBW. According to this result, we verified an overall NP_G of 176 g/kg EBW gain irrespective of degree of maturity, with an uncertainty of ± 12.8 g/kg EBW (Figure 4).

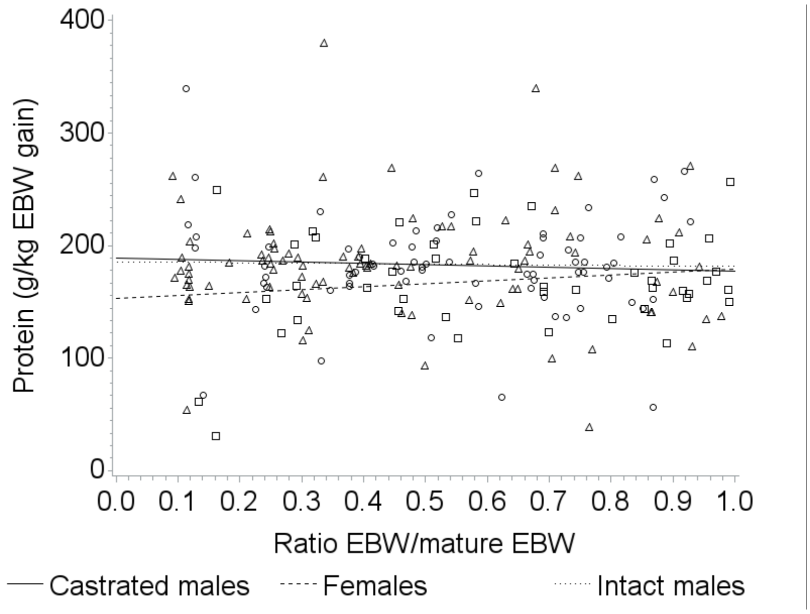

Figure 3. Relationship between Protein (g/kg EBW gain) and ratio EBW/mature EBW of growing dairy goats (○ represents records of castrated males, □ females, and ∆ intact males). Intercepts and slopes were similar between sexes (P > 0.10). The general equation was Protein = 176 (± 12.8) + 3.25 (± 19.0) × Ratio EBW/mature EBW. The slope was not significant (P = 0.86). Estimated study variances (σ^2_s) and the residual variances (σ^2_e) were 156.2 and 2,237, respectively. The observations were adjusted for the study effect.

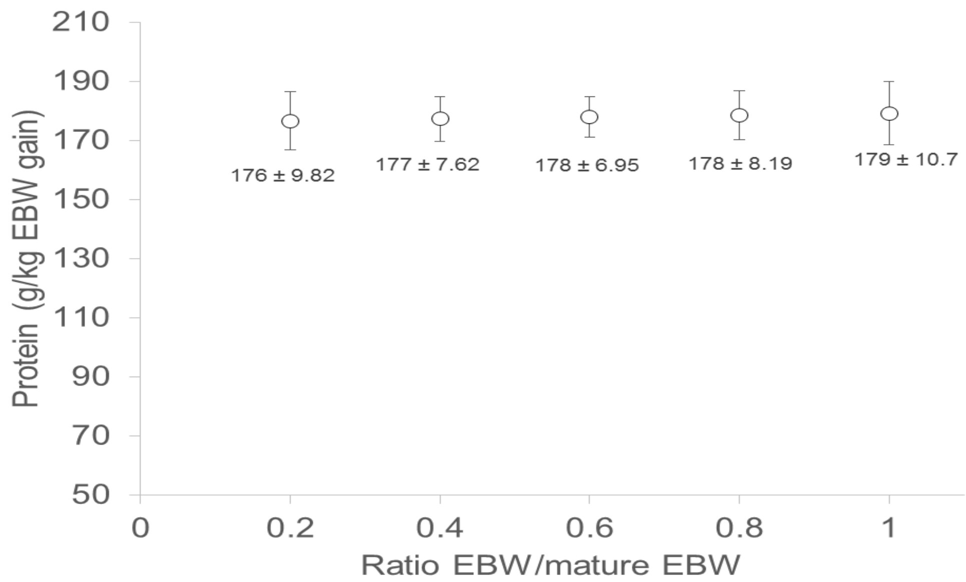

Figure 4. Net protein requirements for growth (g/kg EBW gain; mean ± SD) of growing dairy goats according to degree of maturity, calculated based on the equation Protein = 176 (± 12.8) + 3.25 (± 19.0) × Ratio EBW/mature EBW.

Energy Requirements for Growth

The intercepts and slopes of fat content in EBW equations were different between sexes ($P < 0.05$), therefore we report separate equations to estimate the fat content of EBW for the three sexes (Table 3).

The energy content in EBW differed between sexes ($P < 0.001$; Table 3). Intercepts of the allometric equations for energy contents were similar between castrated males and females ($P = 0.17$) but the slopes were different ($P = 0.008$). Intercept ($P = 0.002$) and slopes ($P < 0.001$) differed between castrated males and intact males. Intercept ($P < 0.001$) and slopes ($P < 0.001$) differed between intact males and females. Therefore, we report separate equations to estimate the energy content of EBW for the three sexes (Table 3). Similar to what are observed for body protein, the allometric equations for body energy on EBW (Figure 5) shows that the model presents a good fit, and the linearization of the model resulted in homoscedastic errors.

The NE_G was estimated as the first partial derivative of the allometric equations for body energy on EBW. Our results show that castrated males, intact males, and females have different NE_G. Therefore, we report separate equations for each of the three sexes:

Castrated males: $NE_G = 1.359 \times 10^{2.873} \times EBW^{0.359}$ [14]

Intact males: $NE_G = 1.240 \times 10^{2.988} \times EBW^{0.240}$ [15]

Females: $NE_G = 1.442 \times 10^{2.820} \times EBW^{0.442}$ [16]

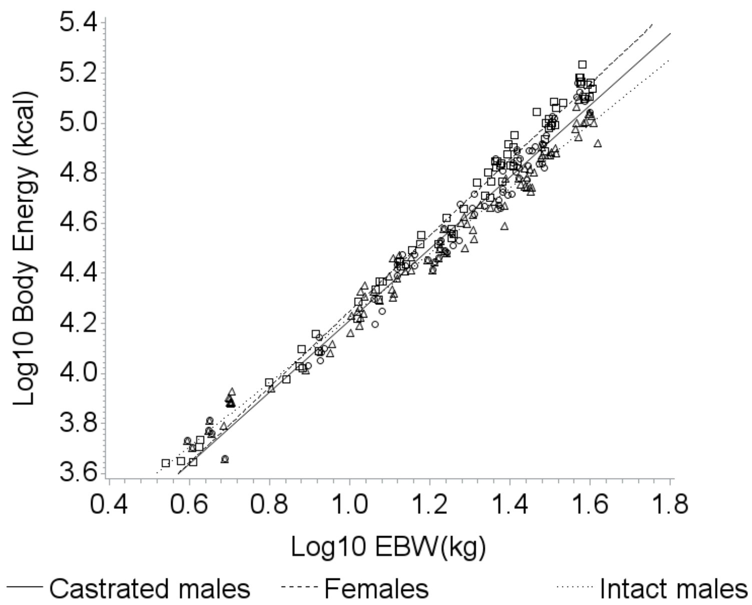

Figure 5. Relationship between Log10 Energy (kcal) and Log10 Empty BW (EBW) (kg) of growing dairy goats (○ castrated males, □ females, and Δ intact males): for castrated males, log10 energy = 2.873 (± 0.0377) + 1.359 (± 0.0165) × log10 EBW; for females, log10 energy = 2.820 (± 0.0377) + 1.442 (± 0.0281) × log10 EBW; for intact males, log10 energy = 2.988 (± 0.0323) + 1.240 (± 0.0238) × log10 EBW. The estimated study variances ($\sigma^2 s$) and the residual variances ($\sigma^2 e$) were 0.000956 and 0.00223. Intercepts and slopes were different between sexes (P < 0.001). The observations were adjusted for the study effect.

As for NP_G, a Monte Carlo method was used to estimate the uncertainty of NE_G estimates (Table 5). Animals at 37.8 kg EBW (45 kg BW) exhibited the greatest uncertainty in NE_G. For castrated males, we found that NE_G increased (mean ± SD) from 1,745 ± 57.78 to 3,751 ± 183.94 kcal/kg EBW gain. For intact males, the increase was from 1,735 ± 61.90 to 2,898 ± 149.6 kcal/kg EBW gain. Lastly, for females the increase was from 1,857 ± 78.7 to 4,766 ± 281.9 kcal/kg EBW gain in goats weighing from 5 to 45 kg BW (Figure 6). When presented in unit of BW as opposed to EBW, the NE_G for castrated males ranged from 1,570 to 3,151 kcal/kg BW gain; for intact males, from 1,561 to 2,434 kcal/kg BW gain; and for females, from 1,671 to 4,003 kcal/kg BW gain.

Similar to NP_G estimates, the uncertainty in the estimates of NE_G when the equations are applied to animals that are not part of the dataset (Table 5) is noticeably greater than when the equations are applied to animals in the dataset.

The NE_G based on degree of maturity was estimated using equation of energy in gain on ratio between EBW and mature EBW (Eq. [17], [18] and [19]; n = 205; σ^2_s = 110,722, σ^2_e = 459,166).

Castrated males: $NE_G = 1{,}421\ (\pm 293) + 2{,}154\ (\pm 453) \times \text{EBW/mature EBW}$ [17]

Intact males: $NE_G = 1{,}375\ (\pm 244) + 2{,}131\ (\pm 400) \times \text{EBW/mature EBW}$ [18]

Females: $NE_G = 1{,}000\ (\pm 332) + 2651\ (\pm 445) \times \text{EBW/mature EBW}$ [19]

Intercepts and slopes were similar between sexes ($P > 0.10$); consequently, we generated a general model for all sexes (Figure 7). Monte Carlo methods were also used to estimate the uncertainty of NE_G and the NE_G increased from (mean ± SD) 1,726 ± 188 to 3,575 ± 197 kcal/kg EBW gain as degree of maturity ranged from 0.2 to 1.0 (Figure 8).

Table 5. Effect of sex on estimated and predicted net energy requirements[1] of growing dairy goats from 5 to 45 kg BW

Sex	BW (kg)	EBW[2] (kg)	Estimation[3]				Prediction[4]			
			Mean	SD	LCI[5]	UCI[6]	Mean	SD	LCI	UCI
Castrated males										
	5	4.5	1,745	57.8	1,699	1,791	1,747	137.4	1,638	1,858
	15	12.0	2,481	70.3	2,440	2,524	2,486	191.0	2,373	2,599
	25	19.8	2,971	104.8	2,899	3,045	2,976	237.3	2,812	3,141
	35	28.8	3,401	145.7	3,319	3,484	3,406	284.1	3,246	3,568
	45	37.8	3,751	183.9	3,615	3,888	3,758	326.0	3,516	3,999
Intact males										
	5	4.5	1,735	61.9	1,703	1,768	1,738	138.8	1,666	1,811
	15	12.0	2,197	78.7	2,161	2,234	2,201	176.1	2,120	2,282
	25	19.8	2,479	102.4	2,363	2,595	2,483	205.3	2,251	2,716
	35	28.8	2,713	127.4	2,612	2,816	2,718	233.0	2,532	2,905
	45	37.8	2,897	149.7	2,813	2,983	2,903	256.6	2,758	3,048
Females										
	5	4.5	1,857	78.7	1,794	1,920	1,860	154.6	1,737	1,984
	15	12.0	2,866	118.1	2,799	2,933	2,871	237.3	2,737	3,005
	25	19.8	3,578	168.9	3,387	3,769	3,584	307.8	3,236	3,933
	35	28.8	4,225	226.9	4,043	4,406	4,232	379.5	3,928	4,536
	45	37.8	4,766	281.9	4,600	4,933	4,775	444.4	4,512	5,037

[1]Net energy requirements expressed in kcal energy per kg EBW gain were calculated as the first derivative of the allometric equation. Castrated males: $NE_G = 1.359 \times 10^{2.873} \times EBW^{0.359}$. Intact males: $NE_G = 1.240 \times 10^{2.988} \times EBW^{0.240}$. Females: $NE_G = 1.442 \times 10^{2.820} \times EBW^{0.442}$.
[2]EBW = Empty BW. The values were calculated from the equation: EBW = 0.76 (± 0.597) + 0.75 (± 0.0209) × BW when 5 < BW < 15 kg, and from the equation EBW = -2.38 (± 0.515) + 0.892 (± 0.0114) × BW when BW ≥ 15 kg.
[3]Estimation refers to the statistics for an animal within the dataset used. It is conditional to the study effect. Hence, the uncertainty related to the study is not factored in.
[4]Prediction refers to statistics for an animal not within the dataset used, for example, a future observation. It is not conditional on a known study effect. Hence, the uncertainty related to the study is factored in.
[5]Lower 95% confidence limit of requirement.
[6]Upper 95% confidence limit of requirement.

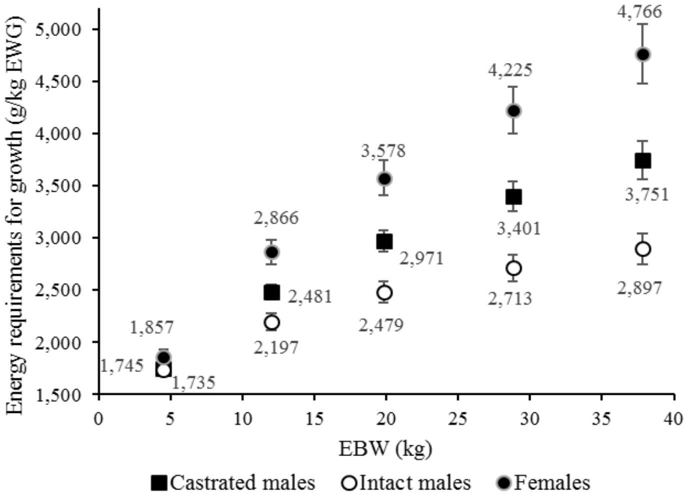

Figure 6. Net energy requirements of growing dairy goats from 5 to 45 kg BW

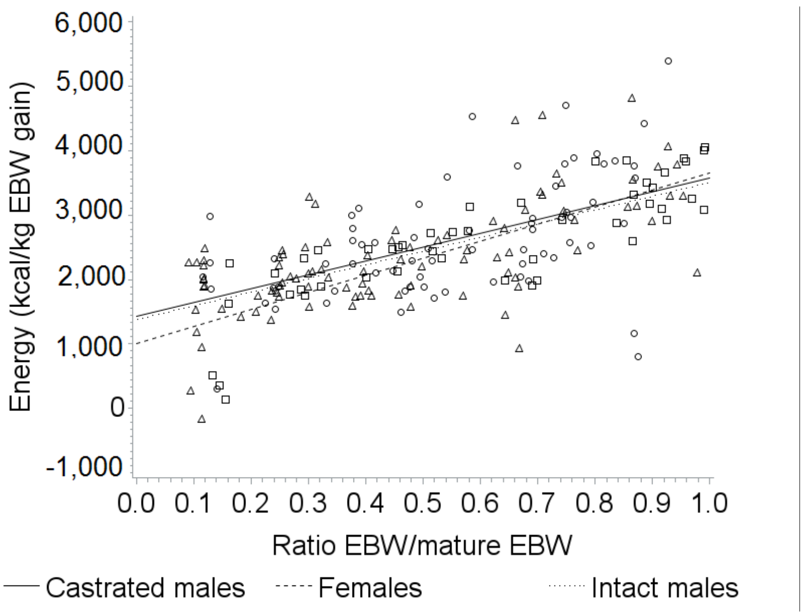

Figure 7. Relationship between Energy (kcal/kg EBW gain) and ratio EBW/mature EBW of growing dairy goats (○ represents records of castrated males, □ females, and Δ intact males). Intercepts and slopes were similar between sexes ($P > 0.10$). The general equation was Energy = 1,265 (± 234) + 2,312 (± 316) × Ratio EBW/mature EBW. The estimated study variances ($\sigma^2 s$) and the residual variances ($\sigma^2 e$) were 110,722 and 459,166, respectively. The observations were adjusted for the study effect.

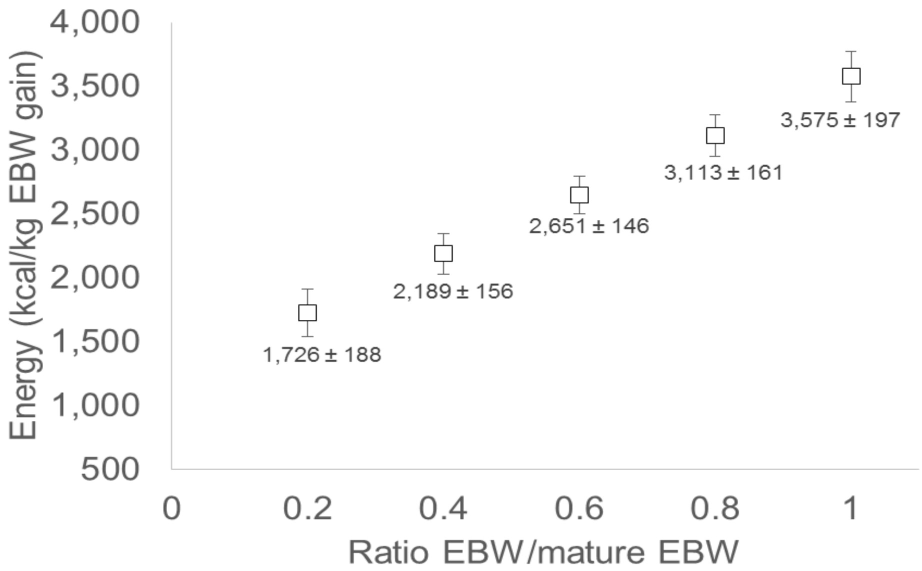

Figure 8. Net energy requirements for growth (kcal/kg EBW gain; mean ± SD) of growing dairy goats according to degree of maturity, calculated based on the equation Energy = 1,265 (± 234) + 2,312 (± 316) × Ratio EBW/mature EBW.

DISCUSSION

The effect of the sex of an animal on its requirements for growth was evaluated in dairy goats that weighed between 5 and 45 kg BW. Allometric equations for estimating protein and energy contents of EBW were developed across sexes, and to estimate net requirements. We found that the sex of an animal affected its protein and energy contents and, consequently, its net requirement for growth.

The allometric equation developed for protein content of EBW resulted in a slope slightly larger than 1.0 for males, leading us to conclude that the body protein showed a slight but non-significant increase in males of increasing EBW. This means that the amount of protein increases at a similar rate to EBW in males. In contrast, for females, the allometric equation resulted in a slope significantly less than 1.0, implying that the amount of protein increases at a lower rate than EBW.

Body protein decreased as BW increased just in females. This decrease occurs in response to a large addition of another constituent in the body, namely, fat. Similarly, Araujo et al. (2015) reported that the protein content in the EBW of castrated dairy goats decreased as BW increased. Their findings are possibly related to the large range of BW evaluated (from 5 ± 1.4 to 84 ± 6.5 kg BW) allowing them to observe fat content up to 396 g/kg EBW (which was similar to the maximum body fat proportion observed in females herein; 390 g/kg EBW).

In this study, body protein (g/kg EBW) remained constant in castrated and intact males. Body composition of male and female growing dairy goats (from 5 to 45 kg BW) was detailed by Almeida et al. (2016) using the same dataset used herein. They observed a similar proportion of protein in the fat-free empty body between sexes as well as a similar ratio between protein and fat in the empty body of castrated and intact males. That leads us to indicate that the pattern observed for protein content in males is a consequence of the proportion of other constituents in the body. In this sense, we consider that the similarity of body protein content between males is mainly because the increase of body fat was not large enough to detect any proportional difference in body protein content. Fat has delayed deposition in relation to body growth, increasing its rate when the deposition of water, protein and minerals is in descending phase (Geay, 1984; Lawrence et al., 2012). Therefore, we could had found differences whether we had evaluated heavier goats, where the body protein content decreases with increasing BW due to the increase of body fat content.In different ruminant species, as well as in pigs, males have greater lean content than females at similar BW (Berg and Butterfield, 1976; Seidman et al., 1982; Geay, 1984). When the results of independent studies included in this dataset were evaluated individually, with more narrow BW range, the authors did not report differences between sexes (Almeida et al., 2015b; Figueiredo et al., 2016a). By combining data from individual animals across studies, our meta-analysis, which covers a much wider range of EBW than independent studies, enhances the power for statistical testing and parameter estimation (Sauvant et al., 2008). Thus, the number of animals used in a wide BW range of 5 to 45 kg was important for detecting differences between sexes.

In this study, we first express the protein and energy requirements per unit of EBW gain. This is because the EBW is the most adequate index of energy and nutrient content of the body, as only tissues are weighed and analyzed, enabling a more accurate comparison (Owens et al., 1995).

Regarding the uncertainty of NP_G, we report an increasing SD of estimates with the EBW. This is a result of the multiplicative errors, and consequently, wider variation in the maximum EBW applied. The multiplicative error model assumes that the measures differ by equal proportion, and is in line with the multiplicative nature of biological processes (Kerkhoff and Enquist, 2009). Unfortunately, this variation is not taken into account in the recommendations of current feeding systems (AFRC, 1998; NRC, 2007), where requirements are commonly reported as a mean value, which might underestimate or overestimate the requirements of some animals. Assuming perfect certainty of requirements when formulating diets, could lead to sub-optimal diet for a proportion of animals. However, the uncertainty in requirements can be incorporated in models and can be solved using stochastic programming techniques (St-Pierre and Harvey, 1986; Yoder et al., 2014).

A direct comparison of our NP_G values with the recommendations made by the most recent feeding systems is difficult due to differences in the methods used. Similar to the present meta-analysis, AFRC (1998) uses comparative slaughter technique to estimate net requirements, although the data used were insufficient to estimate the effect of sex. The AFRC dataset is predominately constituted of data from castrated dairy goats, whose NP_G ranged from 126 – 154 g/kg BW gain in goats weighing between 5 and 45 kg BW, and were quite similar to our NP_G estimates in females (131 to 154 g/kg BW gain).

Body composition clearly affects growth requirements (NRC, 2007): composition of gain provides important information to understand the differences in nutritional requirements between sexes. According to Sahlu et al. (2004), the composition of gain is not reported in most publications on the nutritional requirements for goats and the values reported by NRC (2007) were mostly derived from feeding trials. Based on NRC (2007) the MP for growth (**MP_G**) in dairy goats is 290 g/kg BW gain, which translate to a net requirement of 171 g/kg BW gain (assuming an efficiency of utilization for growth of 0.59 as proposed by AFRC, 1992). Differential requirement of males versus females have not been taken into consideration in this recommendation. In general, the values we obtained for males and females in the present study were lower than the recommendations by NRC (2007), presumably due to differences in techniques used.

Even though prediction and estimation are semantically similar, there is a statistically important distinction between them. An estimate refers to a calculated value for an animal in the dataset (i.e., with a realized study effect). On the other hand a prediction is calculated for an animal not in the dataset, for which the effect of the study is unknown (Fan et al., 2002). Including the effect of the study as a random factor improves the accuracy of parameter estimates in the model (St-Pierre, 2001). Consequently, this reduces the error in the estimated values (estimation). On the other hand, the error in prediction is larger because it applies to an animal not within the dataset that is being used, a future observation for example, where the study effect is unknown. When we evaluate the confidence limits of the estimates of NP_G, the uncertainty is in the range of 1 to 3% of the mean value in female goats. On the other hand, when applying the prediction to animals that are not included in the dataset and the effect of the study is unknown, the uncertainty regarding the requirements increases, and is between 3 to 6% of the mean value.

The allometric equations developed for the energy and fat content of EBW resulted in positive slopes greater than 1.0 for all sexes. This implies that the amount of energy and fat increase at a rate faster than EBW in growing goats. Sex is a factor that determines the composition of growth, where hormonal regulations can establish biological limits for protein and fat deposition (Byers, 1982). In response to changes in absorbed nutrients, the hormonal regulations in females results in greater increase of fat in their body and consequently greater amount of energy than in males. This is possibly because of the earlier fat deposition in abdominal tissues of females, which is an innate preparation of the female for future pregnancy (Berg and Butterfield, 1976). However, there is clearly an increase in fat deposition in castrated males as well. Sexual hormones are involved in the control of many mechanisms and testosterone is one of the hormones that affect the secretion of growth hormone (**GH**), and is also synergistic with estrogen for enhancing deposition of lean tissue (Owens et al., 1993). The importance of GH in modulating lipid metabolism by decreasing glucose transportation and lipogenesis was detailed by Louveau and Gondret (2004). A castrated male clearly has reduced testosterone production compared to an intact male, thus explaining the increase in the lipid content and adipose tissue mass of the EBW that we observe.

We found differences in energy requirement between sexes, with NE_G at a given EBW greater for females than for males. Likewise, castrated males have greater NE_G than intact males. These are directly related to differences in body composition, fat, and energy accretion described in the allometric equations. These results regarding the effect of the sex of an animal on its NE_G are consistent with recommendations in beef cattle, where the NE_G of steers is greater than that of bulls and less than that of heifers (NRC, 2000). Fat is a component of EBW that undergoes greater variation throughout the life of an animal (Berg and Butterfield, 1976), which explains the greater SD in NE_G observed with females.

The estimated NE_G for castrated males in the present study was similar to that reported by AFRC (1998) of 2.2 to 4.1 Mcal/kg EBW gain, but without distinction between sexes in their estimation. The similarity of results may be due to the characteristics of the dataset and the estimation technique used by AFRC (1998) that worked mainly with castrated males, using comparative slaughter technique. NRC (2007) also did not incorporate a sex effect on the energy requirements for growth. In NRC (2007), the requirements are expressed in ME units (**ME_G**; 5.5 Mcal/kg BW gain**)**, making comparisons to our results more difficult, since differences related to composition of gain of different sexes would directly affect the protein and fat deposition efficiencies. Variation in diet components and the ratio of roughage to concentrate has been reported to affect the partial efficiency of energy use for gain in lambs (Galvani et al., 2014). Therefore, energy requirements expressed in net terms should result in a more reliable estimate. Moreover, as females retain a greater proportion of energy as fat, the efficiency may be different from a bioenergetics perspective (Almeida et al., 2015a). More investigations considering the ME intake would be beneficial to evaluate the efficiency of energy use for gain.

When we evaluate the confidence limits for our estimates of NE_G, the uncertainty falls in the range of 3 to 5% of the mean value for growing goats. On the other hand, when it is applied in animals that are not included in the dataset (i.e., with unknown study effect), the uncertainty falls in the range of 5 to 9% of the mean value (prediction). The uncertainty surrounding NE_G is greater than the uncertainty associated with NP_G, which may be related to the considerable variation of body fat content between individuals (Berg and Butterfield, 1976).

Given the body composition changes from birth to maturity, the most remarkable change in the body constituents of growing dairy goats (i.e., body fat content) is dependent on the degree of maturity at certain BW, where females reach maturity earlier than males (Almeida et al., 2016). The earlier fat deposition determines why females require more energy and less protein than males at the same BW, because animals with distinct mature weights present different patterns of growth and consequently body composition (Tedeschi et al., 2004).

Based on this fact it is important to evaluate requirements for growth according to degree of maturity. The results of NP_G and NE_G based on degree of maturity presented herein were similar between sexes. In this sense, we are able to eliminate the effect of sex in the model because we isolate the differences in body composition that goats of different sexes show at same BW. The degree of maturity allows us to confirm that a female at 26.0 kg EBW has similar NP_G and NE_G to a castrated male weighing 34.9 kg EBW or an intact male weighing 42.6 kg EBW because they are at similar degree at maturity. Therefore, they can receive similar diets considering just NP_G and NE_G.

Although in the approach considering the degree of maturity the estimates of NP_G and NE_G show greater variation than when applying the allometric equations, this new approach can be useful for extending the results of requirements for growth obtained herein to different sexes and perhaps breeds. Future studies should focus on evaluating the effect of sex on the body composition of mature goats and its consequence on the nutritional requirements and test if it is still possible to use a general model across sexes for describing the requirements based on the degree of maturity.

Estimates of nutritional requirements that are more accurate should lead to better assessment of diets. To our knowledge, this is the first analysis aimed at quantifying the effect of sex on the energy and protein requirements for growth in dairy goats weighing from 5 to 45 kg BW, as well as considering the degree of maturity on them. The information presented here improves the knowledge about the composition of gain across different sexes in dairy goats and should lead to improved diets for growing dairy goats.

CONCLUSION

Allometric equations for estimating NP_G and NE_G differ between sexes in growing Saanen goats. The NP_G for males is greater than that for females. The NE_G of castrated males is greater than that of intact males, and is less than that of females at similar BW. The effect of sex on the estimative of nutritional requirements for growth should be accounted for by feeding systems. Including the degree of maturity as predictor cancels out those differences across sexes in protein and energy requirements.

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CHAPTER 5. IMPLICATIONS

The knowledge about protein and energy requirements is essential to formulate diets and we focused on evaluating the effect of sex on the protein and energy requirements for growing dairy goats. The main contribution of the results obtained in this study is that sex affects the estimative of protein and energy requirements of dairy goats. Beyond the knowledge about nutritional requirements, it is important to highlight also that we found some relevant aspects about methodologies used herein.

Regarding the protein and energy requirements for growth, we found differences between sexes at similar BW, and we also found that those could be canceled out if we take into account the mature weight of the different sexes. For adopting the net requirements to formulate diets is crucial the knowledge about the efficiency of energy or protein use, which is affected by feed factors as well as animal factors. We evaluated animal factors and found that sex affects the efficiency of energy use for growth, considering that the animals received similar diets in this study, this area should be more investigated in future studies.

Regarding net energy requirements for maintenance, is very interesting the fact that values obtained herein were similar to those reported to other ruminants. We infer that the net energy requirement for maintenance between species are not as distinct as we presumed before, and in future models, those values could be evaluated between species.

There is a huge difference between methods of prediction of the protein requirements for maintenance (i.e., comparative slaughter technique and N balance). It is known that N balance conveys some limitations that tend to overestimate the protein requirements. Our findings suggest that for many years, probably, we have been feeding animals with more protein than they really need. The use of more accurate protein requirements in diets can improve animal performance as well as to reduce problems of N losses in water and soil quality.

Other contribution of this study relates to the methodologies used herein. This was the first dissertation in our graduate program using a large dataset to evaluate nutritional requirements of dairy goats. We verified clearly one of the great advantages of using meta-analysis in the topic evaluated; we had a statistic power that allowed us to verify differences in protein requirements for growth as well as differences in energy requirements for maintenance across sexes, which were not found in previous independent studies. Besides, we provided a summary of those requirements to Saanen goats of different sexes with BW ranging from 5 to 45 kg (Table 1).

Other statistical approaches used in this work contributed to the improvement of scientific field. We used allometric equations (nonlinear equation) to estimate requirements for growth. Contrary to the general concept, the log transformation in allometric equations used for estimating requirements for growth in our situation was still more appropriate based on the residual pattern than the use of nonlinear models in statistical programs.

Additionally, we highlighted one important aspect that is not usually taken into account and should be adopted in animal nutrition that is the evaluation of uncertainty. We cannot feed adequately dairy herds if we do not understand a dimension of the variation between animals. We investigated those aspects in protein and energy requirements using Monte Carlo method, and we understand that it could be adopted in other variables related to ruminant nutrition and in the field routine. We pointed out that in dairy herds, it is not necessarily the best choice to feed animals according to the mean value of those nutritional requirements and the uncertainty can contribute to build applicable stochastic models.

All in all, related to the contribution in the field of protein and energy requirements of dairy goats, it is still necessary to evaluate the relationship between feed and animal factors to better understand the efficiencies of use and to develop more robust models.

We expected that our findings might contribute to improve the accuracy of nutritional recommendations that can bring economic and environmental advantages in this production system.

Table 1. Summary of the protein and energy requirements for growing Saanen goats of different sexes from 5 to 45 kg BW

Sex	BW (kg)	BW gain (g/day)	Protein requirements (g/day)			Energy requirements (Mcal/day)		
			MP_M	MP_G	MP total	ME_M	ME_G	ME total
Castrated male								
	5	0	4.1	0.0	4.1	0.37	0.00	0.37
	5	50	4.1	13.2	17.3	0.37	0.25	0.62
	5	100	4.1	26.4	30.5	0.37	0.49	0.86
	5	150	4.1	39.6	43.7	0.37	0.74	1.11
	5	200	4.1	52.8	56.9	0.37	0.98	1.36
	15	0	9.4	0.0	9.4	0.78	0.00	0.78
	15	50	9.4	11.9	21.2	0.78	0.31	1.09
	15	100	9.4	23.7	33.1	0.78	0.62	1.40
	15	150	9.4	35.6	45.0	0.78	0.93	1.71
	15	200	9.4	47.5	56.8	0.78	1.24	2.02
	25	0	13.8	0.0	13.8	1.13	0.00	1.13
	25	50	13.8	11.9	25.6	1.13	0.37	1.50
	25	100	13.8	23.8	37.5	1.13	0.74	1.87
	25	150	13.8	35.6	49.4	1.13	1.10	2.24
	25	200	13.8	47.5	61.3	1.13	1.47	2.61
	35	0	17.7	0.0	17.7	1.50	0.00	1.50
	35	50	17.7	12.4	30.1	1.50	0.44	1.94
	35	100	17.7	24.8	42.5	1.50	0.87	2.38
	35	150	17.7	37.2	54.9	1.50	1.31	2.81
	35	200	17.7	49.7	67.3	1.50	1.75	3.25
	45	0	21.4	0.0	21.4	1.84	0.00	1.84
	45	50	21.4	12.7	34.1	1.84	0.49	2.34
	45	100	21.4	25.5	46.9	1.84	0.98	2.83
	45	150	21.4	38.2	59.6	1.84	1.48	3.32
	45	200	21.4	51.0	72.3	1.84	1.97	3.81

Sex	BW (kg)	BW gain (g/day)	Protein requirements (g/day)			Energy requirements (Mcal/day)		
			MP_M	MP_G	MP total	ME_M	ME_G	ME total
Female								
	5	0	4.1	0.0	4.1	0.31	0.00	0.31
	5	50	4.1	13.0	17.2	0.31	0.26	0.57
	5	100	4.1	26.1	30.2	0.31	0.52	0.83
	5	150	4.1	39.1	43.2	0.31	0.78	1.10
	5	200	4.1	52.2	56.3	0.31	1.04	1.36
	15	0	9.4	0.0	9.4	0.65	0.00	0.65
	15	50	9.4	11.1	20.5	0.65	0.36	1.01
	15	100	9.4	22.2	31.6	0.65	0.72	1.37
	15	150	9.4	33.4	42.7	0.65	1.07	1.73
	15	200	9.4	44.5	53.8	0.65	1.43	2.09
	25	0	13.8	0.0	13.8	0.95	0.00	0.95
	25	50	13.8	10.7	24.5	0.95	0.44	1.39
	25	100	13.8	21.5	35.2	0.95	0.89	1.84
	25	150	13.8	32.2	46.0	0.95	1.33	2.28
	25	200	13.8	43.0	56.7	0.95	1.77	2.72
	35	0	17.7	0.0	17.7	1.26	0.00	1.26
	35	50	17.7	11.0	28.7	1.26	0.54	1.80
	35	100	17.7	22.0	39.7	1.26	1.09	2.34
	35	150	17.7	33.1	50.8	1.26	1.63	2.89
	35	200	17.7	44.1	61.8	1.26	2.17	3.43
	45	0	21.4	0.0	21.4	1.54	0.00	1.54
	45	50	21.4	11.1	32.5	1.54	0.63	2.17
	45	100	21.4	22.2	43.6	1.54	1.25	2.79
	45	150	21.4	33.3	54.7	1.54	1.88	3.42
	45	200	21.4	44.4	65.8	1.54	2.50	4.04

Sex	BW (kg)	BW gain (g/day)	Protein requirements (g/day)			Energy requirements (Mcal/day)		
			MP_M	MP_G	MP total	ME_M	ME_G	ME total
Intact male								
	5	0	4.1	0.0	4.1	0.37	0.00	0.37
	5	50	4.1	13.2	17.3	0.37	0.30	0.67
	5	100	4.1	26.4	30.5	0.37	0.60	0.97
	5	150	4.1	39.6	43.7	0.37	0.90	1.27
	5	200	4.1	52.8	56.9	0.37	1.20	1.57
	15	0	9.4	0.0	9.4	0.78	0.00	0.78
	15	50	9.4	11.9	21.2	0.78	0.34	1.12
	15	100	9.4	23.7	33.1	0.78	0.68	1.46
	15	150	9.4	35.6	45.0	0.78	1.01	1.79
	15	200	9.4	47.5	56.8	0.78	1.35	2.13
	25	0	13.8	0.0	13.8	1.13	0.00	1.13
	25	50	13.8	11.9	25.6	1.13	0.38	1.51
	25	100	13.8	23.8	37.5	1.13	0.76	1.89
	25	150	13.8	35.6	49.4	1.13	1.13	2.27
	25	200	13.8	47.5	61.3	1.13	1.51	2.65
	35	0	17.7	0.0	17.7	1.50	0.00	1.50
	35	50	17.7	12.4	30.1	1.50	0.43	1.93
	35	100	17.7	24.8	42.5	1.50	0.86	2.36
	35	150	17.7	37.2	54.9	1.50	1.29	2.79
	35	200	17.7	49.7	67.3	1.50	1.72	3.22
	45	0	21.4	0.0	21.4	1.84	0.00	1.84
	45	50	21.4	12.7	34.1	1.84	0.47	2.31
	45	100	21.4	25.5	46.9	1.84	0.94	2.78
	45	150	21.4	38.2	59.6	1.84	1.40	3.25
	45	200	21.4	51.0	72.3	1.84	1.87	3.71

APPENDIX

Chapter 2 – SAS Inputs

The NLMIXED procedure was used to fit nonlinear models. The statistical models included blocks and study as random effect, sex (castrated male, intact male and females) as fixed effect. We used dummy variables approach to assess the effect of sex on the regression parameters. That is, 3 dummy variables (a1, a2, and a3) were created. For castrated males, a1 = 1, a2 = 0, and a3 = 0; for intact males, a1 = 0, a2 = 1, and a3 = 0; and for females, a1 = 0, a2 = 0, and a3 = 1. CONTRAST statements were used for testing whether a regression parameter differed across the 3 sexes.

```
PROC NLMIXED DATA=<dataset name> TECH=newrap CORR COV ECORR ECOV EDER
HESS MSING=<> METHOD=firo;
PARMS    a1 = <> b1 = <>
         a2 = <> b2 = <>
         a3 = <> b3 = <>
s2u1= <>  s2e= <> ;

A = a1*k1+a2*k2+a3*k3;
B = b1*k1+b2*k2+b3*k3;


BOUNDS s2u1 > 0;
pred= (A+u1)*exp(B*MEI);
MODEL HP ~ NORMAL(pred,s2e);
RANDOM u1~ NORMAL(0,s2u1) SUBJECT=block;
PREDICT pred OUT=p;

CONTRAST 'Ma-Fa' a1-a2;
CONTRAST 'Mb-Fb' b1-b2;
CONTRAST 'Ma-Ca' a1-a3;
CONTRAST 'Mb-Cb' b1-b3;
CONTRAST 'Ca-Fa' a3-a2;
CONTRAST 'Cb-Fb' b3-b2;
RUN;
```

The linear regression analyses were computed with MIXED procedure. The statistical models included blocks and study as random effect, sex (castrated male, intact male and females) as fixed effect. When sex was found to be significant (*P* < 0.10), indicating a different intercept for at least 1 sex, 3 CONTRAST statements were used to conduct all 3 pairwise comparisons of sex. Likewise, 3 CONTRAST statements were used to conduct all 3 pairwise comparisons when the interaction between sex and regressor effects was found to be significant (*P* < 0.10), indicating that at least 1 sex had different slope.

```
PROC MIXED DATA=<dataset name>;
 CLASS sex block study;
 MODEL MEI = sex ADG sex*ADG/ RESIDUAL INFLUENCE;
 RANDOM block(study*sex)/;
 LSMEANS sex/at ADG=0;
 ESTIMATE 'b0 for sex=C' Intercept 1 sex 1 0 0;
 ESTIMATE 'b0 for sex=F' Intercept 1 sex 0 1 0;
 ESTIMATE 'b0 for sex=M' Intercept 1 sex 0 0 1;
 ESTIMATE 'b1 for sex=C' ADG 1 sex*ADG 1 0 0;
 ESTIMATE 'b1 for sex=F' ADG 1 sex*ADG 0 1 0;
 ESTIMATE 'b1 for sex=M' ADG 1 sex*ADG 0 0 1;
 CONTRAST 'C vs F' sex -1 1 0;
 CONTRAST 'C vs M' sex -1 0 1;
 CONTRAST 'F vs M' sex  0 -1 1;
 CONTRAST 'C vs F' sex*ADG -1 1 0;
 CONTRAST 'C vs M' sex*ADG -1 0 1;
 CONTRAST 'F vs M' sex*ADG 0 -1 1;
RUN;
```

The multiple linear regression of MEI_G used to calculate the efficiencies of RE as protein (k_p) and as fat (k_f) where MEI_G is the metabolizable energy intake above the maintenance, RE as protein (RE_p) and the RE as fat (RE_f) were calculated as the difference between final and initial BW of the respective body protein or fat.

```
PROC MIXED DATA=<dataset name>;
CLASS sex block study;
 MODEL MEIg = sex REp REf sex*REp sex*REf/ OUTP=<>  noint;
 RANDOM block(study*sex)/TYPE=VC;
 ESTIMATE 'b0 for sex=C' Intercept 1 sex 1 0 0;
 ESTIMATE 'b0 for sex=F' Intercept 1 sex 0 1 0;
 ESTIMATE 'b0 for sex=M' Intercept 1 sex 0 0 1;
 ESTIMATE 'b1 for sex=C' REp 1 sex*REp 1 0 0;
 ESTIMATE 'b1 for sex=F' REp 1 sex*REp 0 1 0;
```

```
ESTIMATE 'b1 for sex=M' REp 1 sex*REp 0 0 1;
ESTIMATE 'b1 for sex=C' REf 1 sex*REf 1 0 0;
ESTIMATE 'b1 for sex=F' REf 1 sex*REf 0 1 0;
ESTIMATE 'b1 for sex=M' REf 1 sex*REf 0 0 1;
CONTRAST 'C vs F' sex -1 1 0;
CONTRAST 'C vs M' sex -1 0 1;
CONTRAST 'F vs M' sex  0 -1 1;
CONTRAST 'C vs F' sex*REp -1 1 0;
CONTRAST 'C vs M' sex*REp -1 0 1;
CONTRAST 'F vs M' sex*REp 0 -1 1;
CONTRAST 'C vs F' sex*REf -1 1 0;
CONTRAST 'C vs M' sex*REf -1 0 1;
CONTRAST 'F vs M' sex*REf 0 -1 1;

RUN;
```

Chapter 3 – SAS Inputs

The MIXED procedure was used to fit linear models to calculate the net protein requirements for maintenance as the intercept of the linear regression of the N retained (g/kg $^{0.75}$ of BW) on the total N intake (g/kg $^{0.75}$ of BW) multiplied by 6.25.

```
PROC MIXED DATA=<dataset name>;
CLASS sex block study;
 MODEL NR_day_BW75 = sex NI_day_BW75 sex*NI_day_BW75 /
 OUTP=ptn;
 RANDOM block(study*sex);
 LSMEANS sex/at NI_day_BW75=0;
 ESTIMATE 'b0 for sex=C' Intercept 1 sex 1 0 0;
 ESTIMATE 'b0 for sex=F' Intercept 1 sex 0 1 0;
 ESTIMATE 'b0 for sex=M' Intercept 1 sex 0 0 1;
 ESTIMATE 'b1 for sex=C' NI_day_BW75 1 sex*NI_day_BW75 1;
 ESTIMATE 'b1 for sex=F' NI_day_BW75 1 sex*NI_day_BW75 0 1;
 ESTIMATE 'b1 for sex=M' NI_day_BW75 1 sex*NI_day_BW75 0 0 1;
 CONTRAST 'C vs F' sex -1 1 0;
 CONTRAST 'C vs M' sex -1 0 1;
 CONTRAST 'F vs M' sex  0 -1 1;
 CONTRAST 'C vs F' sex*NI_day_BW75 -1 1 0;
 CONTRAST 'C vs M' sex*NI_day_BW75 -1 0 1;
 CONTRAST 'F vs M' sex*NI_day_BW75 0 -1 1;

 RUN;
```

Chapter 4 – SAS Inputs

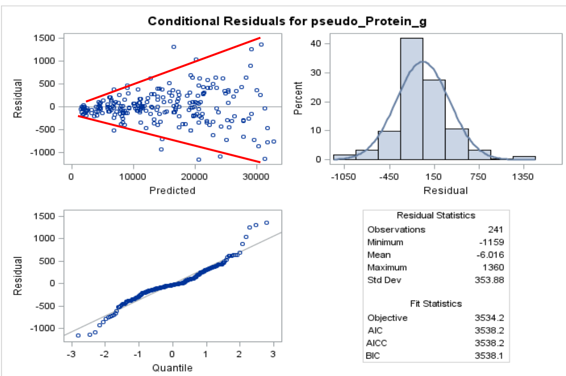
Allometric equations were developed for body contents (protein, fat or energy) as dependent variables and EBW as the allometric predictor. Parameters estimates were obtained using PROC MIXED of SAS and RANDOM effect of the study. A correction of the degrees of freedom was performed using the DDF option in the MODEL statement of MIXED procedure. Whenever the effect of sex was found to approach significance (*P* < 0.10), indicating a different intercept for at least one sex, three CONTRAST statements were used to conduct all three pairwise comparisons of sex. The SAS code used in this analysis is presented below:

```
/* Protein and energy requirements of dairy goats*/

*Importing dataset;
*----------------------;
PROC MIXED DATA=<dataset name>;
 CLASS study sex;
 MODEL <nutrient or energy content> = sex Log_EBW sex*Log_EBW / DDF=<>
OUTP=<>;
 RANDOM study/SOLUTION;
 LSMEANS sex/AT logEBW=0;
 ESTIMATE 'b0 for sex=C' Intercept 1 sex 1;
 ESTIMATE 'b0 for sex=F' Intercept 1 sex 0 1;
 ESTIMATE 'b0 for sex=M' Intercept 1 sex 0 0 1;
 ESTIMATE 'b1 for sex=C' LogEBW 1 sex*LogEBW 1;
 ESTIMATE 'b1 for sex=F' LogEBW 1 sex*LogEBW 0 1;
 ESTIMATE 'b1 for sex=M' LogEBW 1 sex*LogEBW 0 0 1;
 CONTRAST 'C vs F' sex -1 1 0;
 CONTRAST 'C vs M' sex -1 0 1;
 CONTRAST 'F vs M' sex  0 -1 1;
 CONTRAST 'C vs F' sex*LogEBW -1 1 0;
 CONTRAST 'C vs M' sex*LogEBW -1 0 1;
 CONTRAST 'F vs M' sex*LogEBW 0 -1 1;
RUN;
```

The linearized model was adopted based on the pattern of standardized conditional residuals. We analyzed the residual graphs and the residuals were clearly more homogeneous throughout the range of predicted values when the linearized expression of the allometric equation was used. For demonstration, we added the graphs of protein body composition:

Non-transformed expression



Linearized expression (log transformation)