UNIVERSIDADE FEDERAL DA BAHIA – UFBA

PROGRAMA DE DOUTORADO EM ZOOTECNIA

ENDOGENOUS FRACTION OF PURINE DERIVATIVES EXCRETED IN THE URINE OF SMALL RUMINANTS

MÁRCIA PEREIRA DA SILVA

SALVADOR - BA

JULHO – 2023

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Dra. Luciana Louzada Prates
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SUMMARY

ENDOGENOUS FRACTION OF PURINE DERIVATIVES EXCRETED IN THE URINE OF SMALL RUMINANTS

General Introduction

Even though urinary PD excretion is routinely used as an indicator of microbial protein synthesis in ruminants, there are still some factors used in the model that affect PD excretion that are not completely clear (Chen et al., 2003). Among them, DP excretion of endogenous origin, mainly in goats and sheep.

The existence of an endogenous fraction in excreted PD has been confirmed in several experiments using different methods. The use of the intragastric infusion technique and casein infused in the rumen and abomasum, or the use of animals kept in maintenance (Fujihara et al., 1987; Belenguer et al., 2002 and Barbosa et al., 2012) or fasting (Braga et al., 2012). Endogenous DP excretion in fasted goats was reported by Belenguer et al. (2002) the value was 202.2 μmol/$BW^{0.75}$, urinary PD excretion responded linearly to the supply of purine bases along the abomasal cannula, with a mean recovery of 76%.

Chen et al., 1990 examined the relationship between exogenous nucleic acid (NA) purine supply in lambs, fully fed by intragastric infusions of volatile fatty acids (VFA) and casein, endogenous DP excretion was of 0.150 mmol/$BW^{0.75}$. In sheep submitted to total duodenal digesta replacement followed by the administration of a purine-free solution or one enriched with increasing amounts of purines Balcells et al. (1991) found the value of 158 μmol/$BW^{0.75}$.

The endogenous excretion represents an important parameter in the modeling of excretion of PD (Chen and Orskov, 2003; Gonzalez-Ronquillo et al., 2003); however, it is of hard quantification in surgically intact animals (Fujihara et al., 1987), due to technical limitations to eliminate the contribution of ruminal microorganisms under physiological conditions in ruminants (Chen et al., 1990). There is a linear relationship between urinary

excretion of purine derivatives and dry matter intake (Braga et al.,2012). Allowing this excretion to be estimated from the consumption of total digestible nutrients and digestible organic matter.

The use of regression to estimate endogenous PD uptake seems promising, as it avoids the adverse effects of fasting and intragastric feeding (Barbosa et al., 2011 and Braga 2012). However, according to Chen et al. (1990) to estimate the endogenous excretion of PD, the use of the intercept of the linear regression equation between the excretion of purine derivatives in the urine and the levels of dry matter intake did not prove to be an adequate method. In sheep, since the relationship between DP excretion and purine absorption is not linear.

However, it is important that more experiments be conducted to evaluate this excretion in goats and sheep. Endogenous fraction is subtracted from the total excretion of purines to quantify the production of microbial protein and that the recovery of purines absorbed in the urine compose the parameters of the estimation models (Chen and Gomes, 1992). If the value applied to the endogenous losses is inadequate, this can result in an under or overestimation of microbial protein (Andrade-Montemayor et al., 2004), considering that endogenous losses are subtracted from total purine excretion to estimate crude microbial protein production.

Thus, three experiments were carried out aiming to estimate the difference of EP urinary excretion between goats and sheep fed diets containing different protein contents, supplied with different maintenance levels of intake, and submitted to a fasting period to estimate EP fasting losses.

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Chapter 1. DA SILVA, Márcia Pereira, D.Sc., Federal University of Bahia, July 2023. **Effect of dietary protein on digestibility, rumen fermentation, microbial protein synthesis and serum metabolites in goats and small sheep**. Adviser: Stefanie Alvarenga.

ABSTRACT: Goats seem to be more efficient in fiber utilization and nitrogen utilization than sheep, but there are no good explanations or reasons in the literature. Digestibility data for these nutrients comparing the two species are few. The main aim of this study was to evaluate the nutrient digestibility, nitrogen utilization efficiency. Therefore, the digestibility of nutrients, excretion of urinary purine derivatives (PD) and nitrogen (N), estimation of microbial N synthesis and N utilization efficiency were determined in twelve Boer goats (17.3 kg±1.8 BW) and twelve Dorper sheep (20.7 kg±2.8 BW), all males, not castrated and aged four months. The animals were fed 4 diets with different levels of N concentration [6.4, 12.8, 19.2 and 25.6 g N/kg of dry matter (DM)] in 6 simultaneous designs of Latin squares 4 × 4. Only the intake of CP and EE had diet x species interaction. Nutrient intake was significant between diets and species ($P<0.001$). Sheep had higher nutrient intake ($P < 0.01$) than goats. The apparent digestibility of nutrients was similar ($P > 0.05$) in both species. Except for digestibility from CP ($P=0.02$) and aNDFn ($P=0.01$), which was higher for goats. The sheep indicated higher urinary N excretion ($P<0.001$), however, N retention (g/day; $P =0.89$) was similar for both species. The ration between N retained, ingested ($P<0.001$) and apparently digested ($P<0.001$) was higher in goats. Microbial efficiency ($P < 0.001$) increased linearly with the addition of N content in the diet. When fed a low-N diet (40g/kg DM), N consumption, urinary N excretion and N absorption were similar between species. Plasma urea concentration ($P<0.001$) and urinary urea excretion in g/day ($P<0.001$) indicated an increasing linear effect with increasing dietary N and differed ($P<0.001$) between species,

with goats with higher concentration in plasma and lower excretion (g/day) in urine. Small ruminants challenged to low (40 g/kg DM) dietary N supplementation required greater metabolic adaptation to more pronouncedly conserve N as a survival mechanism. Diets with higher CP content (160 g/kg) and 700 g/kg of forage increase the growth of ruminal microorganisms and improve microbial efficiency, resulting in increased intake of dry matter and nutrients, as well as promoting the digestion of fibers and proteins in goats and sheep.

Key words: digestibility, nitrogen balance, purine derivatives, small ruminant

1. INTRODUCTION

Nitrogen (N) conservation is a priority for animals. As a survival mechanism, as they evolved, ruminants developed ways to conserve N more pronouncedly (Detmann et al 2014a). One of the possible high priority metabolic functions is the recycling of nitrogen to the gastrointestinal tract (Batista et al., 2016; De Oliveira et al 2020).

Considering the theoretical concept of "N status", the N available for animal metabolism is used for various metabolic functions that follow an order of priority for individual animals, such as survival, i.e. N recycling, maintenance and production, production of meat and milk, reproduction (Matthews et al., 2019; Rufino et al., 2020; Oliveira et al., 2020). Then, N deposition as tissues or body products would only occur after the highest priority N demands are satisfied (Detmann et al., 2014a).

However, these physiological adaptation responses are still not fully understood in goats and sheep. No definitive information of a similar nature comparing the two species is available in the literature. Therefore, it is necessary to define the limits for reducing dietary N intake for these species and their physiological adaptations to the proposed challenges. Thus, as nitrogen supplementation contributes to the nutrition of the microbial population, increasing forage intake and digestibility, there is interest in accurately quantifying N limits in the diet and the real benefit of nitrogen supplementation in terms of microbial production. This information may provide insight into which species respond best to low-N dietary challenges and how to optimize the use of protein supplementation in both species.

It has been hypothesized that goats and sheep have distinct responses when fed diets containing crude protein levels below the minimum required in the diet to support microbial

growth and efficient digestion of fibrous carbohydrates. As well, when they are fed diets with high levels of protein, they present different responses regarding the efficiency of use N. Thus, the objective was to determine the minimum amount of dietary N intake that enhances microbial growth and increases nutrient intake and digestibility in goats and sheep.

Two simultaneous experiments were then developed to evaluate the effects of diet on intake, digestibility, nitrogen balance, blood parameters, urinary creatinine, rumen fermentation parameters and microbial protein synthesis in goats and sheep. Both experiments, in which the two species are fed diets containing different levels of N, provide a basis for clarification.

2. MATERIAL AND METHODS

2.1. Location and Ethical Considerations

The experiments were approved by the Ethics Committee on the Use of Animals of the Faculty of Veterinary Medicine and Zootechnics of the Federal University of Bahia (approval nº 28/2014), and followed the guidelines established by the National Council for the Control of Animal Experimentation (CONCEA). Both experiments were conducted on the experimental farm of the same institution, located in the municipality of São Gonçalo dos Campos, State of Bahia, Brazil. All chemical analyzes were performed at the Laboratory of Animal Nutrition at UFBA.

All animals were kept individually in metabolic cages (total surface area of 1.2m2), completely covered with a slatted floor and fresh water was provided ad libitum. A 15-d adaptation period allowed the animals to familiarize themselves with the experimental procedures, the staff, during which they were weighed, identified, and dewormed.

In both experiments, the animals were fed corn silage as forage, with the exception of diet 1 (6.4 g N/kg DM), which was composed of sugarcane silage. All diets showed a

forage: concentrate ratio of 70:30. The concentrate was a mixture of ground corn, corn germ meal, soybean meal and specific mineral mixture for goats and sheep. The experimental diet was offered to animals twice daily (09:00h and 16:00h) in similar proportions. Daily intake was adjusted to keep leftovers between 10% to 20% of the offered daily amount of feed in wet basis.

2.2. Experiment 1

2.2.1. *Animals, Experimental Design and Diets*

Twelve Boer goats, whose average initial body weight (BW) was 17.3±1.8 kg, and twelve Dorper sheep, whose average initial BW was 20.7±2.8 kg, all males, not castrated and four months old were used. Four experimental diets were formulated with increasing levels of N, as follow: 6.4, 12.8, 19.2 and 25.6 g N/kg DM (Table 1). The animals were distributed to these diets in six Latin Squares (LS) 4 × 4, in which 3 LS composed by goats and 3 LS composed by sheep. Each LS was composed by 4 animals of the same species, totaling 24 animals. A factorial scheme 4×2 was designed, with the 4 diets and 2 species as main factors.

The experiment was divided into four sub-periods consisting of 8 days for dietary adaptation and 8 days for sample collection comprising 16 day for each period, and 64-d in total.

2.2.2. *Nutrient Intake*

To evaluate the intake of nutritional components, the amounts of feed supplied and refusals were recorded daily. From days 9th to 12th of each experimental period, the silages and refusals where sampled. Silages were sampled directly in the pool removed from the silos for each day and refusals was sampled 20% of the total per animal. The concentrate ingredients were sampled directly from the grain storage of the feed factory in the days when they were mixed. These samples were homogenized to obtain a composite sample for each

animal per period. The material was pre-dried in a forced-air oven at 55 ℃ for 72 h. After drying, each sample was ground in a knife mill (Wiley mill; TECNAL, São Paulo, SP, Brazil) with 2 mm. After that, half of each sample was ground again to pass through a 1-mm screen sieve. Samples were then pooled and was proportionally composited based on dry weight per animal and period. All samples were stored for further chemical laboratory analyses.

2.2.3. *Fecal Collection and Nutrient Digestibility Trial*

The digestibility trial was conducted between the 9th and 12 th days of each experimental period. Total fecal output was measured during a 96-h period, with the aid of collection bags tied to the animals. Feces samples were taken directly into collection bags, twice daily (11.00 and 16.00 h) after homogenization. Then, the total fecal production of each animal was recorded and aliquots of approximately 10% of the total pool were removed, stored in individual plastic bags, labeled, and frozen at -20°C. Fecal samples were oven-dried (55 ◦C), ground and pooled as previously described.

The digestibility coefficient (DC) of the nutritional fractions was calculated by the following formula:

$$DC\ nutrient\ (\%) = \frac{nutrient\ intake\ (g) - Nutrient\ in\ feces\ (g)}{nutrient\ intake\ (g)} \times 100,$$ where Nutrient intake(g) = Nutrient supplied(g) − Nutrient in refusals(g).

2.2.4. *Urine Collection, Nitrogen Balance Assay and Estimation of CP Microbial Yield CP*

On the 11th day of each experimental period, urine collection funnels were attached to the animals for their adaptation. Between the 13th and 16th days, total urine collection was performed using hoses attached to the funnels, which conducted the urine to a plastic

container with 100 mL of 20% sulfuric acid (H2SO4) (v/v) as described by Santos et al. (2017) to preserve N compounds.

At the end of 24h, the urinary pool was weighed, registered, homogenized, and filtered through two layers of cheese cloth, and a 10mL aliquot was diluted in 40 mL of a 0.036N H2SO4 solution (Valadares et al. 1999). Subsequently a composite sample was obtained per period for each animal, and proportionally to the 4 sampling days. Composite samples were stored at -20°C for further analyses of nitrogen, creatinine, purine derivatives (DP), which were uric acid, allantoin, hypoxanthine and xanthine, and urea.

The amount of nitrogen in the feed, leftovers, feces and urine samples were determined according to the protocol described by the National Institute of Science and Technology in Zootechnics (INCT-CA; method N-001/2 - Detmann et al., 2021). The apparent nitrogen balance (NB) was calculated using the following formulas:

$$\text{NB or Nretained} = \text{Nintake} - (\text{Nfeces} + \text{Nurine})$$

$$\text{Nabsorbed} = \text{Nintake} - \text{Nfeces}$$

$$\text{NIntake} = \text{Nsupplied} - \text{Nrefusals}$$

Ruminal MCP synthesis was estimated by the urinary PD method using the following equations of Chen and Gomes (1992):

$$PD = 0.84X + (0.15BW^{0.75}e^{-0.25X})$$

$$\text{MicrobialN (g N/d)} = \frac{\text{X(mmol/d)} \times 70}{0.116 \times 0.83 \times 1000}$$

where: PD (mmol/d) = purine derivatives excreted in urine; X (mmol/d) = absorbed microbial PD. The N content of purines is 70 mg N/mmol. The total purine-N:N ratio in

mixed rumen microbes is taken as 0.116 (11.6/100). Digestibility of microbial purines is assumed to be 0.83, considered the average digestibility value for microbial nucleic acids based on observations reported in the literature.

2.2.5. *Blood Metabolites*

In day 16th, 10-mL blood samples were collected from the animals directly from the jugular using vacuum tubes with coagulation accelerator gel (BD Vacutainer®, SST II Advance, Franklin Lakes, NJ, USA). The samples were immediately centrifuged at 3500 rpm for 15 min to obtain the serum. Subsequently, the obtained serum was transferred to the labeled Eppendorf tubes and stored at -20°C for further analysis.

Serum urea concentration was determined enzymatic-colorimetric method in the presence of salicylate and sodium hypochlorite. Analyses were carried out using commercial kits (Urea – Ref.:27, Labtest)., with readings taken by a semi-automatic spectrophotometer (SBA 200®, CELM, São Caetano do Sul, Brazil) at the respective wavelengths at the Animal Nutrition Laboratory (LANA), Federal University of Bahia (UFBA).

2.3. Experiment 2

2.3.1. *Animals, experimental design, and diets*

Four uncastrated crossbred male sheep with medium BW of 45 kg ± 3 kg, all cannulated in the rumen. The animals were kept in individual pens, equipped with feeders and drinkers with free access to water.

Four experimental diets were formulated with increasing levels of crude protein (CP), as follows: 40, 80, 120 and 160 g CP/kg DM (Table 1). The animals were distributed to these diets in a Latin Square (LS) 4 x 4, with four diets and four periods, each animal representing a diet.

The experiment was divided into four sub-periods consisting of 8 days for dietary adaptation and 8 days for sample collection comprising 16 days for each period and 64 days in total.

2.3.2. *Nutrient Intake and Nutrient Digestibility Trial*

The collection and processing of samples and data for assessing the consumption and digestibility of nutritional components in Experiment 2 were the same as those described in Experiment 1.

2.3.3. *Ruminal fermentation parameters*

Ruminal fluid samples were collected on the first 4th day of the sampling period and were performed at 2-hour intervals. The ruminal fluid was collected immediately before feeding (0 h) and 2, 4 and 6 h after the morning feeding. The ruminal fluid samples were collected from four different points in the ventral region of the rumen and were subsequently homogenized. The digesta was filtered through four layers of cheesecloth, the liquid was homogenized immediately, and the pH was measured by direct reading with a digital potentiometer (Handy lab 12; Xylem Analytics Germany Sales GmbH & Corporation, Weilheim, KG, Germany).

After the pH measurement, a 40-mL aliquot was combined with 1 mL of a H2SO4 solution (9 M) and frozen (-20 ◦C) for later analysis of ruminal ammoniacal nitrogen (RAN) concentration. The RAN concentration was quantified using the colorimetric technique described by Broderick *et al.* (1980).

2.4. *Laboratory analysis*

In Experiments 1 and 2, the samples (duplicates) from ingredients, refusals and feces, were analyzed according to the protocols described by the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2021). The following

method numbers were used: dry matter: (DM) method G-003/1, ether extract (EE) method G-005/2 and ash, method M-001/2. The total N contents were measured by the Kjeldahl method, and crude protein (CP) was calculated as N × 6.25 (CP, method N-001/2).

The neutral detergent fiber (NDF), assayed with a heat stable amylase and without adding sodium sulfite, and acid detergent fiber (ADF) contents were determined according to (Mertens, 2002) and VAN SOEST. (1991). These samples were placed in plastic pots with 100 ml of detergent in the proportion of 1g of sample per 100 ml of detergent and autoclaved at 110°C (Barbosa et al., 2015). The NDF was corrected for ash and nitrogen by incinerating the neutral detergent digestion residues in a muffle oven at 600 ℃ for 4 h, and the correction for protein was performed by the neutral detergent insoluble protein method (Licitra et al., 1996).

Creatinine was quantified in all the urine samples the end point reaction enzymatic-colorimetric method from an alkalinepicrate reaction using commercial kits for analyses (Creatinine – Ref.:35 Labtest, Minas Gerais, Brazil). Urinary purine derivatives (PDs), including Allantoin, xanthine, and hypoxanthine were carried out based in calorimetric method according to Chen and Gomes (1992). Uric acid was quantified by an enzymatic method in uricase and peroxidase with a commercial kit (uric acid– Ref.:140 Labtest, Minas Gerais, Brazil).

2.5. Statistical analyses

Experiment 1 was conducted and analyzed according to six simultaneous 4 × 4 Latin squares with four treatments each (fixed effect), four animals (random effect) and four experimental periods (random effect). A 4 × 2 factorial schemes was formed by the four treatments (40, 80, 120 and 160 g PB/kg DM) and 2 species (goat and sheep). Experiment 2 was conducted and analyzed in a 4 × 4 Latin square with four treatments (fixed effect), four

animals (random effect) and four experimental periods (random effect) with one species (sheep).

Results were subjected to statistical analysis using PROC MIXED (SAS Inst. Inc., Cary, NC) and significances were stated at P < 0.05. The following model was applied:

$$Y_{ijk} = \mu + P + S + li + a(li)j + pk + \text{"ijklm}$$

where Yijkl = dependent variable; μ = overall mean; P = fixed effect of CP level (l = 40, 80, 120, or 160 g/kg DM), S = fixed effect of specie (goat or sheep); li = random effect of Latin square (i = 1 to 6); a(li)j = random effect of animal within Latin square (j = 1 to 24); pk = random effect of period (k = 1 to 4); "ijklm = random error assumed NID~(0, σ2).

In both models, the effect of crude protein inclusion level was assessed by fitting polynomial contrasts to assess linear (-3 -1 +1 +3) and quadratic (+1 -1 -1 +1) effects. The specie effect was evaluated by adjusting polynomial contrast (-1 -1 -1 +1 +1 +1) for Latin square comparison.

Ruminal fermentation variables (pH and RAN) were analyzed in a Latin square, considering the effects of measurements over time, using repeated measures over time. For all evaluations, a 5% probability level for type-I error was considered significant.

3. RESULTS

3.1. Experiment 1

3.1.1. *Intake and digestibility*

There was effect of interaction between diet and species on the CP intake (P=0.03; Figure 1A), EE (P=0.01; Figure 1B) The diet × specie interaction had an effect on the CP and EE intakes. The species did not influence (P=0.52) CP intake when animals were fed

low-protein diets (40 g/kg in DM). However, sheep showed higher CP and EE intakes with the increasing in protein content.

There was an effect of diet × species interaction on EE consumption. The species did not influence (P=0.10) the EE consumption when the animals ingested the diet with 40 g CP/kg DM, presenting 12.1 and 16.6 g EE/kg DM for goats and sheep, respectively. As well, the two species showed a quadratic effect for this variable, with the highest consumption recorded when the animals consumed the diet containing 120 g CP/kg DM.

The DM intake was different between diets and species (P<0.01; Table 2). Diet and species influenced (P<0.01) the OM, aNDFn, NFC and TDN intakes, as well as, the DM/TDN intake and CP/DOM intake, being the CP/DOM ratio influenced only by the diet.

The diet × specie interaction influenced the EE digestibility (P=0.05; Figure 2). The diet influenced (P<0.01) the digestibility of DM, OM, CP, aNDFn and NFC (Table 3). However, the effect of the specie impacted only on the digestibility of CP (P=0.02) and aNDFn (P=0.01).

3.1.2. *Nitrogen balance*

There was an effect of the diet × species interaction on N intake (P=0.01; Figure 3A), urinary N (P=0.03; Figure 3B) and absorbed N (P=0.02; Figure 3C). Diet linearly influenced fecal N (P<0.01) and N retention (P<0.01). The species, on the other hand, had a significant effect on the fecal N variables (P< 0.01), on the N retained from consumed and absorbed N ratios (Table 4).

The addition of CP to the diet linearly increased the proportions of N retained from ingested (-11.0 to 40.8%) and absorbed N (-35.6 to 58.8%). Although the N retained (g/day) was not influenced by the species, goats had higher values in the ratios between N retained/consumed and N retained/absorbed, being 26.9 and 39.8%, respectively.

3.1.3. *Excretion of purine derivatives and microbial efficiency*

Diet and species both influenced (P<0.001, Table 4) microbial nitrogen flux and estimation of microbial protein synthesis. The addition of dietary protein linearly increased microbial protein (g/day) by consumption of TDN (52.0 to 80.0 g/kg) and MOD (53.9 to 85.0 g/kg). Regarding species, sheep showed the highest estimate (74.0 and 80.2 g/kg of NDT and MOD, respectively).

No effect of the diet × specie interaction (P > 0.05; Table 4 and 5) was observed on the excretion of purine derivatives and the estimation of microbial protein synthesis. There was a linear increase (P<0.01) in total excretion of purine derivatives (4.35 to 9.87 mmol/day) and absorbed purines (4.19 to 8.50 mmol/day) with the dietary protein addition.

The total excretion of purine derivatives and absorbed purines was influenced by the species (P<0.01). The highest excretion of purine derivatives (9.49mmol/day) and absorbed purines (7.93 mmol/day) was observed in sheep.

3.1.4. *Urea and creatinine excretion*

There was an effect of diet × species interaction (P=0.02) on urea excretion in urine (g/day). The addition of dietary protein linearly increased (P<0.001) the concentration of urea in urine (mg/dl), however, the species (P=0.75) did not impact this same variable.

There was no effect of diet × specie interaction on creatinine concentration in urine excretion. Dietary treatments had a quadratic effect (P=0.01) on urinary creatinine concentration (mg/dl). When the animals were fed with 40 g CP/kg DM, they had the lowest creatinine concentration (64.9 mg/dl). The highest concentration was obtained when animals were fed with 80 g CP/kg DM (88.2 mg/dl).

The effects of diet (P<0.01) and specie (P<0.01) were observed on creatinine excretion (mg/day), (mg/kg BW) and (mmol/kg BW0.75). There was no effect of diet

(P=0.42), however, there was an effect of specie (P=0.03) on creatinine excretion (mg/kg BW) per OM intake.

4. DISCUSSION

Dietary CP content may enhance the growth of ruminal fibrolytic bacteria (Zhang et al., 2020), resulting in increased ruminal digestion of cellulose, forage intake, and energy utilization of fiber constituent carbohydrates. (Detmann et al., 2009). The main effects of the increase in dietary protein observed in this study related to DM, CP and OM intake, CP digestibility, aNDFn and dietary DOM content.

Overall, increasing CP content increased DM and nutrient intake in goats and sheep. Whereas supplementation was able to increase dietary CP above the minimum required to maximize microbial growth and fiber degradation (60–100 g CP/kg DM; Lazzarini et al., 2009; Detmann et al., 2014a) in cattle, an increase in forage intake is expected. Consistently, in our study, it was observed that reducing the CP content of the diet (40 g CP/kg DM) and 700 g/kg of sugarcane silage-based forage did not provide enough digestible OM to maximize microbial growth, thus resulting in lower efficiency of microbial protein. This may have reduced the solid passage rate, limited DMI (dry matter intake), and restricted the flow of nutrients to be digested and absorbed.

The authors Detmann et al., 2014a ensure the CP:MOD ratio as a reliable marker of protein:energy status. The protein:energy ratio is a reliable parameter for better adjustment of voluntary intake in ruminants, as it better reflects ruminal and metabolic events (Batista et al., 2016; Franco et al., 2017). In the study in question, this proportion varied from 76.3 to 298, a positive response in voluntary intake observed as this proportion increased. Diets with 120 and 160g of CP/kg of DM presented CP:MOD ratio values within the limit considered ideal to maximize voluntary intake in cattle (216 to 288 g/kg of DM; Detmann et al., 2014;

Reis et al., 2016), provided by the better synchronization of energy and protein present in them.

However, no difference observed in this relationship between goat and sheep species in our study, both below the range considered ideal by Detmann et al. (2014a) in cattle. Considering that sheep had a higher consumption of DOM and that there was no significant difference in the PB:MOD ratio, we can state that both species obtained use of CP by DOM. When this relationship is unbalanced, it does not influence DM intake in both species. As some authors (Batista et al., 2016; Oliveira et al., 2020; Sousa et al., 2022) reported the non-existence of significant differences in voluntary consumption, when the diets were unbalanced for this ratio of 74 or 404 g/kg, in cattle.

It known that nutrient digestion differs between ruminant species depending on forage quality. Studies have reported that sheep and goats fed a high-quality diet (with an average of 16% CP and 38% NDF) have apparently similar nutrient digestibility (Askar et al., 2016; Carro et al., 2012; Dos santos et al., 2018). Other studies have shown that goats versus sheep are more efficient at digesting nutrients when fed forage composed of a low CP (12%) and high fiber (50%) content (Askar et al., 2016; Domingue et al., 1991b). In our study there was no diet x species interaction, but the goats showed better digestibility of CP and NDF, which can be justified by the lower intake of DM and better efficiency of nitrogen use, enhancing the use of fiber in the diet by the goats.

Sheep and goats were similar in terms of EED when fed higher quality diets; inconsistencies occurred when fed a low-quality diet (40g CP/kg DM), with greater digestibility demonstrated by goats. We hypothesize that goats can markedly reduce fecal EE excretion when metabolizable energy intake is restricted. This suggests that goats are able to reduce their basal metabolic rate as a mechanism of adaptation to diets low in EE (2.4%), as

reported by Asmare et al. (2006), Helal et al. (2011) and Askar (2015, 2016). These findings agree with those reported by Asmare et al. (2006) and Askar (2015 and, 2016)

The increase in voluntary intake decreased the retention time of digesta in the digestive tract, providing an increase in the passage rate (Mertens et al., 1980, Colucci et al., 1982, Pino et al., 2018) and consequent reduction in the digestibility of DM, OM, aNDFn and CNF. The increase in voluntary intake due to nitrogen supplementation is associated with a sequence of events, such as increases in the rate and efficiency of microbial growth, and rate of NDF digestion (Detmann et al., 2014a). As consequence of the increase in the passage rate in the rumen, there is an increase in the proportion of indigestible fiber and in the ruminal turnover (Allen, 1996).

The positive effects of dietary protein on ruminal transit evidenced by NDF consumption. At the same time that the passage rate increased, it probably increased the production of indigestible fiber, due to the increase in the voluntary consumption of the diets.

However, the Protein:Energy ratio of the diet (120g CP/kg DM) was not synchronized, that is, in this diet the amount of nitrogenous compounds reaching the rumen was very low, limiting the digestibility of potentially degradable neutral detergent fiber (aNDFn). When the animals consumed the diet with 160 g CP/kg DM, digestibility increased again, due to the increase in nitrogenous compounds reaching the rumen proportionally to the amount of aNDFn.

It was demonstrated that the addition of protein in the diet increased CP digestibility, this result be explained by the increase in nitrogenous compounds reaching the rumen, evidenced by the increase in NH_3-N in the rumen, when the level of protein in the diet increased (figure 4b). The digestibility of CP is a direct reflection of the supply of highly degradable nitrogenous compounds in the diet (Lanzzarini, et al, 2009). According to Satter

and Slyter, (1974) nitrogen supplementation increases microbial activity and improves forage energy intake and use. These effects result from the improvement in the rate and extent of degradation of the aNDFn and the decrease in the rumen filling effect (Lazzarini et al, 2009; Costa et al, 2008).

Measuring the intake of digested compounds allows integrating the effects of supplementation on intake and digestibility (Lazzarini et al., 2009). In this context, a quadratic effect of CP levels observed on the consumption of digested DM, digested OM and digested NDF with critical points (minimum response CP 120 g/kg DM).

The low digestibility of CP observed in the animals when they consumed a diet with 40g of CP/kg of DM is a reflection of the high content of aNDFn and low content of CP in the diet. Probably when fed this diet, there was a reduction in rumen degradable protein (RDP). According to Lanzzarini et al.(2009) CP levels of 60 g/kg as the basis of DM are necessary to meet the microbial requirements of nitrogenous compounds, in order to obtain adequate use of NDF in the diet.

Therefore, providing an adequate supply of RDP and, consequently, an adequate concentration of ammoniacal N is the first priority to optimize the fermentative digestion of forage in ruminants (Detmann et al., 2009; Lanzzarini et al., 2009; Satter and Slyter, 1974). This will allow the microorganisms in the rumen to grow efficiently and, through fermentative activity, extract as much energy as possible from the roughage carbohydrates.

CP and aNFDn digestibility also influenced by species. Goats digested nNDF and PB better than sheep. Goats had lower voluntary intake, which explains the results in the digestibility of CP and NDF present in the diet. Possibly, the reduction in DM intake was associated with a prolonged retention time in the ruminoreticulum. N recycling appeared less extensive for sheep than for goats, to the point of negatively affecting fiber digestion. The

greater tissue mobilization of sheep than goats, affecting the amount of N available for recycling, may also have contributed to this finding.

Goats were more efficient in digesting, high-fiber, low-CP diets than sheep. This fact can be attributed to differences between ruminant species in their greater uptake and ability to recycle N in the rumen (Dos santos et al., 2018). Considering that N recycling was more extensive for goats than for sheep (Asmare et al., 2012), there was probably adequate synchronization between protein and energy for microbial growth. Also, greater production of salivary secretion, which influences the recycling of urea, an important factor in fiber digestion (Devendra, 1989).

Sheep consumed greater amounts of N than goats (10.3 vs. 14 g/day, respectively), however, nitrogen digestibility was higher in goats than in sheep (57.6 vs. 53.6%, respectively). Goats conserved more N (39.8% N ret/abs) in the body compared to sheep (17.6% N ret/abs), even though goats ingested less N, N retention was similar between the two species (3.75 vs. 3.75 g/day, respectively).

Given the above, let us assume that the greater digestion of aNDFn in goats provided by the higher concentration of NH_3, which may be indicative of greater efficiency of the species in recycling N or the efficiency of microbial protein synthesis. We conclude that regarding roughage and/or low-quality diets, current evidence suggests that goats are more efficient than sheep in their "utilization".

The pH observed in animals not influenced by the offered diets. However, a marked reduction in pH observed two to four hours after morning feeding in animals consuming a diet with 40g/kg CP. The lower pH (6.58) was likely a mechanism to maintain N supply for microbial growth in the rumen. Lower rumen pH decreases the permeability of the ruminal

epithelium to ammonia (Abdoun et al., 2006), thus preventing an excessive transfer of ammonia into the blood stream.

The CP 40 g/kg DM diet used in this experiment characterized by low cell wall carbohydrate fermentation rates. Low concentrations of NH_3-N (2.16 mg/dL) limited fiber digestion in the rumen. Since 10 to 15.33 mg of NH_3-N/L are required to maintain ruminal microbial growth and for efficient digestion of OM to occur in the rumen (Lazzarini et al., 2009 and Sampaio, 2009). Therefore, it can stated that the microbial growth rate was negatively affected at CP levels below 80 g/kg in DM, which caused changes in the microbial composition, once again corroborating the digestibility pattern of the NDF, as previously discussed.

Observations made by Domingues et al. (1991a) with goats and sheep consuming low quality diets, showed significant interspecies differences, favorable to goats. Goats had a lower rate of N intake; however, they were more efficient in using N, had lower fecal N excretion, making it similar to sheep in absorption when 40g PB/kg MS diets are provided and more efficient in using incoming nitrogen compounds in the rumen.

When the authors Domingues et al. (1991a) observed the ratio g N absorbed/g N ingested, the absorption of NH_3 in the rumen was similar between the species. However, goats recycled more N to the rumen (0.96 vs. 0.74) and greater NAN outflow from the rumen (1.22 vs. 0.99). Domingue et al. (1991b) found higher salivary N secretion rates in goats than in sheep, supporting higher ruminal N recycling rates in this species.

The results of this study indicate that N digested and retained more a function of CP digestibility than N consumption. Sheep consumed greater amounts of N than goats (14.0 vs. 10.3 g/day, respectively) in the however, nitrogen digestibility was higher in goats than in sheep (57.6 vs. 53.6%, respectively), leading to similar digested N between species. N

retention (g/day) was similar, but the percentage of N retained from the total N consumed and absorbed was higher in goats (26.9 and 39.8, respectively). Low protein intake can increase the amount of N that is recycled from the rumen for use, consequently, urinary N losses decrease in an attempt to save N (Batista et al., 2016, 2017; Menezes et al., 2016).

When the level of N in the diet is very low, as observed for the diet with 40g/kg CP, the animal decreases N excretion in the urine and increases the fraction of N in the diet that is recycled to the rumen (Batista et al., 2016). Nitrogen recycling is one of the highest priority metabolic functions in animals. This statement seems to be plausible, as the continuous supply of N for microbial growth in the rumen is an animal survival strategy (EGAN, 1965). The main effect of increased protein may be associated with increased N balance and increased urinary urea excretion.

When nitrogen deficiency becomes more severe, the animal can mobilize tissue N to sustain the mass of recycled nitrogen (Detmann et al., 2014). Based on these findings, we hypothesize that the available liquid N and the intestinal absorption of AA in the animals that consumed the diet with 40g/kg of CP may also be due to the contribution of N from the mobilization of proteins in the skeletal muscle, which may exceed apparent digestible N (Lapierre and Lobley, 2001), this is in part due to N from AA's being released from tissue protein during low N intake.

In the present study, reducing the level from 160 to 40 g PB/kg DM decreased microbial efficiency, probably due to reduced intake and consequent limitation of the amount of diet in the rumen. The amount of microbial nitrogen absorbed in the intestine of ruminants is highly related to the purine derivatives (PD; hypoxanthine, xanthine, uric acid and allantoin) excreted in the urine of these animals (Chen et al., 1990a). This is because in these

animals the nucleic acids (NA) that flow into the small intestine are essentially of ruminal microbial origin (Mcallan and Smith, 1973).

The flow of NA that reaches the intestine is influenced by the nutritional quality of the diet, dry matter intake, energy and protein intake, and animal species (Balcells et al., 1991; Campos et al., 2019). Urinary excretion of PDs in this study consisted mainly of allantoin, some uric acid, and hypoxanthine. According to (Chen et al., 1990a; Chen et al. 1990b), sheep have low xanthine oxidase (OX) activity in digestive tissues, liver and blood and, as a result, xanthine and hypoxanthine are hardly oxidized and excreted as PD in the urine. So far, studies on the activity of the XO enzyme in goats are still scarce. However, in our studies, PD excretion in the urine of these animals was similar to that of sheep. Which leads us to believe in the low activity of XO also in the goat species.

In this study, urinary creatinine excretion (mg/d) not influenced by digestible organic matter intake.We expected to find these facts because other authors have demonstrated that dietary factors do not influence creatinine excretion (Dos Santos et al., 2018). The daily excretion of creatinine (mmol/BW$^{\wedge 0.75}$), corrected for metabolic weight, in this study, was altered as a function of the protein level in the diets. Based on the interpretation of the results obtained in studies by Pereira.(2015), the daily excretion of creatinine (mmol/BW$^{\wedge 0.75}$) in lambs undergoes changes depending on the diet, with the level of dry matter intake (DM) is the main factor causing this alteration, however, Santos et al. (2018) reported that creatinine excretion (mmol/BW$^{\wedge 0.75}$) in goats and sheep is not influenced by DM or organic matter intake. In cattle, no differences were observed between daily creatinine excretion in different diets (Barbosa et al., 2011; Braga et al., 2012).

There are some factors that can cause variation in the concentration of creatinine in the urine, one of them is the volume of urine produced, which is dependent on the water

intake. The alteration of the glomerular filtration rate (GFR) (Pereira, 2015). Diet quality, according to Liu et al. (2006), being reduced in diets with low protein and low energy content. Naqvi et al. (2015) reported that creatinine excretion in lambs can be altered if there is nitrogen deficiency. Proteolysis and endogenous sources of N influence creatinine levels (Caldeira et al. 2007; Kataria and Kataria 2007).

These findings support the conclusion reached from the results of our study. Possibly, when animals consumed very low CP diets (40 g/kg CP based on DM), part of absorbed ammonia or AA or both are from endogenous sources of N, which would mainly be recycled urea N. What leads us to the hypothesis that the large ratio of urea N input rate to digestible N for the animals when consuming diets with 40g CP/kg DM may be due to the contribution of N from skeletal muscle protein mobilization. In such cases, the net N available to the animal and intestinal absorption of AA may exceed apparently digestible N in part due to AA N being released from tissue protein on a liquid basis during ingestion, of protein sub-maintenance (Lapierre and Lobley, 2001).

De Oliveira et al (2020) reported that there were impacts of rumen and post-rumen N supplementation on the metabolic characteristics of cattle fed low quality tropical forage. Non-supplemented animals showed a decrease in the 3MH:creatinine ratio serum, greater muscle catabolism, in an attempt to mobilize N for an additional ruminal supply (Batista et al., 2016; Rufino et al., 2016). In our study, the 3MH analysis was not carried out, however, the creatinine findings are an indication of this tissue N mobilization.

5. CONCLUSIONS

Diets with higher CP content (160 g/kg) and 700 g/kg of forage increase the growth of ruminal microorganisms and improve microbial efficiency, resulting in increased intake

of dry matter and nutrients, as well as promoting the digestion of fibers and proteins in goats and sheep.

Daily creatinine excretion in g/day is not influenced by dietary protein levels, however, daily creatinine excretion per body weight corrected for metabolic weight in goats and sheep varies as a function of protein levels in the diets.

When comparing the results between species, goats showed greater efficiency in the use of N, compared to sheep, in the same challenge.

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Table 1
Proportion of Ingredients and chemical composition of experimental diets.

Item	Diets protein level, g/kg DM			
	40	80	120	160
Ingredients, g/kg of dry matter				
Ground Corn	265	200	60.0	32.0
Soybean meal	-	13.0	110.0	220
Corn germ	-	59.6	105.4	30.0
Mineral	35.0	27.4	24.6	18.3
Sugarcarne Silage	700	-	-	-
Corn Silage	-	700	700	700
Chemical composition, g/kg of dry matter				
Dry matter (DM; g/kg as fed)	410	480	483	479
Organic matter	929	941	939	939
Crude protein	47.8	82.0	120	159
Ether extract	24.2	46.1	58.7	31.4
aNDFn	498	423	434	425
NFC	359	390	326	324

aNDFn - neutral detergent fiber with thermolabile amylase corrected for ash and nitrogen; NFC - non-fiber carbohydrates.

Table 2
Effect of experimental diets and animal species on nutrient intake.

Items	Protein level, g/kg DM				Specie		SEM	P-value			
								Diets		Specie	Diets× Specie
	40	80	120	160	Goat	Sheep		L	Q		
Intake (g/kg of $LW^{0.75}$)											
Dry matter	54.0	61.9	66.7	75.6	59.4	69.7	1.91	<0.001	0.73	<0.001	0.74
aNDFn	24.8	23.8	23.8	29.0	24.0	28.0	0.94	<0.001	0.01	0.003	0.74
Intake (g/day)											
Dry matter	535	620	669	751	548	740	19.0	<0.001	<0.001	<0.001	0.86
Organic matter	497	583	627	704	514	691	18.3	<0.001	<0.001	<0.001	0.87
Ether extract	14.3	31.9	44.6	26.0	25.1	33.3	0.76	<0.001	<0.001	<0.001	0.03
Crude protein	26.7	53.9	88.8	134	64.5	87.3	3.50	<0.001	<0.001	<0.001	0.01
aNDFn	246	238	264	288	221	297	9.28	<0.001	0.02	<0.001	0.83
NFC	208	258	229	256	202	274	7.01	<0.001	0.04	<0.001	0.91
TDN	362	421	451	483	370	488	19.7	<0.001	0.30	<0.001	0.88
DOM	350	386	402	455	344	452	19.3	<0.001	0.47	<0.001	0.89
DM/ TDN	173	199	218	268	177	251	0.01	<0.001	0.06	<0.001	0.62
CP/DOM	76.3	140	222	298	188	193	7.96	<0.001	0.07	0.08	0.86

$LW^{0.75}$ - metabolic weight; aNDFn - neutral detergent fiber with thermolabile amylase corrected for ash and nitrogen; NFC - non-fiber carbohydrates; TC - total carbohydrates; TDN-total digestible nutrientes; DOM - digestible organic matter; SEM - standard error of the mean; L - linear effect; Q - quadratic effect. P<0.05.

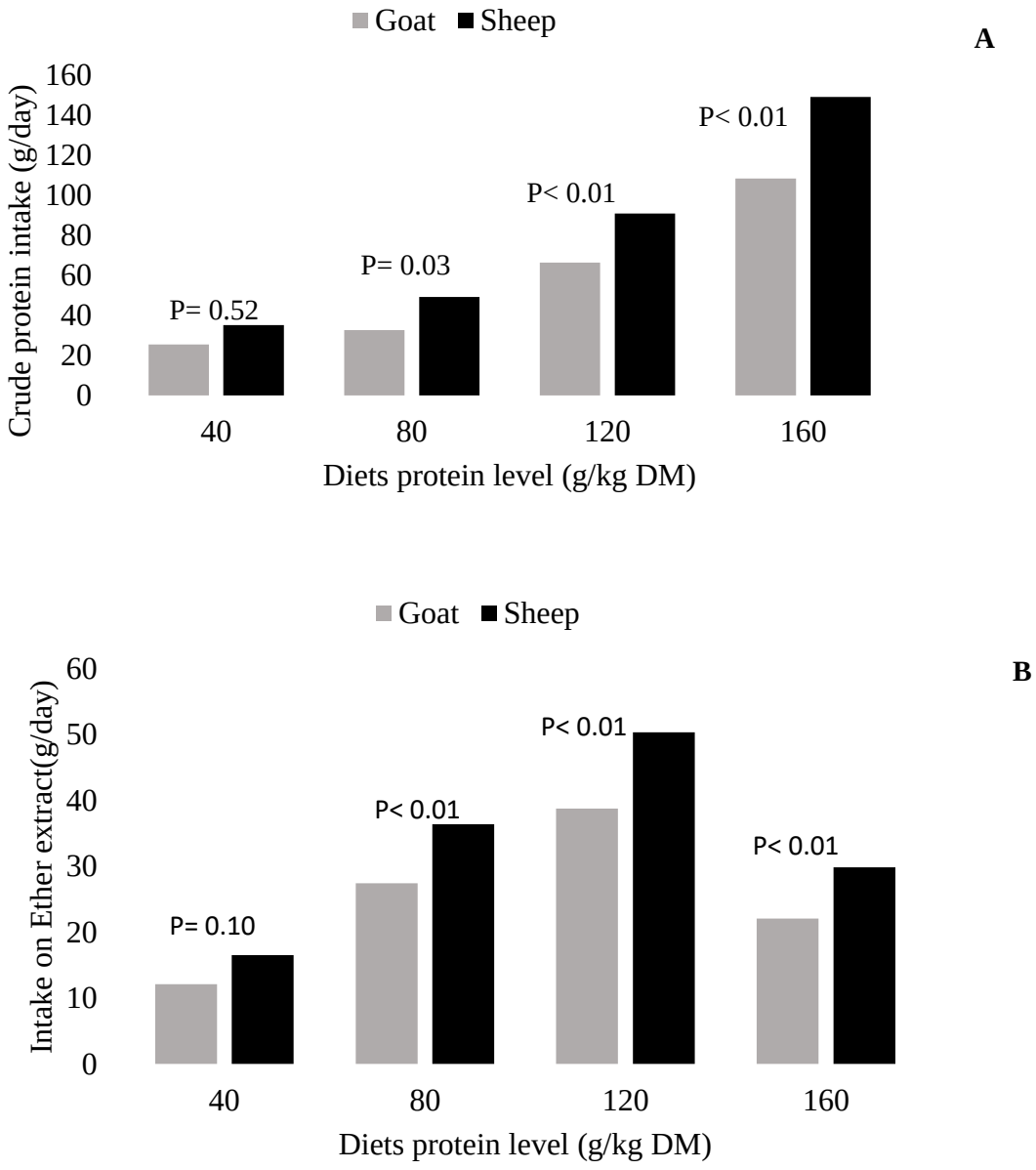


Figure 1. Interaction slicings related to the intake of crude protein (A) and ether extract (B) in goats and sheep, fed diets containing different levels of protein.

Table 3

Effect of experimental diets and animal species on nutrient apparent digestibility.

Digestibility (g/kg)	Protein level, g/kg DM				Specie		SEM	P-value			
								Diets		Specie	Diets *Specie
	40	80	120	160	Goat	Sheep		L	Q		
Dry matter	687	650	629	631	658	641	1.65	<0.001	0.01	0.09	0.54
Organic matter	705	661	642	645	673	654	1.54	<0.001	0.01	0.06	0.60
Ether extract	813	913	890	841	864	864	1.38	0.01	<0.001	0.95	0.05
Crude protein	400	491	641	693	576	536	2.81	<0.001	0.20	0.02	0.64
aNDFn	555	502	449	453	510	470	3.17	<0.001	0.03	0.01	0.69
NFC	911	810	814	814	835	839	1.27	<0.001	<0.001	0.73	0.96
TDN	591	627	631	597	670	650	1.9	<0.001	<0.001	<0.001	0.88

aNDFn - neutral detergent fiber with thermolabile amylase corrected for ash and nitrogen; NFC - non-fiber carbohydrates; TDN-total digestible nutrients; SEM - standard error of the mean; L - linear effect; Q - quadratic effect. P<0.05.

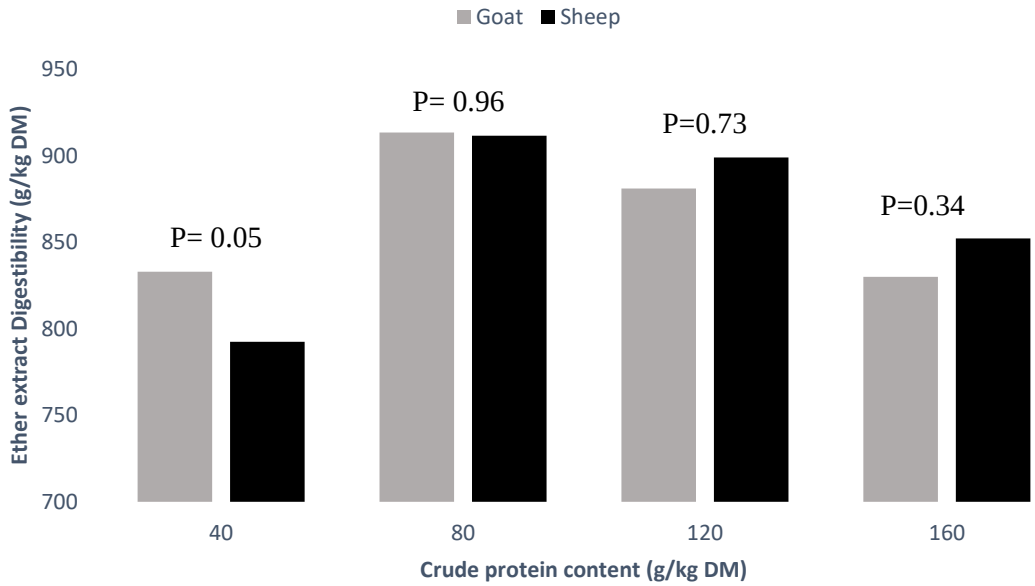


Figure 2. interaction slicings related to apparent digestibility of the ether extract in goats and sheep fed diets containing different levels of protein.

Table 4
Effect of experimental diets and animal species on nitrogen (N) balance, microbial CP synthesis and its efficiency.

Items	Protein level, g/kg DM				Specie		SEM	P-value			
								Diets		Specie	Diets*Specie
	40	80	120	160	Goat	Sheep		L	Q		
Nitrogen balance (g/day)											
Intake	4.28	8.63	14.2	21.5	10.3	14.0	0.56	<0.001	<0.001	<0.001	0.01
Fecal	2.57	4.36	5.15	6.51	3.81	5.48	0.21	<0.001	0.17	<0.001	0.16
Urine	2.18	2.44	4.12	6.14	2.76	4.68	0.19	<0.001	<0.001	<0.001	0.03
Absorbed	1.71	4.26	9.06	14.9	6.51	8.48	0.57	<0.001	<0.001	<0.001	0.02
Retention	-0.48	1.82	4.95	8.81	3.75	3.80	0.52	<0.001	0.01	0.89	0.29
$N_{retention}$ (% N_{intake})	-11.1	20.9	35.8	40.9	26.9	16.3	3.23	<0.001	<0.001	<0.001	0.36
$N_{retetion}$ (% $N_{absorved}$)	-35.6	36.2	55.4	58.8	39.8	17.6	8.3	<0.001	<0.001	<0.001	0.87
Microbial protein (g/day)											
Microbial N	3.04	3.82	4.38	6.18	2.95	5.76	0.32	<0.001	0.08	<0.001	0.36
Microbial CP	19.0	23.9	27.4	38.6	18.4	36.0	1.97	<0.001	0.08	<0.001	0.36
Microbial efficiency											
g microbialCP / TDN	52.0	55.8	59.4	80.0	49.6	74.0	4.56	<0.001	0.07	<0.001	0.98
g microbialCP /DOM	53.9	61.2	66.8	85.0	53.3	80.2	5.00	<0.001	0.27	<0.001	0.97

TDN-total digestible nutrientes; DOM - digestible organic matter; SEM - standard error of the mean; L - linear effect; Q - quadratic effect. P<0.05.

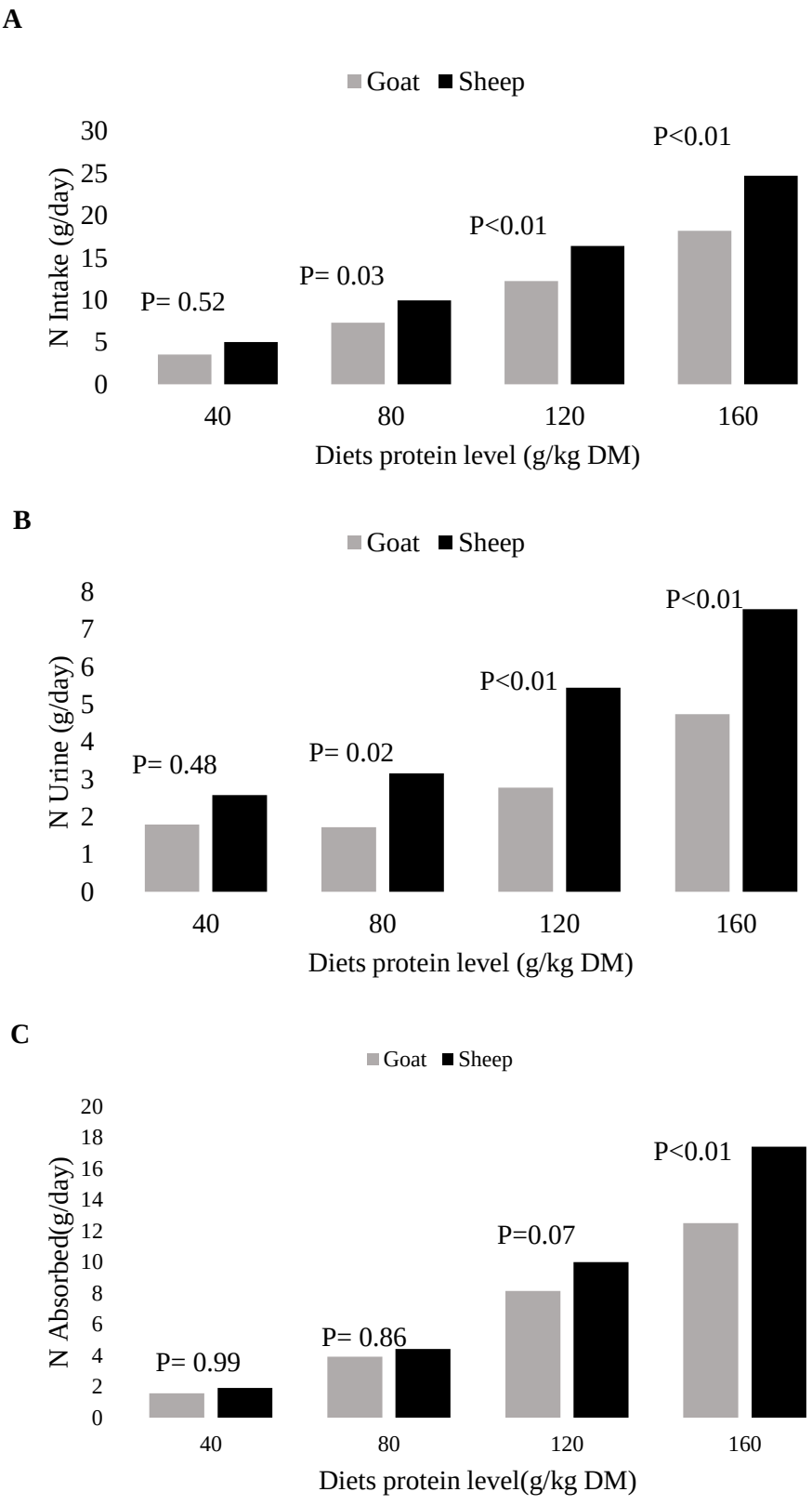


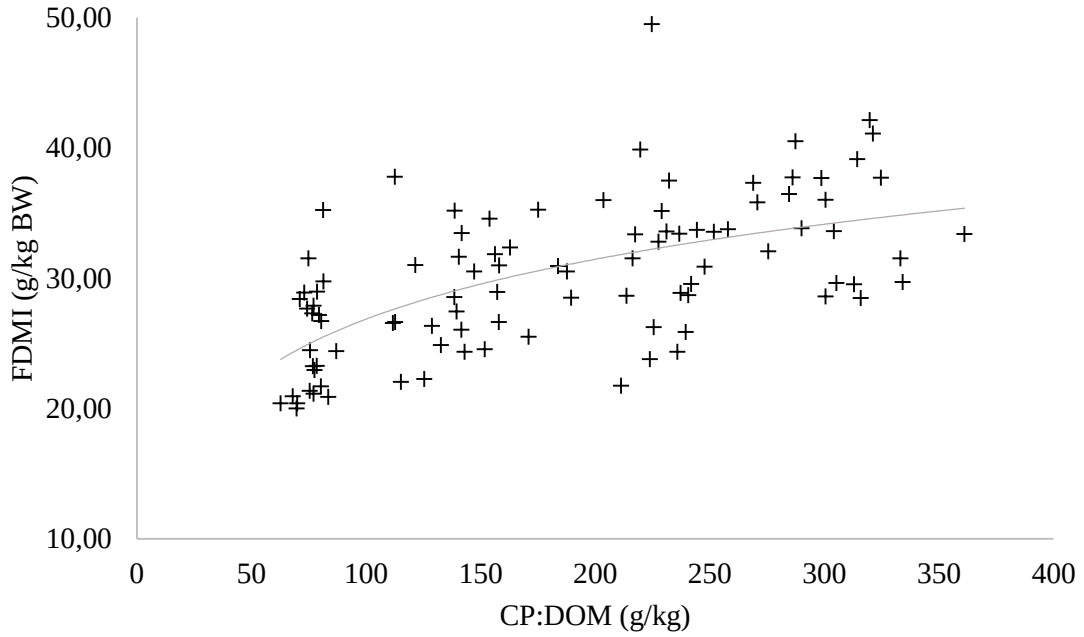
Figure 3. Unfolding of interactions related to the intake(A), absorbed (B) and excretion (C) urine of nitrogen in goats and sheep, fed diets. containing different levels of protein.

Tabela 5
Effect of experimental diets and animal species on plasma urea concentration, excretion of urinary purine derivatives, creatinine and urea.

Items	Protein level, g/kg DM				Specie		SEM	P-value			
								Diets		Specie	Diets×Specie
	40	80	120	160	Goat	Sheep		L	Q		
Plasma urea											
Ureiamg/dL	15.2	22.9	41.6	57.1	36.7	31.6		<.01	0.02	0.05	0.36
Ureia mmol/L	2.51	3.79	6.90	9.48	6.09	5.25		<.01	0.02	0.05	0.36
Purine derivatives (mmoL/day)											
Allantoin	3.47	4.61	5.44	8.02	3.41	7.36	0.46	<.01	0.09	<.01	0.44
Xant. and hipoxant	0.26	0.31	0.35	0.48	0.36	0.50	0.03	<.01	0.19	<.01	0.22
Acid uric	0.62	0.77	0.91	1.37	0.54	1.29	0.09	<.01	0.07	<.01	0.70
Total PD	4.35	5.70	6.70	9.87	4.15	9.49	0.56	<.01	0.07	<.01	0.46
$Purine_{Absorbed}$	4.19	5.26	6.02	8.50	4.05	7.93	0.43	<.01	0.08	<.01	0.36
Purine derivatives (%)											
Allantoin	80.2	81.2	81.2	81.4	81.7	80.0	0.77	0.26	0.58	0.04	0.70
Acid uric	14.0	13.4	13.2	13.7	12.9	14.3	0.65	0.73	0.40	0.06	0.50
Xant. and hipoxant	5.86	5.37	5.64	4.87	5.12	5.75	0.40	0.09	0.69	0.12	0.88
Creatinine											
mg/dl	64.9	88.2	84.7	70.5	105	48.6	9.53	0.64	0.01	<.01	0.11
mg/day	303	353	390	415	304	427	24.8	<.01	0.51	<.01	0.65
mg/kg BW	14.3	16.4	17.9	19.3	15.6	18.3	1.14	<.01	0.69	<.01	0.53
mMol/kg $BW^{\wedge 0,75}$	0.27	0.31	0.34	0.37	0.29	0.36	0.02	<.01	0.64	<.01	0.57
Creatinine/OM Intake	3.01	2.86	2.93	2.79	3.09	2.70	0.23	0.42	0.96	0.03	0.65
Urea											
mg/dl	795	1430	2563	3158	2015	1958	163	<.01	0.90	0.75	0.71
g/day	5.07	8.3	16.2	21.9	6.81	18.9	1.50	<.01	0.39	<0.01	0.02

PD -purine derivates; SEM - standard error of the mean; L - linear effect; Q - quadratic effect. P<0.05.

A



B

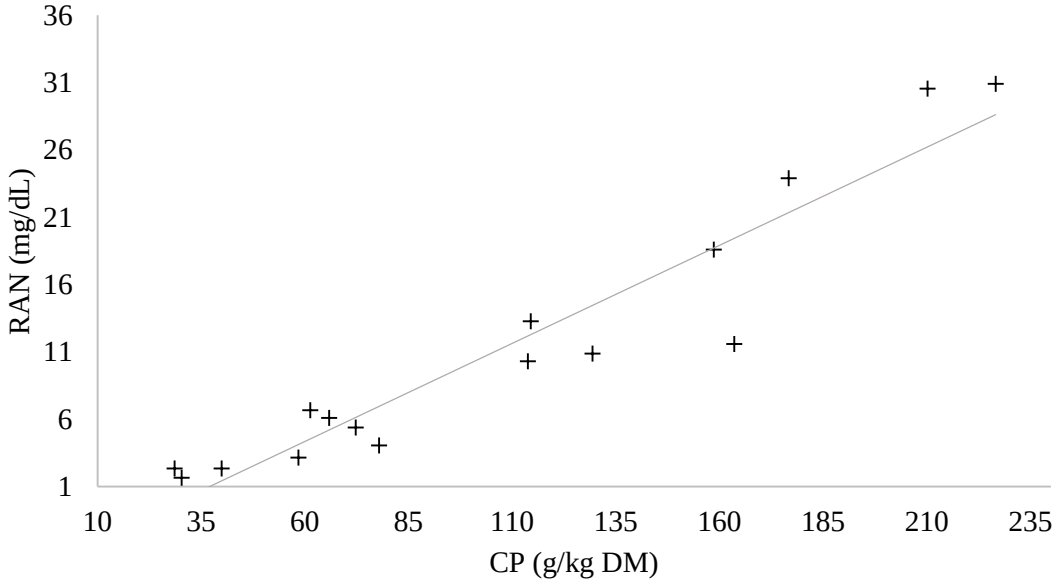


Figure 4. Relationships between the ratio of CP on digestible organic matter content in diet (CP: DOM) and the forage dry matter intake (FDMI) and between crude protein (CP) content in the diet and concentration of rumen ammonia nitrogen (RAN).

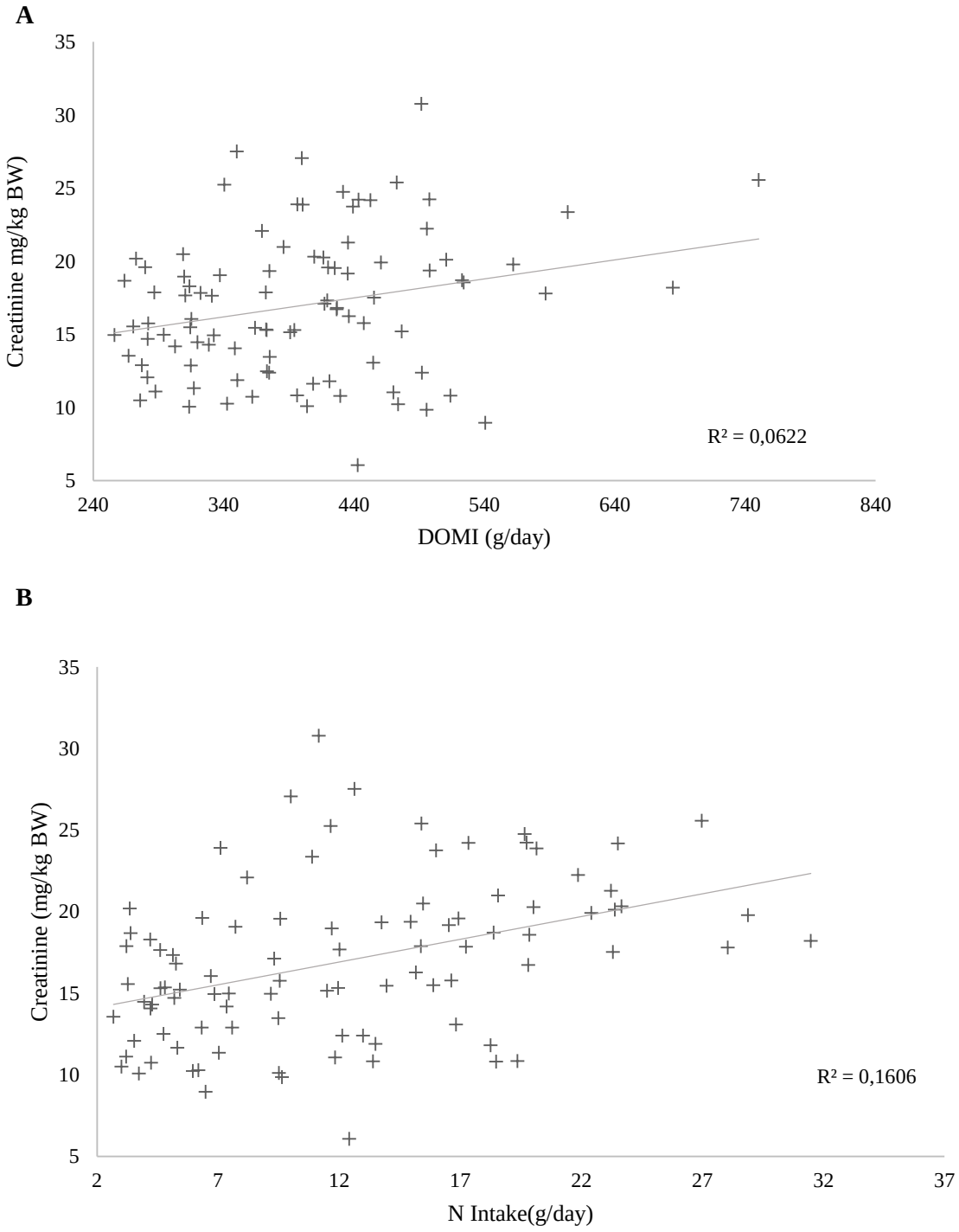


Figure 5. Relationship between urinary creatinine concentration and intake of digestible organic matter (DOM) and nitrogen (N).

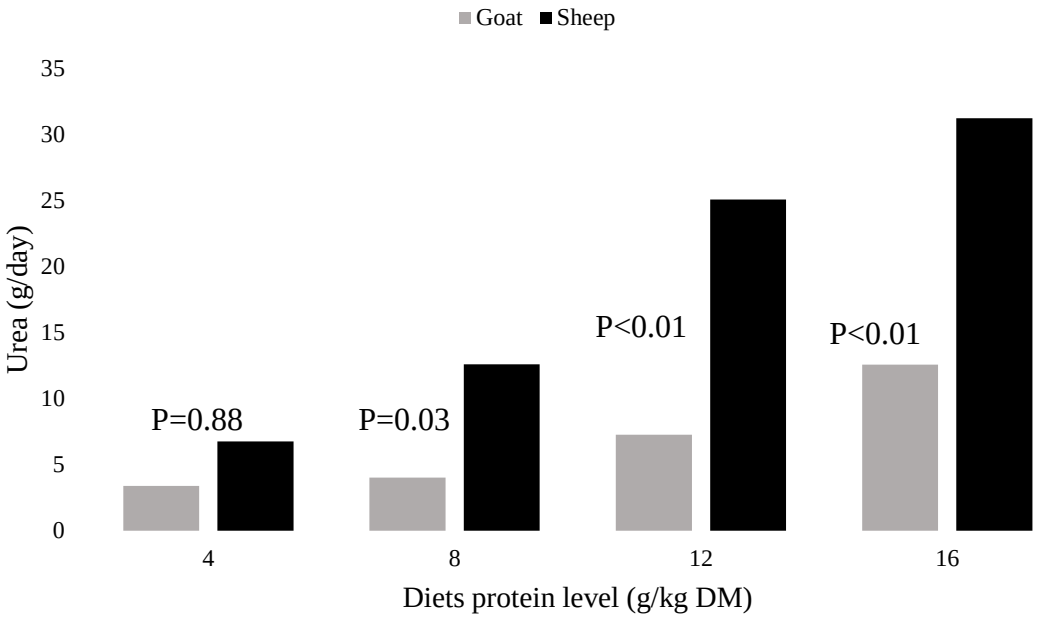


Figure 6. Unfolding of interactions related to urea excretion in g per day in the urine of goats and sheep fed diets containing different levels of protein.

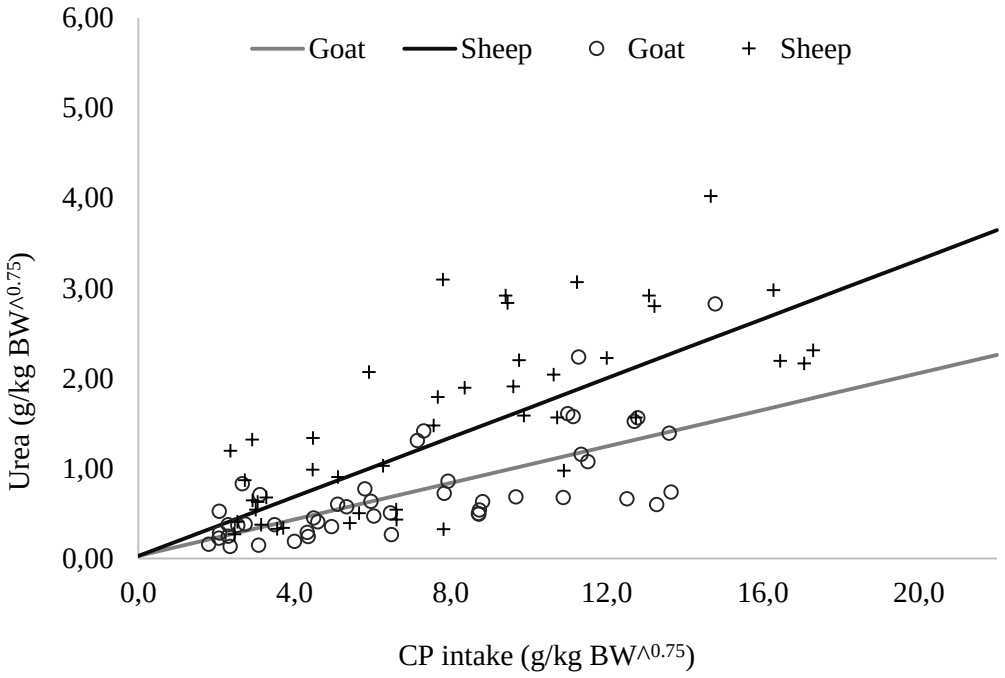


Figure 7. Relationship between urine urea concentration and nitrogen (N) consumption in goats and sheep. Urea (g/kgBW$^{\wedge 0.75}$) = 0.02729 + [0.1014*CPI+ 0.06298 * CPI* (species)] (R2 = 0.62). combined intercept P = 0.4408; combined slope P = 0.0245 (binary =1(sheep) or 0 (goats).

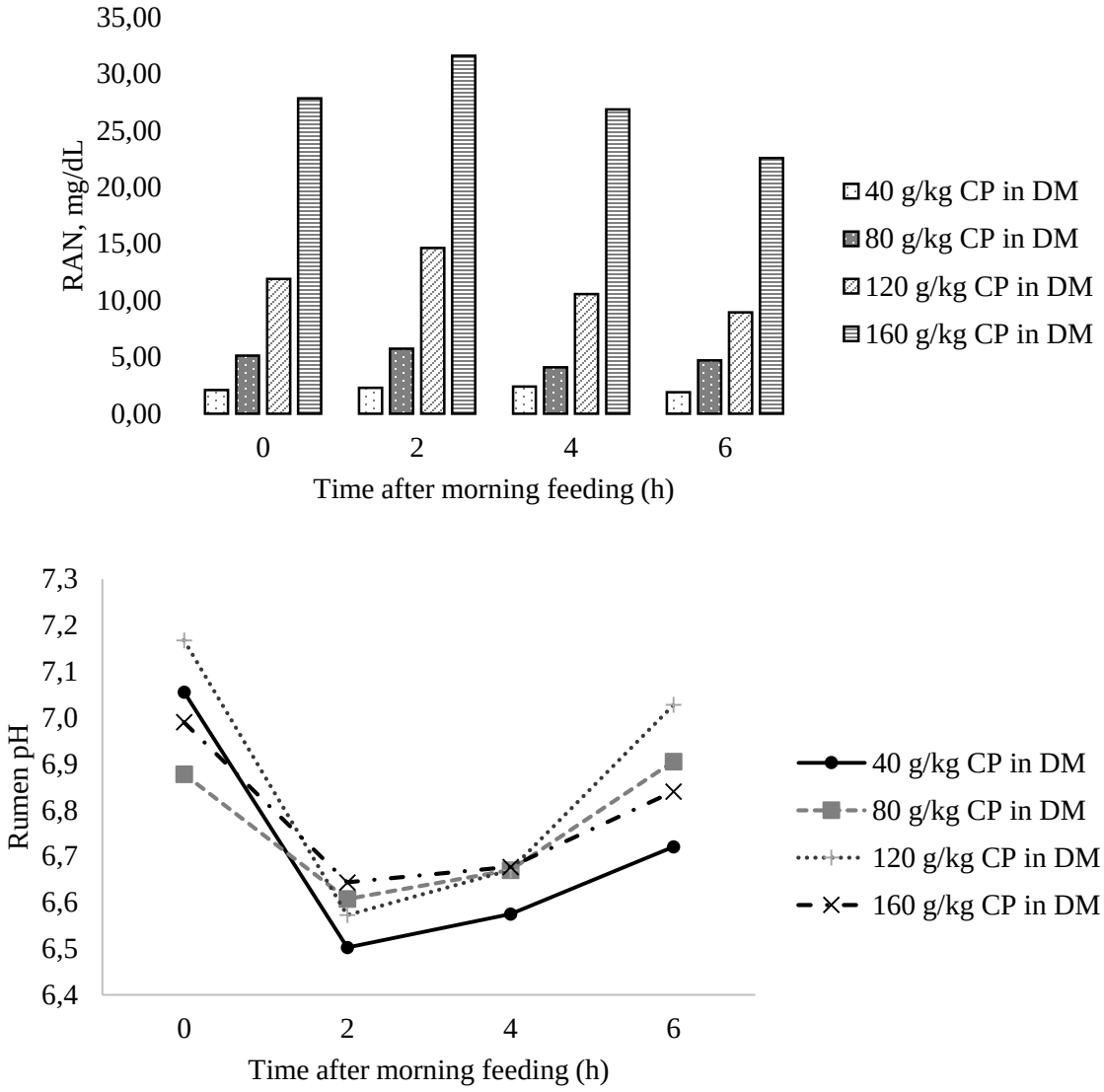


Figure 8. Ruminal ammonia nitrogen content (RAN) and pH values of the ruminal liquid of sheep submitted to diets containing different levels of CP depending on the collection times.

Chapter 2. DA SILVA, Márcia Pereira, D. Sc., Federal University of Bahia, July 2023. **Estimation of urinary excretion of purine derivatives from energy intake in goats and sheep**. Adviser: Stefanie Alvarenga.

ABSTRACT: The excretion of purine derivatives (PD) has been used as a method to estimate microbial protein synthesis in ruminants, showing a positive linear relationship with digestible MO intake (dMOi), in kg/day. However, in goats and sheep an equation to estimate urinary PD excretion from dMO intake is needed. The objectives were to develop equations to estimate the urinary excretion of PD from the energy intake, to evaluate the intake and digestibility of nutrients, nitrogen balance (NB) and microbial efficiency in small ruminants. The experiment was carried out in the same places and conditions described for Exp. I. The animals used in Exp 2 were the same from Exp 1; 12 Boer goats and 12 Dorper sheep, all males and not castrated. After the end of Exp. 1, the animals were kept in the installations to start the Exp. 2, where all the animals were fed with the diet containing 160 g CP/kg DM. Four animals of each species were randomly submitted to one of the three experimental treatments, which were three feeding levels (60, 20 and 0% of feed restriction), totaling eight animals per treatment. The experiment was carried out in a completely randomized design in a 3 × 2 factorial scheme, in which the factors were two species (goats and sheep) and three feeding levels (60, 20 and 0% of feed restriction). The data were submitted to statistical analysis by PROC MIXED, and the species, feeding levels and interaction between species and feeding level were considered a fixed effect. Daily urinary PD excretion (mmol/kg BW) increased linearly ($P < 0.01$) with DM and DOM intake. When PD excretion (mmol/d) was related to the intake of DM and DOM (kg/d), the following equations were obtained: in goats $\mathrm{PD} = 0.01 \times \mathrm{DMI} - 0.03; \mathrm{r}^2 = 0.88$ and sheep $\mathrm{PD} = 0.01 \times \mathrm{DMI} - 0.007; \mathrm{r}^2 = 0.72$ and

goats $PD = 0.02 \times dMOI - 0.51; r^2 = 0.82$ and sheep $PD = 0.02 \times dOMi + 0.47; r^2 = 0.41$, respectively. In conclusion, feed restriction improves the efficiency of nutrient utilization, but decreases the efficiency of microbial synthesis in the rumen. The efficiency of microbial CP based on OM consumption was similar between goats and sheep when submitted to food restriction. Goats are more efficient in using dietary fiber and N conservation than sheep when subjected to food restriction.

Key words: Animal feed, Animal nutrition, feed restriction, protein synthesis,

1. INTRODUCTION

The synthesis of microbial crude protein (MCP) is driven by the energy consumption of the ruminant (Santos et al., 2021). Ruminal microbial fermentation provides MCP by degrading fermentable carbohydrates, non-protein nitrogen and other organic matter in the diet (Liu et al., 2021). Therefore, the synchronism between protein and energy degradation in the rumen is one of the factors that impact MCP efficiency (Zang et al., 2020). Since microbial protein synthesis provides a substantial supply of metabolizable protein to the ruminant (Zhong et al. 2022). Knowledge of the timing of energy and protein release in the rumen is useful to guide diet preparation to increase metabolizable CP synthesis as well as nitrogen utilization efficiency.

The MCP is calculated as a function of the digestible MO (dOM), metabolizable energy (ME) or total digestive nutrients (TDN) with or without discounting subsidies that provide little or no energy for the microbes (AFR,1993; NRC,2006;2007; CSIRO,2001; BRcorte, 2016; NASEM, 2016; INRA, 2018). Since these models can be used to calculate rumen degradable protein (RDP) requirements from metabolizable protein (MP) requirements (Santos et al.,2021), it is of fundamental importance to study of methods to estimate the production of microbial protein quickly, routinely and efficiently in a non-invasive way in goats and sheep, this information for goats and sheep is scarce.

The need to develop non-invasive techniques favored the use of excretion of PD in the urine to determine the production of MCP, as an alternative to determinations using fistulated animals (Braga et al., 2012). Carro et al. (2012) compared methods for estimating MCP in sheep and goats and reported that there were no interspecies differences in the total

excretion of PD in the urine. Santos et al. (2021) stated that MCP synthesis in the rumen can be predicted from energy consumption through combined equations that encompass both sheep and goats. The possibility of estimating PD excretion based on energy intake and using the endogenous excretion obtained in goats and sheep will allow estimating the ruminal production of microbial crude protein under practical feeding conditions.

Therefore, the hypothesis investigated in the present work was that there is a difference in MCP efficiency between sheep and goats, and the possibility of predicting ruminal MCP production from food energy intake by regression equations. Thus, for the present study, goats and sheep fed diets provided from maintenance to voluntary consumption were used to evaluate nutrient intake and digestibility, NB, microbial efficiency and to develop equations to estimate urinary PD excretion from of energy consumption.

2. MATERIAL AND METHODS

2.1. Location and Ethical Considerations

The experiment was approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (protocol nº 28/2014), and followed the guidelines established by the National Council for the Control of Animal Experimentation (CONCEA). The study was carried out in the experimental farm of the same institution, located in the municipality of São Gonçalo dos Campos, State of Bahia, Brazil. All chemical analyzes were performed at the Animal Nutrition Laboratory at Federal University of Bahia.

2.2. Animals, experimental design and diets

The experiment was carried out in the same places and conditions described for Exp. I. The animals used in Exp 2 were the same from Exp 1; 12 Boer goats and 12 Dorper sheep, all males and not castrated. After the end of Exp. 1, the animals were kept in the installations to start the Exp. 2, where all the animals were fed with the diet containing 160 g CP/kg DM from Exp 1 (Table 1). Four animals of each species were randomly submitted to one of the three experimental treatments, which were three feeding levels (60, 20 and 100% of *feed restriction*), totaling eight animals per treatment. The experiment was carried out in a completely randomized design in a 3 × 2 factorial scheme, in which the factors were two species (goats and sheep) and three feeding levels (60, 20 and 100% of *feed restriction*).

All animals were housed individually in metabolic cages (total surface area 1.2 m^2), completely covered with slatted floors equipped with feeders and drinkers, where fresh water was provided *ad libitum*. The animals were adapted to the experimental facilities, and management 15 days before the beginning of the experiment. During the adaptation period

the animals were weighed, identified, and dewormed. The experimental period lasted 15 days, with 11 days for adaptation to the feeding level and 4 days for sample collection.

Corn silage was used as the exclusive source of forage in the experimental diets. The roughage:concentrate ratio was 70:30. The ingredients and its dietary proportions was shown in Table 1. The concentrate was a mixture of ground corn, corn germ meal, soybean meal and a specific mineral mixture for goats and sheep. The experimental diet was offered to the animals twice daily (09:00h and 16:00h) in similar proportions.

Daily intake was adjusted to keep leftovers between 10% to 20% of the daily amount offered, on wet basis, only for animals fed *ad libitum*. The proportional supply of feed for animals with 40 and 80% food restriction was calculated daily in relation to the average intake of animals submitted to ad libitum intake.

2.3. Nutrient Intake

To assess the nutrient intake, the amounts of feed provided and leftovers were recorded daily. From the 1st to the 15th day of the experimental period, silage and leftovers were sampled. Silage was sampled directly from the pool removed from the silo for each day and leftovers were sampled 20% of the total per animal. The concentrate ingredients were sampled directly from the feed factory on the days of ingredient blending. These samples were homogenized to obtain a composite sample for each animal.

The material was pre-dried in a forced air oven at 55 ℃ for 72 h. After drying, each sample was ground in a knife mill (Wiley mill; TECNAL, São Paulo, SP, Brazil) to a thickness of 2 mm. Then half of each sample was ground again to pass through a 1 mm sieve. The samples were then grouped and proportionally composed based on dry weight per animal. All samples were stored for later chemical laboratory analysis.

2.4. Fecal Collection and Nutrient Digestibility Trial

The digestibility test was conducted between the 11th to 15th day of the experimental period. Total fecal production was measured over a period of 96 hours, with the aid of collection bags tied to the animals. Fecal samples were collected directly into collection bags, twice a day (11:00 am and 4:00 pm), after homogenization. Then, the total fecal production of each animal was recorded and aliquots of approximately 10% of the total pool were taken, stored in individual plastic bags, labeled and frozen at -20°C. The fecal samples were dried in a forced air oven (55 ◦C), ground in 2 and 1 mm and grouped as previously described.

2.5. Urine Collection, Nitrogen Balance and Microbial CP estimation

Between 11th to 15th day of experimental period, total urine collection was performed using hoses attached to funnels, which conducted the urine to a plastic container with 100 mL of sulfuric acid (H_2SO_4) 20% (v/v) as described by (Santos et al., 2017) to preserve N compounds. At the end of each 24h, the urinary pool was weighed, registered, homogenized and filtered through two layers of cheese cloth, and a 10mL aliquot was diluted in 40mL of 0.036N H_2SO_4 solution (Valadares et al., 1999). Subsequently, a composite sample was obtained for each animal proportionally to the 4 days of sampling. The composite samples were stored at -20°C for further analysis of nitrogen, creatinine, urea, and purine derivatives (PD), which were uric acid, allantoin, hypoxanthine and xanthine.

The amount of nitrogen in the feed, leftovers, feces and urine samples was determined following the methods (Method N-001/2), proposed by the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2021).

The apparent nitrogen balance (NB) was calculated using the following formulas:

$$\text{NB or Nretained} = \text{Nintake} - (\text{Nfeces} + \text{Nurine})$$

$$\text{Nabsorbed} = \text{Nintake} - \text{Nfeces}$$

$$\text{Nutrient Intake} = \text{Nutrient psupplied} - \text{nutrient efusals}$$

Ruminal MCP synthesis was estimated by the urinary PD method using the following equations of Chen and Gomes (1992):

$$Y = 0.84X + (0.15BW^{0.75}e^{-0.25X)}$$

$$\text{Microbial. N (gN/d)} = \frac{X(\text{mmol/d})70}{0.116 \times 0.83 \times 1000}$$

where: PD (mmol/d) = purine derivatives excreted in urine; X (mmol/d) = absorbed microbial PD. The N content of purines is 70 mg N/mmol. The total purine-N:N ratio in mixed rumen microbes is taken as 0.116 (11.6:100). Digestibility of microbial purines is assumed to be 0.83, considered the average digestibility value for microbial nucleic acids based on observations reported in the literature.

2.6. Blood Metabolites

On day 15th, 10 mL blood samples were collected from the animals directly from the jugular vein using vacuum tubes with clotting accelerator gel (BD Vacutainer®, SST II Advance, Franklin Lakes, NJ, USA). Samples were immediately centrifuged at 3500 rpm for 15 min to obtain serum. Subsequently, the serum obtained was transferred to labeled Eppendorf tubes and stored at -20°C for further analysis of urea.

2.7. Laboratory analysis

The samples of ingredients, refusals and feces, were analyzed according to the protocols described by the INCT-CA; Detmann et al. (2021). For dry matter (DM, method G-003/1), the N contents were measured by the Kjeldahl method and crude protein (CP) was calculated as N × 6.25 (CP, method N-001/2), ether extract (EE, method G-005/2), and ash (method M-001/2).

The neutral detergent fiber (NDF), assayed with a heat stable amylase and without adding sodium sulfite, and acid detergent fiber (ADF) contents were determined according to (Mertens, 2002) and VAN SOEST, 1991 as described in the INCT-CA (Method F-002/2). These samples were placed in plastic pots with 100 ml of detergent in the proportion of 1g of sample per 100 ml of detergent and autoclaved at 110°C (Barbosa et al., 2015). The NDF was corrected for ash and nitrogen by incinerating the neutral detergent digestion residues in a muffle oven at 600 ℃ for 4 h, and the correction for protein was performed by the neutral detergent insoluble protein method (Licitra et al., 1996).

Urinary PDs, including Allantoin, xanthine, and hypoxanthine were carried out based in calorimetric method according to Chen and Gomes. (1992). Uric acid was quantified by an enzymatic method in uricase and peroxidase with a commercial kit (uric acid– Ref.:140 Labtest, Minas Gerais, Brazil). Creatinine was quantified in all the urine samples by the by end point reaction enzymatic-colorimetric method from an alkaline picrate reaction using commercial kits for analyses (Creatinine – Ref.:35 Labtest, Minas Gerais, Brazil).

Serum urea concentration was determined by the enzymatic-colorimetric method in the presence of salicylate and sodium hypochlorite. The analyzes were carried out using commercial kits (Urea – Ref.:27, Labtest)., with readings taken by a semiautomatic spectrophotometer (SBA 200®, CELM, São Caetano do Sul, Brazil) at the respective wavelengths. All laboratory analyzes were performed at the Animal Nutrition Laboratory (LANA), Federal University of Bahia.

2.8. Statistical analyses

The data were subjected to statistical analysis using PROC MIXED (SAS Inst. Inc., Cary, NC), according to the following model was applied:

$$Yijk = \mu + Si + Lj + SLij + eij$$

where: Yij = dependent variable measured in the experimental unit; μ = overall mean; Si = fixed effect of specie; Lj = fixed effect of feeding levels; SL ij = fixed effect of the interaction between specie and feeding level; eij = random error.

The ANOVA F test was conclusive when comparing the two species, while the data for the feeding levels were compared by orthogonal linear (-1 0 1) or quadratic (1 -2 1) contrasts using PROC MIXED of the SAS software (version 9.2), with a probability level of 5% for the occurrence of error type I. In case of significant interaction effect, the SLICE statement (SAS 9.2) was used to evaluate treatment means.

3. RESULTS

3.1. Intake and digestibility

There was no effect of the interaction between diet and species for the intake and digestibility of DM and for the other nutrients (P<0.001; Table 2), with exception for EE digestibility (P=0.02). The DM and nutrient intake by sheep were higher than goats for (P<0.001) all nutrient components. There was a positive linear effect on EE and CP intakes in function of the diets, regardless the specie. A quadratic effect was also observed for the other nutritional components.

The diet x species interaction influenced the EE digestibility (P= 0.02; Table 2). Species showed similar digestibility of this nutrient when DM intake was restricted to 60 and 20 % (Figure 1). However, there was a significant difference between species only when DM intake was 0% of ad libitum, in which goats digested more EE. The animals fed with the ad libitum treatment (0% of feed restriction) presented the lower nutrient digestibility. The DM, OM, and CP and NFC digestibility presented negative linear effect (P<0.001) in function of the diets, regardless the specie. The aNDFn digestibility presented a quadratic effect (P<0.001). Basically, the most restricted diet (60% of feed restriction), presented reduced nutrient intakes and higher digestibility, and the other two treatments (20 and 0% of feed restriction) presented similar values for intake of the nutritional components.

3.2. Nitrogen balance and microbial efficiency

There was no effect of the interaction between diet and species for the variables related to nitrogen balance (P<0.001; Table 3), with exception for urinary N (P<0.001). The N intake, fecal and absorbed N in sheep were higher than goats (P<0.001), as well as the percentage of N retention in function of N intake or N absorbed (P<0.001). There was a positive linear effect of DM intake on N intake, fecal, and absorbed N, regardless the specie.

There was quadratic effect of DM intake on the percentage of N retention in function of N intake or N absorbed (P<0.001). The most restricted diet (60% of feed restriction) presented reduced N retention, and the other two treatments (20% and 0% of feed restriction) presented similar values for N retention in function of N intake or N absorbed.

N excreted in urine was lower (P < 0.001) in goats than in sheep (2.6 and 7.12; respectively), resulting in similar mean N retention in goats and sheep, expressed in g/d (7.85 and 7.50 g/d, respectively), resulting in similar mean N retention in goats and sheep, expressed in g/d (7.85 and 7.50 g/d, respectively). However, goats had higher (P<0.001) percentage of N retained based on N ingested (53.1% and 34.1%) or absorbed (70.3% and 47.4%) compared to sheep.

The intercept of these equations allowed the estimation of fecal metabolic nitrogen in 45 and 115 mg N/kg $BW^{0.75}$ for goats and sheep, respectively (Figure 2A). The regression between N intake and retained N, expressed in g of N/kg $BW^{0.75}$ was described by the following equations, considering goats, the equation obtained is: $N_{retaind} = 0.65 \times N_{intake} - 0.13\ r^2 = 0.98$, considering sheep $N_{retaind} = 0.51 \times N_{intake} - 0.26\ ; r^2 = 0.85$. The intercept of these equations allowed to estimate the endogenous N fraction in 127 and 257 mg N/kg $BW^{0.75}$ for goats and sheep, respectively, (Figure 2B).

There was effect of the interaction between diet and species for the variables related to MCP and microbial efficiency (P<0.001; Table 3), Sheep presented higher microbial N synthesis than goats for all levels of DM intake (Figure 3B). Additionally, goats showed lower (P<0.001) microbial efficiency (g MCP/kg dOMi) than sheep when DM intake was 0% feed restriction, but no differences were observed when DM intake was 60 and 20% of feed restriction (P=0.24 and P=0.79; respectively, Figure 3C). Goats had lower urinary N

losses compared to sheep for all levels of DM intake (Figure 3A). The urinary N loss in sheep showed a linear increase, with the reduction of feed restriction. However, this same parameter showed a quadratic behavior in goats, in which the lowest value observed when DM intake was 20% of feed restriction.

The relationship between fecal N excretion and N intake, expressed in g N/kg $BW^{0.75}$, was described by the following equations, considering goats, the equation obtained is: $N_{fecal} = 0.28 \times N_{intake} - 0.05; r^2 = 0.95$, considering sheep, $N_{fecal} = 0.35 \times N_{intake} - 0.12; r^2 = 0.69$ the equation

3.3. Excretion of urea, creatinine and purine derivatives

Dietary restriction (P=0.16 and P=0.28), species (P=0.18) and their interaction (P=0.48) had no significant effects on blood urea concentration (Table 4). There was no effect of the interaction between diet and species for the variables related to ureic profile (P<0.001; Table 4), with exception for urinary urea excretion (P<0.001). Goats have lower urinary urea excretion compared to sheep (Figure 4) when the feed restriction was 20% (P < 0.001). However, when the feed restriction was 60 and 0%, urinary urea excretion was similar between species (P=0.46 and P=0.4, respectively). When data were reanalyzed by species, goats showed a quadratic effect on urinary urea excretion (g/d) with lower excretion when dietary restriction was 20% of feed restriction.

The relationship between creatinine excretion and the feed restriction did not result in a significant regression (P>0.05). The linear model fitted only a daily mean value of 17.95 mg/kg BW (Figure 5), represented by the mean of the intercepts of the equations, described by the following equations, considering goats, the equation obtained is: $Creatinine = 0.03 \times N_{intake} - 16.79 ; r^2 = 0.34$, considering sheep $Creatinine =$

$0.005 \times N_{intake} - 18.54; r^2 = 0.01$. Thus, it can be said that creatinine excretion was not altered in function of feed intake. The highest means of creatinine (P<0.01 and P=0.05) in mg/day and mg/kg of BW, respectively, were found in sheep than in goats submitted to the same feed systems.

The effect of the interaction between diet and species (Table 4) was detected for allantoin excretion (P=0.02), uric acid (P=0.03), total PD excretion (P=0.01) and absorbed purines (P=0.01; Figures 6A, B, C and D). Goats had lower proportions of allantoin, total PD excretion and absorbed PD than sheep for any level of DM intake (P < 0.05). However, there was no difference in uric acid excretion between goats and sheep when feed restriction was 60% of feed restriction (P = 0.08). Goats had lower amounts (P<0.001) and proportions of xanthine and hypoxanthine (P = 0.03) than sheep. There was a linear effect of the DM intake levels in xanthine and hypoxanthine amounts excreted.

Daily urinary PD excretion (mmol/kg BW) increased linearly (P < 0.001) with DM intake (Figure 7), described by the following equations, considering goats, the equation obtained is: $PD = 0.011 \times IDM - 0.03; r^2 = 0.88$, considering sheep $PD = 0.01 \times IDM - 0.01; r^2 = 0.71$. With dOM intake (Figure 7), described by the following equations, considering goats, the equation obtained is: $PD = 0.02 \times dMO - 0.51; r^2 = 0.82$ considering sheep $PD = 0.02 \times dMO - 0.42; r^2 = 0.47$, and TDN intake (Figure 8), described by the following equations, considering goats, the equation obtained is: $PD = 0.01 \times TDN - 0.50; r^2 = 0.83$ considering sheep $PD = 0.02 \times TDN - 0.15; r^2 = 0.49$.

4. DISCUSSION

When the animals in our study submitted to a feed restriction of 60%, the nutrient utilization efficiency was similar between the two species. According to the authors Pereira

Filho et al. (2005), food restriction practiced at moderate levels maintains food efficiency and reduces food losses. The greater ability of goats to tolerate the feed restriction is a result of a long evolutionary process in natural conditions, where feed availability fluctuates seasonally, as in arid and semi-arid region (Moura et al., 2020). The goats' eating habits also contribute for this resilience. According to Van Soest. (1994; cap 4), intermediate selectors, as goats, have lower metabolic losses than grazing species as sheep.

Studies have compared feed intake and nutrient digestibility between sheep and goats (eg, Abidi et al., 2009; Alciade et al., 2000; Yañez-Ruiz and Molina Alcaide, 2008; Dos santos et al., 2018). The greater digestibility of DM and nutrients with increasing food restriction was due to the lower rate of passage of food through the digestive tract. As there is a negative correlation between intake and digestibility (Mertens et al., 1980, Colucci et al., 1982; Pino et al., 2018), there is a positive correlation between retention time and digestibility (Gindri et al., 2021, Paiva. 2021).

Regarding the greater digestion of NDF for goats compared to sheep, Askar et al. (2016) reported greater efficiency of goats in the use of fiber when compared to sheep. We suggest that the better use of fiber by goats compared to sheep resulted from longer retention times of ruminal digesta leading to a higher rate of fermentation, greater activity of cellulolytic bacteria (Gihad, 1976) and possibly a greater recycling capacity (Esmare et al., 2012) and conserves N in the body (Dos santos et al., 2018).

In our study, the percentage of N retained from consumed and absorbed in goats was higher than in sheep. The greater retention of nitrogen in relation to the percentage of nitrogen consumed and absorbed by goats in relation to sheep, may indicate that there was an intensification of nitrogen recycling and an increase in the conservation of N compounds,

reducing the amount of protein lost to the environment when these animals were submitted to food restriction.

Goats were able to degrade the feed more extensively and more fiber N was released as digestible N, allowing goats to ingest more digestible energy than sheep when consumed the same diet. Potentially, digestible energy intake, amino acid intake, and nutrient synchronization contribute to greater nitrogen retention (Berends et al., 2014). The consequently using AA more efficiently.

The use of nitrogen compounds in restricted animals was marked by a decrease in low urinary nitrogen excretion and urinary urea excretion (g/day) to maintain the plasma urea pool, as a response to the animal's physiological control (Muscher et al., 2010). Indicated by a positive BN, as there an intensification of nitrogen recycling and greater conservation of compounds in sheep submitted to 60% of feed restriction (Pereira et al. 2018; Campos et al., 2019). With low feed intake, certain regulatory mechanisms, such as the breakdown of myofibrillar proteins, are activated to produce energy (Batista et al., 2016). In these cases, tissue protein mobilization provides N for urea production (Zhou et al., 2019a), indicating greater tissue protein mobilization in response to energy deficiency.

The influence of feed restriction on N retained/N ingested and N apparently digested is in line with the study by Campos et al. (2019). In addition, the authors (Campos et al., 2019) observed that greater feed restriction reduced the % of N retained in relation to the N ingested and absorbed in lambs submitted to three feeding levels (0, 30 and 60% of feed restriction). These results may related to greater efficiency in the use of ammonia in response to dietary restrictions.

Ammonia is the most important source of N for microbial amino acid (AA) synthesis in the rumen, and urea is an important source of N for ammonia when dietary N is deficient

(Zhou et al., 2019b). In the present study, there was no change in plasma urea concentration, but urea excretion (g/d) in urine decreased with increasing feed restriction.

The efficiency of urea utilization in ruminants is normally low, due to the faster rate of hydrolyzed ammonia from urea than utilized by bacteria in the rumen (Zhou et al., 2019b). When ruminant animals are subjected to feed restriction, efforts to increase the efficiency of urea utilization are directed towards minimizing NH_3 absorption, aiming to reduce the rate of urea hydrolysis in the rumen (Campos et al., 2019). This increase the ability of rumen microorganisms to assimilate NH_3 (Rodrigues et al., 2016), thus reducing the loss of N by the animal.

In the present study, goats showed lower urinary urea excretion compared to sheep when the feed restriction was 20%, these animals also showed the lowest values of urinary N excretion, ability to reduce urinary N excretion and increase fraction of N that is recycled to the rumen was an attempt to make up for deficiencies. The results found in our study demonstrate the greater efficiency of goats in overcoming the deficiencies imposed by feed restriction.

Microbial N production was similar between goats and sheep when submitted to greater food restriction. The reduction in microbial N from 6.39 to 2.5 g/day in animals subjected to food restriction can be explained by the reduction in DM intake, providing less fermentable substrates for microbial synthesis in the rumen.

Animals subjected to 60% DM restriction showed MCP of 74 g/kg dMOI and 71g/kg of TDN lower than that found by Paiva. (2021) in lambs with 60% DM of 135 g/kg TND, 114 g/kg of TDN (Da silva et al., 2020), found in studies with lambs in tropical regions and recommended by the NRC (2001) of 130 g/kg TDN and greater than 53.10 g/kg TDN (Pereira

et al., 2018) and 46.34 g/kg of MO found by Campos et al.(2019) in lambs subjected to 60% DM restriction. But close to the average value of 76.2 g MCP per kg TDN was obtained in studies with sheep and goats in tropical regions receiving control diets (Fonseca et al.,2006; Carvalho et al., 2010; Pereira et al., 2016). On the other hand, animals fed with 40% DM of food restriction showed the lowest MCP (55.9 and 58.6 g/kg TDN and dMOI, respectively).

The synthesis of microbial proteins is relatively dependent on the availability of carbohydrates and nitrogen in the rumen (NRC, 2001). Probably the oxidation of amino acids through the gluconeogenesis pathway to adjust the available energy in the rumen may have reduced the rate of microbial nitrogen production in animals under feed restriction (Campos et al., 2019). The metabolic activity of ruminants and the degradation rate of nucleic acids is altered when they are subjected to feed restriction (Fujihara et al. 1987), directly interfering with the absorption of purines and the production of microbial N. Corroborating this hypothesis, in our study both the absorption of purines and the synthesis of microbial N decreased linearly with the increase in feed restriction.

Rumen microbes constitute the major source of protein supply to the ruminants. Purines from rumen microbes are metabolized and excreted in the urine as their end products: hypoxanthine, xanthine, uric acid, and allantoin (Chen and Ørskov,2004). Total digestible nutrients were reduced by approximately 10% when intake was transitioned from restricted to voluntary. There is a linear relationship between excretion of purine derivatives and intake of DM, dOM and TDN (Santos et al., 2021; Barbosa et al., 2011; Chen e Gomes, 1992; Braga et al., 2012).

According to Santos et al., (2020) OM consumption is a better marker of available energy in the diet than DM consumption in cattle, as energy is the main limiting factor for ruminal bacterial synthesis. In our study, there was a linear correlation when correlating PD

excretion (mmol/kg BW) with DM intake (g/kg BW), OM (g/d) and TDN (kg/d). DM intake apparently correlated better with PD excretion.

Urinary DPs are metabolic end products of purines, mainly derived from microbial amino acids, since food-borne nucleic acids are completely degraded in the rumen (McAlian et al., 1973; Mcallan and Smith, 1973), the purines of nucleic acids Microbes are then absorbed, degraded and excreted in the urine as their derivatives (Chen and Gomes, 1992).

Urinary PD excretion is used to predict rumen microbial protein synthesis in ruminant. The principle is that duodenal purine bases (PB), as a microbial marker, are efficiently absorbed and the majority of their derivatives excreted via the kidney (Chen and Gomes, 1992). In our study, the species showed different PD excretions. Goats had lower excretion of individual PD, total PD and absorbed purines compared to sheep, as observed by Dos santo et al. (2018) in goats and sheep. Daily urinary PD excretion is correlated with DM intake (Dórea et al., 2017).

In the present study, DM intake was lower in goats, the reduction in DM intake probably reduced fermentable substrates for microbial synthesis in the rumen, decreasing the excretion of DP in the urine of goats. In our study, total PD excretion significantly decreased with feed restriction authors Campos et al. (2020), we also found that PD excretion or ruminal microbial protein production in sheep was proportional to DM intake in sheep subjected to food restriction.

In sheep and goats, purine bases are converted to allantoin, uric acid, xanthine, and hypoxanthine (Chen and Gomes, 1992). Allantoin is the most DP in sheep (Chen et al., 1990) and goats (Belenguer *et al.,* 2002). In our study, the proportions of allantoin, uric acid, and the sum of xanthine plus hypoxanthine for total PD averaged 78.5, 17.3, and 4.0%, respectively; These values are consistent with those reported by (Chen and Gomes, 1992)

(allantoin 60-80%, uric acid 30-10%, xanthine plus hypoxanthine 10-5 %). There was no effect of feed restriction and species on DP proportions, except for the sum of xanthine plus hypoxanthine, which was higher in sheep.

The observed differences in the ratio of xanthine plus hypoxanthine in goats and sheep may be due to higher xanthine oxidase activities in tissues of goats compared to sheep (Chen and Ørskov,2004). Xanthine oxidase breaks down xanthine and hypoxanthine to uric acid and allantoin before excretion in the urine (Chen and Gomes., 1992). Its higher activity in goats probably explains the lower proportion of these derivatives (xanthine and hypoxanthine) in relation to the total PD excreted in the urine of goats.

In our study, daily creatinine excretion as a function of $BW^{0.75}$ was not influenced by DOM intake. An expected result, since there are already studies demonstrating that dietary factors do not influence creatinine excretion in cows cattle (Braga et al., 2012), goat and sheep (Dos santos et al., 2018). Creatinine excretion is a constant function of animal body weight and is often related to muscle tissue Dos Santos et al. (2018).

The results of the present study show that the pattern of creatinine excretion as a function of muscle weight differs between species. Similar to our results, Dos Santos et al. (2018) reported difference in creatinine excretion between goat and sheep species. According to the authors Dos Santos et al. (2018) these results can be justified by differences in body composition, where goats have a lower percentage of lean tissue compared to sheep.

5. CONCLUSION

Feed restriction improves nutrient utilization efficiency but decreases the efficiency of microbial synthesis in the rumen. Microbial CP efficiency based on dOM intake was similar between goats and sheep when subjected to feed restriction.

Goats are more efficient to use dietary fiber and to conserve N than sheep when subjected to feed restriction. However, sheep are more effective than goats, as they consume more and enable greater production of Pbmic and consequently have greater consumption of Mod and TDN in addition to having greater efficiency of Pbmic:MOd

There is a linear relationship between excretion of purine derivatives and dry matter intake, and this excretion can estimated from the intake of total digestible nutrients or digestible organic matter. On the other hand, creatinine excretion is not altered with feed restriction.

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Table 1

Chemical composition of Corn Silage, concentrate and experimental diet.

Chemical composition	Corn Silage	Concentrate	Diet
Dry matter (g/kg as fed)	305	883	478
Ash	42	10	61
Organic matter	958	898	939
Crude protein	75	358	159
Ether extract	22	52	31
aNDFn	548	139	425
NFC	313	345	322
iNDF	228	23	167
TDN	729	713	723

aNDFn - neutral detergent fiber with thermolabile amylase corrected for ash and nitrogen; NFC - non-fiber carbohydrates; iNDF-indigestible neutral detergent fiber; TDN-The total digestible nutrients.

Table 2
Intake and digestibility of nutrients in goats and sheep submitted to feed restriction

Items	Levels of feed restriction (FR)			Specie		SEM	P-value			
							Diets		Specie	Diets × Specie
	60%	20%	0%	Goats	Sheep		L	Q		
Intake (g/kg of $BW^{0.75}$)										
Dry matter	28.7	60.1	74.1	49.5	59.1	1.62	<0.001	0.01	<0.001	0.39
aNDFn	12.15	25.5	27.5	19.6	23.8	0.66	<0.001	<0.001	<0.001	0.41
Intake (g/day)										
Dry matter	315	650	777	466	695	21.2	<0.001	0.01	<0.001	0.14
Organic matter	297	612	730	438	654	24.7	<0.001	0.01	<0.001	0.14
Ether extract	9.94	20.5	28.3	15.9	23.4	0.80	<0.001	0.19	<0.001	0.12
Crude protein	55,6	115	152	86.8	128	4.62	<0.001	0.08	<0.001	0.13
aNDFn	134	275	289	185	280	11.1	<0.001	<0.001	<0.001	0.14
NFC	97.4	201	259	150	221	6.98	<0.001	0.04	<0.001	0.17
TDN	218	441	448	311	435	22.6	<0.001	0.01	<0.001	0.17
DOM	217	441	445	308	428	22.0	<0.001	0.01	<0.001	0.18
CP:DOM	255	260	349	275	301	9.85	<0.001	0.01	0.08	0.12
Digestibility (g/kg)										
Dry matter	711	698	598	693	645	17.4	<0.001	0.11	0.07	0.18
Organic matter	734	721	616	712	668	16.5	<0.001	0.08	0.08	0.18
Ether extract	860	856	805	846	835	5.1	<0.001	0.01	0.15	0.02
Crude protein	769	751	700	758	722	14.0	0.01	0.45	0.09	0.20
aNDFn	610	597	363	561	486	25.4	<0.001	0.01	0.05	0.14
NFC	869	858	825	856	845	9.3	0.01	0.45	0.42	0.43

$BW^{0.75}$ - metabolic weight; aNDFn - neutral detergent fiber with thermolabile amylase corrected for ash and nitrogen; NFC - non-fiber carbohydrates; TDN-total digestible nutrientes; DOM - digested organic matter content in diet; CP: DOM-ratio between dietary CP and dietary DOM; SEM - standard error of the mean; L - linear effect; Q - quadratic effect. P<0.05

Table 3
Nitrogen balance and microbial crude protein synthesis in goats and sheep submitted to feed restriction

item	Levels of feed restriction (FR)			Specie		SEM	P-value			
							Diets		Specie	Diets×Specie
	60%	20%	0%	Goats	Sheep		L	Q		
Nitrogen (g/day)										
Intake	8.89	18.3	24.4	13.9	20.5	0.67	<0.001	0.08	<0.001	0.13
Fecal	2.07	4.55	7.41	3.45	5.90	0.38	<0.001	0.71	<0.001	0.06
Urinary	4.02	5.12	5.44	2.60	7.12	0.27	<0.001	0.29	<0.001	<0.001
Absorbed	6.84	13.8	17.0	10.4	14.6	0.74	<0.001	0.07	<0.001	0.23
Reteined	2.83	8.7	11.5	7.85	7.50	0.64	<0.001	0.10	0.67	0.86
$N_{retention}$ (% N_{intake})	32.7	49.7	48.4	53.1	34.1	2.18	<0.001	0.01	<0.001	0.78
$N_{retetion}$ (% $N_{absorved}$)	42.1	66.2	68.3	70.3	47.4	2.15	<0.001	<0.01	<0.001	0.25
Microbial production (g/day)										
Microbial N	2.50	4.08	6.39	3.33	5.32	0.18	<0.001	0.19	<0.001	0.01
Microbial CP	15.7	25.5	39.9	20.8	33.2	1.13	<0.001	0.19	<0.001	0.01
Microbial efficiency							<0.001			
g MCP / kg TDN	71.1	55.9	84.7	66.9	74.3	2.69	<0.001	<0.001	0.07	0.01
g MCP/ kg dOM intake	74.4	58.6	90.0	70.2	78.5	2.91	<0.001	<0.001	0.06	0.01

N- nitrogen; CP- crude protein; MCP-microbial crude protein; TDN-total digestible nutrients; dOM - digestible organic matter; SEM - standard error of the mean; L - linear effect; Q - quadratic effect. P<0.05.

Table 4

Plasm urea concentration and urinary excretions of PD, urea and creatinine in goats and sheep submitted to feed restriction

Items	Levels of feed restriction (FR)			Specie		SEM	P-value			
							Diets		Specie	Diets×Specie
	60%	20%	0%	Goat	Sheep		L	Q		
Plasma										
Urea mg/dL	47.6	48.9	39.1	48.5	41.9	3.36	0.16	0.28	0.18	0.48
Urea mmol/L	7.90	8.13	6.50	8.06	6.96	0.56	0.16	0.28	0.18	0.48
Urinary excretion										
Urea										
g/day	16.6	23.1	17.7	12.8	25.4	2.47	0.80	0.12	0.01	0.02
g/kg $BW^{0,75}$	1.53	2.04	1.65	1.33	2.15	0.25	0.72	0.16	0.01	0.01
Creatinine										
mg/day	429	427	416	356	493	15.1	0.63	0.85	<0.001	0.91
mg/kg BW	17.8	18.1	18.1	17.8	18.3	0.17	0.31	0.72	0.05	0.49
Purine derivatives (mmoL/day)										
Allantoin	2.5	4.9	8.2	3.9	6.5	0.26	<0.001	0.28	<0.001	0.02
Xant. and hipoxant	0.15	0.29	0.36	0.14	0.39	0.04	0.01	0.47	<0.001	0.12
Acid uric	0.58	1.07	1.77	0.84	1.44	0.07	<.001	0.31	<0.001	0.03

Total PD	3.24	6.26	10.28	4.87	8.33	0.31	<.01	0.29	<0.01	0.01
PurineAbsorbed	3.44	5.61	8.78	4.57	7.32	0.25	<.01	0.19	<0.01	0.01
Purine derivatives (%)										
Allantoin	77.6	78.6	79.5	79.2	77.9	0.61	0.08	0.99	0.14	0.39
Acid uric	17.9	17.1	17.1	17.5	17.2	0.33	0.16	0.46	0.52	0.67
Xant. and hipoxant	4.48	4.30	3.36	3.23	4.86	0.48	0.19	0.60	0.03	0.23

PD-purine derivatives; SEM - standard error of the mean; L - linear effect; Q - quadratic effect. P<0.05.

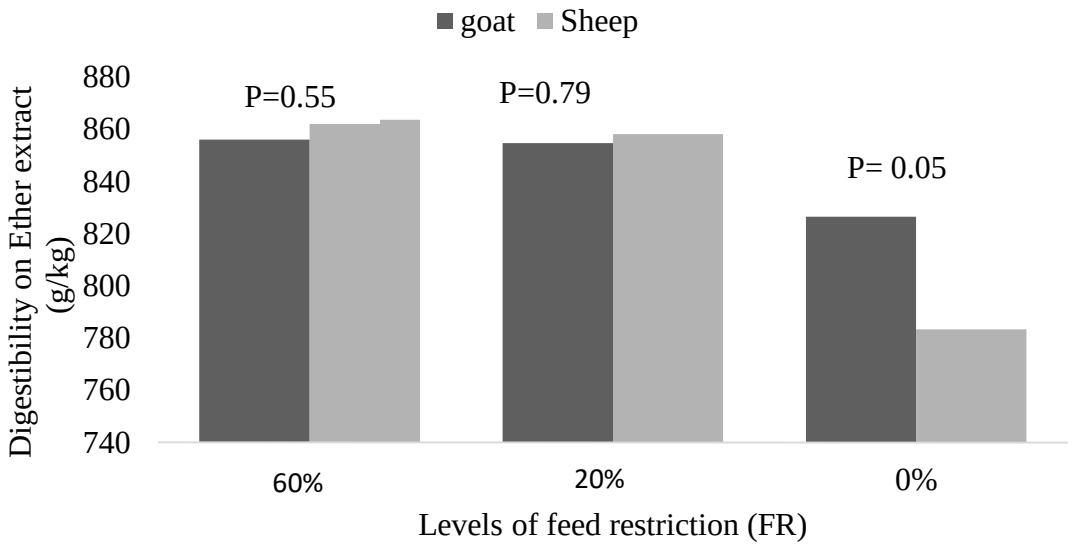

Figure 1. Slicing of the Diet × Specie interaction effect on EE digestibility (g/kg DM) in goats and sheep submitted to feed restriction. 0% or ad libitum intake

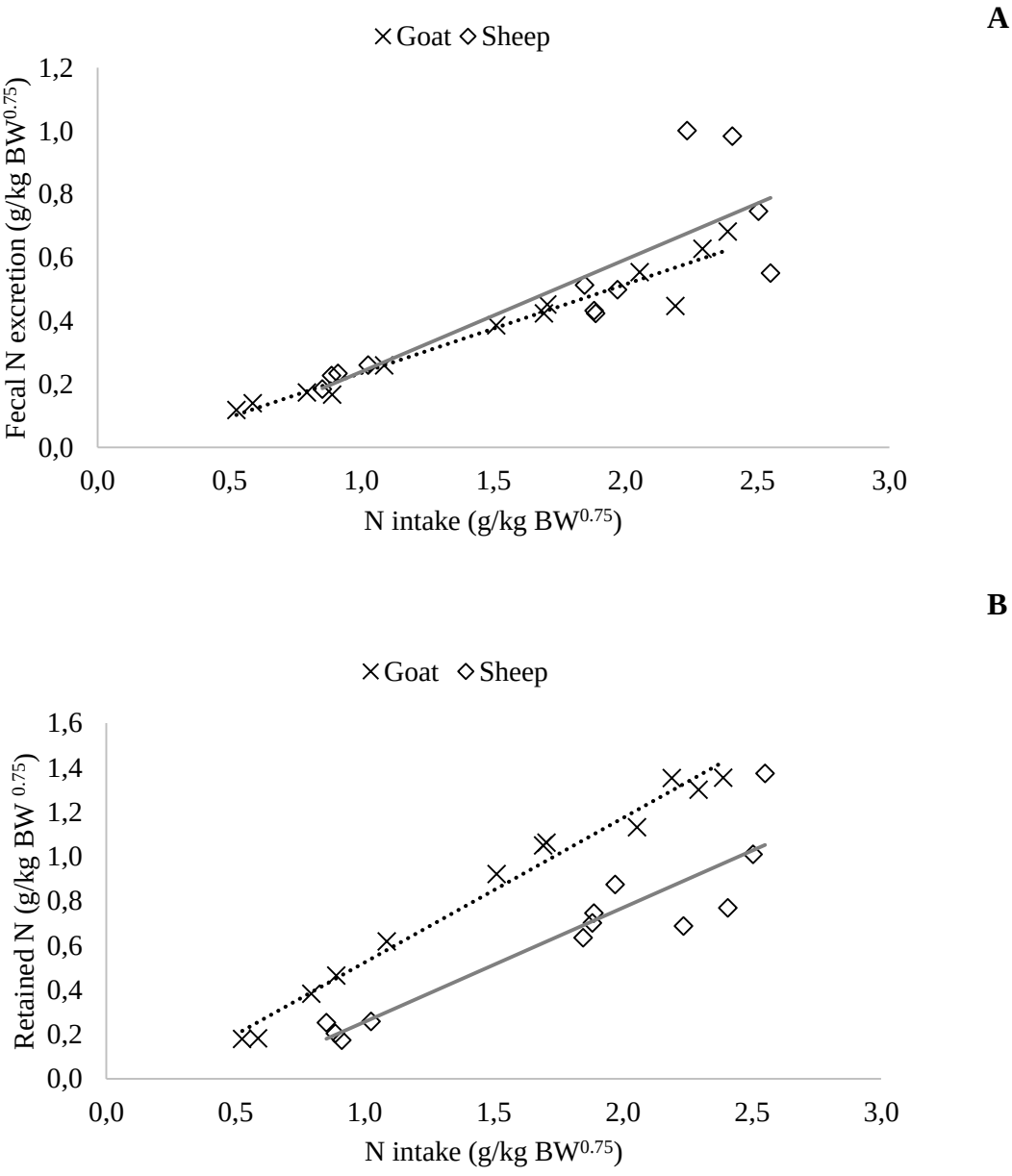

Figure 2. A- Relation between fecal N excretion and N intake in goats (y = 0.2802x - 0.0452; r^2 = 0.95) and sheep (y = 0.3549x - 0.1156; r^2 = 0.69). **B-** Relation between retained N and N intake (g/kg $BW^{0.75}$) in goats (y = 0.6502x-0.127; r^2 = 0.98) and sheep (y = 0.5137x - 0.2575; r^2 = 0.85).

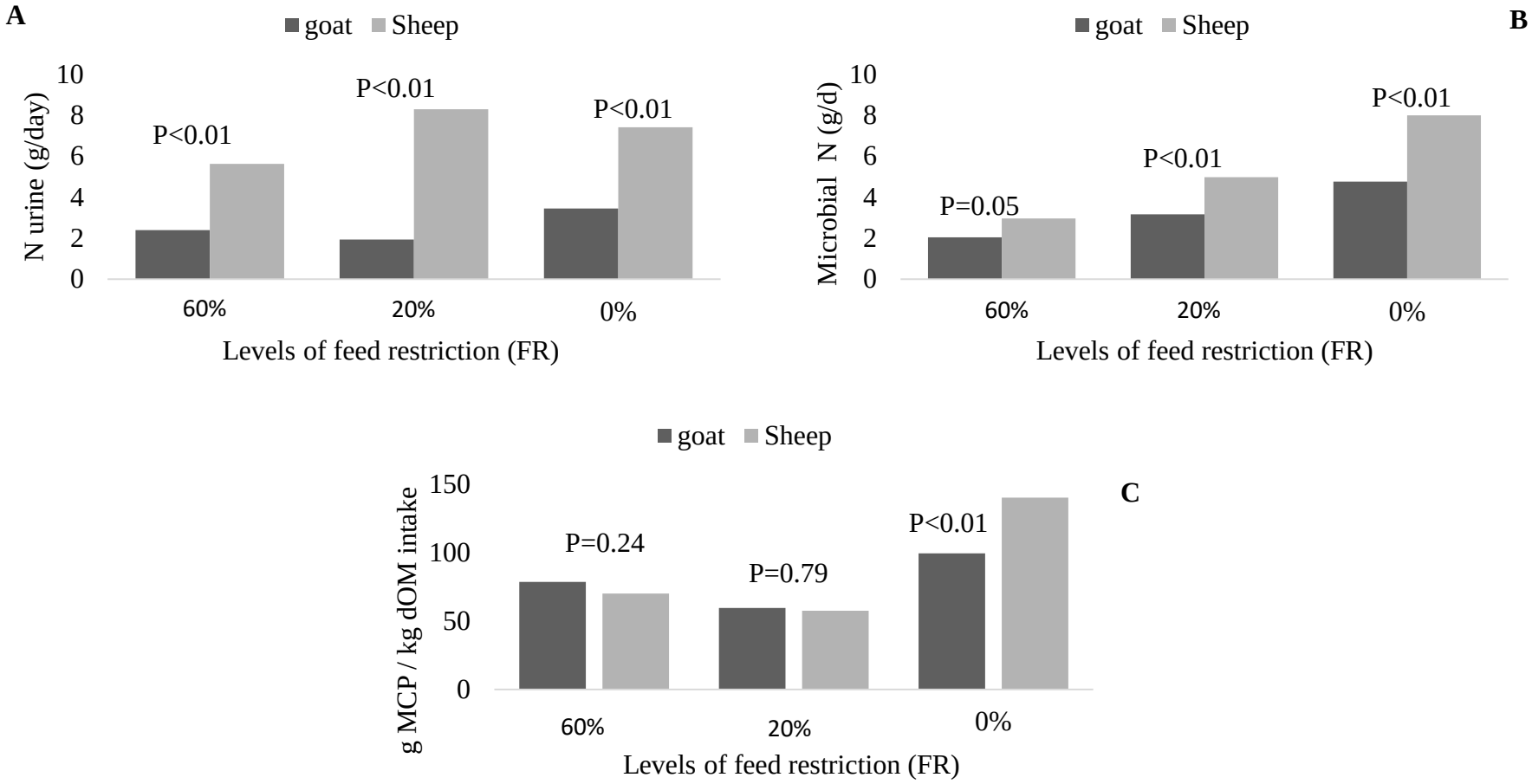

Figure 3. Slicing of the Diet × Specie interaction effect on the urinary excretion of N and urea (A and B); g of microbial N (C) and microbial efficiency in g of microbial N pr kg of digestible OM intake in goats and sheep submitted to feed restriction 0%.

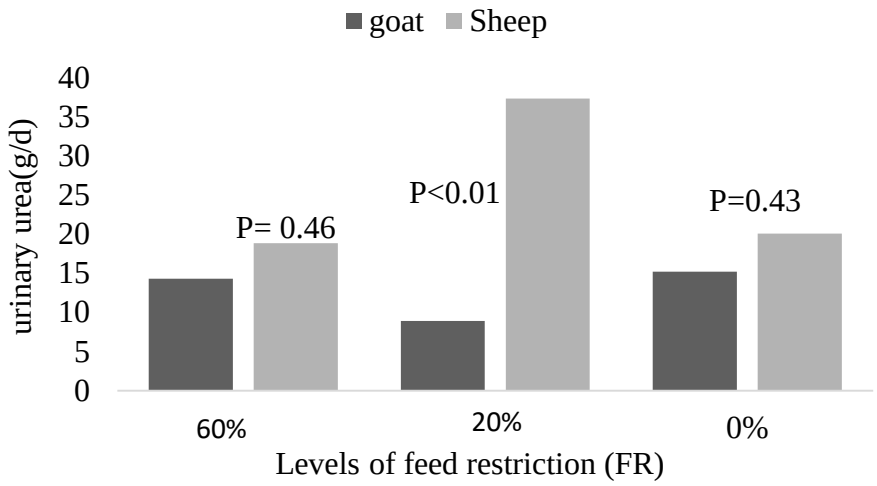

Figure 4. Slicing of the Diet × Specie interaction effect on the urinary excretion of urea in goats and sheep submitted to feed restriction 0%.

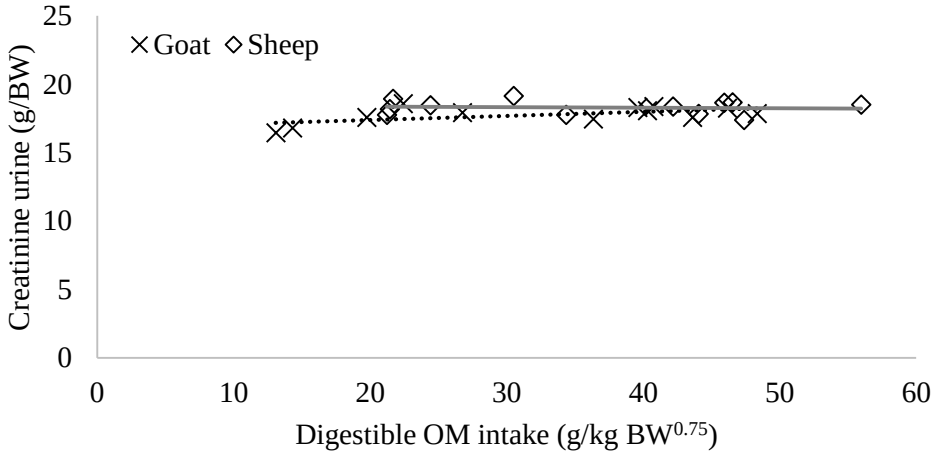

Figure 5. Relation between urinary creatinine excretion and digestible OM intake (g/kg $BW^{0.75}$) in goats $Creatinine = 0.03dMO + 16.79; r^2 = 0.34$ and sheep $Creatinine = -0.005dMO + 18.45; r^2 = 0.01$.

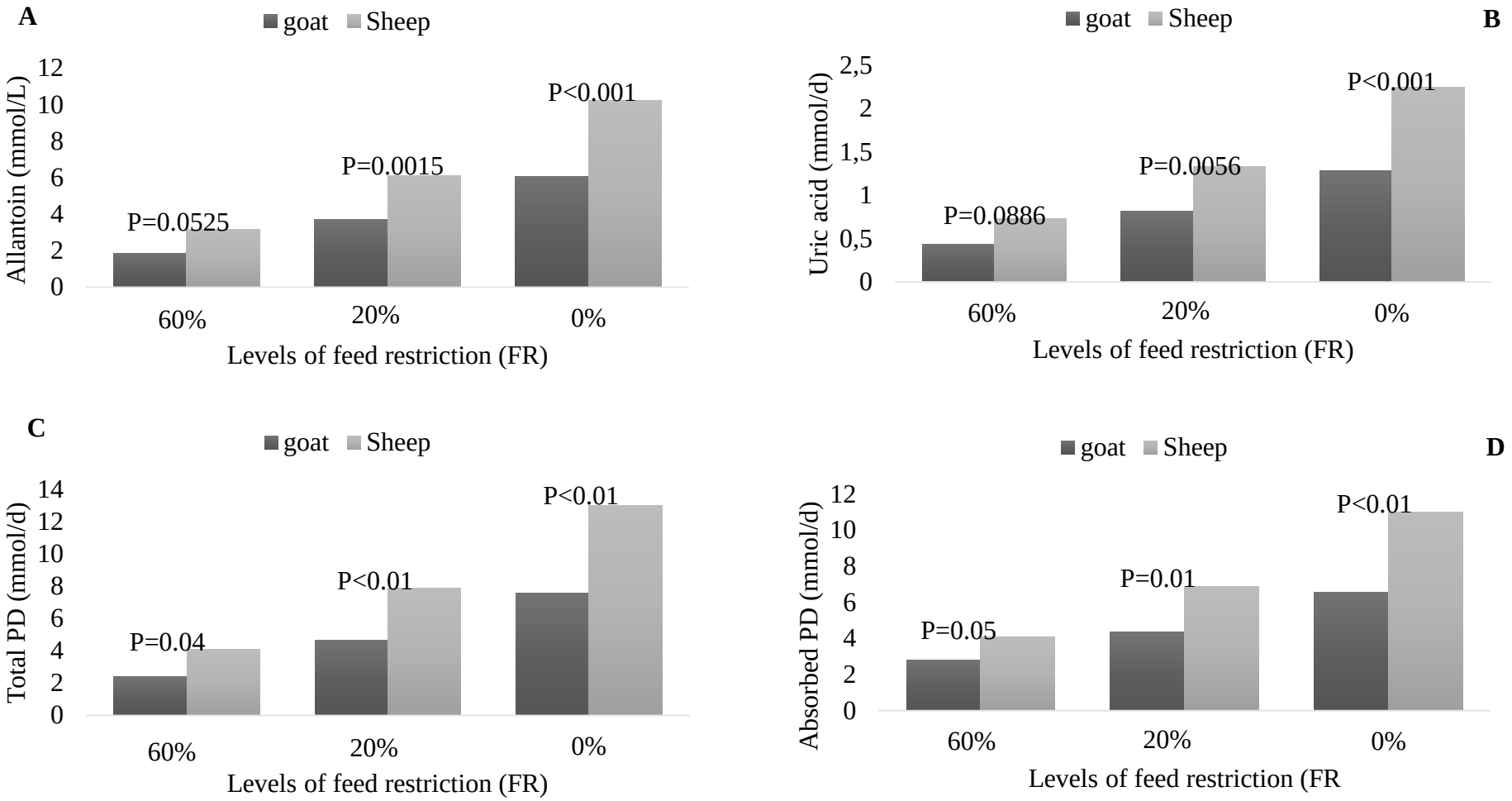

Figure 6. Slicing of the Diet × Specie interaction effect on excretion to the urinary allantoin (A), uric acid (B),total purine derivatives (DP; C) and absorbed DP in goats and sheep submitted to feed restriction 0%.

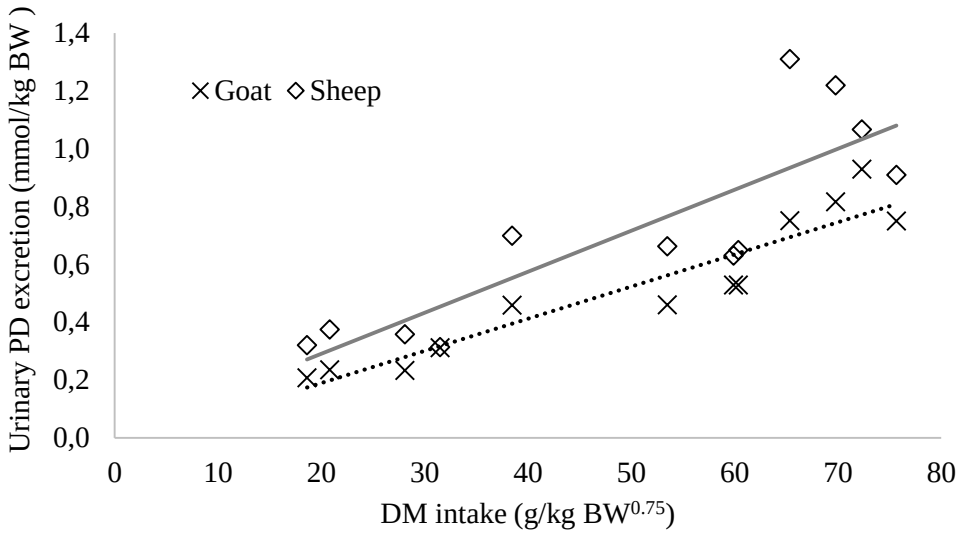

Figure 7. Relation between urinary excretion of purine derivatives (PD) (mmol/day) and digestible organic matter (dOM) intake (kg) in goats (PD =0.0175 × dOMI - 0.512; $r^2 = 0.82$) and sheep (PD = 0.0185 × dOMI + 0.4171; $r^2 = 0.47$) fed with different DM intake levels.

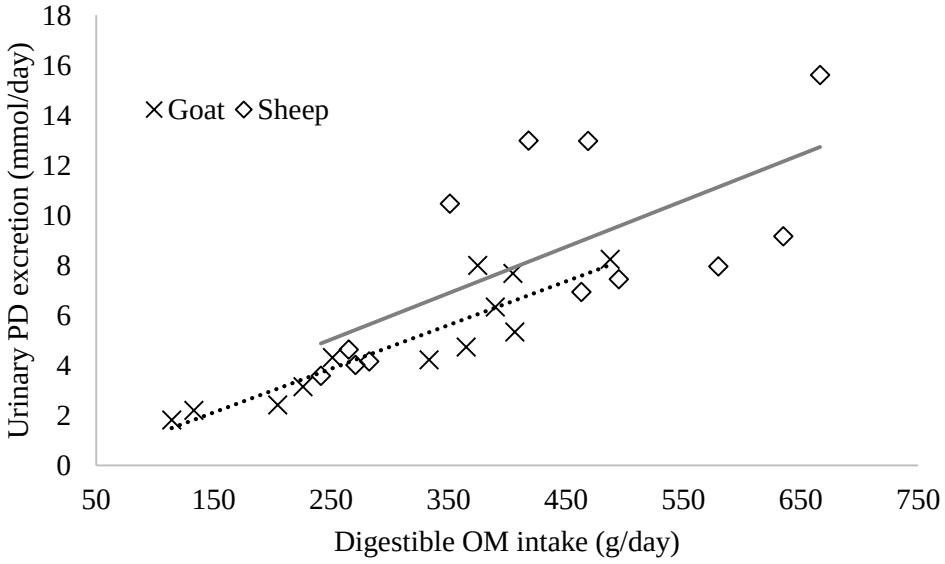

Figure 8. Relation between urinary purine derivative (PD) excretion and dry matter (DM) intake in goats (y = 0.0111x - 0.0339; $r^2 = 0.88$) and sheep (y = 0.0142x + 0.0073; $r^2 = 0.72$).

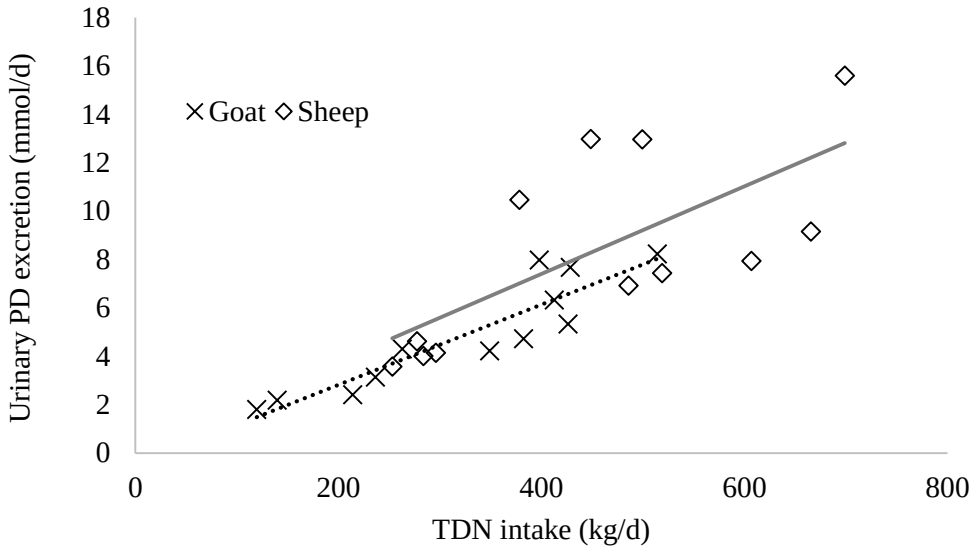

Figure 9. Relation between urinary purine derivative (PD) excretion and total digestible nutrients (TDN) intake in goats $PD = 0.02TDN - 0.50; r^2 = 0.83$ and sheep $PD = 0.02TDN + 0.15; r^2 = 0.49$

Chapter 3. DA SILVA, Márcia Pereira, D. Sc., Federal University of Bahia, July 2023. **Estimation of endogenous fraction of purine derivatives excreted in urine of in sheep and goats using a non-invasive method**. Adviser: Stefanie Alvarenga.

ABSTRACT: Our objective was to estimate the difference in urinary excretion of endogenous purine derivatives (EP) between goats and sheep fed diets containing different protein contents, with different levels of maintenance intake, and submitted to a period of fasting to estimate the losses of Fasting EP. Three experiments were conducted, for both experiments 12 goats and 12 sheep were allocated, all males and not castrated. In Exp. 1, the animals were fed 4 diets with different levels of N concentration [6.4, 12.8, 19.2 and 25.6 g N/kg of dry matter (DM)] in 6 simultaneous designs of Latin squares 4 × 4. The experiment was divided into four sub-periods consisting of 8 days for dietary adaptation and 8 days for sample collection comprising 16 day for each period, and 64-d in total. In Exp. 2, The experiment was carried out in the same location and conditions described for Exp. I, animals were the same as in Exp 1; four animals of each species were randomly submitted to one of the three experimental treatments, which were three feeding levels (40, 80 and 100% of ad libitum intake), totaling eight animals per treatment. The experiment was carried out in a completely randomized design in a 3 × 2 factorial scheme, in which the factors were two species, and three feeding levels. The experimental period lasted 15 days, with 11 days for adaptation to the feeding level and 4 days for sample collection. EP loss was estimated using linear regression between daily urinary purine derivate (PD) excretion and digestible organic matter intake (dOMI), expressed in millimoles per kilogram of $BW^{0.75}$. In Exp. 3. The diets used in this one were the same as in Exp.2, the same animals from exp 1 and 2 were used, lasted for 16d, in

whith all animals were subjected to dietary restriction. During the first eight days the feed was supplied at 1% of the BW (DM basis) and during the ninth to 11th day the feed was supplied at 0.5% BW. During the 12th to 16th day the animals were fasted, with free access to water. The mean excretions of PD. All statistical analyses were performed using the PROC MIXED procedure in SAS. The endogenous losses were obtained by regression between excretion of PD (mmol/BW$^{0.75}$) dOMI and CPI (g/BW$^{0.75}$). The following equations were obtained: $PD = 0.082\pm_{0.046} + 0.161\pm_{0.065} \times ESP + 0.013\pm_{0.002} \times dOMI - 0.002\pm_{0.003} \times (ESP \times dOMI)$; $r^2 = 0.71$ and, $PD = 0.173\pm_{0.071} + 0.265\pm_{0.102} \times ESP + 0.039\pm_{0.008} \times CPI \times (ESP \times CPI); r^2 = 0.34$, respectively. The endogenous PD losses obtained when the animals were fasted for 5 days, with free access to water, were 0.130 mmol/kgBW $^{0.75}$ It is concluded that the urinary excretion of PD can be estimated from the intake of MOD and PB that in animals subjected to fasting the endogenous contribution of purine derivatives is estimated at 0.130 mmol/kg BW0.75 for goats and 0.345 mmol/kg PV0.75 for sheep and that creatinine excretion is not affected by feed restriction.

Keywords: animal nutrition, creatinine, microbial protein, urea

1. INTRODUCTION

Urinary purine derivatives (PD) excretion has been widely used as a method to estimate microbial N flow (MNF) to the duodenum in ruminants. The endogenous purine (EP) fraction is subtracted from the total excretion of PD in urine to quantify the microbial crude protein (MCP) synthesis. If this EP value is applied inadequately, this can result in an under or overestimation of MCP, considering that EP losses are directly subtracted from total PD excretion to estimate MCP synthesis.

Even though urinary PD excretion is routinely used as a marker of MCP synthesis in ruminants, there are still some factors in this model that can affect PD excretion, which are not completely clear (Chen and Ørskov, 2003). Among them, PD excretion of endogenous origin is a key point, mainly in goats and sheep, due to the reduced amount of data available in the literature for these species. Few studies have compared PD excretion in goats and sheep fed with the same diets (Dos Santos et al., 2018; Santos et al., 2021), and information about endogenous contribution is still scarce for both species. The EP contribution, despite being an important parameter in modeling PD excretion in ruminants, is a parameter that is difficult to quantify in intact animals (Fujihara et al., 1987).

The existence of an EP fraction in excreted PD has been confirmed in several experiments using different methods and a variety of studies were carried out to estimate their urinary portion. The use of the intragastric infusion technique, with animals fed with volatile fatty acids and casein infused in the rumen and abomasum, respectively (Fujihara et al., 1987) or the use of animals kept in maintenance (Barbosa et al., 2011; Belenguer et al., 2002) or fasting (Braga et al., 2012) were conducted to estimate EP fraction in ruminant urine. According to Chen et al. (1990a) the measurement of endogenous PD excretion in ruminants under normal physiological conditions has been disturbed by the technical

difficulty of eliminating the contribution of rumen microorganisms. However, this problem can be minimized usingfeed restriction or fasting. The value of 202.2 μmol/$BW^{0.75}$ was reported by Belenguer et al. (2002) as the EP excretion in fasted goats. Theseauthors also reported that urinary PD excretion responded linearly to the supply of purine bases along the abomasal cannula, with a mean recovery of 76%. Chen et al. (1990b) examined the relationship between exogenous nucleic acid and purine supply in lambs, fully fed by intragastric infusions of volatile fatty acids and casein. The authors reported tha EP excretion was 0.150 mmol/$BW^{0.75}$. Balcells et al. (1991) found the value of 158 μmol/$BW^{0.75}$ in sheep submitted to total duodenal digesta replacement, followed by the administration of a purine-free solution or one enriched with increasing amounts of purines.

Several studies have been carried out to determine the endogenous contribution in cattle. According to Pimpa et al. (2001) endogenous PDexcretion was 0.147 mmol/ $BW^{0.75}$ in Bos indicus cattle, using the infusion of purine bases in the duodenum, and extrapolating to the zero level of ingestion. Ojeda et al. (2005), working with Bos indicus x Bos taurus cattle subjected to a 5-day of fasting, found a value 0.277 mmol/ $BW^{0.75}$ for the excretion of EP. The EP excretion in heifers was reported by Barbosa et al. (2011) using two techniques; using RNA infusion into the abomasum and considering PD excretion as a function of RNA flow to the abomasum. The authors obtained 0.301 mmol/$BW^{0.75}$ of EP contribution. . Braga et al. (2012) measured the EP losses obtained in cattle fasted for 5 days, with free access to water. They found the value of 0.332 mmol/$BW^{0.75}$. These authors observed a linear relationship between urinary excretion of purine derivatives and dry matter intake in cattle (Braga et al., 2012), allowing this excretion to be estimated from the intake of total digestible nutrients and digestible organic matter.

The use of regression equations to estimate the EP excretions seems promising, as it avoids the adverse effects of fasting and intragastric feeding. However, according to Chen et al. (1990b), the use of the intercept of the linear regression equation between the urinary PD excretion and DMI would not be an adequate method to estimate the EP excretion, since this relationship would not be linear in goats and sheep. However, it is important that more experiments would be conducted to evaluate if this premise is away valid for goats and sheep in a variety of dietary and experimental conditions. Then, the fasting technique could be combined with regression equations to verify if the linear relationship between PD excretion and nutrient intake could be found in sheep and goats feed different diets.

Thus, three experiments were carried out aiming to estimate the difference of EP urinary excretion between goats and sheep fed diets containing different protein contents, supplied with different maintenance levels of intake, and submitted to a fasting period to estimate EP fasting losses.

2. MATERIAL AND METHODS

2.1. Location and Ethical Considerations

The experiments 1, 2 and 3 were approved by the Ethics Committee on the Use of Animals of the Faculty of Veterinary Medicine and Animal Science of the Federal University of Bahia (approval nº 28/2014), and followed the guidelines established by the National Council for the Control of Animal Experimentation (CONCEA). Both experiments were conducted on the experimental farm of the same institution, located in the municipality of São Gonçalo dos Campos, State of Bahia, Brazil. All chemical analyzes were performed at the Laboratory of Animal Nutrition at UFBA.

All animals were kept individually in metabolic cages (total surface area of 1.2m2), completely covered with a slatted floor and fresh water was provided ad libitum. A 15-d adaptation period allowed the animals to familiarize themselves with the experimental procedures, the staff, during which they were weighed, identified, and dewormed.

2.2. Experiment 1

2.2.1. Animals, Experimental Design and Diets

Twelve Boer goats, whose average initial body weight (BW) was 17.3±1.8 kg, and twelve Dorper sheep, whose average initial BW was 20.7±2.8 kg, all males, not castrated and four months old, were used. Four experimental diets were formulated with increasing levels of crude protein (CP), as follows: 40, 80, 120 and 160 g CP/kg DM (Table 1). The animals were distributed to these diets in six Latin Squares (LS) 4 × 4, in which 3 LS composed by goats and 3 LS composed by sheep. Each LS was composed of 4 animals of the same species, totaling 24 animals. A factorial scheme 4 × 2 was designed, with the 4 diets and 2 species as main factors.

The diets were composed with corn silage as forage, except for diet 1 (40 g CP/kg DM), which consisted of sugar cane silage. The concentrate was a mixture of ground corn, corn germ meal, soybean meal and a specific mineral mix for goats and sheep. All diets had a forage:concentrate proportion of 70:30. The experimental diet was offered to the animals twice a day (09:00h and 16:00h) in similar proportions. Daily intake was adjusted to keep leftovers between 10% and 20% of the daily amount of feed offered on a wet basis.

The experiment was divided into four sub-periods consisting of 8 days for dietary adaptation and 8 days for sample collection comprising 16 days for each period, and 64 days in total.

2.2.2. Nutrient Intake

To evaluate the intake of nutritional components, the amounts of feed supplied, and refusals were recorded daily. From days 9th to 12th of each experimental period, the silages, and refusals were sampled. Silages were sampled directly in the pool removed from the silos for each day and refusals were sampled 20% of the total per animal. The concentrate ingredients were sampled directly from the grain storage of the feed factory in the days when they were mixed. These samples were homogenized to obtain a composite sample for each animal per period. The material was pre-dried in a forced-air oven at 55 °C for 72 h. After drying, each sample was ground in a knife mill (Wiley mill; TECNAL, São Paulo, SP, Brazil) with 2 mm. After that, half of each sample was ground again to pass through a 1-mm screen sieve. Samples were then pooled and were proportionally composited based on dry weight per animal and period. All samples were stored for further chemical laboratory analyses.

2.2.3. Fecal Collection and Nutrient Digestibility Trial

The digestibility trial was conducted between the 9th and 12th days of each experimental period. Total fecal output was measured during a 96-h period, with the aid of

collection bags tied to the animals. Feces samples were taken directly into collection bags, twice daily (11:00 and 16:00 h) after homogenization. Then, the total fecal production of each animal was recorded and aliquots of approximately 10% of the total pool were removed, stored in individual plastic bags, labeled, and frozen at -20°C. Fecal samples were oven-dried (55 ◦C), ground and pooled as previously described.

2.2.4. Urine Collection, estimation of microbial synthesis and quantification of urea and creatinine

On the 11th day of each experimental period, urine collection funnels were attached to the animals for their adaptation. Between the 13th and 16th days, total urine collection was performed using hoses attached to the funnels, which conducted the urine to a plastic container with 100 mL of 20% sulfuric acid (H_2SO_4) (v/v) as described by (Santos et al., 2017) to preserve N compounds.

At the end of 24h, the urinary pool was weighed, registered, homogenized, and filtered through two layers of cheese cloth, and a 10mL aliquot was diluted in 40 mL of a 0.036N H_2SO_4 solution (Valadares et al. 1999). Subsequently a composite sample was obtained per period for each animal, and proportionally to the 4 sampling days. Composite samples were stored at -20°C for further analyses of creatinine, and PD, which were uric acid, allantoin, hypoxanthine and xanthine.

Ruminal MCP synthesis was estimated by the urinary PD method using the following equations of Chen and Gomes (1992):

$$PD = 0.84X + (0.15BW^{0.75}e^{-0.25X})$$

$$MCP\ (g\ N\ (\times 6.25)/d) = \frac{X(mmol/d)70}{0.116\times 0.83\times 1000}$$

where: PD (mmol/d) = purine derivatives excreted in urine; X (mmol/d) = absorbed microbial PD. The N content of purines is 70 mg N/mmol. The total purine-N:N ratio in mixed rumen microbes is taken as 0.116 (11.6/100). Digestibility of microbial purines is assumed to be 0.83, considered the average digestibility value for microbial nucleic acids based on observations reported in the literature.

2.3. Experiment 2

2.3.1. Animals, experimental design, and diets

The experiment was carried out in the same places and conditions described for Exp. I. The animals used in Exp 2 were the same from Exp 1; 12 Boer goats and 12 Dorper sheep, all males and not castrated. After the end of Exp. 1, the animals were kept in the installations to start the Exp. 2, where all the animals were fed with the diet containing 160 g CP/kg DM. Four animals of each species were randomly submitted to one of the three experimental treatments, which were three feeding levels (60, 20 and 0% of feed restriction), totaling eight animals per treatment. The experiment was carried out in a completely randomized design in a 3 × 2 factorial scheme, in which the factors were two species (goats and sheep) and three feeding levels (60, 20 and 0% of feed restriction).

The experimental diet was offered to the animals twice daily (09:00h and 16:00h) in similar proportions. Daily intake was adjusted to keep leftovers between 10% to 20% of the daily amount offered, on a wet basis, only for animals feed ad libitum. The proportional supply of feed for animals with 60 and 20% feed restriction was calculated daily in relation to the average intake of animals submitted to ad libitum intake. The experimental period lasted 15 days, with 11 days for adaptation to the feeding levels and 4 days for sample collections.

2.3.2. Experimental procedures and sample collections

The experiment was carried out in the same places and conditions described for Exp. 1. Feed sampling, urine and feces collection, sample processing, and laboratory determinations were performed as described for Exp. 1. To assess the nutrient intake, the amounts of feed provided, and leftovers (when available) were recorded daily. From the 1st to the 15th day of the experimental period, silage and leftovers were sampled.

The digestibility test was conducted between the 11th to 15th day of the experimental period. Total fecal production was measured over a period of 96 hours, with the aid of collection bags tied to the animals. Fecal samples were collected directly into collection bags, twice a day (11:00h and 16:00h), after homogenization.

Between 11th to 15th day of experimental period, total urine collection was performed using hoses attached to funnels, which conducted the urine to a plastic container with 100 mL of sulfuric acid (H_2SO_4) 20% (v/v) as described by (Santos et al., 2017) to preserve N compounds. Subsequently, a composite sample was obtained for each animal proportionally to the 4 days of sampling.

2.4. Experiment 3

The experiment was carried out in the same locations and conditions described for Exp.1 and 2. The feed sampling, the collection of urine and feces, sample processing, and laboratory determinations were carried out as previously described.

The same animals from exp 1 and 2 were used at the final of Exp.2, where all animals were submitted to the following feed restrictions: restricted to 1% of BW in DM intake in the first eight days, to 0.5% of BW in DM intake from the ninth to the 11th day and fasting from the 12th to the 16th day, with free access to water. The diets used at this step were the same from Exp 2, then a previous adaptation was not necessary.

The experimental diet was offered to the animals twice a day (09:00h and 16:00h) in similar proportions from the first to the 11th day of the experiment. From the 12th to the 16th, when the fasting was applied, total collection of feces and urine from each animal was performed and the samples were stored for later laboratory analysis.

2.5. Laboratory analyses

Ingredient and feces samples from experiments 1, 2 and 3 were analyzed according to the protocols described by the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2021) as described for Exp 1 and 2. All laboratory analyzes were performed at the Animal Nutrition Laboratory (LANA) at the Federal University of Bahia.

Determination of urinary PD were performed by the colorimetric method, according to the technique of Fujihara et al. (1987). The method adopted to measure allantoin was based on the colorimetric method described by Chen and Gomes (1992), kin which allantoin was firstly hydrolyzed under a weak alkaline condition at 100°C to allantoic acid, subsequently degraded to urea and glyoxylic acid in weak acid solution. Glyoxylic acid reacts with phenylhydrazine hydrochloride to produce an acid phenylhydrazone. The product then forms an unstable chromophore with potassium ferricyanide. Color is read at 522 nm.

Determination of xanthine and hypoxanthine was carried out by enzymatic method (Chen and Gomes, 1992). In this method, xanthine and hypoxanthine are enzymatically (by the enzyme xanthine oxidase-XO) converted into uric acid, which is detected by its absorbance at 293 nm. The difference in absorbance of samples with and without XO at 293 nm was used to calculate the amount of uric acid formed based on the uric acid standard curve. Uric acid was quantified by enzymatic method in uricase and peroxidase with a commercial kit (uric acid – Ref.:140 Labtest, Minas Gerais, Brazil).

2.6. Calculations, determination of the endogenous fraction and statistical analysis

First approach: The endogenous excretion of PD was calculated by the sum of allantoin, xanthine, hypoxanthine, and uric acid excretion in the urine, expressed in mmol/day or mmol/kg $BW^{0.75}$ corresponding to the fourth and fifth day of fasting period.

Second approach: Endogenous losses were calculated using the intercept of the linear regression equation between PD excretion in mmol/kg $BW^{0.75}$ as a function of crude protein intake (CPI) from exp 1, and digestible organic matter intake (dOMI) in g/kg $BW^{0.75}$ from exp 2. This approach was made separately from exp 1 and exp 2, considering the variations in protein and energy intake from these experiments, respectively. Absorbed purines (Pabs, mmol/day) were calculated from the excretion of DP (mmol/day) using the equation: Pabs = [PD – (EP × $BW^{0.75}$)] /0.84, where the slope 0.84, represents the recovery of purines as PD in the urine, and EP × $BW^{0.75}$, was considered the EP losses in mmol/kg $BW^{0.75}$ found in this work.

Microbial nitrogen (Nmic) was calculated from the absorbed purines, using the equation: Nmic = (70 × Pabs) / (0.83×0.116×1000), where 70 represents the N content in purines (mg N /mmol); 0.83 the intestinal digestibility of microbial purines and 0.116 the purine N/ total N proportion of ruminal microorganisms (Chen and Gomes, 1992).

The CPI and dOMI (g/kg $BW^{0.75}$), as independent variables, were evaluated in relation to PD excretion (mmoL/kg $BW^{0.75}$), as dependent variable. Total creatinine excretion and urea (mg/kgBW) were also analyzed as dependent variables in the same model basic model was fitted to describe these mathematical relationships, which were linear, with or without the intercept, and were performed with the PROC MIXED, and PROC REG of SAS (version 9.4), adopting 0.05 as the critical level of probability for type I error.

Linear approaches using PD creatinine or urea as independent variables (Y), and CPI, and dOMI as dependent variables (X), were performed by a simple linear regression model:

$$y = \beta_{0+}\beta_1 + \beta_2 X_1 + \beta_3 (D \times X) + \varepsilon$$

On what Y and X = variables considered in the evaluation of the relationship; D= dummy variable referring to the evaluation of the effect of the species on the relationship, where D= 0 for goats and D = 1 for sheep; and ε = random error assuming NID (0; $\sigma^2\varepsilon$).

The regression models were adjusted according to the significance of parameters β1, β2 and β3 using the restricted maximum likelihood method implemented in PROC REG of SAS. All statistical procedures were carried out considering 0.05 as the critical level of probability for type I error.

3. RESULTS

A characteristic of the excretion of purine derivatives was that the excretion of the different derivatives did not change proportionally with fasting. Most of the excretion was with allantoin (75.5%), followed by the proportions of uric acid and xanthine and hypoxanthine (17.4 and 7.0%, respectively; Figure 1).

There was difference between EP obtained in goats and sheep (P=0.01) for approach 1, which was made from fasting in Exp.3. The obtained averages and their respective confidence intervals ((CI) α = 95%) were 0.096 [inferior confidence interval = 0.068 and superior confidence interval = 0.112] mmol/kg $BW^{0.75}$ for goats, and 0.259 [inferior confidence interval = 0.127 and superior confidence interval = 0.392] mmol/kg $BW^{0.75}$.

There was a difference in the intercept (P = 0.02) fitted for goats and sheep in the linear relationship between urinary PD and dOMI, from Exp. 2, and the fasting values of the

Exp. 3(Figure 2). The adjusted slope was similar between species (P = 0.95), and the following equation was obtained:

$$PD = [0.082_{\pm 0.046} + 0.161_{\pm 0.065} \times ESP] + 0.013_{\pm 0.002} \times dOMI;\ r^2 = 0.71$$

Where: ESP = 0 for goats and ESP = 1 for sheep. Thus, considering goats, the equation obtained was $PD = 0.082 + 0.013 \times dOMI$, and considering sheep, the equation was $PD = 0.243 + 0.013 \times dOMI$, where PD was expressed in mmol/kg $BW^{0.75}$ and dOMI in g/kg$BW^{0.75}$.

These values mean that the EP losses was equivalent to 0.082 mmol/kg$BW^{0.75}$ in goats, and 0.243 mmol/kg$BW^{0.75}$ in sheep, when considering the approach 2 referent to the energy dietary variation.

There was difference in the intercept (P = 0.01) fitted for goats and sheep in the linear relationship between microbial N synthesis and dOMI from experiment 2 (Figure 3). The adjusted slope was similar between species (P = 0.95), then only one slope was obtained for both. The following regression equation was obtained to evaluate microbial N:

$$Microbial\ N = [0.124_{\pm 0.026} + 0.094_{\pm 0.036} \times ESP] + 0.006_{\pm 0.002} \times dOMI\ ; r^2 = 0.68$$

where: ESP = 0 for goats and ESP = 1 for sheep. Thus, considering goats, the equation obtained was $Microbial\ N = 0.124 + 0.006 \times dOMI$, and considering sheep, the equation obtained was $Microbial\ N = 0.218 + 0.006 \times dOMI$, where microbial N was expressed in g/kg $BW^{0.75}$ and dOMI in g/kg$BW^{0.75}$.

This means that 0.124 g/kg $BW^{0.75}$ of microbial N in goats and 0.218 g/kg $BW^{0.75}$ could overestimate the microbial N synthesis when the endogenous fraction of PD would not

be discounted in the final estimation of this variable. There was difference in intercept (P<0.01) fitted for goats and sheep, for the relationship between the excretion PD mmol/kgBW$^{0.75}$ in the urine, and the amount of CPI, expressed in g/kgBW$^{0.75}$ from Exp. 1, and the fasting values of the Exp. 3 (Figure 4). The adjusted slope was similar between species (P= 0.12), then only one slope was fitted for both and the following equation was obtained:

$$PD = [0.130_{\pm 0.04} + (0.214_{\pm 0.07} \times ESP)] + 0.044_{\pm 0.006} \times CPI; r^2 = 0.64$$

Where: ESP = 0 for goat and ESP =1 for sheep. Thus, considering goats, the equation obtained was $PD = 0.130 + 0.044 \times CPI$, and considering sheep, the equation was $PD = 0.345 + 0.058 \times CPI$, where PD was expressed in mmol/kg BW$^{0.75}$, and CPI in g/kg BW$^{0.75}$. These values mean that the EP losses was equivalent to 0.130 mmol/kgBW$^{0.75}$ in goats and 0.345 mmol/kg of BW$^{0.75}$ in sheep, respectively, when considering the approach 2 referent to the protein dietary variation.

There was difference in the intercept (P = 0.02) fitted for goats and sheep in the linear relationship between microbial N synthesis and CPI from experiment 1 (Figure 5). The adjusted slope was similar between species (P = 0.10), then only one slope was obtained for both. The following regression equation was obtained to evaluate microbial N:

$$Microbial\ N = [0.150_{\pm 0.026} + 0.113_{\pm 0.036} \times ESP] + 0.023_{\pm 0.003} \times CPI\ ; r^2 = 0.63$$

where: ESP = 0 for goats and ESP = 1 for sheep. Thus, considering goats, the equation obtained was $Microbial\ N = 0.150 + 0.023 \times CPI$, and considering sheep, the equation

obtained was $Microbial\ N = 0.263 + 0.023 \times CPI$, where microbial N and CPI were expressed in g/kg $BW^{0.75}$.

This means that 0.150 g/kg $BW^{0.75}$ of microbial N in goats and 0.263 g/kg $BW^{0.75}$ could overestimate the microbial N synthesis when the endogenous fraction of PD would not be discounted in the final estimation of this variable.

The averages 0.096 [CI = 0.068 to 0.112] mmol of EP/kg $BW^{0.75}$ for goats, and 0.259 [CI = 0.127 to 0.392] mmol of EP/kg $BW^{0.75}$ for sheep were obtained directly using the fasting in Exp. 3. The values obtained from Exp 2, which considered the dOMI to estimate the EP, were inside the CI of the fasting prediction. The EP losses predicted from dOMI were 0.082 mmol/kg$BW^{0.75}$ for goats, and 0.243 mmol/kg$BW^{0.75}$ for sheep, then considering the approach 2 referent to the energy dietary variation. This confirm that this approach was convenient to estimate accurately the EP losses in sheep and goats, tking into consideration varitions in animals consuming 0, 40, 80 and 100% of the *ad ibtum* intake. However, the values obtained from Exp 1 for goats, which considered the CPI to estimate the EP, were above the CI of the fasting prediction. The EP losses predicted from CPI were 0.130 mmol/kg$BW^{0.75}$ in goats and 0.345 mmol/kg of $BW^{0.75}$ in sheep, respectively, when considering the approach 2 referent to the protein dietary variation. The value for sheep was next to the superior confidence interval. This can mean that CPI could overestimate the prediction of EP losses, even considering the fasting values in the model. The, the value obtained from Exp 2, considering dOMI seems to be more adequate to estimate this variable.

The dairy contribution of EP to total DP excretion was estimated using the EP values in the urine of animals obtained in experiments 2 and 3 when dOMI was the selected variable. (Figure 6). The endogenous excretion values found in our study were adapted from Chen et al. (1990a), obtaining the following equations, respectively for goats and for sheep:

$$\text{EP Goats} = (0.082 \times BW^{0.75}) * (e^{-0.25 \times \text{absPD}})$$

$$EP\ sheep = ((0.243 \times BW^{0.75}) * (e^{-0.25 \times \text{absPD}})$$

The magnitude of these contributions was higher as the amount of exogenous microbe available to the animal decreases.

The excretion of the PD is directly related to the purine absorption in goats and sheep, when we take into account the amount of exogenous purines used by the animal, the endogenous net contribution was reduced (Figure7).

There was no correlation with creatinine and dMOI from experiment 3 (Figure 8). Considering that no differences were found between goats and sheep (P = 0.31), a single equation was obtained for this relationship: $Creatinine\left(\frac{mg}{kgBW}\right) = 0.006 \times dMOI + 17.8; r^2 =$ 0.02, where creatinine was expressed in mg/kg BW and dMOI in g/kgBW$^{0.75}$.

There was no difference in the intercept (P= 0.44) adjusted for goats and sheep in the linear relationship between urea and CPI from experiment 1 (Figure 9). Adjusted slope was different between species (P = 0.02), so different slopes were obtained for each species. The following regression equation was obtained to evaluate the urea concentration:

$$Urea = 0.027_{\pm 0.016} + [(0.101_{\pm 0.022} \times CPI) + (0.063_{\pm 0.027} \times CPI \times ESP)];$$

r^2 = 0.62

Where: ESP = 0 for goats and ESP = 1 for sheep. Thus, considering goats, the equation obtained was $\text{Urea} = 0.027 + 0.101 \times \text{CPI}$, and considering sheep, the equation was $\text{Urea} = 0.027 + 0.164 \times \text{CPi}$, where urea was expressed in g/kgBW$^{0.75}$, and CP intake in g/kg BW$^{0.75}$.

4. DISCUSSION

The profile of urinary PD in goats and sheep found in our study was already expected because of the low activity of XO. The PD excreted in the urine of goats and sheep was similar, both were within the range previously observed for the species, allantoin (75.5%), uric acid (17.4%) xanthine and hypoxanthine (7 %), in previous works (Campos et al., 2019; Carro et al., 2012; Chen and Gomes, 1992; Chen et al., 1990; Saeed et al., 2018; Dos Santos, et al., 2018), allantoin, being the dominant PD in sheep and goats.

When estimating microbial N from PD, it is predicted that feed restriction limits substrates in the rumen available for fermentation and microbial growth by significantly reducing microbial N synthesis. Since, the challenge of fasting significantly reduces the excretion of PD in the urine of these animals. Similar results were observed by Campos et al. (2019) by submitting sheep to a feed restriction of 60% of the ad libitum intake and Belenguer et al. (2002) in goats subjected to fasting periods.

Gomes et al. (1994) demonstrated the relationship between microbial N synthesis anddOM intake, by providing levels of soluble starch to sheep. Authors observed linear increase in dOM intake and microbial N synthesis. In our study, an increase in the ruminal synthesis of microbial N and, consequently, an increase in the excretion of PD was observed. In our study, linear regression ($r^2 = 0.71$) confirms the existence of a correlation between PD excretion and dOM intake in goats and sheep.

Among the variables listed in our work, dOM intake represents the sum of all nutrients that can generate energy and that can be accessed by microorganisms, therefore being the most reliable variable to correlate. However, according to Santos et al. (2021) the synthesis of microbial protein in the rumen can be calculated from the energy intake or digestible organic matter, through combined equations that encompass both sheep and goats.

The endogenous fraction in sheep observed in the present study was higher than that found by Chen et al. (1990a) which was 0.142 mmol/kg $BW^{0.75}$, 0.164 mmol/kg $BW^{0.75}$ reported by Fujihara et al. (1987) and 30 mg//kg $BW^{0.75}$ found by (Balcells et al., 1991). However, in goats, endogenous PD excretion was lower than that found by Belenguer et al. (2002) which was 0.202 μmol//kg $BW^{0.75}$ in animals fasted for 6 days.

The existence of an endogenous fraction in bovine PD excreted has already been confirmed in experiments based on the relationship between PD excretion in mmol/kg $BW^{0.75}$ and intake of DM, DOM and TDN (Barbosa et al., 2011; Pellizzoni, 2011; Prates, 2011; Silva Braga et al., 2012). The endogenous excretion in our study was determined from the results of the period in which the animals were submitted to fasting, evaluating the fourth and fifth days of fasting. It was observed that the excretion of purine derivatives was 0.082 mmol/kg $BW^{0.75}$ in goats and 0.243 mmol/kg $BW^{0.75}$ in sheep.

Even though there was no evidence that the flow of CP in the duodenum is interrupted during fasting, Fujihara et al. (1987) stated that this method can be an alternative to reduce exogenous ruminal CP production, when there is no other methodology available. The results obtained in fasted Kelantan cattle (274 mmol/ BW; Liang et al., 1999) were also similar to values reported by Verbic et al. (1990) and Orellana et al. (2001), using Holstein heifers with intragastric feeding (0.385 mmol/kg $BW^{0.75}$) or using the N^{15} isotope dilution technique in cows (235 μmol/$BW^{0.75}$), respectively.

The endogenous contribution, despite being an important parameter in modeling PD excretion in ruminants, is a parameter that is difficult to quantify in intact animals (Fujihara et al., 1987), due to the technical difficulty of eliminating the contribution of microorganisms from the rumen (Chen et al.,1990a). It appears that values of endogenous excretion of goats and sheep in the literature are still scarce, which can be attributed to limitations in the use of

available methods, either due to high costs or impracticability of performing the technique in the species. The use of a linear regression equation seems promising, avoiding the adverse effects of fasting and intragastric feeding.

The model proposed by Chen and Gomes (1992) to estimate the supply of microbial protein in sheep using DP excretion was the same that has been used in goats. The authors Andrade-Montemayor et al. (2004) compared different models for estimating absorbed purine bases in goats. The purine bases and the microbial N flux was different between the models It seems that the models proposed for sheep (Balcells et al., 1991) or cattle (Chen and Gomes, 1992) underestimated the predicted purine base absorbed values for goats (Belenguer et al., 2002).

Enzymatic activity may be similar between sheep and goats, but the variability between the factors used in escapes to estimate purine bases and microbial N flux within and between species limits the use of models for cattle and sheep in goats (Andrade-Montemayor et al., 2004). The purine basis estimated was affected by the recovery of PD and the endogenous fraction of PD in urine.

Therefore, recovery from urinary PD is variable within and between species (Andrade-Montemayor et al., 2004). In sheep, it ranged from 80 to 93% (Balcells et al., 1991; Chen and Gomes, 1992) and in goats from 74 to 94% (Belenguer et al.,2002; Lindberg, 1991). Therefore, recovery from urinary PD is variable within and between species (Andrade-Montemayor et al., 2004). In sheep, it ranged from 80 to 93% (Balcells et al., 1991; Chen and Gomes, 1992) and in goats from 74 to 94% (Belenguer et al.,2002; Lindberg, 1991).

The difference found between the two species in our study can be partially attributed to differences in XO activity between these species. The low activity of XO in the intestinal

mucosa, in the liver and in the plasma, determines a low range of irreversible oxidation of PD of tissue nucleotides (Belenguer et al., 2002) and, therefore, would justify the low level of endogenous excretion (mmol/kg $BW^{0.75}$) determined in goats. The difference in the endogenous excretion of these DP between these species could be explained by the difference in the efficiency of saving these DP for the synthesis of the new one.

Therefore, the data suggest that a single model cannot be applied to both species, as goats seem to have greater activity of the xanthine oxidase enzyme compared to sheep, attributed to this factor a higher PD salvage rate. However, further studies to assess possible differences between goats and sheep in endogenous DP excretion are needed to improve estimates of microbial N synthesis from DP excretion.

DP excretion is directly related to purine absorption in goats and sheep. According to the authors (Chen and Gomes, 1992), with the knowledge of the purine-N: N-total ratio in the microbial biomass, the microbial absorption of N can be calculated from the amount of purine absorbed, which is estimated from of urinary excretion of PD.

In our study, when we take into account the amount of exogenous purines used in goats and sheep, the endogenous net contribution was reduced, as demonstrated in the work of (Chen et al., 1990a) in sheep. So, we agree that as the amount of exogenous purine available to the animal increases, the endogenous net contribution decreases. The response curve of DP purine excretion in relation to absorbed purine was not linear in goats and sheep, however it was performed using the same equation described by Chen et al. 1990, adapted with the endogenous excretion values found in our study, for goats (0.082 mmol/kg$BW^{0.75}$) and sheep (0.243 mmol/kg$BW^{0.75}$).

The amount of purines required to be synthesized again to compensate for the need to replace endogenous purine loss is represented by the endogenous contribution. During

purine-absent feeding, endogenous purine loss is completely replaced by de novo synthesis (Chen et al., 1990b). Conversely, when the animal receives an increasing exogenous supply, biosynthesis is gradually replaced by utilization of exogenous purines and becomes completely inhibited with an abundant supply of exogenous purines (Chen et al., 1990).

Considering the data from this experiment, the absorbed purines (AP) would be calculated as: $Pabs = (PD - (0.082 \times BW^{0.75}) \div 0.84$, for goats and $Pabs = \left(PD - (0.243 \times BW^{0.75})\right) \div 0.84$ for sheep. Comparing this equation with the one proposed by (Chen et al., 1990a). where $Pabs = \left(PD - (0.150 \times BW^{0.75})\right) \div 0.84$, if a 20 kg animal with a daily DP excretion of 10 mmol was considered, the Pabs would be estimated at 10.2 mmol, in the case of our study it would be 11 mmol if 0.082 were used and 9.18 mmol using 0.243. That is, the purines absorbed in goats would be approximately 7.3% higher if the EP found in our study were used, while in sheep this value would be 9 % lower.

Creatinine excretion was closely related to body weight and is proportional to muscle mass (Dos Santos et al., 2018; Santos et al., 2017). Our results confirmed that daily creatinine excretion is not influenced by DM or DOM intake, with an average of 17,872 mg/kg BW. As creatinine is a by-product of muscle tissue metabolism (Santos et al., 2017), we did not expect to find differences in creatinine excretions between species, since the body weight of the animals was similar.

The authors (Belenguer et al., 2002), also subjected the sheep to fasting for 6 days and observed that creatinine excretion remained constant even with feed restriction, being 266. 8 μmol/kg BW $^{0.75}$. The authors Santos et al. (2017), reported that the daily excretion of primary creatinine for growing goats is 17.39 mg/kg of body weight. In studies by Santos,

dos et al. (2018) creatinine excretion was 17.28 and 22.22 mg/kg of BW in goats and sheep respectively.

The authors also found no difference in the slope of the regressions between urinary creatinine excretion and DM or OM intake. Both found no effect of dietary components on creatinine excretion. Corroborating these findings, the regression equations demonstrated that creatinine excretion is not related to DOM intake.

Urea elimination in the urine is one of the fates of urea synthesized in the liver and released into the blood, and factors such as N intake are factors that affect this elimination (Batista et al., 2017). The significant slope in the correlation between protein intake and urea excretion for goats and sheep demonstrated a greater efficiency of goats in retaining N when both species were submitted to the challenge. The need to retain N at low levels of N intake results in changes in renal regulation of urea excretion (Spek et al., 2013). In our studies, sheep showed higher urea excretion during the fasting period.

Specific kidney mechanisms such as glomerular filtration rate and tubular reabsorption in goats were more efficient to reduce urea excreted in urine when N intake was reduced. One of the possible high-priority metabolic functions is the recycling of nitrogen to the gastrointestinal tract (Detmann et al., 2014). This is because a continuous supply of nitrogen for microbial growth in the rumen is a strategy for animal survival.

According to Batista et al. (2017), when there is nitrogen deficiency, the animal is able to decrease the urinary excretion of urea and increase the recycling of urea to the rumen. The low concentration of urea in the urine of both goats and sheep on restriction or no intake of dietary CP demonstrates the remarkable ability of ruminants to conserve N through urea recycling mechanisms in the face of severe N deficiency.

5. CONCLUSION

The relationship between DP excretion and purine absorption in goats and sheep is not linear. However, the relationship between DP excretion and MOD and PB consumption is close to linear, allowing the use of the intercept of the linear regression equation to estimate DP excretion.

Urinary PD excretion can be estimated from CP and MOD intake. The excretions of purine derivatives during the fasting period make it possible to estimate an endogenous contribution of 0.082 mmol/kg $BW^{0.75}$ for goats and 0.243 mmol/kg $PV^{0.75}$ for sheep.

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Table 1
Chemical composition of the experimental diets of experiments 1 and 2.

Chemical composition	40CP[1]	80CP[2]	120CP[3]	160CP[4]
Dry matter (g/kg as fed)	271	379	380	379
Ash	70.6	59.0	60.7	61
Organic matter	929	941	939	939
Crude protein	47.8	82.0	120	159
Ether extract	24.2	46.1	58.7	31
aNDFn	498	423	434	425
Total carbohydrates	856	811	758	747
NFC	356	387	324	322
Lignin	59.7	34.3	34.7	35
iNDF	249	164	166	167
TDN	698	734	727	723

[1]- 40 g crude protein/kg DM; [2]- 80 g crude protein/kg DM; [3]- 120 g crude protein/kg DM; [4]- 160 g crude protein/kg DM; aNDFn - neutral detergent fiber with thermolabile amylase corrected for ash and nitrogen; NFC - non-fiber carbohydrates; iNDF-indigestible neutral detergent fiber; TDN-The total digestible nutrients.

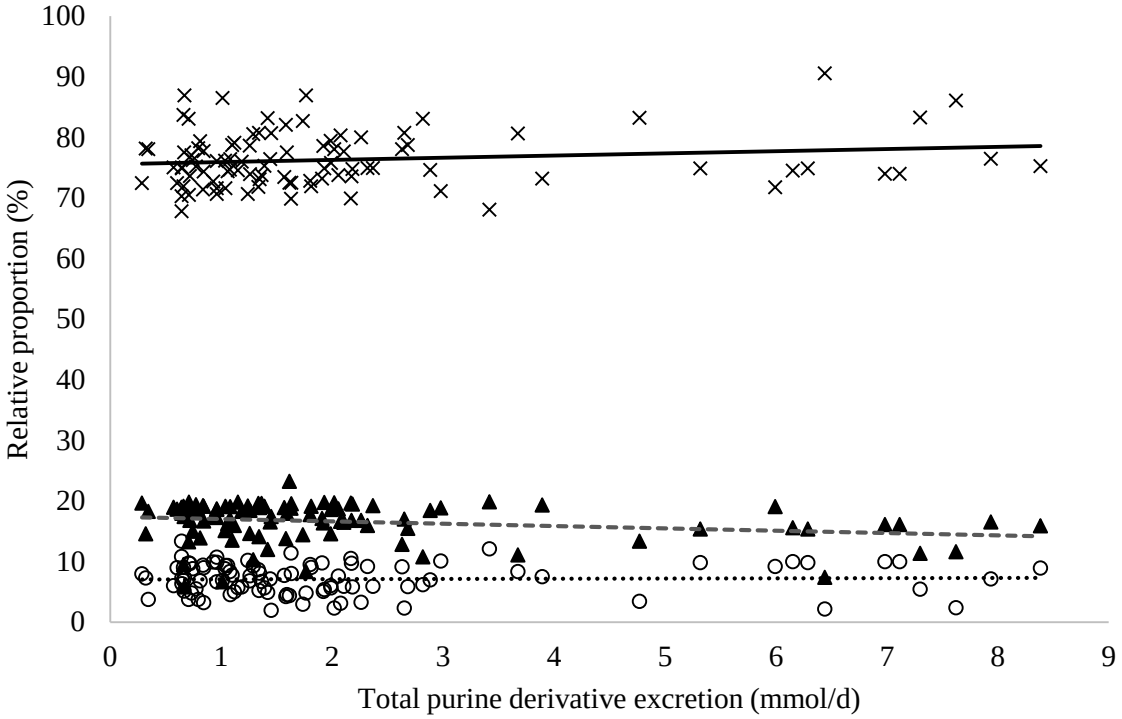

Figure 1. The relative proportions of allantoin (×; $y = 0.36X + 75.57$), uric acid (O; $Y = -0.38X + 17.39$) and xanthine plus hypoxanthine (▲;$Y = 0.03X + 7.04$) excreted in the urine of goats (a) and sheep (b) on days 1 and 5 of fasting (Exp. 3).

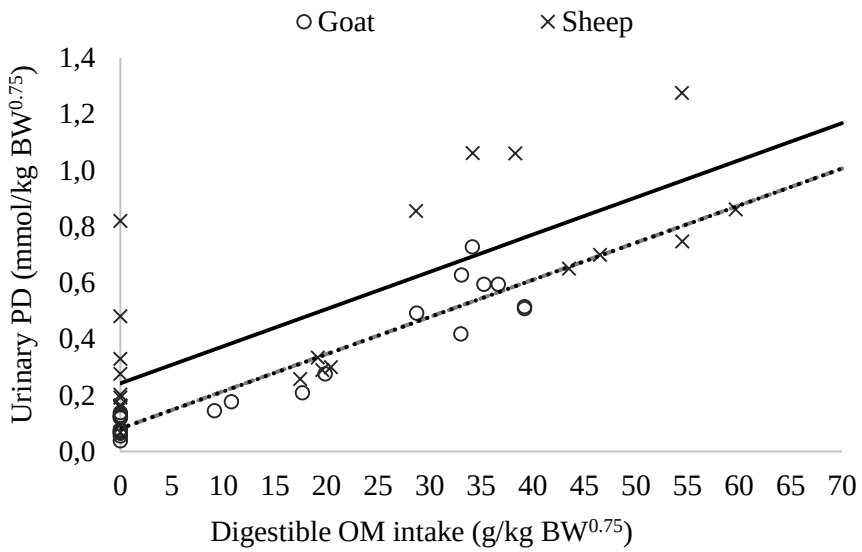

Figure 2. Relationship between the excretion of purine derivatives (PD) in the urine mmol/kgBW$^{0.75}$, from experiment 2 and the intake of digestible organic matter (OM) (dOMI) in g/kg BW$^{0.75}$.Adjusted equation: $PD = (0.082 + 0.161 \times (ESP) + 0.013 \times dOMI); r^2 = 0.71$; intercept (P=0.02) and slope (P=0.95). ESP=0 for goat and ESP=1 for sheep. Data from PD in fasting was added to data from experiment 2 to improve the accuracy of intercept estimation.

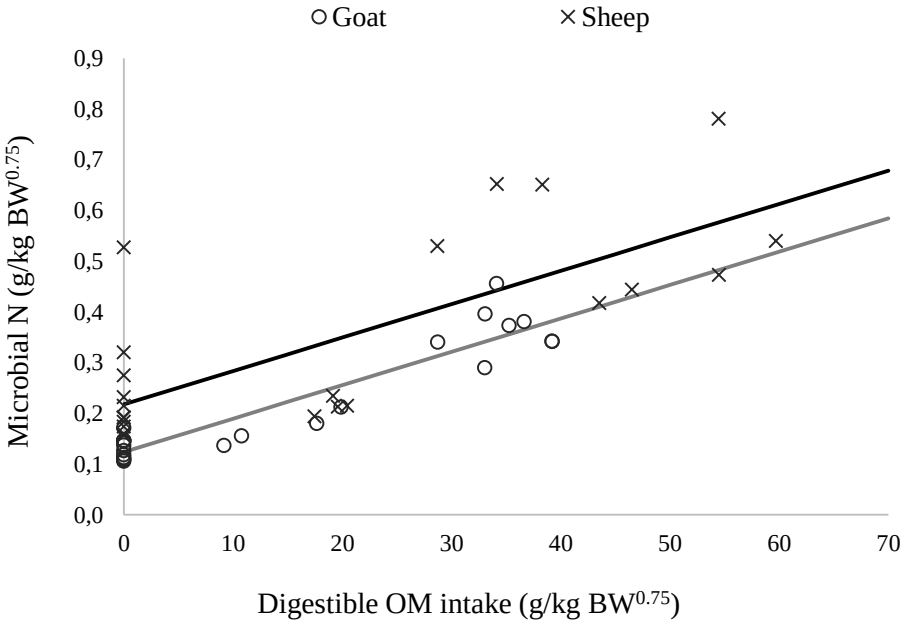

Figure 3. Relationship between microbial nitrogen (N) g/kgBW$^{0.75}$, from experiment 2, and the intake of digestible organic (OM) matter (dOMI) in g/kg BW$^{0.75}$. $Microbial\ N = \big(0.124 + 0.094 \times (ESP)\big) + 0.006 \times dOMI; r^2 = 0.68$, intercept (P=0.01) and slope (P=0.95). ESP: 0 for goat e 1 for sheep. Data from Microbial N in fasting was added to data from experiment 2 to improve the accuracy of intercept estimation.

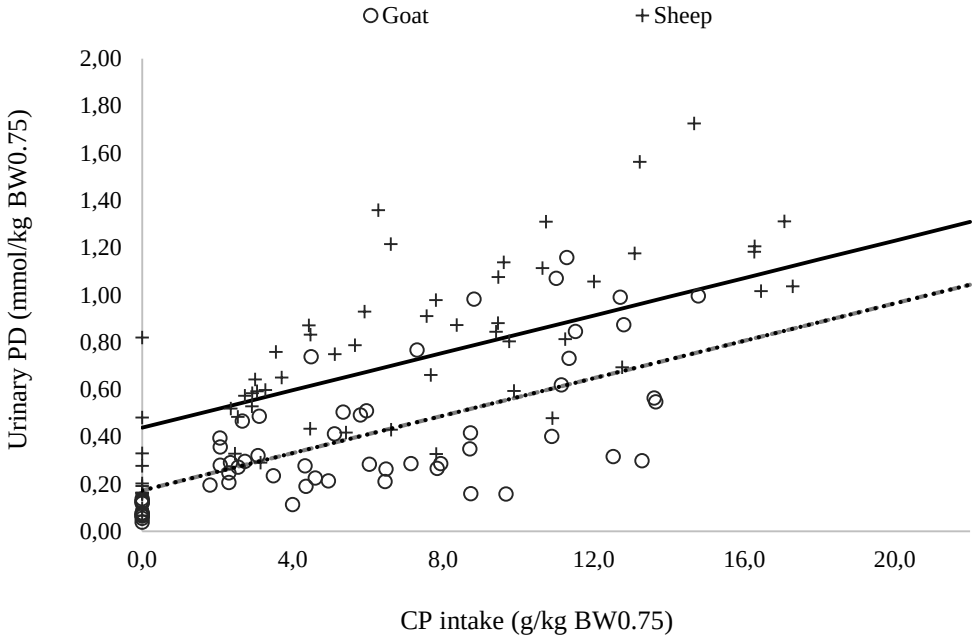

Figure 4. Relationship between the excretion of purine derivatives (PD) in the urine mmol/kgBW$^{0.75}$, from experiment 1, and crude protein intake (CPI) g/kg BW$^{0.75}$. $DP = \big(0.130 + 0.214 \times (ESP)\big) + 0.044 \times CPI; r^2 = 0.64$, intercept (P<0.01) and slope (P=0.12). ESP: 0 for goat e 1 for sheep. Data from Microbial N in fasting was added to data from experiment 2 to improve the accuracy of intercept estimation.

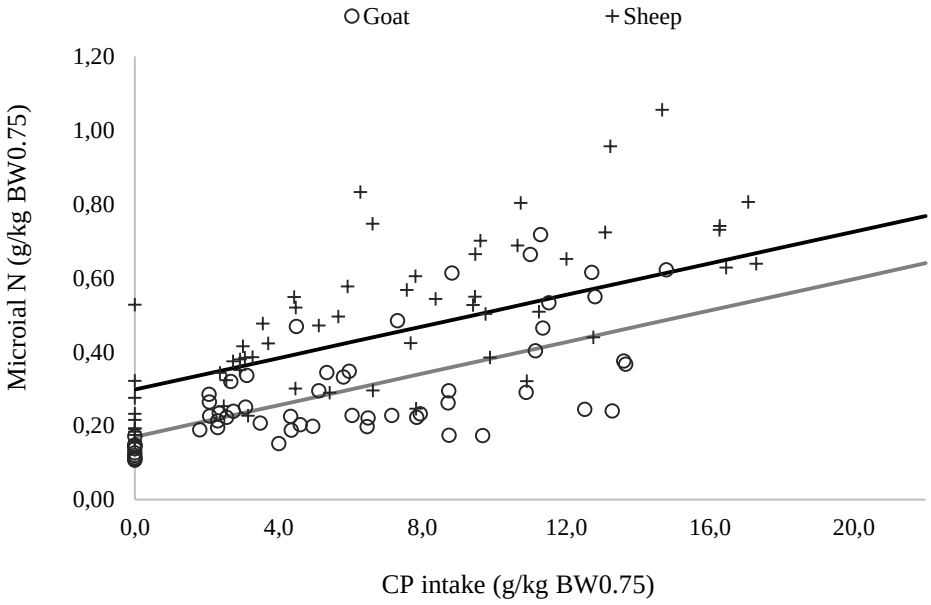

Figure 5. Relationship between microbial nitrogen (N) g/kgBW$^{0.75}$, from experiment 1, and crude protein intake (CPI) g/kg BW$^{0.75}$. $Microbial\ N = \big(0.150 + 0.113 \times (ESP)\big) + 0.023 \times CPI; r^2 = 0.64$. Intercept (P=0.02) and slope (P=0.10). ESP: 0 for goat e 1 for sheep. Data from Microbial N in fasting was added to data from experiment 2 to improve the accuracy of intercept estimation.

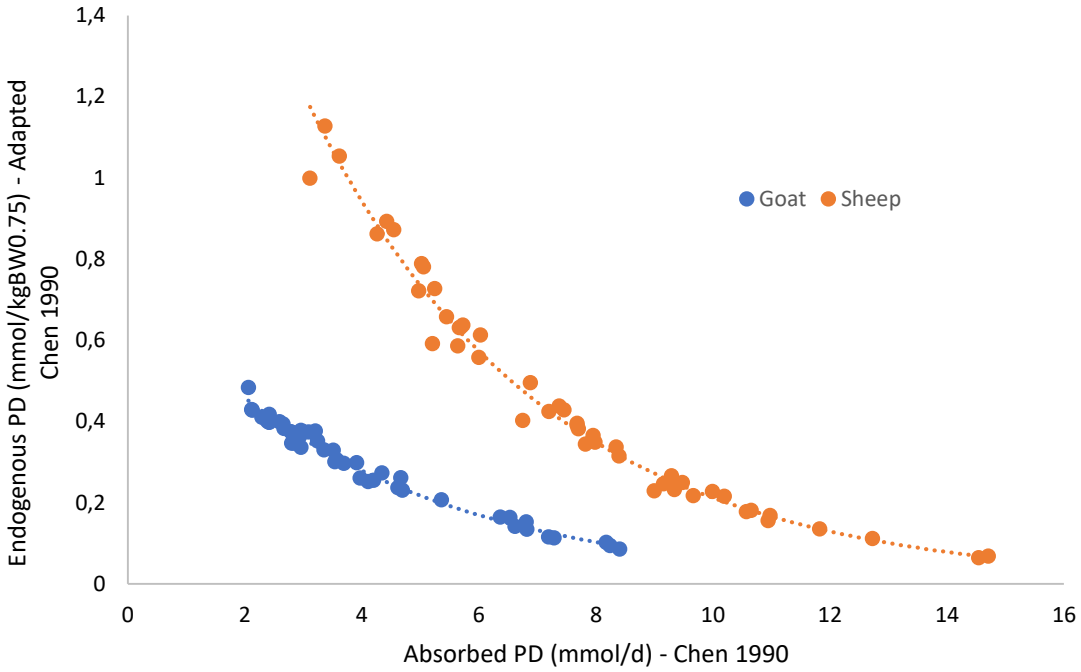

Figure 6. Relationship between the endogenous excretion of purine derivatives (PD) mmol/kgBW$^{0.75}$ in the urine of goats and sheep, and the amount of absorbed microbial purines g/kgBW$^{0.75}$. Plot from the equation: EP $= ((0.082 \times BW^{0.75}) * (\text{EXP}(-0.25 \times \text{absPD})))$, in goat and $EP = ((0.243 \times BW^{0.75}) * (\text{EXP}(-0.25 \times \text{absPD})))$, for sheep. Being the estimated endogenous contribution of 0.082 and 0.243 mmol/kg of BW$^{0.75}$ for goats and sheep, respectively; shown in figure 2. The endogenous excretion values found in our study were adapted to the Chen.1990 equation: 0.150W$^{0.75}$ e$^{-0.25X}$, substituting 0.150 mmol/kg of BW$^{0.75}$ of endogenous excretion value in sheep found by Chen.1990.

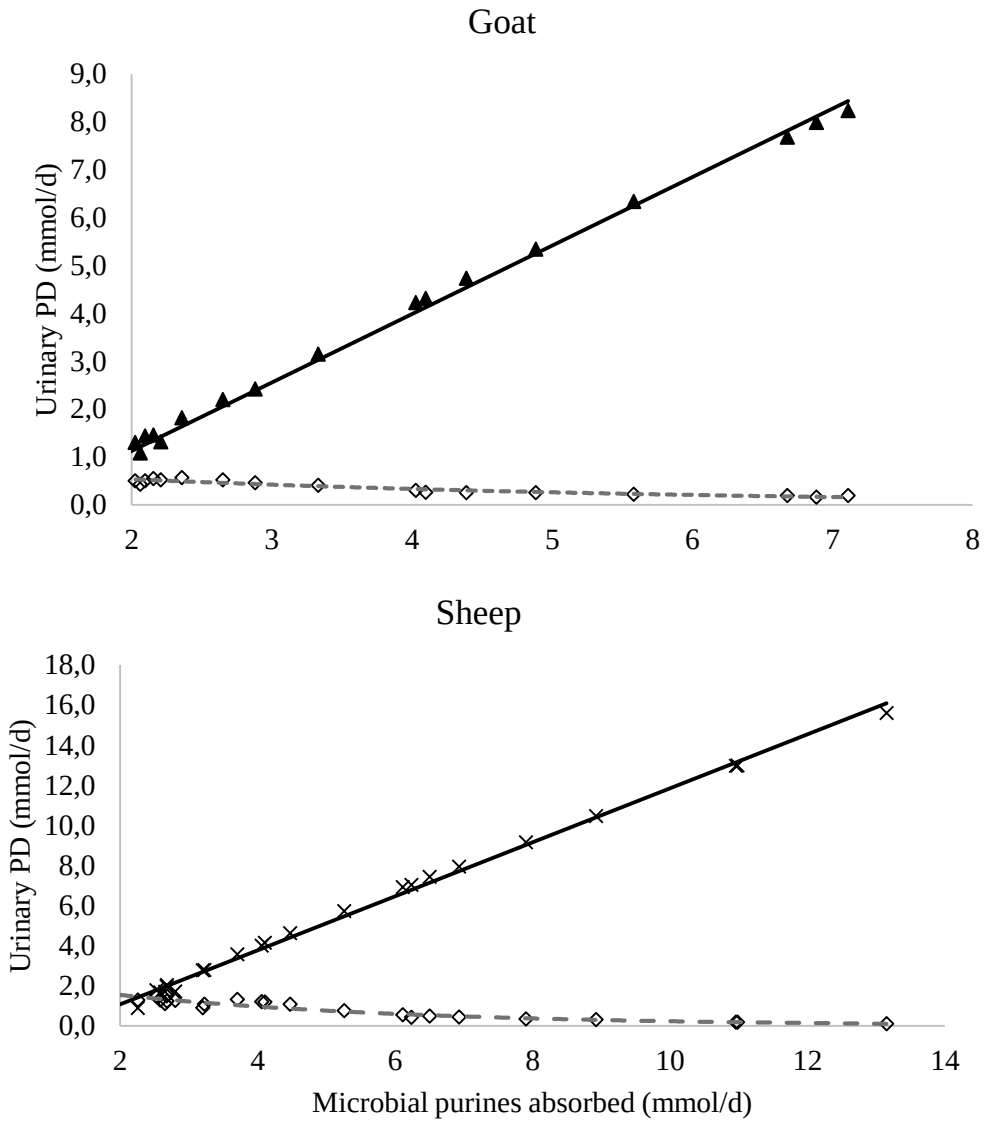

Figure 7. Urinary excretion of purine derivatives in goat and sheep in relation to the amount of microbial purines absorbed (Pabs). The dotted line represents the net contribution of purine derivatives from degradation of tissue nucleic acids. Plot from the equation $DP = 0.86e^{-0.24Pabs}$; $r^2 = 0.93$,in goat and $DP = 2.48e^{-0.23Pabs}$; $r^2 = 0.96$. Data from PD in fasting was added to data from experiment 1 and 2 to improve the accuracy of intercept estimation.

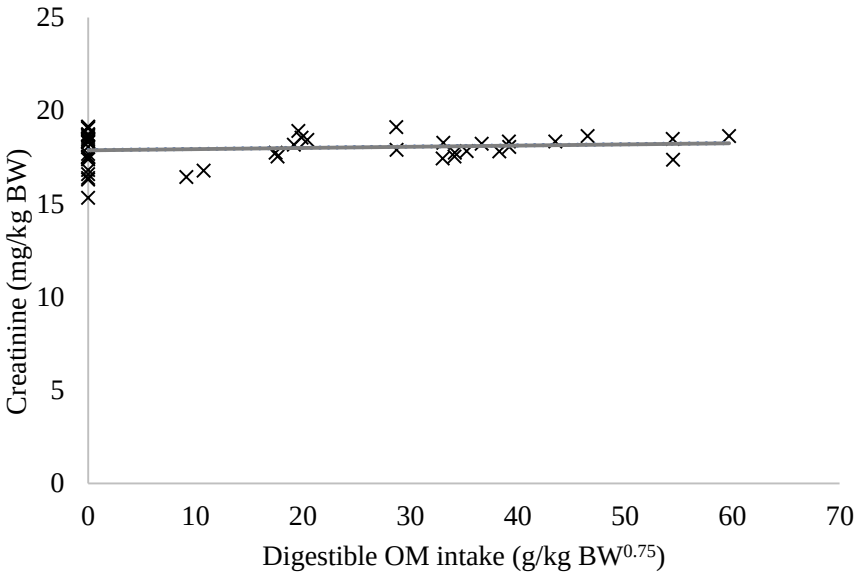

Figure 8. Relationship between creatinine in urine, mg/kg BW and1 the intake of digestible organic matter (dOMI) in g/kg $BW^{0.75}$; $Creatinine = 0.006 \times dMOI + 17.87$. Urinary creatinine was obtained when the animals were fasted for 5 d. The 17.87 mg/kg BW represents the creatinine excreted diary in the urine of goats and sheep (P= 0.31).

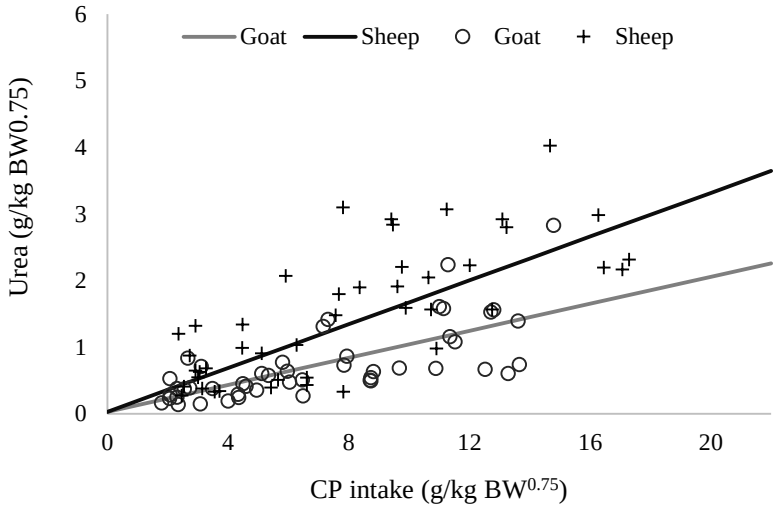

Figure 9. Relationship between urea in urine, g/kg$BW^{0.75}$, and CP intake (CPI) g/kg $BW^{0.75}$. $Urea = 0.027 + (0.101 \times CPI) + (0.063 \times CPI \times ESP); r^2 = 0.62$, intercept (P=0.44) and slope (P=0.02). ESP: 0 for goat e 1 for sheep. Data from urea in fasting was added to data from experiment 1 to improve the accuracy of intercept estimation.

7. GENERAL CONCLUSIONS

Based on the results obtained in this thesis, we conclude:

1- Goats are more efficient in digesting low-quality diets, rich in fiber and low in CP, they are more efficient in using and recycling N in the rumen than sheep, with lower urinary excretion of urea and N. Diets for goats and sheep with a level of 40 g CP/kg DM reduce intake, limiting the amount of diet in the rumen, consequently decreasing microbial efficiency;

2- When subjected to food restriction, goats are more efficient in the use of dietary fiber and N conservation than sheep. In goats and sheep, food restriction improves the efficiency of nutrient utilization, however it decreases the efficiency of microbial synthesis in the rumen;

3- In animals subjected to fasting, the excretion of creatinine is not altered;

4- There is a linear relationship between urinary excretion of purine derivatives and dry matter intake, and this excretion can be estimated from digestible organic matter intake;

5- The excretions of purine derivatives during the fasting period allow estimating an endogenous contribution of 0.082 mmol/kg BW0.75 for goats and 0.243 mmol/kg BW0.75 for sheep.

6- These results will contribute to the nutrition of small ruminants, such as the use of less invasive methods in the determination of DP. As well as, to prevent the flow of microbial N from being over or underestimated in goats and sheep.

7- Knowing that goats and sheep are very important for the security and food supply of the world, and the ability of these animals to use fibrous materials, their efficiency in

recycling N, making them more adapted to the conditions of arid and semi-arid regions as a feed restriction. More research evaluating protein levels and feed system in the area of animal nutrition (small ruminants) is of paramount importance. The results of these studies can lead to an answer to the producer, helping him when it comes to planning feed management, to save resources and reduce costs.