UNIVERSIDADE FEDERAL DA BAHIA
PROGRAMA DE PÓS GRADUAÇÃO EM ZOOTECNIA

NUTRITIONAL ENERGY AND PROTEIN REQUIREMENTS, IMPROVEMENT OF IN SITU INCUBATION METHODS IN GOATS AND SHEEP

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Tese apresentada ao Programa de Pós-Graduação em Zootecnia, da Universidade Federal da Bahia como requisito parcial para obtenção do título de doutor em Zootecnia.

Área de concentração: Produção Animal

Orientador: Profª. Drª. Stefanie Alvarenga Santos

BAHIA
DEZEMBRO 2021

Ficha catalográfica elaborada pela Seção de Catalogação e Classificação da Biblioteca Central da UFBA

Santos, Antônio Carneiro Santana dos.
Nutritional energy and protein requirements, improvement of in situ incubation methods in goats and sheep / Antônio Carneiro Santana dos Santos. - 2022.
86 f.: il.

Orientadora: Profa. Dra. Stefanie Alvarenga Santos.
Tese (doutorado) - Universidade Federal da Bahia, Escola de Medicina Veterinária e Zootecnia, Salvador, 2022.

1. Zootecnia. 2. Nutrição animal. 3. Proteínas na nutrição animal. 4. Caprinos - Alimentação e rações. 5. Ovinos - Alimentação e rações. I. Santos, Stefanie Alvarenga. II. Universidade Federal da Bahia. Escola de Medicina Veterinária e Zootecnia. III. Título.

CDD - 636.3085
CDU - 636.3

ENERGY AND PROTEIN NUTRITIONAL REQUIREMENTS, AND IMPROVEMENT OF *IN SITU* INCUBATION METHODS IN GOATS AND SHEEP

Antônio Carneiro Santana dos Santos

Tese defendida e aprovada para obtenção do grau de Doutor em Zootecnia

Salvador, 21 de dezembro de 2021

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Dedico este trabalho, a meus pais, Ana e Antônio,
a minha irmã, Ana Paula (*in memoriam*),
a meus avós paternos e maternos,
a toda família.

SUMÁRIO

NUTRITIONAL ENERGY AND PROTEIN REQUIREMENTS, IMPROVEMENT OF IN SITU INCUBATION METHODS IN GOATS AND SHEEP

GENERAL INTRODUCTION

Brazilian northeast has an old tradition in goats and sheep breeding. These animals are used primarily for the production of meat, milk and wool. According to IBGE (2018), the number of sheep represents approximately 19 million heads, accompanied by 11 million goats. The northeast and south regions are the territories that hold the largest number of heads of these species, being: 10 million vs 220 thousand heads of goats and 13 million vs 4 million heads of sheep, for the northeast and south regions, respectively.

In general, the nutrition efficiency, which reflects on the animal production, may be related to two points: the first is the appropriate knowledge of the chemical composition of the ingredients, which will impact in nutrient digestion and retention; and secondly, the knowledge in daily nutritional requirements for energy and protein. Thus, diet formulation that reach the nutritional requirements of goats and sheep can avoid economic losses, reduce the unnecessary supply of nutrients, and promote a decrease in the excretion of pollutants into the environment.

The body composition is different between animals, and it varies according to growth (accumulations of water, protein, fat and minerals), according to the animal category, genetics, sex, food and environmental factor (Irshad et al., 2013; AFRC, 1993). However, age is the most influential factor that impact adipose tissue changes (Sanz Sampelayo et al., 1987). Once age advances, there is an increase in the participation of fat and a reduction in protein content in the empty body composition.

According to Berg & Butterfield (1976), muscle tissue in the postnatal period has accelerated growth, generating higher proportions of protein and water in the carcass, while adipose tissue has less growth in the same period, acquiring higher rates of growth as the animal reach the maturity. For Berg & Butterfield (1976), feed restriction can

promote an effect on the accumulation proportions of different tissues, resulting in greater losses of adipose tissue compared to muscle tissue. These authors state that in conditions of severe feed restriction, the development of muscle tissue can be compromised, ultimately generating animals with greater proportions of fat in relation to muscles, after the period of feed scarcity.

The determination of body composition can be carried out by different methodologies. These can be carried out with the live animal, or after its slaughter. The methodologies performed with the live animal, allow numerous determinations of body composition in the same animal, however, part of these methodologies have repeatability inconsistency, high cost and are justified only in specific environmental conditions (Miller et al., 1988; Stanford et al., 1998).

Stanford et al. (1998), in a review, listed the methods that use live animals to determine body composition in sheep and cattle, which are:

I. Ultrasound (Berg et al., 1996; Edwards et al., 1989): In this technique, the animals' body composition can be performed based on the thickness of fat and the measured area of some muscles. However, the time for reading is long and requires an experienced operator.

II. Live weight, (Cameron & Smith, 1985; Jones et al., 1982): This method taken into account that the tissues follow predictable patterns of development, from birth to maturity. However in a negative way, gastrointestinal filling and individual variations between animals make this technique limited.

III. Dilution with urea (Kock & Preston, 1979), deuterium (Martin & Ehle, 1986), or tritium (Panaretto & Till, 1963): This method consists of infusing a known concentration of marker that diffuses into the animal's body water. Marker concentration

at a given time can be used to estimate the size of the compartment in which the marker was infused. However, there are negative points for this method, which are the radioactivity of tritium, the high cost of deuterium and the metabolism of urea.

According to Berg & Butterfield (1978), ARC (1980) and Lerch et al., (2021), the methods that require slaughter of the animal and chemical analysis of the entire body after emptying to estimate body composition are more accurate, being highlighted as references. However, they have a high cost with equipment, in addition to allowing only one evaluation per animal. Information in Brazil on the body chemical composition of different breeds and physiological stages of goats and sheep is still limited due to the complexity of the methodology.

Lofgreen and Garret (1968) developed the comparative slaughter method, which consists of determining the animals' body gain composition. In this method, the animals are separated into two groups: the reference group, slaughtered on day zero; and the performance group, composed by animals in ad libitum feeding, slaughtered at the end of the experimental period. Thus, with the difference between these groups, we can estimate the body composition of gain.

In Brazil, in order to estimate the body composition of ruminant animals, some researchers have been using slaughter, followed by the grinding of part or the entire body of these animals, where finally sampling is carried out for future laboratory analyzes (Alleoni et al., 1997 ; Silva et al., 2010; Regatas Filho et al., 2011; Costa et al. 2013; Perreira et al., 2014; Cutrim et al., 2016; Teixeira et al., 2017). According to AFRC (1993), the two main requirements to be determined are energy and protein, which are divided into maintenance and gain. The maintenance requirement can be defined as the energy required by the animal that provides the maintenance of its vital processes,

allowing the balance between catabolism and anabolism. Then, the loss or gain of tissue must be null, without changes in the body composition of the animal (NRC 1985).

The energy requirements of the animals are difficult to be evaluated, as the efficiency of energy use for the different physiological activities is variable. Factors such as genotypes, sex, age, consumption, environmental factors and physiological stage can influence the growth curve, which can result in a change in the energy requirement for maintenance (Blaxter, 1962; Freetly et al., 2002; Cannas et al., 2004; NRC, 2007). According to Lofgreen & Garret, (1968), the net energy for maintenance (NEm) corresponds to the heat production of the daily animal (kcal / kg EBW0.75) in the post-absorptive state, being obtained through extrapolation to zero level of metabolizable energy intake (MEI) in kcal/kg EBW0.75, referring to the regression equation of the logarithm of heat production (HP) as a function of MEI.

As a NEm requirement, the AFRC (1998) suggests an average of 75 kcal/kg BW0.75, whereas the NRC (2007) establishes the value of 62 kcal/kg fasting animal body weight (FBW0.75), respectively for goats and sheep. Salah et al. (2014) based on data from animals raised in a tropical environment, performed a meta-analysis and suggested an average of 108 and 110 kcal/kg BW0.75/day, of metabolizable energy for maintenance (MEm), for goats and sheep. The net energy for gain / growth (NEg) is defined as the amount of energy retained in the animal's tissues, according to empty body weight gain (EBG), being directly related to the chemical composition of the gain, with regard to protein and fat (Lofgreen & Garret 1968; NRC, 2000). According to the NRC (2006), the net energy for gain can vary according to breed, diet, maturity stage and level of feed intake. In this way, it can be understood that the energy requirements for body weight

gain, growth or fattening are the answer based on the proportions in which protein, fat and water are retained in the animal's body.

Important variables in nutritional requirement studies are: the efficiency of using metabolizable energy for maintenance (km), and the efficiency of using metabolizable energy for weight gain (kg). According to Harris (1970), the km can be expressed by the ratio between the HP on fasting and the MEI in maintenance. The AFRC (1993) uses the following equation to estimate the km, as follow: km = (0.35 x qm) + 0.503; and to estimate kg, the equation, kg = (0.78 x qm) + 0.006, with qm being defined as the metabolizable energy of the diet. Nie et al. (2015) estimate kg as the slope of the regression between NEg and MEI of animals in weight gain.

The requirement for protein in ruminants may be impacted by sex, species and physiological status. Once body weight increases, there is an increase in the participation of fat and a reduction in the percentage of protein in the body composition (AFRC, 1995). Different terms are used to refer to the protein requirement, they are: crude protein, digestible protein, degradable protein in the rumen, non-degradable protein in the rumen, metabolizable protein and liquid protein.

According to the AFRC (1993) and NRC (2007), the requirement for liquid protein for maintenance (LPm) is the result of losses of fecal metabolic nitrogen, urinary endogenous nitrogen, desquamation of the gastrointestinal tract, and of the leather nitrogen, which can be estimated by the comparative slaughter procedure. The NRC (2007) considered that to estimate endogenous urinary nitrogen (NUE), the following equation can be used: EUN = 3.375 + 0.147 × BW (kg). On the other hand, to determine the endogenous fecal nitrogen (EFN), just considered the value of approximately 15.2g of fecal nitrogen per kg of DM consumed per day. Liu et al. (2004) and Moore et al.

(2004) worked with goats, and these authors recommended the following equations to estimate EUN and basal fecal nitrogen: EUN = 0.165 x BW, and EFN = (0.0267 x IDM) / 6.25.

It is necessary to know the body composition of the animal at the beginning and at the end experimental dynamics to determine the energy requirement for gain, and to quantify the liquid protein requirement for gain (NPg) (Lofgreen and Garrett, 1968). According to Attaix et al. (2005), approximately 50% of the protein synthesized daily in the animal's body is destined for organs. This fact is justified due to the high rate of protein turnover in the animal organism.

According to the AFRC (1993), the NPg for whole male sheep with 20 kg of BW and 200 g in ADG, is 28.7 g/day. In contrast, the NRC (1985) establishes a PLg of 47.8 g/day, for lamb weighing 20 kg and 250 g ADG. It is worth mentioning two important variables that concern the efficiency of use of the protein, they are: the efficiency of use of the metabolizable protein for maintenance (Kpm) and the efficiency of use of the metabolizable protein for weight gain (Kpg). According to Marcondes et al. (2009), the Kpm allow to convert the net protein requirement into metabolizable protein requirement, this being the protein portion truly absorbed in small intestine.

Another gap observed in the nutrition of goats and sheep is related to the evaluation of the type of tissue and number of bags incubated simultaneously in the rumen of these animals with the objective of estimating the iNDF of feed and feces of ruminants. Some studies have already been carried out evaluating the types of bags made by different tissues incubated in ruminants (Valente et al., 2011; Valente et al., 2011b). However, these studies were carried out only in cattle. Reis et al. 2017 evaluated the in-situ degradability of feed and ruminant feces in cattle and sheep. These authors did not

recommended in situ incubations in sheep, due to the long incubation time to obtain the indigestible fraction of feed and feces, greater than 288h. However, there is also a need to evaluate the effect of the number of bags incubated simultaneously in sheep in the estimation of iNDF of food and feces.

In this sense, it would be important to elucidate these questions regarding the nutrition of goats and sheep raised in tropical climate.

REFERENCES

AFRC. **Agricultural and Food Research Council: Energy and protein requirements of ruminants**. Wallingford: CAB International, 1993, 159 p.

AFRC. **Agricultural and Food Research Council: Energy and protein requirements of ruminants**. Wallingford: CAB International, 1993, 159 p.

AFRC. **Agricultural and Food Research Council: The nutrition of goats**. Wallingford: CAB INTERNATIONAL, 1998. 118p.

ALLEONI, G.F.; BOIN, C.; LEME, P. R., et al. Avaliação da gravidade específica e de outras medidas corporais e da carcaça para estimar a composição corporal de novilhos Nelore. **Revista Brasileira de Zootecnia**, v.26, n.2, p.375-381, 1997.

ARC. **AGRICULTURAL RESEARCH COUNCIL: The nutrient requirements of ruminant livestock**. London: Commonwealth Agricultural Bureaux, 1980, 351p.

ATTAIX, D., MOSONI, L., DARDEVET, D., COMBARET, L., MIRAND, P. P., & GRIZARD, J. Altered responses in skeletal muscle protein turnover during aging in anabolic and catabolic periods. **The international journal of biochemistry & cell biology**, v. 37, n. 10, p. 1962-1973, 2005.

BERG, E. P., NEARY, M. K., FORREST, J. C., THOMAS, D. L., & KAUFFMAN, R. G. Assessment of lamb carcass composition from live animal measurement of bioelectrical impedance or ultrasonic tissue depths. **Journal of Animal Science**, v. 74, n. 11, p. 2672-2678, 1996.

BERG, R. T. & R. M. BUTTERFIELD. **New Concepts of Cattle Growth,** Sydney University Press. 240p, 1976.

BLAXTER, Kenneth Lyon et al. The energy metabolism of ruminants. **The energy metabolism of ruminants.**, 1962.

CANNAS, A., TEDESCHI, L. O., FOX, D. G., PELL, A. N., & VAN SOEST, P. J. A mechanistic model for predicting the nutrient requirements and feed biological values for sheep. **Journal of Animal Science**, v. 82, n. 1, p. 149-169, 2004.

COSTA, M. R. G. F. et al. Body composition and net energy and protein requirements of Morada Nova lambs. **Small Ruminant Research,** v. 114, p. 206-213, 2013.

CUTRIM, D. O., ALVES, K. S., DOS SANTOS, R. D. C., DA MATA, V. J. V., OLIVEIRA, L. R. S., GOMES, D. Í., & MEZZOMO, R. Body composition and energy and protein nutritional requirements for weight gain in Santa Ines crossbred sheep. **Tropical animal health and production**, v. 48, n. 3, p. 683-688, 2016.

EDWARDS, J. W., CANNELL, R. C., GARRETT, R. P., SAVELL, J. W., CROSS, H. R., & LONGNECKER, M. T. Using ultrasound, linear measurements and

live fat thickness estimates to determine the carcass composition of market lambs. **Journal of Animal Science**, v. 67, n. 12, p. 3322-3330, 1989.

FILHO, S. D. C., GOMES, D. I., & FIGUEIRAS, J. F. Evaluation of ruminal Freetly, H. C., Nienaber, J. A., & Brown-Brandl, T. Relationships among heat production, body weight, and age in Finnsheep and Rambouillet ewes. **Journal of animal science**, v. 80, n. 3, p. 825-832, 2002.

IRSHAD, A., KANDEEPAN, G., KUMAR, S., ASHISH, K. A., VISHNURAJ, M. R., & SHUKLA, V. Factors influencing carcass composition of livestock: A review. **Journal of Animal Production Advances**, v. 3, n. 5, p. 177-186, 2013.

JONES, S. D. M., WALTON, J. S., WILTON, J. W., & SZKOTNICKI, J. E. The use of urea dilution and ultrasonic backfat thickness to predict the carcass composition of live lambs and cattle. **Canadian Journal of Animal Science**, v. 62, n. 2, p. 371-379, 1982.

KOCK, S. W.; PRESTON, R. L. Estimation of bovine carcass composition by the urea dilution technique. **Journal of Animal Science**, v. 48, n. 2, p. 319-327, 1979.

LERCH, S., DE LA TORRE, A., HUAU, C., MONZIOLS, M., XAVIER, C., LOUIS, L., ... & PIRES, J. A. Estimation of dairy goat body composition: A direct calibration and comparison of eight methods. **Methods**, v. 186, p. 68-78, 2021.

LOFGREEN, G.P & GARRET, W.N.A system for expressing net energy requirements and feed values for growing and finishing beef catle. **Journal of Animal Science**, v.27, n.3, p.793-806, 1968.

MARCONDES, M. I., VALADARES FILHO, S. D. C., PAULINO, P. V. R., VALADARES, R. F. D., PAULINO, M. F., NASCIMENTO, F. B., & FONSECA, M. A. Nutrient requirements of protein, energy and macrominerals of Nellore cattle of three genders. **Revista Brasileira de Zootecnia**, v. 38, n. 8, p. 1587-1596, 2009.

MARTIN, R. A.; EHLE, F. R. Body composition of lactating and dry Holstein cows estimated by deuterium dilution. **Journal of dairy science**, v. 69, n. 1, p. 88-98, 1986.

MOORE, J. E. et al. Prediction of fecal crude protein excretion of goats. **Small Ruminant Research**, v. 53, n. 3, p. 275-292, 2004.

NIE, H. T., ZHANG, H., YOU, J. H., & WANG, F. Determination of energy and protein requirement for maintenance and growth and evaluation for the effects of gender upon nutrient requirement in Dorper× Hu Crossbred Lambs. **Tropical animal health and production**, v. 47, n. 5, p. 841-853, 2015.

NRC. NATIONAL RESEARCH COUNCIL. **Nutrient requirements of beef cattle**. 7. ed. Washington, D.C.: National Academic Press, 2000. 242p.

NRC. **Nutrient requirements of domestic animals:** Nutrient requirements of sheep. 6. ed. Washington: National Academy Press, 99 p, 1985.

NRC. **Nutrient requirements of small ruminants**. National Academies Press 2006, 362p.

NRC. **Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids**. National Academies Press, 2007.

PANARETTO, B. A.; TILL, A. R. Body composition in vivo. II. The composition of mature goats and its relationship to the antipyrine, tritiated water, and N-acetyl-4-aminoantipyrine spaces. **Australian Journal of Agricultural Research**, v. 14, n. 6, p. 926-943, 1963.

PEREIRA, E. S., FONTENELE, R. M., SILVA, A. M., OLIVEIRA, R. L., FERREIRA, M. R., MIZUBUTI, I. Y., ... & CAMPOS, A. C Body composition and net energy requirements of Brazilian Somali lambs. **Italian Journal of Animal Science**, v. 13, n. 4, p. 3583, 2014.

REGADAS FILHO, J. G. L., PEREIRA, E. S., VILLARROEL, A. B. S., PIMENTEL, P. G., FONTENELE, R. M., COSTA, M. R. G. F. Efficiency of metabolizable energy utilization for maintenance and gain and evaluation of Small Ruminant Nutrition System model in Santa Ines sheep. **Revista Brasileira de Zootecnia**, v. 40, p. 2558-2564, 2011.

REIS, M. J., SANTOS, S. A., PRATES, L. L., DETMANN, E., CARVALHO, G. G. P., SANTOS, A. C. S., ... & COSTA, E. Comparing sheep and cattle to quantify internal markers in tropical feeds using in situ ruminal incubation. **Animal Feed Science and Technology**, v. 232, p. 139-147, 2017.

SALAH, N., SAUVANT, D., & ARCHIMÈDE, H. Nutritional requirements of sheep, goats and cattle in warm climates: a meta-analysis. **Animal**, v. 8, n. 9, p. 1439-1447, 2014.

SILVA, A. M., DOS SANTOS, E. M., PEREIRA, J. M., FILHO, O. A. B., NETO, S. G., & DA COSTA, R. G. Body composition and nutritional requirements of protein

and energy for body weight gain of lambs browsing in a tropical semiarid region1. **R. Bras. Zootec**, v. 39, n. 1, p. 210-216, 2010.

STANFORD, K., JONES, S. D. M., & PRICE, M. A. Methods of predicting lamb carcass composition: A review. **Small Ruminant Research**, v. 29, n. 3, p. 241-254, 1998.

TEIXEIRA, I. A. M. A., FERNANDES, M. H. M. R., FILHO, J. P., CANESIN, R. C., GOMES, R. A., & RESENDE, K. T. Body composition, protein and energy efficiencies, and requirements for growth of F1 Boer× Saanen goat kids. **Journal of animal science**, v. 95, n. 5, p. 2121-2132, 2017.

VALENTE, T. N. P., DETMANN, E., QUEIROZ, A. C. D., VALADARES FILHO, S. D. C., GOMES, D. I., & FIGUEIRAS, J. F. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. **Revista Brasileira de Zootecnia**, v. 40, p. 2565-2573, 2011.

VALENTE, T. N. P., DETMANN, E., VALADARES FILHO, S. D. C., CUNHA, M. D., QUEIROZ, A. C. D., & SAMPAIO, C. B. In situ estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. **Revista Brasileira de Zootecnia**, v. 40, p. 666-675, 2011b.

CHAPTER ONE: COMPARING NUTRIENT REQUIREMENTS OF ENERGY AND PROTEIN BETWEEN SHEEP AND GOATS

ABSTRACT:

Then, the objective of this study was to determine the nutritional requirements of energy and protein in goats and sheep, under tropical condition, and to compare the dietary requirement estimates between these species. A total of 34 male goats and 34 male sheep were used, with an initial average body weight (BW) of 21 ± 3.9 kg, and age close to 110 days. The experiment lasted 109 days. The experiment was conducted in completely randomized design. After the end of the adaptation period, four animals of each specie were randomly chosen and slaughtered, in order to determine the empty body weight (EBW) and initial body chemical composition. After slaughter, these eight animals composed the group called reference, representing the initial body composition of the other animals that remained in the experiment. After that, another four animals of each specie were randomly designated to another group called maintenance. The remained 26 animals (13 goats and 13 sheep) were included in the group for ad libtum intake, and they were slaughtered at the end of the experiment together with the maintenance group. The comparative slaughter method was applied to evaluate the nutrient requirements. There was difference ($P < 0.05$) between species for all intake variables (kg/d and g/kg of BW), but this effect was not observed ($P>0.05$) for digestibility of the nutrients evaluated. There was greater ($P < 0.01$) microbial CP synthesis in sheep compared to goats (Table 6). However, the microbial efficiency expressed in g/kg of digestible OM ($P = 0.23$) and g/kg of TDN ($P = 0.24$) did not differ between species. The NEm was 0.0683 Mcal/EBW0.75/day.

The requirement of metabolizable energy for maintenance (MEm) was 0.115 Mcal/kg EBW0.75/day, and the km was 0.599, for both species. The estimated NEg for uncastrated male dorper goats was 0.545 Mcal/day, considering a body weight of 30 kg and ADG of 200 g/day, while the estimated NEg for uncastrated lambs with the same body weight and ADG was 0.585 Mcal/day. The kg obtained was 0.113 and 0.699 for goats and sheep respectively. The NPm was 0.318 g/kg EBW0.75/day. The k that allows to convert NPg in MPg was represented by β1= 0.09794, which was obtained from the equation to estimate NPm. Similarity is observed in the energy requirement for maintenance in goats and sheep. However, species show differences in energy requirements for gain and efficiency of energy use for gain.

Keywords: Energy, Goat, Protein, Requirement, Sheep

INTRODUCTION

Some of the main objectives of an animal production system are the maximum efficiency, maintaining product quality and guaranteeing the lowest cost. In Brazil, efforts have been made to make the production systems for goats and sheep more efficient according to the production indexes (feed conversion, body weight gain, carcass yield and milk production), focusing on the interests regarding genetic, sanitary, reproductive, and nutritional improvements. The maintenance and growth requirement of goats, sheep, or any other ruminant specie are quite specific, as it involves several factors, such as: specie, breed, physiological status, environment, and among other factors (Fernandes et al., 2008). Therefore, when data for specific animal group are scarce, most field technicians may extrapolate estimates from similar species concerning metabolic size, such as goat and sheep. Nevertheless, even for the relatively similar species, it is not appropriate to use a single nutritional requirement table to estimate the energy and protein requirements.

Brazil has only one nutritional requirement table, BR-CORTE 2016, which is intended for zebu and crossbred beef cattle. However, due to the lack of national data concerning sheep and goats, the most diets formulated for these species, in a tropical environment, are formulated based on the requirement presented in international tables, such as the National Research Council (NRC), Agricultural and Food Research Council (AFRC), Institut National de la Recherche Agronomique (INRA), and Commonwealth Scientific and Industrial Research Organization (CSIRO). It is known that all these tables were prepared for temperate climate countries. These data are not applicable ruminants reared in tropical climate countries, and their usage can cause productive results different from those described in these international tables. Oliveira et al., (2018) carried out a

meta-analysis to evaluated the energy and protein requirements of growing sheep raised in Brazil. These authors stated that the nutritional needs of sheep reared in tropical regions are different from those recommended by NRC (2007) and AFRC (1993).

Recently, studies have been carried out in order to determine body composition, energy and protein requirement for goats and sheep, reared in a tropical environment (Fernandes et al., 2008; Costa et al., 2013; Perreira et al., 2014; Souza et al., 2014; Cutrim et al., 2016; Perreira et al., 2017; Souza et al., 2017; Teixeira et al., 2017; Costa et al., 2018; Martins et al., 2019 and Souza et al., 2019), which allow to improve the knowledge about nutrient requirement of small ruminants under tropical conditions, but, more information about the difference between these species are required

Due to this scientific gat in the goats and sheep nutrition raised in a tropical climate, it would be necessary to increase research that allows to estimate the nutritional requirement of these animals, under these conditions. Then, the objective of this study was to determine the nutritional requirements of energy and protein in goats and sheep, under tropical condition, and to compare the dietary requirement estimates between these species.

MATERIAL AND METHODS

Location and approval of the ethics committee

The experiment was carried out at the Experimental Farm of São Gonçalo dos Campos – Bahia State, School of Veterinary Medicine and Animal Science of the Federal University of Bahia (UFBA). All procedures performed were clearly presented to the ethics committee for animal usage, being approved with protocol number, 01/2019.

Animals, experimental design and diet

A total of 34 male goats and 34 male sheep were used, with an initial average body weight (BW) of 21 kilograms (kg) ± 3.9 kg, and age close to 110 days. The experiment lasted 109 days, with 15 days for adaptation to diet, handling and facilities, and the remaining 95 days destined to carry out the experimental periods. During the adaptation period all animals were individually identified by earrings, weighed and treated against ectoparasites and endoparasites, and finally placed in individual stalls (1.2 meters (m) x 1.2m x 1.3m), built at a height of 1m from the ground, equipped with feeder and drinker.

The experiment was conducted in completely randomized design, and the animals' distribution to each experimental group was random. After the end of the adaptation period, four animals of each specie were randomly chosen and slaughtered, in order to determine the empty body weight (EBW) and initial body chemical composition. After slaughter, these eight animals composed the group called reference, representing the initial body composition of the other animals that remained in the experiment. After that, another four animals of each specie were randomly designated to another group called maintenance, which were fed with approximately 5% of metabolic weight ($BW^{0.75}$). The remained 52 animals (26 goats and 26 sheep) were included in the group for *ad libtum* intake, throughout the experiment, according to the NRC (2007).

The animals' diet consisted of sorghum silage and concentrate, containing: ground corn, soybean meal, corn germ, urea + ammonium sulfate and mineral mixture (Table 1). The total diet was based in 50:50 forage and concentrate ration, based on dry matter (DM). The concentrate was composed of 713 g/kg ground corn, 217 g/kg soybean meal, 30 g/kg corn germ, 20 g/kg urea + ammonium sulfate and 20 g/kg of mineral mixture (containing 147 g/kg of sodium, 120 g/kg of calcium, 87 g/kg of phosphorus, 18 g/kg of sulfur, 3.8

g/kg of zinc, 1.8 g/kg of iron, 1,3 g/kg of manganese, 0.5 g/kg of copper, 0.3 g/kg of molybdenum, 0.08 g/kg of iodine, 0.04 g/kg of cobalt, 0.02 g kg of chromium and 0.015 g/kg of selenium). The diet was provided at 8:30 a.m and 3:30 p.m. in similar proportions. The total intake of DM was adjusted to keep leftovers between 10 - 15%, of the daily amount offered, with exception for the maintenance group.

Table 1 - Chemical composition of sorghum silage, concentrate and diet.

Nutrient (g / kg DM)	Sorghum Silage	Concentrate	Diet
Dry matter[1]	321	869	595
Organic matter	942	940	941
Crude Protein	96	242	169
Ether Extract	31	37	34
apNDF[2]	494	123	308
Non-fibrous carbohydrates[3]	320	535	425
iNDF[4]	259	21	140
Lignin	53	21	37

[1]g/kg natural material;
[2]Neutral detergent fiber corrected for ash and protein;
[3]Detmann e Valadares Filho (2010): NFC = 100 - [(%CP - CP% of urea + % of urea) +% apNDF + %EE + %ASH], where apNDF is corrected for ash and protein.
[4]Indigestible neutral detergent fiber.

Experimental procedures and sample collections

Samples of the sorghum silage supplied and the leftovers of each animal were obtained daily and subsequently stored at -20 ° C. Weekly, a composite sample of sorghum silage and leftovers was subjected to partial drying in a ventilation oven under a temperature of 55 °C for 72 hours. After drying, the samples were ground in a knife mill (Willey mill; TECNAL, São Paulo, SP, Brazil) in 2 mm and 1 mm sieves, and finally composed proportionally based on dry weight, per animal and per period. The ingredients that composed the concentrate were sampled on the days of ration mixing.

Two periods were used to collect samples during the experiment. Each period consisted of six days, where during the 1st to 2nd day, spot samples of urine were performed, 4h after feeding (Santos et al., 2018). A 10 mL aliquot of each urine sample

was filtered through cheesecloth and diluted in 40 ml of 0.036N H_2SO_4 (Santos et al., 2018), in order to avoid multiplication of microorganisms and degradation of the purine derivatives, (Chen & Gomes et al., 1992). Subsequently, a composite sample was obtained to correspond to two days, per animal and per period. The samples were stored in a freezer at -20 °C, for further analysis of creatinine, purine derivatives (uric acid, allantoin, hypoxanthine and xanthine), urea and total nitrogen.

To determine the digestibility coeficient, 3 days were used to collect feces and leftovers, as proposed by Lazzarini et al. 2016. These samples were subjected to partial drying in a ventilation oven under a temperature of 55 °C for 72 hours. Subsequently, the samples were ground in a knife mill (Willey mill; TECNAL, São Paulo, SP, Brazil) with 2 mm (Valente et al., 2015) and 1 mm sieves and proportionally composed, based on the dry weight per animal for each period.

The iNDF content in ingredients, leftovers and feces was obtained by an in situ incubation trial. Due to the presence of small particles adhered to the empty NWT bags, they were washed before receiving the samples in a solution containing neutral detergent (Mertens, 2002), at 100 ° C for 15 minutes, then rinsed in hot water. Later, to remove any residue associated with water, the bags were immersed in acetone and finally in distilled water (Detmann et al., 2012). After washing, the bags were dried in a ventilation oven at 55 ° C for 72 hours. Then, all the bags were sent to forced circulation oven, at a temperature of 105 ° C for 2 hours (method 934.01, AOAC, 2005). After obtaining the weight of the bags, the sample mass was added to suit the proportion of 20 mg/cm2 of surface (Nocek, 1988), after which all the bags were thermally sealed.

Bags were tied together with a metal chain with a weight at its end, thus allowing the total immersion of the bags with samples in the ruminal content of the animals. The incubation time used to estimate the indigestible neutral detergent fiber of the samples was 288 hours (Reis et al., 2017). After removing the rumen bags, they were washed in running water, until the water was completely cleared. After cleaning, all bags were transferred to a ventilation oven under a temperature of 55 ° C, where they were kept for 72 hours. Then, the bags were packed in polyethylene bags and grouped in plastic boxes, for further fiber analysis.

Comparative slaughtering procedure

All animals were weighed before and after 16-hour fasting period. The registration made before removing the feed was to taken to obtain full body weight. (FuBW). Sixteen hours after removing the feed, the same animals were weighed again, then computing the fasting body weight (FBW). The slaughtering was humanitarian, starting with a penetrating captive dart pistol, followed by bleeding through the carotid + jugular section and evisceration, as described in Ordinance No. 214, of May 2018, of the Ministry of Agriculture, Livestock and Supply.

The blood of each animal was collected, weighed and sampled. The gastrointestinal tract (rumen, reticulum, omasum, abomasum, duodenum, small intestine, large intestine) was washed and after drying it was weighed to calculate to empty body weight (EBW). The following parts were also weighed and collected: tongue, esophagus, trachea, lungs, heart, spleen, liver, kidneys, empty bladder and gallbladder, reproducer organs (testicles + penis), body parts (head, leather, paws and syrup) and fats (mesenteric, perirenal and omental). The weightings of the hot carcasses (HCW) and cold carcasses (after 24h of slaughter – CCW) were obtained, and then they were sectioned in two half-

carcasses, which were cooled to - 4 °C. All half carcasses were dissected and weighed in muscle, fat and bones, in order to measure the proportions of these components in the carcass in relation to the cold carcass weight.

After slaughtering, the animals' bodies were divided into five parts: 1) Blood; 2) Muscles + carcass fat; 3) Viscera + Brain Tissue; 4) Bones; and 5) Leather. With the exception of blood and bones, all the parts described above were ground separately in a low-speed meat mill, homogenized, sampled and added in plastic containers. The bones were cut using a band saw, into small pieces and stored in a -20ºC freezer.

Laboratory analysis

For the analysis of chemical composition, samples of sorghum silage, concentrate feeds, leftovers and feces were subjected to pre-drying in a ventilation oven at 55 °C for 72 hours. All samples were ground in a knife mill (Willey TE-650/1, TECNAL, São Paulo, Brazil), with 2 mm sieves (Valente et al., 2015) for rumen incubations and 1 mm for other analysis.

The samples of feed, leftovers and feces were analyzed for dry matter (DM) and ash (MM) according to the official method 934.01, and 942.05, at 600 °C for 4 hours (AOAC, 2005), respectively. Organic matter (OM) was quantified by subtracting 100 - percentage of MM observed in the sample. Total Nitrogen (N) was quantified through three-step analysis of the micro Kjeldhal (digestion with sulfuric acid, basic distillation and acid titration, by the official method 968.06 (AOAC, 1995), and this value was multiplied by 6.25 to obtain the crude protein (CP) value. The content of ether extract (EE) was quantified by gravimetry after extraction with petroleum ether according to method 920.39 (AOAC, 2005).

For the analysis of neutral detergent fiber (NDF), samples were initially placed in 100 ml self-cleaning bottles, following a ratio of 1 gram (g) per 100 ml of neutral detergent solution (Mertens, 2002), using α- thermostable amylase, without addition of sodium sulfite, autoclaved at 110 °C for 60 minutes at 4.3-5.7 psi (Barbosa et al., 2015). Then, the ash content (Mertens, 2002) and residual N (Licitra et al. 1996) were analyzed, making it possible to quantify the neutral detergent fiber corrected for ash and protein (apNDF). The washing and filtering procedures for NDF and acid detergent fiber followed the protocol described by Barbosa et al. (2015). Lignin was measured according to the official method 973.18 (AOAC, 2005) using 72% v/v sulfuric acid, and the indigestible NDF (iNDF) content was evaluated after incubating the samples at 2 mm in situ for 336 h (Reis et al., 2017).

In urine, creatinine was analyzed by the enzymatic method from the reaction with alkaline picrate, using a commercial kit (Creatinine - K016, Bioclin, Minas Gerais, Brazil). Urinary and plasma urea was quantified by the enzymatic method in the presence of sodium salicylate and hypochlorite, using the commercial kit (enzymatic urea - K047, Bioclin, Minas Gerais, Brazil). Allantoin, xanthine and hypoxanthine were determined according to Chen and Gomes (1992). Uric acid was quantified by enzymatic method in uricase and peroxidase, using the commercial kit (Monoreagent uric acid - K139, Bioclin, Minas Gerais, Brazil). Total urinary nitrogen (N) was quantified using the micro Kjeldhal by the official method 968.06 (AOAC, 1995).

The samples obtained from the animal bodies as blood, viscera, leather, and muscle + fat were pre-dried in a lyophilizer (LV 2000 - Terroni), and subsequently ground in a mill. The bone pieces were pre-dried in a forced ventilation oven, under a temperature of 60 ºC, remaining in it until the weight remained constant, then they were ground. All

samples were analyzed for DM and MM according to the official method 934.01, and 942.05, at 600 ° C for 4 hours (AOAC, 2005), respectively. The organic matter OM was quantified by subtracting 100 - percentage of MM observed in the sample. To obtain the CP value, the total N was initially quantified using the micro Kjeldhal (by the official method 968.06 (AOAC, 1995), and multiplied by 6.25. The EE content was quantified by gravimetry after extraction with petroleum ether according to method 920.39 (AOAC, 2005).

Calculations and statistical analysis

Net energy for maintanance (NEm)

The net energy requirement for maintenance (NEm) (Mcal/EBW 0.75 /day) was calculated from the intercept (β0) of the exponential regression between heat production (HP) and MEI. The model used was as follows:

$$(1)\ HP = \beta 0 \times e^{(\beta 1 \times MEI)}$$

Where: HP = heat production; MEI = metabolizable energy intake; B0 = net energy for maintance ; B1 = model parameter without biological meaning.

Metabolizable energy for maintenance (MEm)

The MEm (Mcal/EBW 0.75/day) was determined by an iterative method using the above equation, where MEm was estimated as the value at which the HP is equal to the MEI. The efficiency of utilization of metabolizable energy for maintenance (km) was obtained from the relationship between the net and metabolizable energy requirements for maintenance (NEm/MEm).

Net energy for gain (NEg)

A regression equation between the retained energy (RE) and the daily gain of EBW (EBWG) was adjusted for a given metabolic EBW (kg 0.75) for the animals in maintenance and performance, using the following model:

$$(2)\ RE = a \times EBW^{0,75} \times EBWG^{b}$$

where RE = retained energy (Mcal/day) or ELg; EBW0.75 = metabolic empty body weight, EBWG = empty body weight gain (kg/day) and 'a' and 'b' are regression parameters.

Metabolizable energy for gain (MEg)

The efficiency coefficient for energy for gain (kg) was determined by linear regression of retained energy (RE) as a function of MEI for gain, where B1 is assumed to represent kg.

$$(3)\ RE = \beta 0 + MEI \times \beta 1$$

Where RE = retained energy in Mcal/d; MEI = metabolizable energy intake (Mcal/EBW0.75/day); B1 represents the kg and B0 = model parameter without biological meaning.

Metabolizable energy for gain (MEg) was determined by NEg/kg.

Net protein for maintenance (NPm)

The net protein requirement for maintenance (NPm, g/$PCVZ^{0.75}$/day) was calculated through the regression between retained protein as a function of metabolizable protein intake.

$$(4)\ RP = \beta 0 + MPI \times \beta 1$$

where RP = retained protein (g/EBW$^{0.75}$/day), MPI = metabolizable protein intake (g/EBW 0.75 /day) and β0 and β1 are regression parameters. The β0 represented the NPm (g/EBW$^{0.75}$/dia) and the β1 represented efficiency of usage from metabolizable protein for gain to net protein for gain (k).

Metabolizable protein for maintenance (MPm)

The requirement of metabolizable protein for maintenance was calculated considering the linear regression between the metabolizable protein intake and the empty body weight gain (EBWG) for the animals in performance and maintenance.

$$(5)\ MPI = \beta0 + EBWG \times \beta1$$

Where MPI = metabolizable protein intake (g/day), EBWG = empty body weight gain (kg/day) and β0 and β1 are regression parameters. The division of the β0 by the mean metabolic weight of the animals was used to estimate the metabolizable protein requirements for maintenance (MPm)

Net protein for gain (NPg)

The requirement of net protein for gain was estimated from the linear regressions between retained protein (RP) as a function retained energy (RE) and EBWG, without the use of intercept.

$$(6)\ RP\ (NPg) = \beta1 \times RE + \beta2 \times EBWG$$

Where RP = retained protein (g/day), which was extrapolated to estimated NPg directly; RE = retained energy (Mcal/day); EBWG = empty body weight gain (kg/day); β1 and β2 was regression parameters without biological meaning.

Metabolizable protein for gain (MPg)

The requirement of metabolizable protein for gain (MPg) was estimated by dividing NPg estimated in equation (5) per k, which was estimated from equation (4)

(7) MPg = NPg/k

The data of intake, digestibility, performance, nitrogen balance, microbial synthesis of the confined animals, were analyzed in a completely randomized design, according to the statistical model.

$$Yij = \mu + ti + eij$$

Where: Yij is the observed value of the dependent variables measured in the experimental unit; μ is the general average; *ti* is the treatment effect represented by specie effect, eij is the random error. The results were statistically interpreted through analysis of variance and regression, using the Statistical Analysis System (SAS, version 9.4). The linear models were built using the PROC REG, and for the non-linear models the PROC NLIN, both for SAS. All tests were used 0.05 as a critical probability level to verify the significance of the model's parameters. The comparison between the two species was performed by inserting a binary variable in the structures of the regression models and adjusted.

RESULTS

There was a difference ($P < 0.05$) between species for all intake variables (kg/d and g/kg of BW), but this effect was not observed ($P>0.05$) for digestibility of the nutrients evaluated (Table 2).

There were differences ($P < 0.05$) between species for all performance parameters (Table 3). Average daily gain (ADG), final body weight (BW) hot carcass weight (HCW) and cold carcass weight (CCW) were higher ($P < 0.05$) for sheep compared to goats. The amounts (kg) of bones ($P = 0.08$) did not differ between species. However, goats had a higher ($P < 0.01$) percentage of muscle in the carcass than sheep. All variables in percentage expressed according to PCF were different among species ($P < 0.05$).

There was a difference ($P < 0.01$) between species for variables referent to intake efficiency (carcass and muscle conten), with a trend ($P = 0.06$) in the intake efficiency in the ADG according to dry matter intake (Table 4).

Sheep had higher N intake than goats ($P < 0.01$). In these animals there was greater N excretion in feces ($P < 0.01$), urine ($P < 0.01$), endogenous losses ($P < 0.01$) and also greater N retention (g/day) ($P < 0 .01$) compared to goats (Table 5).

Sheep had higher excretions (mmoL/d) of allantoin ($P = 0.01$), uric acid ($P < 0.01$) compared to goats. As expected, the absorbed purines were higher ($P = 0.01$) in sheep compared to goats. There was greater ($P < 0.01$) microbial CP synthesis in sheep compared to goats (Table 6). However, the microbial efficiency expressed in g/kg of digestible OM ($P = 0.23$) and g/kg of TDN ($P = 0.24$) did not differ between species.

The NEm was calculated using only the intercept of the exponential regression that used the variables heat production and the MEI: $HP = 4.5268_{(\pm 0.2755)} \times e^{(0.0683\ (\pm 0.0054)\ x\ MEI)}$, being 0.0683 Mcal/$EBW^{0.75}$/day the NEm. There was no effect of specie ($P<0.05$) when the test of model identity was applied to verify this effect. Then, only one equation was presented to estimate NEm (Figure 1). The requirement of metabolizable energy for maintenance (MEm) was 0.115 Mcal/kg $EBW^{0.75}$/day, and the km was 0.599, for both species.

There was difference in the estimate of the NEg, based on the test of model identity ($P<0.05$), then two equations were fitted to estimate NEg. The equations generated to estimate NEg (Mcal/ kg $EBW^{0.75}$/day) were $0.0735 \times EBW^{0.75} \times EBWG^{0.6746}$ for goat and $0.0432 \times EBW^{0.75} \times EBWG^{0.3227}$ for sheep. The estimated NEg for uncastrated male dorper goats was 0.318 Mcal/day, considering a body weight of 33 kg and ADG of 180 g/day, while the estimated NEg for uncastrated lambs with the same body weight and ADG was 0.342 Mcal/day. The kg obtained was 0.113 and 0.699 for goats and sheep respectively.

The equations suggested to estimate the metabolizable energy requirement for gain MEg (Figure 2) are: $RE = 0.00705_{(\pm 0.00162)} + MEI \times 0.11302_{(\pm 0.00943)}$ for goats and $RE = 0.00705_{(\pm 0.00162)} + MEI \times 0.06998\ _{(\pm 0.01131)}$ for sheep. The two equations were fitted with the same intercept once no statistical difference was found ($P = 0.08$) when a dummy variable were placed associated to $\beta 0$. In the case of $\beta 1$, there was difference for dummy variable relative to specie ($P < 0.01$), then, two slopes were adopted.

The NPm was estimated as a single equation for both species: $RP = MPI \times 0.09794\ _{(\pm 0.01604)} - 0.31871\ _{(\pm 0.1653)}$. This equation was fitted with only one value for intercept once

no statistical difference was found (P = 0.29) when a dummy variable for species was placed associated to β0. The same was found for slope, once no difference was found for β1 also (P = 0.08). The NPm was 0.318 g/kg EBW0.75/day. Two equations were suggested to estimate the requirement of metabolizable protein for maintenance (MPm): MPI = 38.28 $_{(\pm 5.9529)}$ + 318.07 $_{(\pm 30.788)}$ × EBWG for goat, and MPI = 38.28 $_{(\pm 5.9529)}$ + 516.06 $_{(\pm 46.723)}$ × EBWG, for sheep. The two equations were fitted with the same intercept once no statistical difference was found (P = 0.12) when a dummy variable were placed associated to β0. In the case of β1, there was difference for dummy variable relative to specie (P < 0.01), then, two slopes were adopted.

The requirements of net protein for gain (NPg) were estimated by only one model for both species, which was RP (NPg) = 0.04709$_{(\pm 1.8658)}$ + 27.6724 $_{(\pm 16.2871)}$ × EBWG + 11.3121 $_{(\pm 12.1537)}$ × ER. This equation was fitted with only one value for intercept once no statistical difference was found (P = 0.93) when a dummy variable for species was placed associated to β0. The same was found for slopes, once no difference was found for β1 (P = 0.16) and β2 (P = 0.11). The k that allows to convert NPg in MPg was represented by β1= 0.09794, which was obtained from the equation to estimate NPm.

DISCUSSION

We can see from our results that there area differences in nutrient intake between goats and sheep fed a similar diet. In this study, the diets provided for the species consisted of sorghum silage as a forage source. Domingue et al. (1991a) fed alfalfa hay for goats and sheep, and these authors observed that sheep spent less time feeding (-3h) and had higher feed rate (62%) (DM intake per minute). Thi may explain the differences of intake observed between these species. Studies such as the one by Arslan et al. (2007) and Ferreira et al. (2013), also report that sheep are less selective animals compared to goats. In this sense, we suggest that the lower dry matter intake of goats compared to sheep is partly due to the longer time spent in feed selection by these animals. Van Soest (1994) reports that sheep are strictly grazer animals, however goats have a more flexible feeding habit. In this sense, the NRC (2007) described that the rumen-reticulum of sheep have a greater volume compared to goats. This corroborates with our data regarding to the higher DM and NDF intakes according to body weight in sheep.

Despite the higher nutrient intake observed for sheep, no differences were found in apparent nutrient digestibility between goat and sheep. It is recognized that nutrient digestibility may differ between ruminant species, and this result is normaly dependent on the quality of the forage source (Soto-Navarro et al., 2014; Nasrullah et al., 2015; Garry et al., 2021). Some studies reported that goat and sheep fed with high quality forage have similar apparent nutrient digestibility (Askar et al., 2016; Candyrine et al., 2019; Lunesu et al., 2021). However, other research reports demonstrated that goats vs. sheep are more efficient in terms of nutrient digestibility when they are fed a forage source with high fiber and low protein content. This situation is quite common in tropical conditions (Domingue et al., 1991b; Tesk et al., 2018). Therefore, our results suggest that goat and sheep have similar capacity to digest diets that consist of sorghum silage as forage.

It is already understood that variables such as nutrient availability and their synchronization in the rumen are important and provide efficient use of ruminal substrates (Hackmann & Firkins 2015). We can observe that, regardless of the lower availability of nutrients in the rumen of goats, these animals show similar microbial efficiency when compared to sheep. In this sense, we can point out that the species do not present similarity regarding ruminal N recycling, and assuming that the recycling of n is apparently bigger in goat compared to sheep (Alam et al., 1985; Asmare et al., al., 2011; Asmare et al., 2012; Nair et al., 2021). This event can promote the timely synchronization between protein and energy, favoring microbial growth in the rumen.

It is known that the level of nutrient intake directly influences performance (Ciu et al., 2019; Saro et al., 2020), we already expected that the higher nutrient intake would generate greater average daily gain (mean = 234 g/day) in sheep compared to goat (mean = 170 g/day). Sen et al., 2004 reported greater average daily gain in sheep (57.40 g/day) compared to goats (39.64 g/day) in semiarid conditions. However, Mahgoub and Lodge (1998) and Santos et al. (2018) observed daily gains of 179 g/day and 160 g/day vs 111 g/day and 100g/day for sheep and goats fed a higher quality diet. Mahgoub and Lodge (1998) state that the average age at which goats reach slaughter weight is 190 days and sheep goats 120 days. We observed in this study that at 205 days of age, 95 days of confinement, goats have 15.1% lower final body weight compared to sheep, which indicates that these animals have a lower growth rate.

Research has reported differences in carcass fat deposition between the two species (Mahgoub and Lodge, 1998; San et al., 2004). Goat being leaner compared to sheep (Devendra and Burns, 1983). Goat generally deposit less subcutaneous fat but more internal fat compared to sheep (Shija et al., 2013; Brand et al., 2019; Gama et al., 2019).

These reports corroborate our results, which demonstrate greater muscle deposition (%) and lower fat deposition (%) in goat carcasses compared to sheep.

Although sheep have higher N retention (approximately 49.30%) than goats, it is important to report that sheep showed a significant increase in urinary (22.4%) and fecal (46.9%) excretion. We can assume that faecal and urinary excretion of nitrogen is influenced by the increase in nitrogen intake, where as the intake decreases, its excretion decreases linearly (Kreuzer and Kirchgeßner, 1985; Fanchone et al., 2013; Schuba et al., 2017). We were also able to verify similar relationship between nitrogen retained according to nitrogen absorbed in both species, which supports the hypothesis that goats use nitrogen more efficiently compared to sheep.

Several studies evaluating the nutritional requirement of energy and protein for goats and sheep kept in a tropical environment have been carried out, thus generating a lot of knowledge on the subject. It is already known that variables such as genetics, sex; region's climate and food quality can influence nutritional requirements. (Souza et al., 2007; Souza et al., 2017; Oliveira et al., 2018; Goetsch et al., 2011). We believe that our study may be beneficial in predicting the nutritional requirement of energy and protein for growing goats and sheep. We could observe that the NEm obtained in this research was 0.0683 Mcal/EBW$^{0.75}$/day for both species. At a comparative level, for goat species, this value is 30% lower, compared to the 0.0985Mcal/kg EBW$^{0.75}$/day, reported by Teixeira et al. 2017. Barcelos et al. 2020 obtained a value of 0.0704Mcal/kg EBW$^{0.75}$ / day in sheep fed a diet with the same standard as used in our study.

The NEm observed in our research, for the sheep species, is 3% lower than that observed by this author, being also close to that reported by Perreira et al. 2017, (0.0575 Mcal/kg EBW$^{0.75}$/day). The NRC 2007 reports that the Nem is around 0.062 Mcal / kg EBW$^{0.75}$ /day, so our result is consistent, being compared with this manual. The

maintenance metabolizable energy requirement was similar (0.115 Mcal/kg $EBW^{0.75}$/day) among species, a result that is very similar to those observed (0.0867; 0.0854 Mcal/kg $EBW^{0.75}$/day) by Souza et al. 2014 and Galvani et al. 2004, respectively for goats and sheep. Once again comparing our result with the one described by the NRC 2007, once again we pass consistency in our value suggestion. In this sense, we realize that goats and sheep have similar energy requirements for maintenance.

Considering that different equations were obtained for the species regarding the nutritional requirement of energy for gain. In a hypothesis of a goat and lamb both weighing 33 kg of BW and an expected ADG of 180 g/day, the estimated NEg would be 0.545 and 0.585 Mcal/day, respectively. Mendes et al. 2021 describe a hypothesis of lamb with 30kg of body weight and GMD of 200 g / day, in this sense the estimated NEg is 0.736 Mcal / day, being 19% lower than the recommended estimate (0.91 Mcal / day) by the NRC 2007.

Thus, we see that our average NEg for the two species is approximately 25% lower than that observed by Mendes et al. 2021, and 38% lower than suggested by the NRC 2007. According to Garrett, 1980 the Kg, the composition of the weight gain and a can vary according to the composition of the diet, weight gain composition, genetic group, state physiological and environmental factors. In our study we observed different Kg rates, being 0.133 and 0.699 for goats and sheep respectively, in this sense we can understand that goats are less efficient in converting metabolizable energy into body weight gain.

CONCLUSION

In this study, goats and sheep are similar in terms of energy requirement for maintenance, however, they diverge in terms of energy requirement for body weight gain and the efficiency of converting metabolizable energy to weight gain.

REFERENCES

AFRC - AGRICULTURAL AND FOOD RESEARCH COUNCIL: **Energy and protein requirements of ruminants**. **An advisory manual prepared by the AFRC Technical Committee on Response to Nutrients**. Wallingford, UK: CAB International, 1995. 159p.

AFRC. **Agricultural and Food Research Council: Energy and protein requirements of ruminants**. Wallingford: CAB International, 1993, 159 p.

AFRC. **Agricultural and Food Research Council: Energy and protein requirements of ruminants**. Wallingford: CAB International, 1993, 159 p.

ALAM, M. R., POPPI, D. P., & SYKES, A. R. Comparative intake of digestible organic matter and water by sheep and goats. In: **Proceedings of the New Zealand Society of Animal Production**. 1985. p. 107-111.

AOAC - Association of Official Analytical Chemists 2. **Official methods of analysis of the association of analytical chemists international**. A Official methods, Gathersburg, MD, 2005.

AOAC. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY. **Official methods of analysis**. 16.ed. Arlington: AOAC International, 1995. 1025p.

ARSLAN**,** C**.** Feeding Behaviors Of Sheep And Goats. **J. Fac. Vet. Med. Istanbul Univ,** v33, n3, 77-88, 2007.

ASKAR, A. R., NASSAR, M. S., BADAWY, H. S., EID, E. Y., GUADA, J. A., & FARID, M. F. A. Recovered energy and efficiency of digestion in sheep and goats fed Atriplex nummularia compared to alfalfa hay. **Livestock Science**, v. 194, p. 1-6, 2016.

ASMARE, A., PUCHALA, R., TESFAI, K., DETWEILER, G. D., DAWSON, L. J., ASKAR, A. R., ... & GOETSCH, A. L. Effects of small ruminant type and restricted protein intake on metabolism. **Small Ruminant Research**, v. 98, n. 1-3, p. 111-114, 2011.

BARBOSA, M. M., DETMANN, E., ROCHA, G. C., FRANCO, M. D. O., & FILHO, S. D. C. V. Evaluation of laboratory procedures to quantify the neutral detergent fiber content in forage, concentrate, and ruminant feces. **Journal of AOAC International**, v. 98, n. 4, p. 883-889, 2015.

BARCELOS, S., ALVES, K. S., MEZZOMO, R., LUZ, J. B., CUTRIM, D. O., GOMES, D. I., ... & GIONBELLI, M. P. Energy and protein requirements of woolless sheep under tropical conditions. **Livestock Science**, v. 231, p. 103856, 2020.

BRAND, T. S., VAN DER MERWE, D. A., SWART, E., & HOFFMAN, L. C. The effect of finishing period and dietary energy content on the carcass characteristics of Boer goats. **Small Ruminant Research**, v. 174, p. 110-117, 2019.

CAMERON, N. D.; BRACKEN, J. Selection for carcass lean content in a terminal sire breed of sheep. **Animal Science**, v. 54, n. 3, p. 367-377, 1992.

CANDYRINE, S. C. L., JAHROMI, M. F., EBRAHIMI, M., CHEN, W. L., REZAEI, S., GOH, Y. M., ... & LIANG, J. B. Oil supplementation improved growth and diet digestibility in goats and sheep fed fattening diet. **Asian-Australasian journal of animal sciences**, v. 32, n. 4, p. 533, 2019.

CHEN, X. B., & GOMES, M. J. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: an overview of the technical details.1992.

COSTA, R. G., LIMA, H. B., MEDEIROS, A. N., CRUZ, G. R., PEIXOTO, M. G., & SILVA, J. K. Net protein and energy requirements for weight gain of Santa Inês and Morada Nova sheep. **Livestock Science**, v. 214, p. 288-292, 2018.

DELLOW, D. W., & BARRY, T. N. The efficiency of chewing during eating and ruminating in goats and sheep. **British Journal of nutrition**, v. 65, n. 3, p. 355-363, 1991.

DETMANN, E., SOUZA, M. D., VALADARES FILHO, S. D. C., QUEIROZ, A. D., BERCHIELLI, T. T., SALIBA, E. D. O., ... & AZEVEDO, J. A. G. Métodos para análise de alimentos. **Visconde do Rio Branco: Suprema**, v. 214, 2012.

DEVENDRA, Canagasaby et al. Goat production in the tropics. **Goat production in the tropics.**, n. 19, 1970.

DOMINGUE, B. F., DELLOW, D. W., WILSON, P. R., & BARRY, T. N. Comparative digestion in deer, goats, and sheep. **New Zealand journal of agricultural research**, v. 34, n. 1, p. 45-53, 1991.

DOS SANTOS, A. C. S., SANTOS, S. A., CARVALHO, G. G. P., MARIZ, L. D. S., TOSTO, M., VALADARES FILHO, S. C., & AZEVEDO, J. A. G. A comparative study on the excretion of urinary metabolites in goats and sheep to evaluate spot sampling applied to protein nutrition trials. **Journal of animal science**, v. 96, n. 8, p. 3381-3397, 2018.

FANCHONE, A., NOZIERE, P., PORTELLI, J., DURIOT, B., LARGEAU, V., & DOREAU, M Effects of nitrogen underfeeding and energy source on nitrogen ruminal metabolism, digestion, and nitrogen partitioning in dairy cows. **Journal of Animal Science**, v. 91, n. 2, p. 895-906, 2013.

FERNANDES, M. H. M. R. et al. Predicting the chemical composition of the body and the carcass of 3/4Boer× 1/4Saanen kids using body components. **Small Ruminant Research**, v. 75, n. 1, p. 90-98, 2008.

FERREIRA, L. M. M., CELAYA, R., BENAVIDES, R., JÁUREGUI, B. M., GARCÍA, U., SANTOS, A. S., ... & OSORO, K. Foraging behaviour of domestic herbivore species grazing on heathlands associated with improved pasture areas. **Livestock Science**, v. 155, n. 2-3, p. 373-383, 2013.

GALVANI, D. B., PIRES, C. C., KOZLOSKI, G. V., & WOMMER, T. P. Energy requirements of Texel crossbred lambs. **Journal of Animal Science**, v. 86, n. 12, p. 3480-3490, 2008.

GAMA, K. V. M. F., PEREIRA FILHO, J. M., SOARES, R. F., CORDÃO, M. A., CÉZAR, M. F., BATISTA, A. S. M., BEZERRA, L. R. Fatty acid, chemical, and tissue composition of meat comparing Santa Ines breed sheep and Boer crossbreed goats submitted to different supplementation strategies. **Tropical animal health and production**, v. 52, n. 2, p. 601-610, 2020.

GARRY, B., MCGOVERN, F. M., KENNEDY, E., BAUMONT, R., BOLAND, T. M., WRIGHT, M. M.; LEWIS, E. Comparison of sheep and dairy cows for in vivo digestibility of perennial ryegrass. **Animal**, v. 15, n. 6, p. 100258, 2021.

GOETSCH, A. L., MERKEL, R. C., & GIPSON, T. A. Factors affecting goat meat production and quality. **Small Ruminant Research**, v. 101, n. 1-3, p. 173-181, 2011.

HACKMANN, T. J., & FIRKINS, J. L. Maximizing efficiency of rumen microbial protein production. **Frontiers in microbiology**, v. 6, p. 465, 2015.

HARRIS, Lorin E. et al. Nutrition research techniques for domestic and wild animals. 1970.

LAZZARINI, Í., DETMANN, E., DE CAMPOS VALADARES FILHO, S., PAULINO, M. F., BATISTA, E. D., DE ALMEIDA RUFINO, L. M., ... & DE OLIVEIRA FRANCO, M. Nutritional performance of cattle grazing during rainy season with nitrogen and starch supplementation. **Asian-Australasian Journal of Animal Sciences**, v. 29, n. 8, p. 1120, 2016.

LICITRA, G.; HERNANDEZ, T. M.; VAN SOEST, P. J. Standardization of procedures for nitrogen fractionation of ruminant feeds. **Animal feed science and technology**, v. 57, n. 4, p. 347-358, 1996.

LUNESU, M. F., DECANDIA, M., MOLLE, G., ATZORI, A. S., BOMBOI, G. C., & CANNAS, A. Dietary Starch Concentration Affects Dairy Sheep and Goat Performances Differently during Mid-Lactation. **Animals**, v. 11, n. 5, p. 1222, 2021.

MAHGOUB, O., & LODGE, G. A. A comparative study on growth, body composition and carcass tissue distribution in Omani sheep and goats. **The Journal of Agricultural Science**, v. 131, n. 3, p. 329-339, 1998.

MARTINS, A. A., HÄRTER, C. J., VENTURINI, R. S., MOTTA, J. H., TEIXEIRA, W. S., MACARI, S., ... & PIRES, C. C. Energy and protein requirements for maintenance of Texel lambs. **animal**, v. 13, n. 9, p. 1865-1873, 2019.

MENDES, M. S., SOUZA, J. G., HERBSTER, C. J. L., BRITO NETO, A. S., SILVA, L. P., RODRIGUES, J. P. P., PEREIRA, E. S. Maintenance and Growth Requirements in Male Dorper× Santa Ines Lambs. **Frontiers in Veterinary Science**, v. 8, p. 574, 2021.

MERTENS, D. R., & COLLABORATORS: ALLEN M CARMANY J CLEGG J DAVIDOWICZ A DROUCHES M FRANK K GAMBIN D GARKIE M GILDEMEISTER B JEFFRESS D JEON CS JONES D KAPLAN D KIM GN KOBATA S MAIN D MOUA X PAUL B ROBERTSON J TAYSOM D THIEX N WILLIAMS J WOLF M. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. **Journal of AOAC international**, v. 85, n. 6, p. 1217-1240, 2002.

NAIR, M. R., SEJIAN, V., SILPA, M. V., FONSÊCA, V. F. C., DE MELO COSTA, C. C., DEVARAJ, C., ... & BHATTA, R. Goat as the ideal climate-resilient animal model in tropical environment: revisiting advantages over other livestock species. **International Journal of Biometeorology**, v. 65, n. 12, p. 2229-2240, 2021.

NASRULLAH, M., JAVED, K., BHATTI, J. A., KHOSA, A. N., MARGHAZANI, I. B., SALES, J., ... & AHMAD, N. Nutrient intake and digestibility of various winter fodders fed to beetal goats and lohi sheep. **J. Anim. Plant Sci**, v. 25, p. 1206-1209, 2015.

NOCEK, J. E. In situ and other methods to estimate ruminal protein and energy digestibility: a review. **Journal of Dairy Science**, v. 71, n. 8, p. 2051-2069, 1988.

NRC. **Nutrient requirements of domestic animals:** Nutrient requirements of sheep. 6. ed. Washington: National Academy Press, 99 p, 1985.

OLIVEIRA, A. P., PEREIRA, E. S., BIFFANI, S., MEDEIROS, A. N., SILVA, A. M. A., OLIVEIRA, R. L., & MARCONDES, M. I. Meta-analysis of the energy and protein requirements of hair sheep raised in the tropical region of Brazil. **Journal of animal physiology and animal nutrition**, v. 102, n. 1, p. e52-e60, 2018.

PEREIRA, E. S., LIMA, F. W. R., MARCONDES, M. I., RODRIGUES, J. P. P., CAMPOS, A. C. N., SILVA, L. P.; OLIVEIRA, R. L. Energy and protein requirements of Santa Ines lambs, a breed of hair sheep. **Animal**, v. 11, n. 12, p. 2165-2174, 2017.

SANZ SAMPELAYO, M.R.; MUNOZ, F.J.; LARA, L. et al. Factors affecting pre-and post weaning growth and body composition in kid goats of the granadina breed. **Animal Production**, v.45, p.233-238, 1987.

SARO, C., MATEO, J., CARO, I., CARBALLO, D. E., FERNÁNDEZ, M., VALDÉS, C., ... & GIRÁLDEZ, F. J. Effect of Dietary Crude Protein on Animal Performance, Blood Biochemistry Profile, Ruminal Fermentation Parameters and Carcass and Meat Quality of Heavy Fattening Assaf Lambs. **Animals**, v. 10, n. 11, p. 2177, 2020.

SCHUBA, J., SÜDEKUM, K. H., PFEFFER, E., & JAYANEGARA, A. Excretion of faecal, urinary urea and urinary non-urea nitrogen by four ruminant species as influenced by dietary nitrogen intake: A meta-analysis. **Livestock Science**, v. 198, p. 82-88, 2017.

SEN, A. R., SANTRA, A., KARIM, S. A. Carcass yield, composition and meat quality attributes of sheep and goat under semiarid conditions. **Meat science**, v. 66, n. 4, p. 757-763, 2004.

SHIJA, D. S., MTENGA, L. A., KIMAMBO, A. E., LASWAI, G. H., MUSHI, D. E., MGHENI, D. M., SAFARI, J. G. Chemical composition and meat quality attributes of indigenous sheep and goats from traditional production system in Tanzania. **Asian-Australasian journal of animal sciences**, v. 26, n. 2, p. 295, 2013.

SILVA, A. M. A., SOBRINHO, A. S., TRINDADE, I. A. C. M., RESENDE, K. T. D., & BAKKE, O. Net and metabolizable protein requirements for body weight gain in hair and wool lambs. **Small Ruminant Research**, v. 67, n. 2-3, p. 192-198, 2007.

SOTO-NAVARRO, S. A., LOPEZ, R., SANKEY, C., CAPITAN, B. M., HOLLAND, B. P., BALSTAD, L. A., & KREHBIEL, C. R. Comparative digestibility by cattle versus sheep: effect of forage quality. **Journal of animal science**, v. 92, n. 4, p. 1621-1629, 2014.

SOUZA, A. P., MEDEIROS, A. N., CARVALHO, F. F. R., COSTA, R. G., RIBEIRO, L. P. S., BEZERRA, A. B., ... & SILVA JR, C. G. Energy requirements for maintenance and growth of Canindé goat kids. **Small Ruminant Research**, v. 121, n. 2-3, p. 255-261, 2014.

SOUZA, A. P., MEDEIROS, A. N., CARVALHO, F. F. R., COSTA, R. G., RIBEIRO, L. P. S., BEZERRA, A. B., ... & SILVA JR, C. G. Energy requirements for maintenance and growth of Canindé goat kids. **Small Ruminant Research**, v. 121, n. 2-3, p. 255-261, 2014.

SOUZA, A. P., ST-PIERRE, N. R., FERNANDES, M. H. M. R., ALMEIDA, A. K., VARGAS, J. A. C., RESENDE, K. T., & TEIXEIRA, I. A. M. A. Energy requirements and efficiency of energy utilization in growing dairy goats of different sexes. **Journal of dairy science**, v. 103, n. 1, p. 272-281, 2020.

SOUZA, A. P., ST-PIERRE, N. R., FERNANDES, M. H. R. M., ALMEIDA, A. K., VARGAS, J. A. C., RESENDE, K. T., & TEIXEIRA, I. A. M. A. Sex effects on net protein and energy requirements for growth of Saanen goats. **Journal of Dairy Science**, v. 100, n. 6, p. 4574-4586, 2017.

TESK, C. R. M., PEDREIRA, B. C., PEREIRA, D. H., PINA, D. D. S., RAMOS, T. A., & MOMBACH, M. A. Impact of grazing management on forage qualitative characteristics: a review. **Embrapa Agrossilvipastoril-Artigo em periódico indexado (ALICE)**, 2018.

VALENTE, T. N. P., DETMANN, E., & SAMPAIO, C. B. Recent advances in evaluation of bags made from different textiles used in situ ruminal degradation. **Canadian Journal of Animal Science**, v. 95, n. 4, p. 493-498, 2015.

VAN SOEST, Peter J. **Nutritional ecology of the ruminant**. Cornell university press, 1994.

Table 2 - Effect of species on nutrient intake and apparent digestibility of nutrients.

Item[1]	Species		SEM[2]	*P value*
	Goat	Sheep		Species
	Intake (kg/d)			
Dry matter	0,84	1,30	0,05	< 0,01
Organic matter	0,79	1,22	0,04	< 0,01
Crude protein	0,15	0,22	0,01	< 0,01
Ether extract	0,03	0,05	0,01	< 0,01
Neutral detergent fiber ap[1]	0,21	0,35	0,01	< 0,01
Non-fibrous carbohydrates	0,37	0,57	0,02	< 0,01
Total digestible nutrients	0,64	0,93	0,03	< 0,01
	Intake (g/kg de BW)			
Dry matter	24,05	32,50	0,97	< 0,01
Neutral detergent fiber ap[1]	5,95	8,71	0,28	< 0,01
	Digestibility (g/kg)			
Dry matter	714	711	3,74	0,61
Organic matter	730	722	3,65	0,15
Crude protein	718	713	4,29	0,37
Ether extract	828	829	4,90	0,87
Neutral detergent fiber ap[1]	465	479	6,95	0,16
Non-fibrous carbohydrates	856	854	4,30	0,75
Total digestible nutrients	729	720	3,74	0,12

[1]Neutral detergent fiber ap = Neutral detergent fiber corrected for ash and protein; [2]SEM = Standard error of the mean

Table 3 - Species effect on carcass performance and tissue composition.

Item[1]	Species		SEM[2]	*P value*
	Goat	Sheep		Specie
Performance (kg)				
Final BW	38,73	45,53	1,10	<0.01
Weight total gain	16.19	22.22	0.93	<0.01
ADG	0.170	0.234	0.01	<0.01
HCW	17.33	20.56	0.44	<0.01
CCW	17.21	20.46	0.43	<0.01
Weight of tissue (kg)				
Muscle	9.89	10.95	0.35	0.04
Fat	2.31	4.03	0.14	<0.01
Bone	4.18	4.50	0.13	0.08
Tissue ratio				
Muscle:bone	2.35	2.46	0.08	0.33
Fat:bone	0.56	0.88	0.03	<0.01
Fat:muscle	1.12	2.15	1.11	0.52
Weight of tissue (% CCW)				
Muscle	57.00	53.74	0.93	0.02
Fat	13.58	19.41	0.66	<0.01
Bone	24.70	22.19	0.60	<0.01

[1]Final BW = Final body weight; ADG = Average daily gain; HCW = Hot casting weight; CCW = Cold carcass weight. [2]SEM = Standard error of the mean

Table 4 - Efficiency of gains in relation to intake

Item[1]	Species		SEM	P *value*
	Goat	Sheep		Species
Dry matter	ADG/intake (kg)			
	0.211	0.174	0.13	0.06
	Gain of Carcass/intake (kg)			
	0.23	0.16	0.01	<0.01
	Gain of muscle/intake (kg)			
	0.13	0.08	0.01	<0.01
	Gain of fat/intake (kg)			
	0.03	0.03	0.01	0.84
OMd	Gain of fat/intake (kg)			
	0.05	0.05	0.01	0.88
Crude protein	Gain of muscle/intake (kg)			
	0.75	0.50	0.05	<0.01

[1]OMd = digestible organic matter

Table 5 - Effect of species on nitrogen balance and retention

Item	Specie		SEM[1]	P - *value*
	Goat	Sheep		Specie
Intake nitrogen (g/day)	24.08	35.42	1,24	<0,01
Excreted Nitrogen (g/day)				
Feces	6.86	10.08	0.34	<0.01
Urine	7.60	9.30	0.42	<0.01
Endogenous Nitrogen (g/day)	5.35	5.86	0.08	<0.01
Nitrogen Balance (g/day)	9.98	16.00	0.67	<0.01
Retained Nitrogen (g/day)	4.66	10.58	0.54	<0.01
% of ingested	18.45	26.60	1.47	<0.01
% of absorbed	25.59	40.02	1.65	<0.01

[1]SEM = Standard error of the mean

Table 6- Effect of species on excretion of purine derivatives and microbial efficiency.

Item[1]	Specie		SEM[2]	P *value*
	Goat	Sheep		Specie
Urinary excretions (mmol/day)				
Allantoin	6.17	8.39	0.47	<0.01
Uric acid	0.83	1.30	0.11	<0.01
Xanthine + hypoxanthine	2.00	2.31	0.12	0.09
Absorbed purines (mmol / day)	10.42	14.15	0.67	<0.01
Purine derivatives (% TP)				
Allantoin	68.08	68.73	1.48	0.77
Uric acid	8.89	11.16	0.76	0.05
Xanthine + hypoxanthine	23.57	20.04	1.26	0,06
Microbial synthesis (g/day)				
Microbial N	7.42	10.30	0.49	<0.01
Microbial PB	46.37	64.38	3.04	<0.01
Microbial efficiency (g / kg)				
CPM/OM digestible[1]	81.83	74.26	4.32	0.23
CPM/TDN[1]	77.12	70.14	4.07	0.24

[1]Microbial crude protein / digestible organic material intake; [1]Microbial crude protein / total digestible nutrient intake; [2]SEM = standard error of the mean

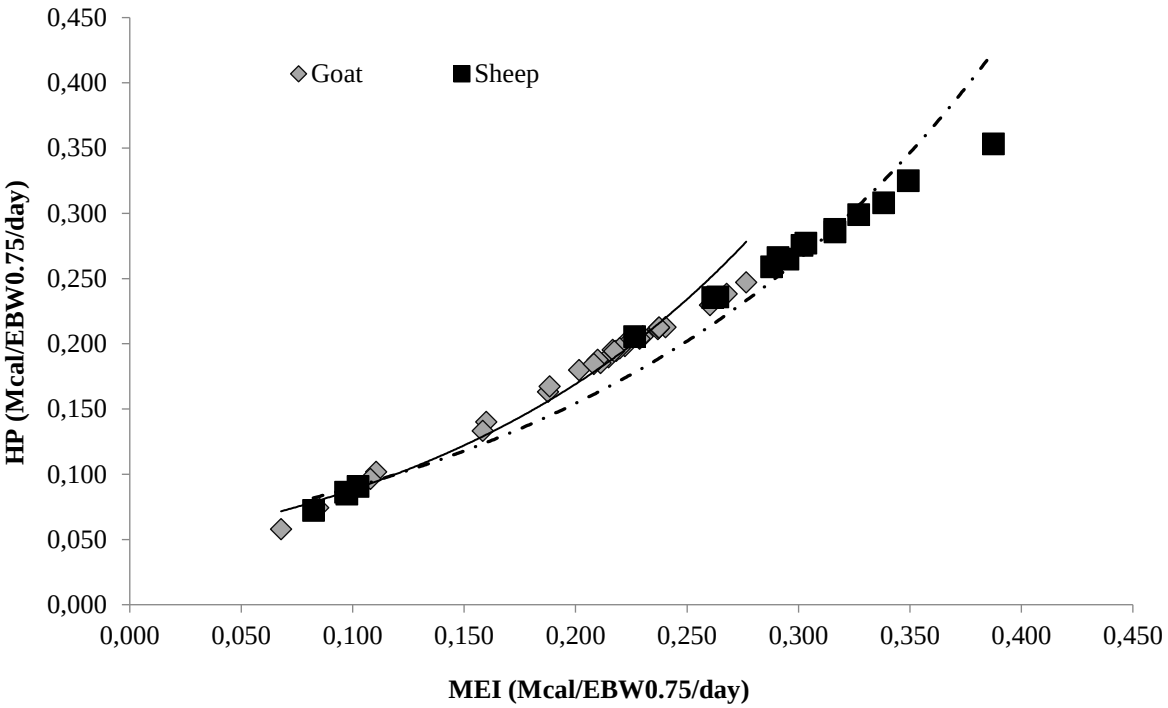

Figure 1 – Relationship between heat production (Mcal/EBW$^{0.75}$/day) and metabolizable energy intake (Mcal/EBW$^{0.75}$/day), which gave rise to equation to estimate net energy for maintenance (NEm) in sheep and goats. Exponential regression equation fitted for both species: $HP = 4.5268_{(\pm 0.2755)} \times e^{(0.0683\ (\pm 0.0054)\ \text{x MEI})}$

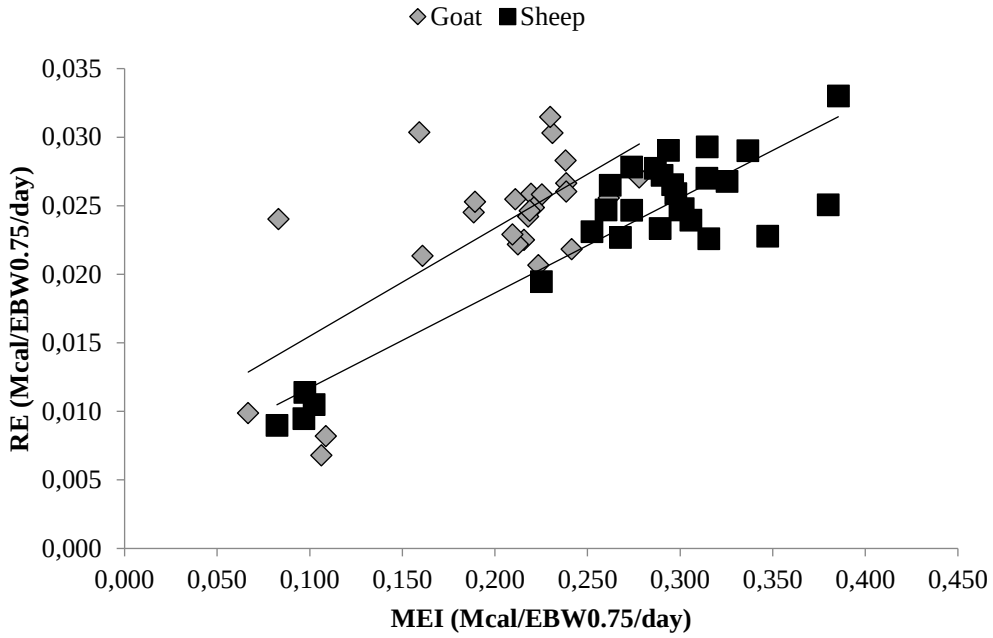

Figure 2 – Relationship between retained energy (Mcal/EBW$^{0.75}$/day) and metabolizable energy intake (Mcal/EBW$^{0.75}$/day), which gave rise to equation to estimate metabolizable energy for gain (MEg) in sheep and goats. Linear regression equations fitted were: RE = $0.00705_{(\pm 0.00162)}$ + MEI × $0.11302_{(\pm 0.00943)}$ for goats and RE = $0.00705_{(\pm 0.00162)}$ + MEI × $0.06998_{(\pm 0.01131)}$, for sheep.

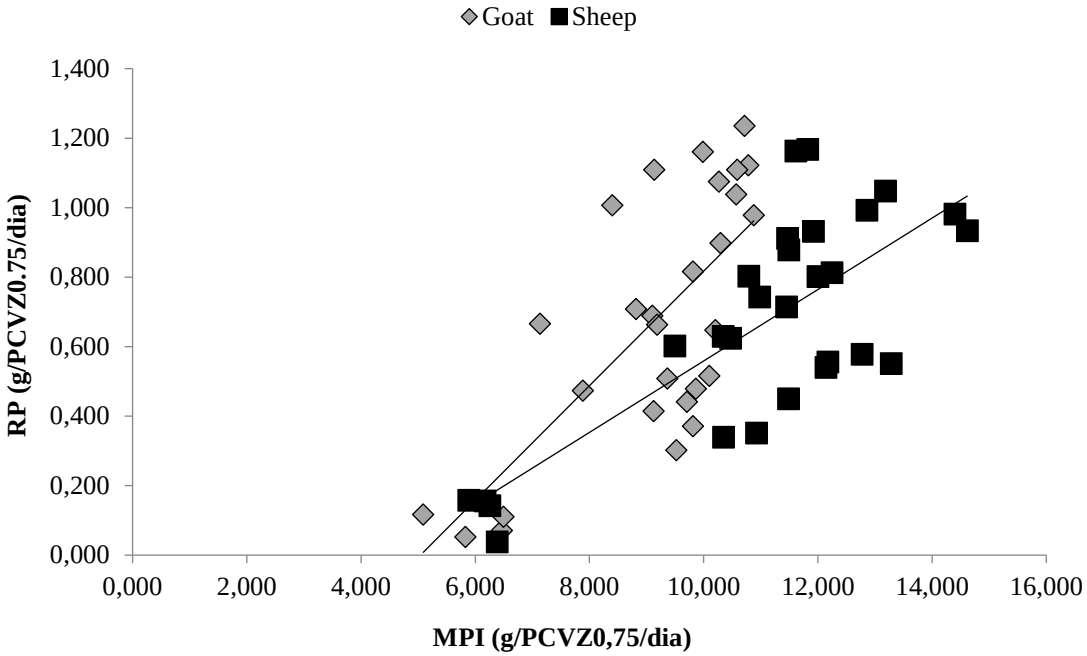

Figure 3 – Relationship between retained protein (g/EBW$^{0.75}$/day) and metabolizable protein intake (g/EBW$^{0.75}$/day), which gave rise to equation to estimate net protein for maintenance (NPm) in sheep and goats. A single linear regression equation was fitted: RP = MPI × 0.09794 $_{(\pm 0.01604)}$ - 0.31871 $_{(\pm 0.1653)}$, for both species.

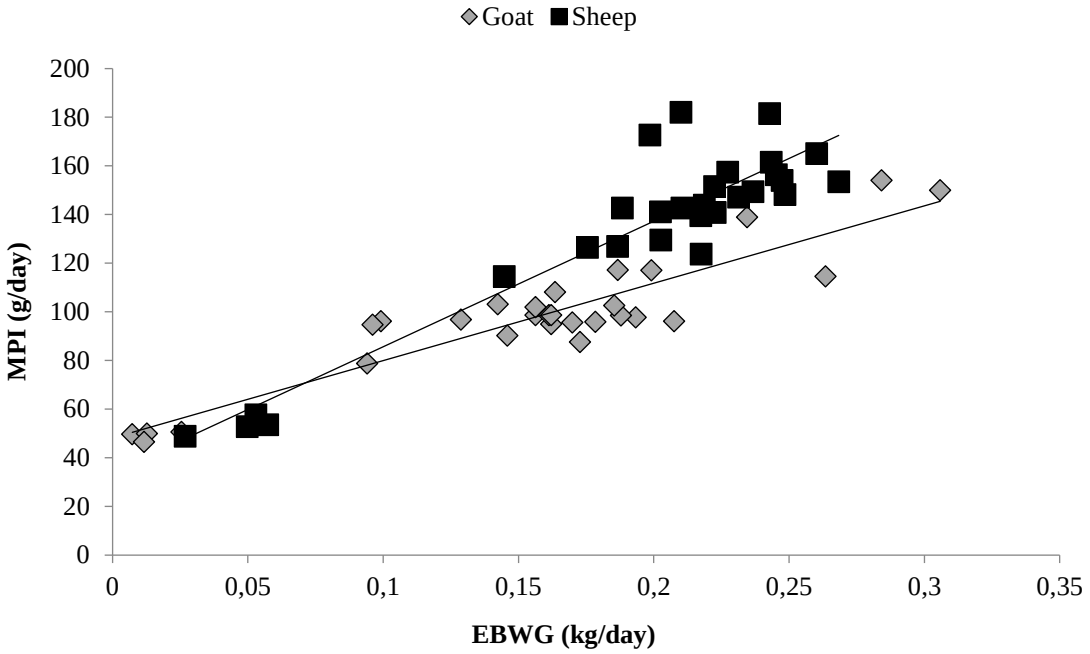

Figure 4 – Relationship between empty body weight gain (g/day) and metabolizable protein intake (g/ day), which gave rise to two equations to estimate metabolizable protein for maintenance (MPm): MPI = 38.28 $_{(\pm 5.9529)}$ + 318.07 $_{(\pm 30.788)}$ × EBWG for goat, and MPI = 38.28 $_{(\pm 5.9529)}$ + 516.06 $_{(\pm 46.723)}$ × EBWG for sheep.

CHAPTER TWO: INVESTIGATING AMOUNT OF BAGS AND ITS FABRIC WOOF USED IN RUMEN INCUBATION PROCEDURES APPLIED TO *IN SITU* ESTIMATION OF INDF FOR SHEEP

ABSTRACT:

The type of textile and the amount of bags incubated simultaneously in the rumen may lead to different indigestible neutral detergent fiber (iNDF) estimates in sheep. This study aimed to evaluate the effect of these variables in *in situ* incubations carried out in sheep. Six ruminally-cannulated non-castrated Santa Ines sheep, aged between 9 to 15 months and an average initial body weight (BW) of 46 ± 3 kg, were used. This research was carried out using a 6 × 6 Latin square experimental design. Two types of textiles were used for the rumen incubations: Ankon F57 and non-woven textile (NWT). Three samples commonly used in ruminant incubations to estimate iNDF was used as feed control: sorghum silage, ground corn and feces from sheep. Each type of bags was incubated in a total number of 4; 8 or 12 units per sample. Thus, a total of 144 bags were in situ incubated per period. At the end of each incubation period, the bags were removed from rumen, washed, oven dried and directed for iNDF analysis. It is observed that the textile types and the amount that were incubated did not promoted different estimates ($P>0.05$) in the iNDF concentration. According to our result, F57 fabric and the NWT can be incubated in the rumen of sheep, when it is desired to estimate the iNDF of feeds or feces.

Keywords: degradation, F57, non-woven textile, ovine, ruminant

INTRODUCTION

Internal markers are natural components of feed and are associated with diet, which are neither digested or absorbed by animals. The main internal markers used in sheep nutrition trials are the indigestible neutral detergent fiber (iNDF) and the indigestible acid detergent fiber (iADF) (Carvalho et al., 2013; Santos et al., 2018; Assis et al., 2018 and Lopes et al., 2020). The iNDF and iADF can be measured by *in situ* incubations. The results of the *in situ* technique could be varied (Oskov et al., 1980; Van Der Koelen et al., 1992; Casali et al., 2008; Casali et al., 2009). Technical aspects such as bag porosity, sample volume, sample size, washing procedure after incubation; total incubation time, bag size and the animal's basal diet can influence the results (Lindberg, 1985; Nocek, 1988; Madsen & Hvelplund, 1994; Huntington & Givens, 1995; Vazant et al., 1998). Then, a significant number of standardizations have been proposed over the years (Vanzant et al., 1988; Wang et al., 2021), to reduce the variation between studies.

The implementation of Ankom® bags made of F57 fabric for the analysis of fiber content and indigestible compounds in vitro and in situ is very usable (Coblentz et al., 2019; Defeo et al., 2020; Tassone et al., 2020). However, a factor that goes against the popularization of the F57 bag is the inherent cost of using this technology, preventing its massive use in feed analysis. Alternatives were proposed to replace the F57 fabric, so bags made from non-woven textile (NWT) were considered. These bags are made from a synthetic polymer called polypropylene (Casali et al., 2009 and Valente et al. 2011).

In this context, there are few studies that performed in situ incubation to estimate iNDF in sheep. Reis et al. (2017) evaluated *in situ* incubation in cattle and sheep. The authors showed that iNDF and iADF evaluated in NWT, incubated in cattle, were estimated in fewer hours when compared to sheep. Moreover, it must be emphasized that both species received the same amount of bags incubated in each rumen. Then it is clear

that the validated methods made for cattle could not be adapted for small ruminants, mainly due to rumen volume, which could impact in digestion rate and time to reach the indigestible fraction.

Given the lack of a standardized methodology regarding the amount and type of bags recommended for in situ incubation in sheep, we aimed to investigate the effect of fabric woof types (F57 vs. NWT) and amount of bags incubated simultaneously in sheep, and their effect on indigestible residue (iNDF) of sorghum silage, ground corn and feces from sheep.

MATERIAL AND METHODS

Location and ethical standards

The experiment was carried out at the Experimental Farm of São Gonçalo dos Campos – BA, in the research unit for goats and sheep, belonging to the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (UFBA).

All procedures were performed with authorization and strict accordance with the Committee for Animal Use (CEUA) of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (Protocol number: 02/2019).

Animals and dietary handling

Six ruminally-cannulated non-castrated Santa Ines sheep, aged between 9 to 15 months and an average initial body weight (BW) of 46 ± 3 kg, were used. The animals were housed in covered shed individual 1.0 m² pens (1.0 × 1.0m) with suspended and slatted wood floors, equipped with drinking fountains and feeders. Water supply and experimental diets were supplied ad libitum.

During incubations, ewes were treated in separate pens. The animals were fed diets composed of sorghum silage (*Sorghum bicolor* (L.) Moench) as the forage source, chopped into particles of approximately 5-cm in length and composed 80% of the diet. The concentrate used in the current study was comprised of ground corn, soybean meal, corn germ, urea, ammonium sulfate and mineral mixture, on dry matter (DM) basis (Table 1). According to the weight of the animals, a basal diet was formulated with approximately 12 g/kg crude protein according to the recommendations of the National Research Council (NRC, 2007), for animals in maintenance.

Basal control diet consisted of 355 g/kg of ground corn, 450 g/kg of soybean meal, 135 g/kg of corn germ, 10 g/kg of urea with ammonium sulfate and 50 g kg of mineral mixture 175 g/kg of sodium, 140 g/kg of calcium, 60 g/kg of phosphorus, 13 g/kg of sulfur, 3 g/kg of zinc, 1.5 g/kg of iron, 2 g/kg of manganese, 0.05 g/kg of molybdenum, 0.06 g/kg of iodine, 0.02 g/kg of cobalt, and 0.01 g/kg of selenium.

Diets were offered daily twice a day (8:30 and 15:30 h), and divided equally into two meals, to allow between 10 and 15% of refusals. Daily, before offering the morning diet, the refusals were collected and weighed to determine DM intake and feed adjustment.

Feed samples preparation

Samples of 2 types of feed (sorghum silage and ground corn grain) and 1 samples of feces from fed with 50:50 forage concentrate ratio, were used (Table 2). The sorghum silage and feces samples from sheep fed standard diet were dried in a forced-air oven (55°C for 72 hours), ground in Wiley cutting mill (Willey TE-650/1, TECNAL, São Paulo, Brazil) equipped with a 2-mm mesh sieve for in situ incubation (Valente et al., 2015; Reis et al., 2017) and at 1-mm for subsequent laboratory analysis.

Experimental design

Two types of textiles were used for the rumen incubations: Ankon F57 and non-woven textile (NWT) with the three samples described above. Each type of bags was incubated in a total number of 4; 8 or 12 units per sample. For the incubation procedure, a 6 x 6 Latin square design was used. The experimental period lasted 94 days, with 10 days being designated to adaptation to diet, daily handling and facilities, and the remaining 84 days being used to perform the experimental periods, which lasted 14 days each. In each of the six periods, a round of incubation was performed on the animals (Table 3), using 336 hours (14 days) as limit time to estimate the iNDF of the samples (Reis et al., 2017). The type of tissue (F57 vs NWT) and the amount of bags incubated in situ per period were evaluated, corresponding to sorghum silage, ground corn and sheep feces samples (Table 3). Thus, a total of 144 bags incubated in situ per period were used. At the end of the test, a total of 864 bags were used, with 144 bags x 6 periods.

Incubation procedure

All samples were stored in the two types of tissue (F57 and NWT), following the respective number of repetitions for each type of sample (4, 8 and 12). The F57 bags were purchased directly from the manufacturer, (Ankom®). The bags made using NWT with density reference 100 g/m2, (Casali et al., 2009), were cut with a dimension of 4 x 5 centimeters (cm), and filled with a proportion of 20 mg of sample per cm^2 (Nocek, 1988).

Due to the presence of small particles adhered to the NWT bags, they were washed before receiving the samples in a solution containing neutral detergent (Mertens, 2002), at 100°C for 15 minutes, then rinsed in hot water. Later, to remove any residue associated with water, the bags were immersed in acetone and finally in distilled water (Detmann et al., 2012). After washing, the bags were dried in a forced ventilation oven at 55°C for 72 hours. Then, all the bags were oven-dried at 105°C for 2 hours (method 934.01, AOAC,

2000). After obtaining the weight of the bags, the sample mass was added to suit the proportion of 20 mg/cm^2 of surface (Nocek, 1988), after which all the bags were thermally sealed.

Bags were tied together with a metal chain with a weight at its end, thus allowing the total immersion of the bags with samples in the ruminal content. The incubation time used to estimate the iNDF of the samples was 336 hours (Reis et al., 2017). After removing the rumen bags, they were washed in running water, until the water was completely cleared. After cleaning, all bags were transferred to a ventilation oven with 55°C, where they were kept for 72 hours. Then, the bags were packed in polyethylene bags and grouped in plastic boxes, for further fiber analysis.

Chemical analyses

For the analysis of chemical composition, the total diet and the incubated samples of sorghum silage, ground corn and feces, were pre-dried in a ventilation oven at 55°C for 72 hours. All samples were ground in a knife mill (Willey TE-650/1, TECNAL, São Paulo, Brazil), with 2 mm sieves (Valente et al., 2015) for incubations and 1 mm for chemical analysis.

The feed and fecal samples were analyzed for dry matter (DM) and ash according to the official method 934.01, and 942.05, at 600°C for 4 hours (AOAC, 2005), respectively. Organic matter (OM) was quantified by subtracting 100 from the percentage of ash quantified in the sample. For the analysis of neutral detergent fiber (NDF), the samples were initially placed in 100 ml self-cleaning bottles, following a ratio of 1g per 100 ml of neutral detergent solution (Mertens, 2002), using of thermostable α-amylase (Ankom Technology, Tecnoglobo Equipamentos, Curitiba, Brazil), without the addition of sodium sulfite, autoclaved at 110°C for 60 minutes at 4.3-5.7 psi (Barbosa et al., 2015).

The neutral detergent fiber corrected for ash and protein was obtained after correction of the residues for the ash content (Mertens, 2002) and residual N (Licitra et al. 1996). The content of ether extract (EE) was quantified by gravimetry, after extraction with petroleum ether according to method 920.39 (AOAC, 2005). Total Nitrogen (N) was quantified through three-step analysis of the micro Kjeldhal procedure with digestion with sulfuric acid, basic distillation and acid titration, by the official method 968.06 (AOAC, 1995). The N value was multiplied by 6.25 to obtain the crude protein content (CP). The lignin was measured according to the official method 973.18 (AOAC, 2005) using sulfuric acid with a concentration of 72%. The content of non-fibrous carbohydrates (CNF) was calculated according to Detmann e Valadares Filho (2010): NFC = 100 - [(%CP - CP% of urea + % of urea) +% apNDF + %EE + %ASH], where NDFcp is NDF corrected for ash and protein.

The remained bags after the incubations were analyzed for the quantification of iNDF, immersed in a neutral detergent solution (Mertens, 2002), without the use of thermostable α-amylase and without the addition of sodium sulfite. Then they were directed to the fiber analyzer (Ankom, 200 Fiber Analizer, Ankom Technology). Subsequently, the bags were dried in a forced ventilation oven (55°C for 72 h), and then oven-dried at 105°C for 2 h. Afterward, they were stored in a desiccator in a proportion of (20 bags/desiccator) and weighed. The quantification of the indigestible fraction (iNDF) of each sample inserted in the bags was estimated by difference between the dry bag filled, after inclusion of the samples before the analytical procedure, and dry bag weight after the analytical procedure.

Statistical analyses

The experimental design was 6 x 6 Latin square design, and statistical procedures were undertaken using the SAS software (version 9.4). The obtained data were analyzed by the PROC MIXED procedure (Statistical Analysis System - SAS Institute Inc., Cary, NC, USA) using the following statistical model:

$$Y_{ijkl} = \mu + N_i + T_j + (NT)_{ij} + a_k + p_l + e_{ijk}$$

Where: μ = overall mean; N_i = fixed effect of the amount of bags *i*; T_j = fixed effect of textile type *j;* (NT) *ij* = fixed effect of the interaction between amount and type of tissue, a_k = random effect of animal *k*; p_l = random effect of experimental period *l*; e_{ijk} = random error between experimental plots. The effects of the treatments were compared through analysis of variance adopting a significance level of 5%.

RESULTS AND DISCUSSION

It is observed that the textile types did not promote different estimates (P>0.05) in the iNDF concentration (Table 4). In the current study, it was observed that the F57 tissue and the NWT can be incubated in the rumen of animals of the sheep species, when it is desired to estimate the iNDF of foods and samples commonly used in digestibility tests, without divergences in the final result of the estimates. Similar results were obtained when iNDF data were evaluated by samples. The iNDF in Sorghum silage, ground corn and feces (P>0.05) from sheep were similar when incubated in Ankon or TNT bags, as well as 12 24 or 36 bags inside rumen (P>0.05). Standard deviations for sorghum silage and ground corn obtained from 36 Ankon bags incubated were the smallest estimate (Figure 1). Feces from sheep presented the smallest standard deviation when 24 bags were also incubated in Ankon bag (Figure 1).

The result of the current study is in agreement with Valente et al. (2011), who reported that F57 can be replaced by NWT in an *in-situ* procedure for iNDF analysis. According to the authors this result is based on the premise that the tissues evaluated promote a similar estimate of the iNDF of foods.

Casali et al. (2009) evaluated the use of these types of tissues in cattle and observed statistical results similar to those found in our study, for corn silage and ingredients used in the composition of concentrated feeds (wheat bran and soybean husks), with the result of similarity among the estimates, also evidenced by Valente et al. (2011b), which also in this context in animals of the bovine species. It is noteworthy that studies such as the one developed here are scarce in sheep, which would require further studies and interpretations.

It is widely commented that the pore size and texture of the bags used for *in situ* incubations can influence the efflux of the material and their interaction with the rumen content (Kitessa et al., 1999). For Valente et al. (2011) the structural surfaces of NWT and F57 are similar, where the two types of fabrics present an unequal dimension of their textile fibers, resulting in similar porosities. Thus, this author states that these types of tissue promote the influx of microorganisms without loss of material, increasing degradation without draining the sample from the bag (Nozière & Michalet-Doreau, 2000).

Chen et al. (2011) reported that there are few studies comparing the density of bags simultaneously incubated in the rumen. Valente et al. 2011 reported that in long periods of in situ incubation, the bags can resist, leading to sample losses and consequently erroneous estimates of iNDF. Thinking in this sense, we could expect that the increase in the amount of bags incubated simultaneously in the rumen of these animals could influence the estimates of iNDF of the samples.

It was observed in the present study that the amount of bags inserted in the rumen of sheep simultaneously did not cause a difference (P>0.05) in the estimation of iNDF (Figure 1). Reis et al. (2017) stated that variations between results may be related to differences inherent in the particle size of the food and the bags used in the incubation procedure. This reality was not found in this research, considering that there was standardization of the grinding of the samples (2 mm), the samples were inserted into the bags obeying a density of 20mg/cm^2, the NWT bags were made in a standardized way and carefully selected for introduction into the incubation march.

In theory, it was expected that animals that underwent *in situ* incubations with the highest amount of bags would generate overestimated estimates for iNDF. This effect is related to the fact caused by the higher volume of bags present within the rumen, which in theory could impair the influx of fluids bags and, as a consequence, it does not favor the degradation of the samples by microorganisms from the ruminal site. However, in this study, the similarity of the iNDF estimates regarding the amount of bags incubated simultaneously in sheep may be associated with the length of time the bags remain in the rumen of the animals and the type of diet used. According to Rinne et al (1997) and Rinne et al. (2002) the longer the incubation period, the lower the iNDF estimate, until reaching the asymptote (Reis et al. 2017).

According to Meyer & Mackie (1986) the microenvironment inside the bags during *in situ* incubation may be different from that observed inside the rumen, thus reducing microbial activity. Krizsan & Huhtanen (2013) reported that diets with a high concentration of starch and a low proportion of forages influence the final concentration of iNDF in foods incubated in situ in ruminants.

However, Mertens (1993) already reported that there is little evidence that this is the only causal factor. Broadly, NWT and F57 tissues showed similar behavior in

analytical terms. However, due to the similarity of the results obtained when comparing NWT and F57, we can infer that in situ incubation in sheep using the NWT bag is more interesting due to its lower cost (US$ 1.75/m^2) compared to F57 ($256.00/box with 200 units).

CONCLUSION

The use of NWT and F57 tissues in *in situ* incubation procedures in sheep for determination of iNDF was similar. Taking into account the cost of each type of bag, it is clear that the NWT bag can be an option, without compromise the iNDF estimate. In this study, using sheep fed a control basal diet and low inclusion of non-fibrous carbohydrates, the volume of 36 bags incubated simultaneously in the rumen of these animals had no effect on the estimation of iNDF, regardless of the type of bag. If more accurate data is necessary, feces can be incubated up to 24 bags, but the incubation of 36 bags do not compromise the iNDF mean.

As there are few studies on *in situ* incubation procedures evaluating types and amount of bags, aiming at the determination of iNDF in feed used in digestibility tests in sheep, further evaluations are suggested regarding this topic.

REFERENCES

AOAC. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY. Official methods of analysis. 16.ed. Arlington: AOAC International, 2005.

AOAC. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY. **Official methods of analysis**. 16.ed. Arlington: AOAC International, 1995. 1025p.

BARBOSA, M. M., DETMANN, E., ROCHA, G. C., FRANCO, M. D. O., & FILHO, S. D. C. V. Evaluation of laboratory procedures to quantify the neutral detergent

fiber content in forage, concentrate, and ruminant feces. **Journal of AOAC International**, v. 98, n. 4, p. 883-889, 2015.

CASALI, A. O; DETMANN, E; VALADARES FILHO, S.C; HERIQUE, L.T; DE FREITAS, S.G. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos in situ. **Revista Brasileira de Zootecnia**, v. 37, p. 335-342, 2008

CASALI, A.O.; DETMANN, E.; VALADARES FILHO, S.C. PERREIRA, J.C.; CUNHA, M, DETMANN, K.S.C.; PAULINO, M.F. Estimação de teores de componentes fibrosos em alimentos para ruminantes em sacos de diferentes tecidos. **Revista Brasileira de Zootecnia**, v.38, p.130-138, 2009.

CHEN, Y., PENNER, G. B., LI, M., OBA, M., & GUAN, L. L. Changes in bacterial diversity associated with epithelial tissue in the beef cow rumen during the transition to a high-grain diet. **Applied and environmental microbiology**, v. 77, n. 16, p. 5770-5781, 2011.

COBLENTZ, W. K., AKINS, M. S., OGDEN, R. K., BAUMAN, L. M., & STAMMER, A. J. Effects of sample size on neutral detergent fiber digestibility of triticale forages using the Ankom DaisyII Incubator system. **Journal of dairy science**, v. 102, n. 8, p. 6987-6999, 2019.

DE CARVALHO, G. G. P., GARCIA, R., PIRES, A. J. V., SILVA, R. R., DETMANN, E., OLIVEIRA, R. L., & RIBEIRO. Long-term bias of internal markers in sheep and goat digestion trials. **Asian-Australasian Journal of Animal Sciences**, v. 26, n. 1, p. 65, 2013.

DE M. ASSIS, D. S., DE A. SILVA, A. M., DO NASCIMENTO JÚNIOR, N. G., BEZERRA, L. R., PEREIRA FILHO, J. M., OLIVEIRA, R. L., ... & DE MOURA, J. F. Dorper × Santa Ines F1 crossbred lambs under different grazing times and supplement

levels in tropical regions: performance and macromineral requirements. **Italian Journal of Animal Science**, v. 17, n. 4, p. 931-940, 2018.

DEFEO, M. E., SHAMPOE, K. V., CARVALHO, P. H., SILVA, F. A., & FELIX, T. L. In vitro and in situ techniques yield different estimates of ruminal disappearance of barley. **Translational Animal Science**, v. 4, n. 1, p. 141-148, 2020.

DETMANN, E.; VALADARES FILHO, S. C.; BERCHIELLI, T. T. **Métodos para analises de alimentos. Instituto Nacional de Ciência e Tecnologia de Ciência Animal**. Visconde do Rio Branco, MG: Suprema, 2012. 201p.

DOS SANTOS, A. C. S., SANTOS, S. A., CARVALHO, G. G. P., MARIZ, L. D. S., TOSTO, M., VALADARES FILHO, S. C., & AZEVEDO, J. A. G. A comparative study on the excretion of urinary metabolites in goats and sheep to evaluate spot sampling applied to protein nutrition trials. **Journal of animal science**, v. 96, n. 8, p. 3381-3397, 2018.

DOS SANTOS, A. C. S., SANTOS, S. A., CARVALHO, G. G. P., MARIZ, L. D. S., TOSTO, M., VALADARES FILHO, S. C., & AZEVEDO, J. A. G. A comparative study on the excretion of urinary metabolites in goats and sheep to evaluate spot sampling applied to protein nutrition trials. **Journal of animal science**, v. 96, n. 8, p. 3381-3397, 2018.

HALL, M.B. **Neutral detergent-soluble carbohydrates**: nutritional relevance and analysis, a laboratory manual. University of Florida. (Extension Bulletin, 339), April, 2000.

HUHTANEN, P.; KAUSTELL, K.; JAAKKOLA, S. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. **Animal Feed Science and Technology**, v.48, p.211-227, 1994.

KITESSA, S., IRISH, G. G., & FLINN, P. C. Comparison of methods used to predict the in vivo digestibility of feeds in ruminants. **Australian Journal of Agricultural Research**, v. 50, n. 5, p. 825-842, 1999.

KRIZSAN, S. J.; HUHTANEN, P. Effect of diet composition and incubation time on feed indigestible neutral detergent fiber concentration in dairy cows. **Journal of Dairy Science**, v. 96, n. 3, p. 1715-1726, 2013.

LICITRA, G..; HERNANDEZ, T.M..; van SOEST, P.J. Standardization of procedures for nitrogen fractionation of ruminant feeds. **Animal Feed Science and Technology**, v.57, p.347-358, 1996.

LINDBERG, J. E. Estimation of rumen degradability of feed proteins with the in sacco technique and various in vitro methods: a review. **Acta Agriculturae Scandinavica**, suppl. 25, p. 65-97, 1985.

LOPES, Levi Auto et al. Intake, digestibility, and performance of lambs fed spineless cactus cv. Orelha de Elefante Mexicana. **Asian-Australasian journal of animal sciences**, v. 33, n. 8, p. 1284, 2020.

MADSEN, J., & HVELPLUND, T. Prediction of in situ protein degradability in the rumen. Results of a European ringtest. **Livestock Production Science**, v. 39, n. 2, p. 201-212, 1994.

MERTENS, D. R. et al. Rate and extent of digestion. **Quantitative aspects of ruminant digestion and metabolism**, v. 2, p. 13-47, 1993.

MERTENS, D.R. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. **Journal of AOAC International**, v.85, p.1217-1240, 2002.

MEYER, J. H., & MACKIE, R. I. Microbiological evaluation of the intraruminal in sacculus digestion technique. **Applied and environmental microbiology**, v. 51, n. 3, p. 622-629, 1986.

NOCEK, J. In situ and other methods to estimate ruminal protein and energy digestibility: a review. **Journal of Dairy Science**, v.71, p.2051-2069, 1988.

NOZIÈRE, P., & MICHALET-DOREAU, B. In saco methods. **Farm animal metabolism and nutrition**, p. 233-254, 2000.

NRC. 2007. **Nutrient Requirements of Small Ruminants**: Sheep, Goats, Cervids, and New World Camelids. 6th. ed. Washington, DC: National Academy Press, 384 p.

ØRSKOV, E. R., HOVELL, F. D., & MOULD, F. T. A. P. The use of the nylon bag technique for the evaluation of feedstuffs. **Tropical animal production**, v. 5, n. 3, p. 195-213, 1980.

REIS, M. J., SANTOS, S. A., PRATES, L. L., DETMANN, E., CARVALHO, G. G. P., SANTOS, A. C. S. & COSTA, E. Comparing sheep and cattle to quantify internal markers in tropical feeds using in situ ruminal incubation. **Animal Feed Science and Technology**, v. 232, p. 139-147, 2017.

RINNE, M., HUHTANEN, P., & JAAKKOLA, S. Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. **Journal of animal science**, v. 80, n. 7, p. 1986-1998, 2002.

RINNE, M; JAAKKOLA, S; HUHTANEN, P. Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. **Animal Feed Science and Technology**, v. 67, n. 1, p. 1-17, 1997.

TASSONE, SONIA; FORTINA, RICCARDO; PEIRETTI, PIER GIORGIO. In Vitro techniques using the DaisyII incubator for the assessment of digestibility: A review. **Animals**, v. 10, n. 5, p. 775, 2020.

VALENTE, T. N. P., DETMANN, E., QUEIROZ, A. C. D., VALADARES FILHO, S. D. C., GOMES, D. I., & FIGUEIRAS, J. F. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. **Revista Brasileira de Zootecnia**, v. 40, p. 2565-2573, 2011.

VALENTE, T. N. P., DETMANN, E., VALADARES FILHO, S. D. C., CUNHA, M. D., QUEIROZ, A. C. D., & SAMPAIO, C. B. In situ estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. **Revista Brasileira de Zootecnia**, v. 40, p. 666-675, 2011b.

VALENTE, T.N.V.; DETMANN, E.; SAMPAIO, C.B. Review: Recent advances in evaluation of bags made from different textiles used in situ ruminal degradation. **Canadian Journal of Animal Science**. v.95, p.493-498, 2015.

VAN DER KOELEN, C.J.; GOEDHART, P.W.; VAN VUUREN, A.M.; SAVOINI, G. Sources of variation of the in situ nylon bag technique. **Animal Feed Science and Technology**.V.38 p.35-42, 1992.

VANZANT, Eric S.; COCHRAN, Robert C.; TITGEMEYER, Evan C. **Standardization of in situ techniques for ruminant feedstuff evaluation. Journal of animal science**, v. 76, n. 10, p. 2717-2729, 1998.

WANG, W. K., WANG, Y. L., LI, W. J., WU, Q. C., YANG, K. L., LI, S. L., & YANG, H. J. In situ rumen degradation characteristics and bacterial colonization of whole cottonseed, cottonseed hull and cottonseed meal with different gossypol content. **AMB Express**, v. 11, n. 1, p. 1-11, 2021.

Table 1 - Chemical composition of sorghum silage, concentrate and diet.

Nutrient (g / kg DM)	Sorghum silage	Concentrate	Basal diet
Dry matter[1]	321	897	436
Organic matter	942	951	943
Crude protein	96	304	137
Ether extract	31	72	39
apNDF[2]	494	122	419
Non-fibrous carbohydrates[3]	320	471	349
iNDF[4]	259	24	212
Lignin	53	25	47

[1]g/kg natural material;

[2]Neutral detergent fiber corrected for ash and protein;

[3]Hall (2000): NFC = 100 - (cpNDF - CP - EE - ASH), and Detmann e Valadares Filho (2010): NFC = 100 - [(%CP - CP% of urea + % of urea) +% apNDF + %EE + %ASH] where cpNDF is corrected for ash and protein.

[4]Indigestible neutral detergent fiber.

Table 2 - Chemical composition of sorghum silage, ground corn grain and sheep feces.

Chemical composition (g / kg DM)	Sorghum silage	Ground corn	Sheep Feces
Dry matter[1]	280	896	308
Organic matter	939	984	883
Crude Protein	107	84	192
Ether Extract	31	34	18
apNDF[2]	480	63	545
Nonfibrous carbohydrates[3]	318	801	126
Neutral detergent fiber	526	117	617
Acid detergent fiber	274	59	311
Lignin	54	19	74

[1]g/kg natural matter;

[2]Fiber in neutral detergent corrected for ash and protein;

[3]Hall (2000): NFC = 100 - (cpNDF - CP - EE - ASH), where cpNDF is corrected for ash and protein.

Table 3 - Schematic representation of treatments

Period	Animal					
	1	2	3	4	5	6
P1	[1]T1	[2]T2	[3]T3	[4]T4	[5]T5	[6]T6
P2	T2	T3	T4	T5	T6	T1
P3	T3	T4	T5	T6	T1	T2
P4	T4	T5	T6	T1	T2	T3
P5	T5	T6	T1	T2	T3	T4
P6	T6	T1	T2	T3	T4	T5

T = Treatment. [1]T1 = 4 NWT bags per sample, totaling 12 bags; [2]T2 = 8 NWT bags per sample, totaling 24 bags; [3]T3 = 12 NWT bags per sample, totaling 36 bags; [4]T4 = 4 F57 bags per sample, totaling 12 bags; [5]T5 = 8 F57 bags per sample, totaling 24 bags; [6]T6 = 12 F57 bags per sample, totaling 36 bags. The incubated samples were: Sorghum silage, ground corn and sheep feces.

Table 4 - Effect of the tissue type and amount of in situ incubated bags on the estimate of indigestible neutral detergent fiber in sheep.

Item	Textile Woof		Amount of bags			SEM	P-Value[3]		
	F57	NWT	12	24	36		TW	NB	TW x NB
General	23.95	23.78	23.45	23.29	23.85	2.30	0.95	0.98	0.96
Sorghum silage	26.89	26.54	26.27	26.80	27.08	1.48	0.68	0.74	0.16
Ground corn	2.45	2.47	2.10	2.56	2.72	0.24	0.97	0.27	0.48
Sheep Feces	41.52	41.37	41.01	42.55	40.78	1.31	0.91	0.51	0.57

[1]iNDF = indigestible neutral detergent fiber (g/kg DM);
[2]SEM = standard error of the mean;
[3]TW x NB = interaction between the factors: textile woof and amount of bags

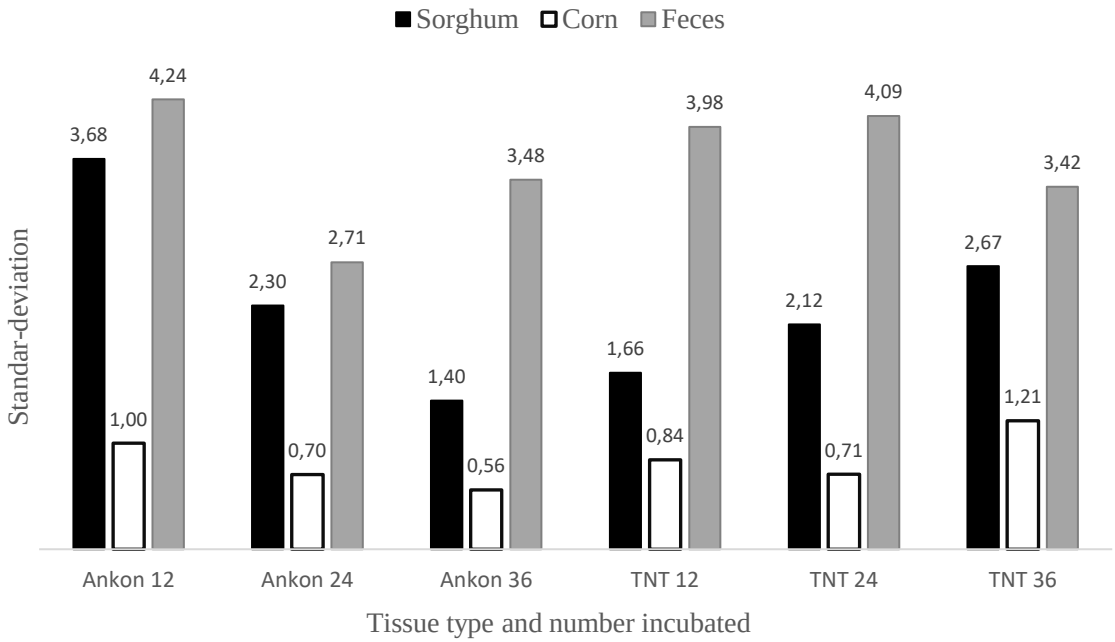

Figure 1 – Standard deviation of iNDF obtained from different tissue type and amount of in situ incubated bags in sheep rumen for sorghum silage, ground corn and feces from sheep