

# Intake, total apparent digestibility, and microbial efficiency of sheep fed pineapple waste silage in different planes of nutrition

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**ABSTRACT** - The study aimed to nutritionally evaluate the silage of pineapple crop waste in sheep feeding in different planes of nutrition (L). We used eight growing sheep and four male castrated adults, in individual metabolic cages distributed in a switch-back design with two treatments and three periods. The treatments were the different planes of nutrition:  $L = \text{MEI}/M_m$ ,  $\text{MEI}/1.5M_m$ , and  $\text{MEI}/2.5M_m$ , in which  $L = \text{MEI}/M_m$ , MEI is the energy amount of the feed intake and  $M_m$  is the maintenance. We performed a digestibility trial of the diet composed of silage of pineapple crop waste, ground corn, and soybean meal. Data were analyzed using regression analysis. Nutrient intake behaved linearly according to the increase in the L, except for organic matter, which presented a quadratic behavior. Planes of nutrition did not affect protein and fiber digestible fractions. However, digestible fractions of fat and non-fibrous carbohydrates (NFC) increased with L. The indigestible fractions displayed a quadratic behavior with an increase in the L. Regarding the microbial synthesis efficiency, we observed a linear decrease with the increase in L. Thus, the silage of pineapple crop waste is a feed very rich in NFC. Besides, silage of pineapple crop waste presented a good alternative roughage during forage shortages. Diet inclusion of 2.5x the maintenance does not compromise the sheep performance.

**Keywords:** byproduct, nutritional evaluation, ruminant

## 1. Introduction

The expansion of fruit farming combined with investments in agroindustries results in increased amounts of byproducts, which may increase operating costs for companies and potentially become an environmental problem depending on how the byproducts are discarded (Santos et al., 2014). Brazil is among the largest pineapple-producing countries (*Ananas comosus* (L.) Merrill) in the world, contributing 9.89% of global production (CONAB, 2020). The pineapple crop generates a large number of residues, from the plant that remains in the field to the fruit processing to obtain pulp (Antunes, 2018). From planting to selling pineapple, two types of waste are generated: industrial and crop residue, which comprises leaves, stems, and roots (Lallo et al., 2003).

Nevertheless, the largest amount of pineapple crop waste matches the time of great availability of grazing fields with good nutritional value and relatively low cost, reducing dairy and meat producers' interest in using this feed resource in its fresh form. Therefore, there is a need to preserve this crop residue for times of feed scarcity (Alves et al., 2016). Fresh fruit byproducts, such as waste from fresh

pineapple cannery, are rich in water (about 90%) and soluble carbohydrates (e.g., pectin) and decay quickly (Ososanya et al., 2014). Of the most common feed preservation techniques applicable to pineapple crop waste, controlled fermentation (through ensiling techniques) is particularly popular.

Thus, determining the nutritional value of pineapple crop waste represents an important step for this feed resource to be used with greater assurance in ruminant feeding, providing a feed alternative to increase the efficiency of the production system and minimize feed costs and nutrient losses to the environment (Russell et al., 1992; Sniffen et al., 1992). An important factor in determining nutrient utilization is the relationship between intake and digestibility of the diet, which is necessary for the measurement of the interaction between these. Digestive efficiency predicted from measurements of nonproducing ruminant animals overestimate by at least 12% the digestibility of the same ration given to the producing ruminants. Besides, much of the reduced digestive efficiency of the lactating dairy cows is correlated with the amount of diet consumed per unit of time (Tyrrel and Moe, 1975). However, information obtained empirically can be of great value in detecting anomalies (e.g., physiological state of the animal) in predicting intake and digestibility. Using the nutritional plan can get around this problem because it refers to the energy amount gained from the feed intake and the energy requirement of animals (ARC, 1980).

Although few studies have been carried out using pineapple crop waste in ruminant feeding, they have shown very promising results, apparent digestibility of dry matter (DM) of 665.80 g/kg, and digestibility of neutral detergent fiber of 548.6 g/kg (Santos et al., 2014). Thus, we hypothesized that pineapple crop waste can be used as a roughage source in sheep feeding in times of feed scarcity. So, the present study aimed to nutritionally evaluate the silage of pineapple (*Ananas comosus* (L.) Merrill) crop waste in sheep feeding on different planes of nutrition.

## 2. Material and Methods

Research on animals was conducted according to the Institutional Ethics Committee on the Use of Experimental Animals (Protocol 207/2013).

The experiment was carried out in Campos dos Goytacazes, Rio de Janeiro, Brazil, in a circumscribed area defined by the coordinates 21°45'14" S, 41°19'26" W, and elevation of 14 m above sea level.

The maintenance diet was calculated based on the maintenance requirement of metabolizable energy for sheep according to the Agricultural and Food Research Council (AFRC, 1993). To formulate the diet, we used Microsoft Excel Solver® nonlinear programming with the Newton resolution method proposed by Lasdon et al. (1978). The diet was composed of silage of pineapple (*Ananas comosus* L., Merrill, var. Pérola) crop waste, ground corn (DM = 856.30; crude protein (CP) = 84.93; neutral detergent fiber (NDF) = 90.0), and soybean meal (DM = 869.04; CP = 496.9; NDF = 140.0) as expressed in g/kg of DM, except the DM expressed on fresh basis. The diet in L = 1 consisted of: 757 g/kg silage of pineapple crop waste, 213 g/kg ground corn, and 30 g/kg soybean meal; L = 1.5 consisted of 768 g/kg silage of pineapple crop waste, 221 g/kg ground corn, and 12 g/kg soybean meal; and L = 2.5 consisted of 771 g/kg silage of pineapple crop waste, 223 g/kg ground corn, and 6.0 g/kg soybean meal. The ingredients of the diet and chemical composition are presented in Table 1.

The treatments were three planes of nutrition (L), in which  $L = MEI/M_m$ , MEI is the metabolizable energy supplied by the diet, and  $M_m$  is the metabolizable energy requirement by the animal (AFRC, 1993). The three levels were planned:  $MEI/M_m$ ,  $MEI/1.5M_m$ , and  $MEI/2.5M_m$ . We calculated how many times the energy required for maintenance would be ingested; this value is represented by L.

We used twelve male sheep (castrated [Dorper × Santa Inês] and dewormed; eight growing sheep with an average age of two years and an initial body mass of  $32 \pm 2.18$  kg [standard deviation] and four adult rams with an average age of 3.5 years and an initial body mass of  $51 \pm 2.45$  kg). The animals were housed in individual metabolic cages provided with troughs for the feed supply and water *ad libitum*. The animals were fed twice a day (at 8:00 and 16:00 h).

The growing animals were distributed in a switch-back design with two treatments and three periods. Each experimental period lasted 21 days, in which the first 14 days were the adaptation of the animals to cages and treatments and the last seven days were for samplings. Animals were weighed on the first day of each period to adjust the planes of nutrition.

For the ARC (1980), maintenance is considered constant when there is a ratio between body mass and feed intake without any change in body composition. Therefore, adult animals were weighed every three days until they reached body mass stability and feed intake. The beginning of the samplings was determined when the animals' body mass and feed intake (on a fresh basis) were stable in the last 15 days of the initial phase (Figure 1). The experimental period lasted 65 days, the first 58 days comprised the initial phase (stabilization), and the last seven days were used for the samplings.

Total fecal collections were performed using collector bags. These bags were checked every hour to avoid overfilling, which could cause discomfort to the animals, due to excessive weight. At the end of each 24 h of collection, feces were weighed and homogenized, and a sample corresponding to 10% of the total fresh weight was taken. Feces samples were then placed in properly identified polyethylene bags and stored at  $-18^{\circ}\text{C}$  in a freezer for subsequent chemical analyses.

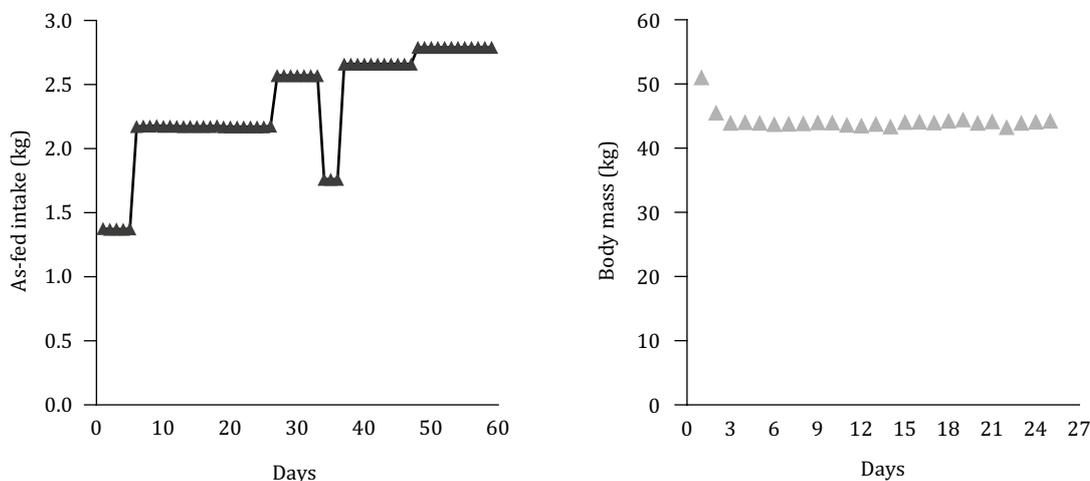
Simultaneously, urine collections were performed using plastic buckets (5 L) with filters (monofilament nylon screen) adapted to avoid contamination by impurities. In each bucket, 100 mL

**Table 1** - Chemical composition of the feeds supplied in the planes of nutrition, expressed in g/kg of DM

Variable	Silage <sup>1</sup>	Concentrate <sup>2</sup>
Dry matter (as-fed)	170.40	897.09
Organic matter	160.1	799.37
Crude protein	81.1	97.72
Ash	10.3	4.57
Lignin	54.20	64.47
Crude fat	30.94	48.50
Neutral detergent fiber	540.75	102.07
Acid detergent fiber	350.8	31.96
Non-fibrous carbohydrates	323.01	709.3

<sup>1</sup> Silage of pineapple crop waste.

<sup>2</sup> The concentrate was composed of ground corn (dry matter = 856.30; crude protein = 84.93; neutral detergent fiber = 90.0) and soybean meal (dry matter = 869.04; crude protein = 496.9; neutral detergent fiber = 140.0) as expressed in g/kg of DM, except the dry matter, expressed on fresh basis.



**Figure 1** - Stability of as-fed intake (n = 58 records) and body mass (n = 26 records) over time.

of 20% sulfuric acid solution (v:v) were added. At the end of 24 h, an aliquot of 10% of the total amount collected in each bucket was sampled, packed in polyethylene terephthalate (PET) bottles, and stored in a freezer at  $-18\text{ }^{\circ}\text{C}$  for further chemical analyses. These samplings were taken from each animal for five consecutive days.

The diet and ort samples from each treatment were weighed and recorded daily to determine the nutrient intake. They were stored in a freezer at  $-18\text{ }^{\circ}\text{C}$  for further chemical analyses.

To determine digestible energy (DE, MJ/kg), metabolizable energy (ME, MJ/kg), and total digestible nutrients (TDN, g/kg), we used the equations described in the NRC (1996, 2001):

$$\text{DE (MJ/kg)} = (\text{tdNFC}/100) \times 4.2 + (\text{tdNDF}/100) \times 4.2 + (\text{tdCP}/100) \times 5.6 + (\text{tdCF}/100) \times 9.4 - 0.3 \quad (\text{Eq. 1})$$

$$\text{ME (MJ/kg)} = \text{DE} \times 0.82 \quad (\text{Eq. 2})$$

$$\text{TDN (g/kg)} = \text{DE} \times 4.409 \quad (\text{Eq. 3})$$

The energy values were expressed in joule ( $\text{Mcal} \times 4.184$ ).

Diet, Orts, and feces samples were dried at  $55\text{ }^{\circ}\text{C}$  for 72 h in a forced-air oven. After that, the samples were ground in a knife mill with a 1-mm sieve and then homogenized to form composite samples per period (the composite samples represented the individual sample of each animal in each treatment).

Samples of diets, Orts, and feces were analyzed for DM (AOAC 967.03; AOAC, 1990), crude fat (CF; AOAC 2003.06; Thiex et al., 2003), and ashes (ASH; AOAC 942.05; AOAC, 1990). The CP content was obtained by digesting the samples (0.25 g) in 100 mL tubes, using aluminum digestion blocks according to the guidelines described in the AOAC 984.13 and AOAC (1990). We used 5 mL of  $\text{H}_2\text{SO}_4$  and 1 g of a mixture with a 56:1 ratio of  $\text{Na}_2\text{SO}_4$  and  $\text{Cu}_2\text{SO}_4 \cdot 5\text{H}_2\text{O}$ , including N recovery with  $\text{NH}_4\text{H}_2\text{PO}_4$  and Lysine-HCl certification (AOAC, 1990; Thiex et al., 2002). The soluble fiber fraction was analyzed with sodium sulfite and two additions of a standardized heat-stable amylase solution, excluding the ash according to the AOAC (2002.04; Mertens, 2002). The non-fibrous carbohydrate (NFC) content was estimated as  $\text{NFC (g/kg)} = 1000 - \text{CP} - \text{CF} - \text{Ash} - \text{NDF}$ . The analyses of fiber soluble in acid detergent (ADF) and lignin were determined according to the descriptions of Silva and Queiroz (2006).

Urine composite samples were formed per animal per period. In the urine samples, nitrogen (N urine) was determined according to the method by Thiex et al. (2002).

The purine, allantoin, and uric acid derivatives were calculated as described by Chen and Gomes (1992). Digestible organic matter fermented in the rumen (DOMR, kg/day) was estimated by the equation:

$$\text{DOMR (kg/day)} = \text{DMI} \times \text{DM} \times \text{OM} \times \text{dOM} \times 0.65, \quad (\text{Eq. 4})$$

in which DMI is the dry matter intake (kg/day), DM was the dry matter content, OM is the organic matter content, and dOM is the digestibility of organic matter.

Purine absorption (Pabs, mmol/day) was determined using microbial nitrogen (MN, g/kg):

$$\text{Pabs} = \text{MN}/0.727 \quad (\text{Eq. 5})$$

The MN was 32 g/kg DOMR (ARC, 1980). The excreted amount of total purine derivatives (DPe, mmol/day) was calculated using the equation:

$$\text{DPe} = 0.84 \times \text{Pabs} + 2 \quad (\text{Eq. 6})$$

For sheep, Chen and Gomes (1992) assumed that the endogenous contribution is equal to 2 mmol/day. Allantoin excretion ( $\text{Ae}$ , mmol/day) was considered 0.85 of DPe and, for uric acid excretion ( $\text{UAe}$ , mmol/day), 0.15 of DPe. The efficiency of microbial protein synthesis (EMPS) was estimated according to the equation:

$$\text{EMPS (g MN/kg TDN)} = [(0.629 \times \text{Pabs}) \times 6.25] / \text{TDNI} \quad (\text{Eq. 7})$$

in which TDNI is the total digestible nutrient intake and 0.629 represents the purine absorbed without considering the contribution of the endogenous fraction.

The statistical model proposed by Tempelman (2004) for animal growth analysis was used:

$$y_{ijk} = \mu + \alpha_i + \beta_j + a_k + \alpha\beta_{ij} + e_{ijk}, \quad (\text{Eq. 8})$$

in which  $y_{ijk}$  is the observation in the  $k$ -th animal receiving treatment  $i$  in the  $j$ -th period;  $\mu$  is the overall mean;  $\alpha_i$  represents the effect of the  $i$ -th treatment, for  $i = 1$  and  $2$ ;  $\beta_j$  represents the effect of the  $j$ -th period, for  $j = 1, 2$ , and  $3$ ;  $a_k$  represents the effect of the  $k$ -th animal; and  $e_{ijk}$  represents the random error.

Data were analyzed using regression analysis via MIXED procedure of SAS (Statistical Analysis System, University Edition), with restricted maximum likelihood (REML) as the estimation method and a 0.05 significance probability. The nutrient intake data were converted to  $W^{3/4}$  metabolic size and analyzed as a repeated measure over time. The repeated command was used with  $a_k$  as subjects.

The following variance and covariance structures were tested: variance components, compound symmetry, first-order autoregressive model, and heterogeneous first-order autoregressive model (Littell et al., 2006).

### 3. Results

The variance and covariance structure that best fitted the intake data of DM, CP, CF, ASH, lignin (LIG), and OM was the heterogeneous first-order autoregressive model (AR(1)). As for the intake of fiber (NDF) and NFC, it was the first-order autoregressive model. The digestibility of CP and fat had a better fit with the first-order autoregressive model. However, the digestibility of fiber, NFC, and OM, TDN, DE, ME, the indigestible fractions of CP, CF, fiber, and NFC fitted better with the heterogeneous first-order autoregressive model. The DOMR, microbial nitrogen, Pabs, PDe, allantoin excretion (Ae), uric acid excretion (UAe), and EMPS also had better fit with the heterogeneous first-order autoregressive model.

In the present study, there was no interaction effect (treatment  $\times$  period) on the variables (Table 2). The planes of nutrition ( $L = 1$ ,  $L = 1.5$ , and  $L = 2.5$ ) linearly affected the intake of DM ( $P < 0.001$ ), CP ( $P < 0.001$ ), NDF ( $P < 0.001$ ), CF ( $P < 0.001$ ), ASH ( $P < 0.001$ ), and LIG ( $P < 0.001$ ). However, the intake of NFC ( $P = 0.002$ ) and OM ( $P = 0.001$ ) presented curvilinear behavior (Tables 2 and 3). Animals in  $L = 2.5$  ingested 38.88% (4.15/6.79) more CP (Table 3) than those in  $L = 1.5$  and 40.50% (4.04/6.79) more than those in  $L = 1$ .

The increase in planes of nutrition linearly increased digestible CF ( $P < 0.001$ ) and digestible NFC ( $P = 0.005$ ), but did not affect digestible CP ( $P > 0.05$ ), digestible NDF ( $P > 0.05$ ), and digestible OM ( $P > 0.05$ ) (Tables 2 and 4). In energy content, there was a linear trend for the values of TDN ( $P = 0.051$ ), DE ( $P = 0.051$ ), and ME ( $P = 0.051$ ) (Tables 2 and 4). When analyzing indigestible nutrient fractions, the behavior that best fitted was curvilinear for CP ( $P = 0.016$ ), NDF ( $P = 0.029$ ), CF ( $P = 0.001$ ), and NFC ( $P = 0.008$ ) (Tables 2 and 4). Despite the higher CP intake of animals in  $L = 2.5$ , the digestibility did not differ from the other planes of nutrition. However, there was an increase in protein excretion of 45.90% in  $L = 2.5$  compared with that in  $L = 1.5$  and 29.67% compared with that in  $L = 1$  (Table 4). In the NFC, we observed the opposite, animals in  $L = 1$  ingested more NFC and excreted 29% less than  $L = 2.5$ , for example (Table 4).

The DOMR ( $P = 0.002$ ), MN ( $P = 0.003$ ), Pabs ( $P = 0.003$ ), PDe ( $P = 0.003$ ), Ae ( $P = 0.003$ ), and UAe ( $P = 0.003$ ) were affected by planes of nutrition in a curvilinear form and EMPS ( $P < 0.001$ ) presented a linear behavior (Tables 2 and 5).

**Table 2** - P-values related to the measured variables analyzed for the effects of planes of nutrition, periods, and treatment by period interaction

Variable	P-values associated with the effects of the statistical model				
	Treatment	Period	Interaction	L	Q
DMI <sup>1</sup>	<0.001	0.496	0.223	<0.001	0.007
CPI <sup>1</sup>	<0.001	0.424	0.239	<0.001	0.012
NDFI <sup>1</sup>	<0.001	0.912	0.395	<0.001	<0.001
CFI <sup>1</sup>	<0.001	0.761	0.592	<0.001	0.007
AshI <sup>1</sup>	<0.001	0.470	0.402	<0.001	0.275
LIGI <sup>1</sup>	<0.001	0.524	0.149	<0.001	0.002
NFCI <sup>1</sup>	0.019	0.623	0.127	0.106	0.002
OMI <sup>1</sup>	<0.001	0.470	0.402	<0.001	0.001
dOM <sup>2</sup>	0.103	0.626	0.427	0.077	0.588
dCP <sup>2</sup>	0.531	0.265	0.123	0.598	0.879
dNDF <sup>2</sup>	0.787	0.268	0.365	0.861	0.848
dCF <sup>2</sup>	0.004	0.929	0.065	<0.001	0.428
dNFC <sup>2</sup>	0.004	0.166	0.362	0.005	0.437
TDN <sup>2</sup>	0.164	0.240	0.284	0.051	0.578
DE <sup>3</sup>	0.164	0.240	0.284	0.0511	0.579
ME <sup>3</sup>	0.163	0.241	0.285	0.051	0.580
indCP <sup>4</sup>	<0.001	0.136	0.139	<0.001	0.016
indNDF <sup>4</sup>	<0.001	0.099	0.080	<0.001	0.029
indCF <sup>4</sup>	<0.001	0.268	0.127	<0.001	0.001
indNFC <sup>4</sup>	<0.001	0.730	0.148	0.003	0.008
DOMR <sup>5</sup>	<0.0001	0.0589	0.132	<0.001	0.002
MN <sup>5</sup>	<0.0001	0.0815	0.083	<0.001	0.003
Pabs <sup>6</sup>	<0.0001	0.2816	0.084	<0.001	0.003
PDe <sup>6</sup>	<0.0001	0.2865	0.086	<0.001	0.003
Ae <sup>6</sup>	<0.0001	0.2817	0.087	<0.001	0.003
UAe <sup>6</sup>	<0.0001	0.2839	0.081	<0.001	0.003
EMPS <sup>7</sup>	<0.0001	0.1843	0.137	<0.001	<0.001

L - linear model, Q - quadratic model.

<sup>1</sup> Intakes of dry matter (DMI), crude protein (CPI), neutral detergent fiber (NDFI), crude fat (CFI), ashes (AshI), lignin (LIGI), non-fibrous carbohydrates (NFCI), and organic matter (OMI), all expressed in g/W<sup>0.75</sup>.

<sup>2</sup> Digestible organic matter (dOM), digestible crude protein (dCP), digestible neutral detergent fiber (dNDF), digestible crude fat (dCF), digestible non-fibrous carbohydrates (dNFC), and total digestible nutrients (TDN), all expressed in g/kg of DM.

<sup>3</sup> Digestible energy (DE) and metabolizable energy (ME) contents expressed in MJ/kg of DM.

<sup>4</sup> Indigestible crude protein (indCP), indigestible neutral detergent fiber (indNDF), indigestible crude fat (indCF), and indigestible non-fibrous carbohydrates (indNFC), all expressed in g/kg of DM.

<sup>5</sup> Digestible organic matter fermented in the rumen (DOMR) and microbial nitrogen (MN) expressed in g/day.

<sup>6</sup> Purine absorption (Pabs), purine derivatives excretion (PDe), allantoin excretion (Ae), and uric acid excretion (UAe), all expressed in mmol/day.

<sup>7</sup> Efficiency of microbial protein synthesis expressed in g MN/kg TDN.

## 4. Discussion

It is extremely important to determine the nutritional value of this feed. For Van Soest (1994), evaluating the nutritional value of a feed or a diet requires determining feed intake, digestibility, and efficiency of nutrient utilization. Intake represents most of the variations in the feed quality, as it depends on the total amount of ingested nutrients the animal receives for its maintenance, growth, reproduction, and production. Likewise, the amount of nutrients absorbed depends on the interaction between intake and digestibility (Van Soest, 1994; Pulina et al., 2013).

**Table 3** - Intake of nutrients in the different planes of nutrition, expressed in g/W<sup>3/4</sup>

Variable	Plane of nutrition			SEM	Regression equation		
	1	1.5	2.5		$\beta_0 \pm SD$	$\beta_1 \pm SD$	$\beta_2 \pm SD$
DM	41.98	43.11	71.4	0.623	7.17±5.752	26.27±2.975	
CP	4.04	4.15	6.79	0.06	0.849±0.574	2.42±0.304	
NDF	10.89	11.9	22.05	0.197	-1.75±1.380	9.79±0.604	
CF	1.74	1.78	3.24	0.029	-0.12±0.249	1.40±0.115	
Ash	2.25	2.87	5.1	0.043	-0.19±0.088	2.13±0.198	
LIG	2.68	2.71	4.72	0.041	0.09±0.366	1.91±0.173	
NFC	16.18	12.2	14.3	0.139	33.07±6.023	-23.61±7.102	6.45±1.911
OM	91.91	86.71	69.83	0.353	109.49±1.775	-15.79±1.001	

SEM - standard error of the mean;  $\beta_0$  - intercept;  $\beta_1$  and  $\beta_2$  - slopes; SD - standard deviation; DM - dry matter; CP - crude protein; NDF - neutral detergent fiber; CF - crude fat; LIG - lignin; NFC - non-fibrous carbohydrates; OM - organic matter.

**Table 4** - Nutrient digestibility by Dorper × Santa Inês sheep in the different planes of nutrition

Variable	Plane of nutrition			SEM	Regression equation		
	1	1.5	2.5		$\beta_0 \pm SD$	$\beta_1 \pm SD$	$\beta_2 \pm SD$
dOM <sup>1</sup>	693.06	732.45	758.51	3.693			
dCP <sup>1</sup>	54.82	55.82	54.16	0.719			
dNDF <sup>1</sup>	130.75	135.54	132.96	2.572			
dCF <sup>1</sup>	28.37	31.67	35.07	0.269	25.35±1.278	3.96±0.656	
dNFC <sup>1</sup>	479.11	509.42	536.33	2.444	457.25±14.969	31.95±7.859	
TDN <sup>1</sup>	577.79	611.74	634.76	4.352	562.18±26.981	29.20±14.179	
DE <sup>2</sup>	13.1	13.87	14.4	0.099	12.75±0.662	0.66±0.312	
ME <sup>2</sup>	10.75	11.38	11.81	0.081	10.45±0.502	0.54±0.263	
indCP <sup>3</sup>	31.85	24.5	45.29	0.983	77.92±24.445	-69.13±28.299	22.42±7.486
indNDF <sup>3</sup>	99.41	84.53	165.82	3.699	227.89±86.951	-200.67±100.47	70.294±26.504
indCF <sup>3</sup>	9.76	5.82	10.74	0.246	29.01±5.783	-27.61±6.738	8.11±1.799
indNFC <sup>3</sup>	59.15	44.76	83.32	1.904	147.55±47.630	-132.25±55.495	42.61±14.819

SEM - standard error of the mean;  $\beta_0$  - intercept;  $\beta_1$  and  $\beta_2$  - slopes; SD - standard deviation.

<sup>1</sup> Digestible organic matter (dOM), digestible crude protein (dCP), digestible neutral detergent fiber (dNDF), digestible crude fat (dCF), digestible non-fibrous carbohydrates (dNFC), and total digestible nutrients (TDN), all expressed in g/kg of DM.

<sup>2</sup> Digestible energy (DE) and metabolizable energy (ME) expressed in MJ/kg of DM.

<sup>3</sup> Indigestible crude protein (indCP), indigestible neutral detergent fiber (indNDF), indigestible crude fat (indCF), and indigestible non-fibrous carbohydrates (indNFC), all expressed in g/kg of DM.

**Table 5** - Urinary excretion of purine derivatives and microbial efficiency of Dorper × Santa Inês sheep in the different planes of nutrition

Variable	Plane of nutrition			SEM	Regression equation		
	1	1.5	2.5		$\beta_0 \pm SD$	$\beta_1 \pm SD$	$\beta_2 \pm SD$
DOMR <sup>1</sup>	177.27	99.2	141.99	2.455	534.42±70.151	-489.81±80.153	133.07±20.781
MN <sup>1</sup>	5.89	3.16	4.47	0.082	18.12±2.503	-16.739±2.852	4.509±0.736
Pabs <sup>2</sup>	8.11	4.35	6.15	0.113	24.929±1.294	-23.026±3.922	6.203±1.012
PDe <sup>2</sup>	8.81	5.65	7.17	0.095	22.941±2.924	-19.343±3.295	5.211±0.850
Ae <sup>2</sup>	7.49	4.8	6.09	0.081	19.499±2.458	-16.441±2.801	4.429±0.723
UAe <sup>2</sup>	1.32	0.85	1.07	0.014	3.440±0.433	-2.901±0.494	0.781±0.127
EMPS <sup>3</sup>	71.46	46.63	35.55	0.883	68.383±4.867	-11.353±0.907	

SEM - standard error of the mean;  $\beta_0$  - intercept;  $\beta_1$  and  $\beta_2$  - slopes; SD - standard deviation.

<sup>1</sup> Digestible organic matter fermented in the rumen (DOMR) and microbial nitrogen (MN) expressed in g/day.

<sup>2</sup> Purine absorption (Pabs), purine derivatives excretion (PDe), allantoin excretion (Ae), and uric acid excretion (UAe), all expressed in mmol/day.

<sup>3</sup> Efficiency of microbial protein synthesis expressed in g MN/kg TDN.

The DMI and nutrient intake were affected with increased planes of nutrition, a behavior that was expected (Table 3). However, the animals in L = 1.5, despite the higher supply of feed, showed a small increase in the DMI of 2.62% ( $41.98 / 43.11 \times 100$ ) compared with animals in L = 1 (Table 3). This increase in DMI probably occurred due to the difference between the body masses of growing and adult animals ( $32 \pm 2.18$  and  $51 \pm 2.45$ , respectively). For sheep, the AFRC (1993) recommends a single equation for all categories depending on metabolic body mass ( $W^{3/4}$ ), but for growing animals, we suggested a correction based on the ME concentration of the diet. However, the energy requirement for maintenance is taken as the oxygen consumption of the body, in which half of these needs is used by the walls of the gastrointestinal tract and liver for the absorption and metabolism of the digested nutrients, one third through the skin, kidneys, and nervous tissue and the remainder for basic muscle activities (Seal and Reynolds, 1993; Oliveira et al., 2013). Variations in the activity levels of these tissues, depending on the genotype, age, physiological status, planes of nutrition, urea secretion, and environmental conditions, modify the energy requirements for maintenance, as observed in this study.

In this study, we used the equations proposed by the AFRC (1993) to formulate the diet of the different planes of nutrition and observed the strong effect of body mass on nutrient intake. To obtain the different planes of nutrition, we first calculated the requirement for maintenance of animals using equations 39, 42, and 50 of the AFRC (1993):

$$M_m \text{ (MJ/d)} = F + A/k_m$$

in which  $F$  is the fasting metabolism requirement,  $A$  is the activity allowance as defined below, and  $k_m$  is the efficiency of utilization for maintenance:

$$F \text{ (MJ/d)} = C1[0.23 \times (W/(1.08)^{3/4})]$$

in which  $C1 = 1.0$  for females and castrates:

$$A \text{ (MJ/d)} = 0.0067 \times W$$

Thus, despite using the metabolic size  $W^{3/4}$ , the nutrient intake of animals in L = 1.5 was slightly above that of animals in L = 1. According to the ARC (1980), the maintenance requirements of an animal are related to the amount of nutrients or energy it uses so that the vital processes of its body remain normal. To be in this state, the animal cannot change its body composition; for that, intake and body mass should not vary.

The indigestible fractions of CP (indCP) and NDF (indNDF) of the animals in L = 1.5 were smaller concerning the other planes of nutrition. This can be explained by the decrease in the passage rate of feed particles through the rumen-reticulum and the long digesta retention time through the gastrointestinal tract, facilitating the access of microbiota to feed particles, thereby probably increasing the degradation rate of feed in this organ and directly influencing the amount of fecal indCP excreted (NRC, 2001; Clauss et al., 2016). On the other hand, the increase in indCP and indNDF of animals in L = 2.5 was possibly due to the increase in the passage rate due to a high flow pressure, caused by the presence of more feed in the gastrointestinal tract (Van Soest, 1994; Clauss et al., 2016). The increase in the concentrations of TDN ( $P = 0.051$ ), DE ( $P = 0.051$ ), and ME ( $P = 0.051$ ) in the diet with increased levels in the planes of nutrition may have occurred due to the higher energy supply resulted from high values of dCF and dNFC fractions (Koscheck et al., 2013).

Regarding the digestibility coefficients, it should be noted that the increasing levels in planes of nutrition did not influence the CP and NDF digestibility (Table 4). However, the indigestible fractions of CP and NDF of the animals in L = 1.5 were smaller. This can be explained due to the decrease in the passage rate of feed particles through the rumen-reticulum and the long digesta retention time through the gastrointestinal tract, which favors the access of the microbiota to feed particles, which probably increased the degradation rate of the feed in this organ, directly influencing the amount of fecal indCP excreted (NRC, 2001; Clauss et al., 2016). On the other hand, the increase in indigestible

fractions of CP and NDF of animals in L = 2.5 was possibly due to the increase in the passage rate due to a high flow pressure caused by the ingestion of more feed and the presence of potentially digestible OM in the gastrointestinal tract (Van Soest, 1994; Clauss et al., 2016). The increase in TDN, DE, and ME concentrations in the diets with the increasing levels in planes of nutrition may be due to the higher energy supply resulting from the increases in the values of CF and NFC digestibility coefficients.

The NFC corresponds to a feed fraction with a high rate of rumen digestion (Sniffen et al., 1992) and contributes to the energy supply of ruminant animals. Non-fibrous carbohydrates and nitrogen availability in the rumen are important for maximizing microbial growth, which contributes to the microbial protein supply in the small intestine (Seo et al., 2013). In the present study, the amount of NFC ingested by the animals in L = 1 was higher than that of the animals in the other planes of nutrition, but the dNFC was lower (Table 4). Nevertheless, a higher microbial synthesis and better microbial efficiency were observed in the animals in L = 2.5 (Table 5). This was probably because the pineapple crop waste is rich in pectin, which is a rapidly fermentable carbohydrate in the rumen (Van Soest, 1994). Another factor that maximized the microbial synthesis and efficiency was the high OM intake by the animals in L = 1 (Table 5), directly impacting the fermentable OM in the rumen (Table 5). According to the ARC (1980), the amount of OM fermented in the rumen is a good estimate of the flow of microbial proteins reaching the intestine; the animals in L = 2.5 had 17.8% more than those in L = 1 and 27.67% more than those in L = 1.5.

The higher amount of OM fermented in the rumen of animals in L = 1 probably occurred due to the high intake of OM combined with its better use, resulting in the greater microbial efficiency of the animals in L = 1 (Table 5). However, the microbial efficiency observed in this study was lower than that established by the NRC (2001) of 130 g N/kg TDN. On the other hand, the value observed in L = 1 was 71.46 g N/kg TDN, a value very close to that found by Almeida et al. (2019) of 80.6 g N/kg TDN.

## 5. Conclusions

The silage of pineapple crop waste is a very rich non-fibrous carbohydrates (pectin, mainly) feed. Besides, this silage presented a good alternative roughage during forage shortages. The diet inclusion of 2.5 times the maintenance does not compromise the sheep performance.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: A.M. Fernandes and T.S. Oliveira. Data curation: C.C. Cordeiro, A.M. Fernandes and T.S. Oliveira. Formal analysis: T.S. Oliveira and L.S. Glória. Investigation: C.C. Cordeiro, A.M. Fernandes, M.G. Camilo, D.F. Baffa and S.E.E. Bernardo. Methodology: A.M. Fernandes and T.S. Oliveira. Project administration: A.M. Fernandes and T.S. Oliveira. Resources: A.M. Fernandes. Supervision: A.M. Fernandes. Writing-original draft: C.C. Cordeiro, A.M. Fernandes, T.S. Oliveira and L.S. Glória. Writing-review & editing: A.M. Fernandes and T.S. Oliveira.

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