

1 Physical and biochemical characteristics of tropical grass and legume
2 pastures grazed by lambs

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1 **Abstract**

2

3 Large areas of tropical grass pastures are not grazed by lambs because of the difficulties
4 in managing high growth rate swards with high selective animals. The objective of this
5 study was to evaluate the physical and biochemical characteristics of tropical pastures
6 offered to lambs in continuous grazing. An upright grass and a shrub legume were set out
7 in three pasture types and grazed by lambs: 1) aruana grass monoculture (AG – *Panicum*
8 *maximum* Jacp. cv. IZ-5), 2) pigeon pea monoculture (PP – *Cajanus cajan* (L.) Millsp.
9 cv. Anão) and 3) contiguous swards (CS), half of the paddock with AG (CSAG) and half
10 with PP (CSPP). The pastures were evaluated for structural characteristics, production,
11 nutritional composition and antioxidant concentrations in 4 periods over 92 days of
12 continuous grazing by lambs. Hand plucking samples, similar to animal's diet, were
13 collected for chemical analysis. Regarding height, the PP legume monoculture after 42
14 days of grazing had uncontrollable growth by the lambs, reaching 1.2 m in height. This
15 same legume but as a contiguous sward beside of AG (CSPP) was maintained at lower
16 and similar height throughout the experimental periods. In general, the Leaf:Stem ratio
17 of the different pastures decreased over the experimental periods from 0.7 to 0.2. In most
18 periods, the CS showed intermediary nutritional quality, comparing to AG and PP. The
19 alpha-tocopherol content was similar among swards, with an average of 137.2 ± 13.67
20 mg/kg of green matter ($P > 0.05$). Pigeon pea showed the highest levels of total tannin
21 and condensed tannin at 63 d and 92 d ($P < 0.05$). The use of tropical grass together with
22 a legume provide a better physical structure of the pigeon pea for grazing lambs than
23 when monoculture of this species.

24 **KEYWORDS:** green panic, pigeon pea, shrub legume, tannin, tocopherol

1 1 | INTRODUCTION

2

3 Tropical grasslands are one of the largest terrestrial biomes (Török et al., 2021), it covers
4 more than 25% of the terrestrial earth's surface (Török & Dengler, 2018). The C4
5 photosynthesis of tropical grasses can turn these areas one the most ruminant production
6 areas of the world. Brazilian beef production is an example of this production potential.
7 Brazil is one of the greatest beef cattle meat exporters and most of this production happens
8 in these pastures. The primary challenge in this production system lies in ensuring
9 efficient plant growth and optimal consumption by animals through proper pasture
10 management. According to Costa et al. (2021), the intensity of defoliation by cattle in
11 tropical pastures under continuous stocking must maintain a pasture height between 20-
12 40cm. Under intermittent stocking, this defoliation intensity must be 40 to 50% of the
13 pasture height. Maintaining these pasture structures results in greater bovine performance
14 and productivity. However, sheep production in these areas is still much below its
15 potential (Hermuche et al., 2013). One of the main reasons is due to the lack of knowledge
16 of how the pasture physical and the animal's diet biochemical characteristics change
17 during the plants' growing season, especially when comparing different swards formed
18 by tropical grass and/or legume.

19 The growth dynamics of pastures in the pastoral ecosystem is directly affected by
20 the action of grazing (Carvalho et al., 2001; Fajardo et al., 2015). In tropical pastures,
21 most of the studies have been carried out with cattle, focusing on maximizing sward leaf
22 area index in order to optimize assimilate production and energy supply to plant growth
23 (Silva et al., 2015). However, Silva et al. (2020) suggested that for lambs, the grazing
24 height must be below what is recommended for cattle. Small ruminants that graze tropical
25 pastures show a very challenge management once these animals have great diet selection

1 and low pasture growth control (Poli et al., 2020). This selective grazing behavior can
2 lead to uneven utilization of available forage, potentially resulting in overgrazing of
3 preferred species and underutilization of others. Understanding these changes in tropical
4 pasture structure in grazing systems is essential to outline the adequate management and
5 to determine strategies that guarantee the productive efficiency of the herd, offering
6 quality and availability of forage.

7 In addition to the productive and nutritional capacity, tropical swards may have
8 biochemical characteristics that favor the production of ruminants. The presence of
9 secondary compounds with antioxidants, such as tocopherols and condensed tannins, can
10 have a significant impact on the production and quality of meat (Tontini et al., 2019).
11 Many studies associate alpha-tocopherol with the ability for retarding lipid oxidation and
12 losses due to carcass exudation, as well as, enhancing meat color stability, significantly
13 increasing its shelf life (Liu et al., 2012; López-Bote et al., 2001; Soares, 2002). The
14 condensed tannins and tocopherols are compounds that can also improve utilization
15 efficiency of plant crude protein during rumen fermentation, thereby improving overall
16 digestibility, and performance (Patino et al., 2015). As a result of changes in the digestive
17 process, condensed tannins can mitigate environmental impact by reducing rumen
18 methane emission, increasing urea recycling efficiency in the rumen, having anthelmintic
19 properties that reduce infection by gastrointestinal parasites (Athanasiadou et al., 2001;
20 Pathak et al., 2013; Tedeschi et al., 2014).

21 Therefore, understanding the growth dynamics of these pastures when grazed by
22 lambs becomes essential to increase the efficiency of ruminant production in regions of
23 tropical and subtropical climates. The objective of this study was to evaluate the plant
24 structural growth dynamics and the animal's diet biochemical characteristic variations of

1 upright tropical forage species, offered for lambs under continuous grazing in
2 monocultures or in a combined sward.

3 4 2 | MATERIAL AND METHODS

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6 The experimental protocol was approved by the Ethics Committee on the Use of Animals
7 of the Universidade Federal do Rio Grande do Sul Project N° 27830.

8 9 2.1 | Experimental site: location and climatology

10 The study was conducted at the Agricultural Experiment Station (EEA) of the
11 Universidade Federal do Rio Grande do Sul (UFRGS), Brazil, located 46 m above sea
12 level in southern region of Brazil (30°05'11" S, 51°39'09" W). The experiment was
13 carried out during 92 d from 10th January to 12th April, 2016. The climate is classified as
14 subtropical humid (Cfa classification, Köppen) with a long-term average rainfall of 1.440
15 mm distributed evenly throughout the year. During the experimental period, the average
16 temperature recorded was 24.1 °C and the average monthly precipitation was 143.5 mm.
17 These data were obtained from a meteorological station located 2 km from the
18 experimental area. The climatic conditions during the experimental period are shown on
19 Figure 1.

20 21 2.2 | Experimental design

22 The experiment was set out in a randomized complete block design with three
23 replications. The block was used to control the differences between soil characteristics.
24 Paddocks of 0.2 ha constituted the experimental units in order to understand the sward
25 structural growth dynamics and the lamb diet biochemical characteristic variations of

1 three different pasture types (Treatments): 1) monoculture of aruana grass (*Panicum*
2 *maximum* Jacq. cv. IZ-5; AG); 2) monoculture of pigeon pea legume (*Cajanus cajan* (L.)
3 Millsp. cv. anão, PP) and 3) contiguous swards (CS), half the paddock with AG (CSAG)
4 and half with PP (CSPP) (see Figure 2).

5 The trial was established in 2015. Three months preceding the sowing of the
6 experiment, the area was sprayed twice with 1.5 L/ha of glyphosate (480 g a.i./ha) and
7 the soil was ploughed, incorporating 200 kg/ha of a N–P–K fertilizer formulated with 5%
8 of N, 30% of P₂O₅ and 15% of K₂O. In November of 2015, 70 days before the beginning
9 of the experiment, 10 kg/ha of Aruana grass seed was over-sowed by hand and 30 kg/ha
10 of pigeon pea seeds was sown using a seeder with lines spaced of 34 cm between them.
11 In December 2015, 150 kg N/ha was applied over the Aruana Grass swards, using urea.
12 Approximately one month before starting the experiment, the paddocks were mowed at
13 20 cm high to make the plots uniform.

14

15 2.3 | Pasture characteristics

16 Fifty sward height random points were obtained every 21-d (21, 42, 63 and 92 days) in
17 each paddock, using a sward stick. In order to estimate herbage mass (HM), six 0.25-m²
18 quadrats were hand-clipped at ground level from each paddock: three representing the
19 average sward height and three random samples. From these samples, random subsamples
20 of approximately 200g were collected to place in a forced air oven at 65°C until constant
21 weight in order to estimate the dry matter content (DM). A second subsample was taken
22 to separate leaves from stems (stem plus sheath of Aruana grass) and senescent material.
23 After separation, this material was dried in a forced air oven at 65°C until constant weight,
24 and weighted.

1 Herbage allowance was adjusted every 21 days in order to provide the same
2 amount of leaf blade in the different paddocks, according to methodology described by
3 Sollenberger et al. (2005). To calculate the herbage allowance, it was necessary to know
4 the growth rate of the pasture through the methodology described by Kinglmann et al.
5 (1943). Daily forage accumulation rate was measured using three grazing exclusion cages
6 per paddock. The objective of the evaluation was to measure the daily rate of pasture
7 growth. The daily pasture growth rate was estimated by the difference between the sample
8 cut inside the cage in the present period, and the forage mass cut in the previous period
9 outside the cage, divided by the number of days in the period.

10 11 2.4 | Animals

12 Fifty-four Corriedale x Texel male castrated lambs, aged between 3-4 months and with
13 an average live weight (LW) of 20.4 ± 3.97 kg were allocated to the treatments. The
14 animals were allocated based on weight, ensuring that all paddocks had similar weight
15 among the animals. Prior to the commencement of experiment, all animals were
16 vaccinated against clostridiosis (Ourovac® 10 TH, 2 mL). In addition, gastrointestinal
17 parasite infection was monitored using fecal egg count (FEC) and the FAMACHA
18 method. Ten days after the start of the experiment, all animals received anthelmintic
19 treatment (Closantel 10%, 1mL/10 kg of body weight). Throughout the experiment,
20 animals were treated whenever their FEC exceeded 1000. Lambs had access to shade, as
21 well as water and mineral salt in *ad libitum*.

22 During 92 days of assessment, six tester lambs grazed continually each paddock
23 (n=18) with variable number of regulating animals in order to maintain the same herbage
24 allowance of 10% green leaf in all treatments [10 kg leaf blade dry matter (DM) per 100
25 kg of animal body weight (BW)/day]. The herbage allowance was adjusted every 21-d

1 using the “put-and-take” technique (Mott & Lucas, 1952). According to this technique,
2 there were two groups of animals, one called “testers”, represented by the animals that
3 remain throughout the experimental period in grazing and express the effects of
4 treatments, and the other group called “regulators”, used only to control the growth of the
5 pasture and keep the herbage allowance at the desired level.

6 7 2.5 | Sampling of the chemical composition and analysis

8 Three weeks before the beginning of the experiment, the animals were used to the
9 presence of people close to them. Representative samples of the forage consumed by the
10 lambs were collected by grazing simulation termed “hand plucking” technique, described
11 by Euclides et al. (1992). The lambs (n=6 per paddock) were followed by a trained
12 evaluator during grazing, and samples, similar to what animals were grazing, were
13 collected. Sampling was carried out twice a day, obeying the lambs' grazing peaks (at
14 dawn and dusk). Each sampling lasted an average of 2 hours, where the evaluator
15 alternated the grazing simulation between the six “testers” animals in each paddock. The
16 samples collected were similar in the quality consumed and the quantity collected by the
17 animals in each mouthful. The samples were conditioned in a thermal box, with ice and
18 without light.

19 After hand pluck sampling, each sample was divided into three subsamples. One
20 subsample was used for tocopherol analysis, one was freeze dried for tannin
21 determination, and one was dried in an oven at 65 °C for 72 hours for the determination
22 of the pasture quality.

23 Alpha-tocopherol content was determined following the methodology of Val et al.
24 (1994). Extraction was done with acetone and ascorbic acid, and the determination was
25 performed using high performance liquid chromatography (HPLC) (Waters Acquity

1 UPLC CLASS), MeOH: H₂O 93:7 (v:v) as mobile phase and a NovaPak4 μm 30-cm
2 column (Górnaś et al., 2014).

3 The subsamples for pasture tannin content were ground in a Wiley mill fitted with
4 a 0.5-mm screen. Total (TT), hydrolysable (HT) and condensed (CT) tannin
5 concentrations were determined by adapting Grabber et al. (2013) and Makkar (2000)
6 methods. The results were expressed as gram equivalent (eq-g) of leucocyanidine/kg of
7 DM, following the regression: eq-g leucocyanidine (L)/kg DM = {absorbance x [10x
8 (dilution volume in ml) / (460 x sample weight)]/(DM, in kg)} x 10.

9 The subsamples for assessing pasture quality were ground in a Wiley mill fitted
10 with a 0.5-mm screen. Chemical analysis for estimating pasture quality included DM
11 (method n° 930.15), organic matter (OM) and ashes (method n° 942.05), and crude protein
12 (CP, method n° 984.13), according to the Association of Official Analytical Chemists
13 (AOAC, 1995); ether extract (EE; Silva & Queiroz, 2002); neutral detergent fiber (NDF;
14 Van Soest et al., 1991), acid detergent fiber (ADF) and acid detergent lignin (ADL;
15 Goering & Van Soest, 1970). In situ dry matter digestibility (ISDMD) was determined
16 using a rumen fistulated bovine, according to Soto-Navarro et al. (2014) and Ørskov &
17 McDonald (1979). The dried samples were weighed and incubated in polyester filter bags
18 (porosity 41 μm) for 48 h.

19

20 2.6 | Statistical analysis

21 The physical and biochemical characteristics of the pasture species and swards were
22 analyzed with the MIXED procedure of SAS statistical software, using repeated measures
23 in time at a significance level of 5%, in which pasture species or sward type, blocks and
24 period (repeated measure over time) and their interaction were considered as fixed effects.

1 The paddocks and error effects were random. Each paddock was considered as an
2 experimental unit.

3 The normality of the residues was tested and covariance structures were analyzed
4 to fit the models, based on Bayesian information criterion (BIC), from which the structure
5 "component of variance (VC)" was selected. The means were compared by Tukey test at
6 5% significance using least-square means. The variables related to chemical composition
7 and antioxidants of the pastures were also submitted to Pearson correlation analysis.

8

9 3 | RESULTS

10

11 3.1 | Pasture characteristics

12 The physical characteristics of the pasture for each treatment throughout the experimental
13 periods are shown in Figure 3. The variables pasture height (Figure 3A), herbage mass
14 (HM, Figure 3B), and stem mass (SM, Figure 3C) were different only at 63 d and 92 d.
15 PP showed to be the tallest species ($P < 0.05$; Figure 3A), while AG, CSAG and CSPP
16 pastures had similar heights among them, throughout the experimental periods ($P > 0.05$).
17 At the 63 d and 92 d, the CSPP showed the smallest HM ($P < 0.05$; Figure 3B), while the
18 others swards had similar HM ($P > 0.05$). SM data (Figure 3C) had a similar variation to
19 HM (Figure 3B). The CSPP pasture, at the 63 d and 92 d, showed the lowest SM ($P <$
20 0.05 ; Figure 3C), while AG, PP and CSAG pastures were similar in the amount of SM (P
21 > 0.05). Treatments with the presence of Aruana grass (AG and CSAG) show the highest
22 leaf mass values at the 42d and 63d of the experiment compared to CSPP ($P < 0.05$;
23 Figure 3D). The CSPP pasture, at the 42 d, showed the lowest leaf:stem ratio (L:S ratio)
24 ($P < 0.05$; Figure 3E), the CSAG the greatest, and AG and PP pastures had similar L:S
25 ratio ($P > 0.05$). It can be observed that there was, in general, a reduction of the L:S ratio

1 over the experimental periods. The CSPP pasture, at the 92 d, showed the smallest growth
2 rate ($P < 0.05$; 28.50 kg DM/ha/day; Figure 3F). This growth rate was 73% lower
3 compared to PP, and 56% lower compared to AG and CSAG. On average, the PP pasture
4 showed the greatest growth rate (106.4 kg DM/ha/day), and AG and CSAG pastures had
5 similar ($P > 0.05$) and intermediate values (64.95 kg DM/ha/day).

6 7 3.2 | Pasture quality

8 There was no significant interaction between type of pastures and sampling periods ($P >$
9 0.10) for DM, ISDMD, EE and ADF, as shown in Table 1. However, there were
10 significant interaction between pasture type and period for OM, ashes, NDF, ADL ($P <$
11 0.05), and a tendency of this interaction effect for CP ($P = 0.0946$) (Table 1; Figure 5).

12 All three pasture types showed to be similar ($P > 0.10$; Table 1) for DM and
13 ISDMD, but sampling date had effect on them ($P < 0.01$; Figure 4). The first sampling
14 period presented the highest levels of DM and ISDMD (30 ± 0.88 and $62 \pm 2.4\%$,
15 respectively), compared to the others periods (average of the last three periods: 21.3 ± 1.2
16 and $55.6 \pm 2.1\%$, respectively). The EE content was similar ($P > 0.10$) among the three
17 pasture types and it kept steady through all sampling date (Table 1). However, the pasture
18 type, independent of the period, affected the ADF content ($P < 0.05$; Table 1), being the
19 greatest values in the AG and the lowest in PP ($P < 0.05$), whereas the CS pasture did not
20 differ from both.

21 The effect of the interaction between sampling period and sward type on the
22 biochemical composition is shown in Figure 5. It was observed only significant
23 differences between pasture types on concentrations of OM (Figure 5A), ashes (Figure
24 5B) and ADL (Figure 5C) at 63 d and 92 d. The modification of the chemical composition
25 along the experiment showed that CS pasture, most of the time, had an intermediate value

1 between AG and PP. At 63 d and 92 d, PP pasture showed the greatest concentration of
2 OM and ADL ($P < 0.05$, Figures 5A and 5C), the smallest concentration of ashes (Figure
3 5B) and tended to have the greatest CP content ($P = 0.09$, Figure 5E). The CP content
4 tended to differ between pastures in the 63d and 92d sampling period, PP tended to show
5 the greatest CP content, while AG had the lowest (Figure 5E; $P = 0.0946$). The NDF
6 content varied between treatments at 21 d sampling (Figure 5D), showing that AG had
7 the greatest NDF content and PP the lowest ($P < 0.05$), whereas CS pasture had
8 intermediate concentration, without differing from the other treatments ($P > 0.05$).

9

10 3.3 | Antioxidants composition

11 The alpha-tocopherol content was similar among the different pasture types, with an
12 average of 137.2 ± 13.67 mg/kg of green matter (GM) ($P > 0.05$), but the sampling day
13 had effect on its content ($P < 0.05$), with greater contents in 21 and 42 d (155.1 ± 15.54
14 mg/kg GM), and the lowest content was recorded in the last two sampling periods (119.3
15 ± 14.76 mg/kg GM). This variation of alfa-tocopherol significantly fitted in a linear
16 regression (Figure 6) along the time ($\hat{y} = 197.51 - 0.89x$; $R^2 = 0.81$; $P < 0.01$). Every day
17 it is expected a reduction of 0.89 mg/kg alpha-tocopherol in the forage.

18 There was significant interaction between type of pasture and sampling periods
19 on TT and CT contents ($P < 0.05$). The variation of the TT and CT levels in the three
20 pastures studied are shown in Figure 7. At the beginning of the study, 21 d and 42 d, there
21 were not significant differences among the pastures ($P > 0.05$), but from 63 d until the
22 end of the study (92 d), the PP had the greatest TT (Figure 7A) and CT (Figure 7B)
23 concentration, whereas the chemical composition of AG and CS were similar ($P > 0.05$,
24 Figures 7A and 7B). The HT content was similar in all treatments and periods ($P > 0.05$),
25 with an average of 2.34 ± 0.37 eq-g of leucocianidine/kg of DM.

1

2 4 | DISCUSSION

3

4 The association, or not, of a shrubby tropical legume, as *Cajanus cajan* (PP), with an
5 upright grass, as *Panicum maximum* (AG), showed to affect the pasture structure when
6 grazed by lambs. When associated with a grass the shrubby legume did not show an
7 excessive height. When the animals were grazing a monoculture of the legume, the
8 average height was greater than one meter, making the access to leaves by animals very
9 difficult. Apparently, it demonstrates that the animals have a more important effect on
10 legume height when associated to grass than when it is by itself. This result complement
11 of what was found by Tontini et al. (2021) that PP height affected the grazing time and
12 lamb performance.

13 Tontini et al. (2021) showed that height is the main variable responsible for the
14 increase in grazing time of lambs in a monoculture of the legume *Cajanus Cajan*. The
15 developed model estimates that if PP height is lower than 76 cm, grazing time will be on
16 average 329 minutes/day; however, if PP height is ≥ 76 cm, lamb grazing time will
17 increase to 455 minutes/day. According to the authors, the increase in grazing time is a
18 strategy expressed by herbivores to maintain the intake. In this present study, the
19 difficulty for the animals reach the leaves might make them to spend more time to harvest
20 enough leaves for the daily food. Although the legume HM when associated to a grass
21 was significantly lower, after 42 days of grazing, it was possible to observe a similar L:S
22 ratio among pastures. Therefore, these studies indicate that maintaining a shorter structure
23 of the tropical legume with a shrubby growth habit associated with a grass, can make the
24 legume structure more adequate for grazing lamb.

1 The monoculture of PP under young lamb grazing demonstrated the characteristic
2 development of a tropical shrubby legume with woody stems, exhibiting a fast growth
3 habit and rapid maturation rate. The challenge posed to the lambs in grazing this legume
4 differed depending on its presentation form (PP or CSPP). The lower height observed in
5 CSPP (Figure 3A) compared to the monoculture of PP, after 63 days of grazing, may be
6 associated with the grazing pressure that occurred in this treatment due to the smaller
7 legume area compared to the PP treatment. This way, lambs in contiguous pasture had
8 better control of CSPP height, which meant that this legume area had a lower growth rate.

9 Although there is no treatment effect on digestibility of the forage, it was observed
10 an important increase of lignin content of PP after 42 days of continuous grazing. This
11 result might be related to the type of carbohydrate and lignin concentrations found in
12 tropical grasses and legumes (Van Soest, 1994), and to the variations on plant structures.
13 As expected, pigeon pea showed greater concentration of protein and greater
14 concentration of lignin than aruana grass during the grazing period (Castro-Montoya &
15 Dickhoefer, 2020). Despite the lower leaf-to-stem ratio and maturity of pastures observed
16 over the experimental period, the protein concentration increased in the legume treatment
17 and remained more constant in the CS and grass monoculture treatments. It is important
18 to reaffirm that these nutritional values are based on samples that simulate animal grazing
19 (hand plucking), indicating that the decrease in nutritive value with overall pasture
20 maturation does not necessarily imply lower dietary quality for animals. Given the
21 opportunity, lambs exhibit selective grazing behavior, targeting the most nutritious parts
22 of the plant. One of the primary objectives motivating producers to intercrop a legume
23 with a grass is to improve the nutritional quality of pasture provided to animals,
24 particularly by increasing the protein content of the diet. In this study, a comparison with

1 the grass monoculture revealed a trend towards a 16% higher protein concentration in the
2 adjacent intercropped pasture system (Table 1).

3 This study shows variations of biochemical characteristics over time between
4 tropical grass and legumes. It can be observed that when the legume and grass is offered
5 together, the animals have a better opportunity to have a balanced diet than if only one
6 pasture species is offered. In addition, Castro-Montoya & Dickhoefer (2020) state that
7 the higher lignin concentration of legumes, compared to grasses, may be an important
8 limiting factor for their inclusion at high levels in the diets of ruminants when they are
9 offered in the form of hay or silage. This fact shows that offering the legume with the
10 grass allows the animal to balance its consumption. According to a thorough review of
11 Castro-Montoya & Dickhoefer (2020), there is limited information for several nutrients'
12 characteristics of tropical legumes, indicating the need for future research.

13 The use of different types of pastures formed by monocultures of a legume or a
14 grass, or the association of both were not able to influence the concentrations of alpha-
15 tocopherol in lamb's diet (See results in Antioxidants composition Section). The possible
16 causes of this result are the similar leaf:stem ratio between pasture species (Figure 3E). It
17 contributed to the concentration of tocopherol in the animal diet being similar between
18 treatments. Usually, tocopherol concentration is higher in leaves than in the stems
19 (DellaPenna & Last, 2006; Livingston et al., 1968; Lynch et al., 2001). The greater
20 leaf:stem ratio and sampling period contributed to the higher concentration of the
21 tocopherol concentration in the first two sampling periods (Figure 3E). The amount of
22 alpha-tocopherol found in the present study is above of usually found in unstressed
23 photosynthetic plant tissues (below 50 g/kg, according to DellaPenna & Last, 2006). The
24 alpha-tocopherol content found in all pastures is enough to cover the daily requirements

1 of grazing lambs (NRC, 2007) and might promote an important reduction of meat lipid
2 oxidation (Ripoll et al., 2011; Ripoll et al., 2013).

3 This experiment shows a small decrease of tocopherol concentration in the lambs'
4 diet over time, with advancing plant phenological stages (Figure 6). This small variation
5 of tocopherol concentration in the diet over time might represent that the animals select
6 plants parts. Munné-Bosch et al. (2001) reported a marked reduction of tocopherol
7 concentration with the senescence, losing antioxidant defenses and oxidative stress of the
8 photosynthetic membranes. However, very little studies have been carried out relating the
9 pasture tocopherol concentration and animal selection, performance and product quality.

10 Tropical legumes are known to have high concentrations of condensed tannin
11 (CT) (Pereira et al., 2018). High concentration of CT (> 50 g/kg) can have an adverse
12 effect on animal performance, as it is considered an anti-nutritional factor, negatively
13 affecting food consumption and digestibility (Naumann et al., 2017). This may be one of
14 the reasons for the low use of tropical legumes with grazing ruminants. However, in this
15 study, the concentration of condensed tannin in the legume was less than 20 g/kg of DM.
16 The concentration that usually does not cause an adverse effect when consumed by
17 ruminants (Naumann et al., 2017). In addition, this concentration is close to what is
18 considered ideal to bring benefits to ruminant animals. According to Muir (2011), diets
19 containing moderate levels of CT (from 20 to 50 g/kg of DM) can bring benefits to the
20 performance of animals, usually related to the protection of dietary protein from ruminal
21 degradation (by-pass), with a consequent increase in the absorption of amino acids in the
22 animal's small intestine, improving performance and with a potential anthelmintic effect.

23 The highest CT concentration of lambs' diet in PP was found in the last two
24 sampling periods of assessment. There are different reasons for a plant to have a greater
25 concentration of tannin in the end of the growth season, during this period there is an

1 increase in the biosynthesis of secondary metabolites and the development of
2 inflorescences and seed production. Barbehenn & Constabel (2011) affirm that plants can
3 also increase their tannin concentration due to the herbivory, some plants may protect
4 their reproductive phase by increasing the tannin content, thus mitigating herbivory.
5 Kraus et al. (2003) mention that tannin concentrations in plants vary in response to several
6 environmental conditions. The Figure 1 shows that there were no important environment
7 variations during the experiment period, as water availability or temperature. In fact, it
8 can be observed that when the animals were able to choose their diet with, or without
9 tannin, they balance it in order to have an intermediate amount of the tannin. In CS
10 treatment (see Figure 7), different from PP treatment, there is no important variation of
11 CT concentration throughout the experiment periods. This ingestive behavior coincide
12 with different studies that animals balance their diet as a function of their physiological
13 needs (Newman et al., 1992; Poli et al., 2018; Provenza, 1995) and the diet characteristics
14 (Barry et al., 2001).

15 Therefore, there is an important benefit of using a tropical legume together with a
16 grass. The use of the two species can favor a better diet balance of nutrients and tannins.
17 In addition, this study shows that beyond of benefits related to the nutrient's concentration
18 in the diet, the use of different species is important to have better sward physical
19 characteristics for small animals, allowing the swards to be better used for long period by
20 lambs. Upright legume, as *Cajanus cajan*, can easily become too tall for grazing small
21 ruminants.

22

23 5 | CONCLUSION

24

1 We can conclude that in tropical and subtropical areas with grazing lambs, the association
2 of an upright tropical grass, as *Panicum maximum*, with a shrub legume, as *Cajanus*
3 *cajan*, promotes a better biochemical characteristic of the animals' diet and a better
4 pasture physical structure for grazing lambs than when in grass monoculture. However,
5 in tropical pasture, the levels of tocopherol in the animal's diet is more associated to the
6 presence of green leaves than to the fact that the pasture is a monoculture or not. Unlike
7 *Panicum maximum*, *Cajanus cajan* is an important source of condensed tannin, and lambs
8 are able to have an intermediate amount of tannins in their diet when there is association
9 with grass.

10

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1

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1 **TABLE 1** Average chemical composition and dry matter digestibility according to the pasture
 2 types: Aruana grass (*Panicum maximum* cv. IZ-5), Pigeon pea (*Cajanus cajan*), CS (half of the
 3 paddock with *Panicum maximum* cv. IZ-5 and half with *Cajanus cajan*) under grazing of lambs

Variables ¹	Pasture Types			MSE ²	Probability		
	Aruana grass	Pigeon pea	CS		Forage	Sampling Period	Forage * Period
DM	22.51	24.17	23.5	0.88	0.4878	0.0001	0.4709
EE	2.52	3.04	3.22	0.4	0.2900	0.1878	0.8869
ADF	31.55 a	27.70 b	29.99 ab	0.66	0.0482	0.1638	0.1598
OM	88.35 b	90.5 a	88.92 ab	0.49	0.0433	0.0050	0.0086
Ash	11.33 a	9.09 b	10.80 ab	0.38	0.0247	0.0248	0.0065
NDF	62.54 a	47.83 b	56.49 ab	2.27	0.0359	0.0102	0.0012
ADL	3.60 b	7.73 a	5.02 ab	0.54	0.0142	0.0009	0.0003
CP	14.00	20.00	16.18	1.47	0.0973	0.0415	0.0946
ISDMD	58.24	58.85	53.76	2.4	0.4076	0.0086	0.4700

4 ¹DM (%): Dry matter content; EE (%): Ether extract; ADF (%): Acid detergent fiber; OM (%):
 5 Organic matter; NDF (%): Neutral detergent fiber; ADL (%): Acid detergent lignin; CP (%):
 6 Crude protein; ISDMD (%): *In situ* dry matter digestibility (ISDMD).

7 ²MSE: mean square error; a, b Values within a row with different letters differ significantly at
 8 Tukey test ($P < 0.05$).

9

1 Figure legends

2

3 **FIGURE 1** Average precipitation, Average temperature, Maximum temperature and
4 Minimum temperature during the experimental period from 10 January to 12 April, 2016
5 in southern Brazil (30°05'11" S, 51°39'09" W).

6

7 **FIGURE 2** Treatment paddocks layout: 1) monoculture of aruana grass (*Panicum*
8 *maximum* cv. IZ-5); 2) monoculture of pigeon pea legume (*Cajanus cajan* cv. anão) and
9 3) contiguous swards, half the paddock with aruana grass (CSAG) and half with pigeon
10 pea legume (CSPP), there is no fence between the two pasture species. This figure
11 represents one block. Total area of each paddock = 0,2 ha.

12

13 **FIGURE 3** Physical characteristics of tropical pastures species (monoculture of Aruana
14 grass (*Panicum maximum* cv. IZ-5; AG); monoculture of Pigeon pea legume (*Cajanus*
15 *cajan* cv. Anão; PP); and contiguous swards, half the paddock with AG (CSAG) and half
16 with PP (CSPP)) under grazing lambs throughout the experimental period.

17 ^{a, b} Letters different within a same period for each of pasture species differ significantly
18 at Tukey test ($P < 0.05$).

19

20 **FIGURE 4** Dry matter (DM) and *In situ* dry matter digestibility (ISDMD) of tropical
21 pastures over time under continuous grazing by lambs in southern Brazil.

22

23 **FIGURE 5** Chemical characteristics of tropical pastures species (monoculture of Aruana
24 grass (*Panicum maximum* cv. IZ-5; AG); monoculture of Pigeon pea legume (*Cajanus*
25 *cajan* cv. Anão; PP); and contiguous swards (CS), half the paddock with AG and half
26 with PP) under grazing lambs throughout the experimental period.

1 ^{a, b} Different Letters within a same period for each of pasture species differ significantly
2 at Tukey test $P < 0.05$ (OM, Ashes, ADL and NDF). The CP variable does not have
3 different letters as it is a trend with a probability greater than $P > 0.05$ and less than $P <$
4 0.10 ($P = 0.0946$).

5

6 **FIGURE 6** Alpha-tocopherol content between tropical pasture type and sampling period.

7 CS= contiguous swards.

8

9 **FIGURE 7** Concentration of total and condensed tannins of tropical pastures
10 (monoculture of Aruana grass (*Panicum maximum* cv. IZ-5; AG); monoculture of Pigeon
11 pea legume (*Cajanus cajan* cv. Anão; PP); and contiguous swards (CS), half the paddock
12 with AG and half with PP) under grazing lambs throughout the experimental period.

13 ^{a, b} Different letters within a same period for each sward type differ significantly at Tukey
14 Test $P < 0.05$.