Supplementary Material S1

Material and methods

Treatments, subject animals and experimental design

Two experimental treatments were compared. One treatment was dwarf elephant grass (DEG) pasture receiving nitrogen fertilization (150 kg N/ha as ammonium nitrate), and one treatment was DEG pasture intercropped with peanuts, with an adjacent area of peanuts (DEG + P) that was accessible to grazing animals for 5 h/day (from 0700 to 1200 h). The chemical compositions of the pastures are presented in Table 1.

Twelve Charolais steers aged 10 to 12 months were assigned into four homogeneous groups, two groups per treatment, according to body weight (BW) (213 ± 8.9 kg) measured one day before the start of experiment. The treatments were applied using a completely randomized design replicated during three grazing cycles from January to April 2012. Each grazing cycle was 21 days on average. Animals did not change treatment groups during the experiment.

Paddock management

The pastures were cultivated at the Epagri experimental farm of Ituporanga (27°38'S and 49°60'W, Santa Catarina State, Brazil). The climate is humid subtropical with an average annual temperature of 17.0°C. The mean annual rainfall is 2190 mm. The soil of the experimental area was classified as a Cambisol (FAO, 1998). Two uniform areas were used. These areas included a 1-ha area planted with a monoculture of DEG in October 2004 and a 1.5-ha area planted with a 1-ha mixture of DEG and peanut in November 2004 and with a 0.5-ha monoculture of peanuts in October 2005. Each pasture was subdivided into 16 paddocks, forming two replicates with eight paddocks of approximately 600 m2 of DEG in monoculture, two replicates with eight paddocks of approximately 600 m2 of DEG intercropped with peanuts and eight paddocks of approximately 300 m2 of peanuts in monoculture.

Fertilizer was applied to all experimental pastures in April 2011. The areas intercropped with forage peanuts or pure peanuts each received 3.2 tons/ha of turkey litter bedding and 300 kg/ha of natural phosphate, totaling 100 kg/ha of phosphorus (P), 50 kg/ha of potassium (K) and 60 kg/ha of nitrogen (N). The monoculture DEG stands were fertilized with 25 kg/ha of N as ammonium nitrate, 100 kg/ha of P as triple superphosphate and 50 kg/ha of K as potassium chloride. In November 2011, the areas were mowed to achieve uniformity. In the DEG pastures, a total of 150 kg of N/ha as ammonium nitrate was applied in three fractions (once after mowing and once after each of the first two grazing cycles). For the mixed pastures and the peanuts in monoculture, no additional fertilizer was applied.

Pasture management

The animals were managed under intermittent stocking, using a pregrazing management target of 90-100 cm for the DEG-pasture-based paddocks (DEG or DEG+P). The postgrazing management target was 45-50 cm or greater. The management of the forage, pure-peanut paddocks was synchronized with the management of the DEG-pasture-based paddocks. The pre- and postgrazing heights varied between the two paddock types; this variation was considered to be a consequence of the current grazing interval. The number of days of occupancy varied among the paddocks, with the grazing cycles averaging 35 ± 2 days. The grazing period - defined as the length of time that grazing livestock occupy a specific area (Allen et al., 2011) - was 2.5 ± 0.5 days per paddock, with a rest period of 32.75 ± 2.25 days. A sufficient number of paddocks were not available for the animals to be stocked throughout each grazing cycle on the experimental pastures. Therefore, during the intervals between grazing cycles (15 ± 2 days) the animals were all gathered in a *Panicum maximum* pasture.

Pasture measurements

For DEG + P, dry matter of green leaves (DMGL) was estimated before and after grazing by a comparative yield method (Haydock and Shaw, 1975). In each grazing cycle, four squares of 1 m2 were used to construct a standard range of 4 values. For each standard, an area with a similar biomass of green leaves was sampled and dried in an oven at 60°C with forced ventilation. Regression equations for estimating DMGL/ha, depending on the patterns (1-4), were constructed, and for each paddock, DMGL was estimated from 20 sites assessed by three trained evaluators**.** Pregrazing leaf mass was calculated using only living leaf blades; for the after-grazing forage mass, leaf blades that accumulated on the ground during paddock occupancy were also included. For the peanut pastures in monoculture, the aboveground pregrazing DMGL (petiole + foliole) was estimated from the sward height, as measured with a rising plate meter (Farmworks®, F200 model, Feilding, New Zealand), and from the amount of DMGL present in the plate area (0.1 m2). For each grazing cycle, regression equations were obtained to estimate the forage mass (kg DM/ha) as a function of sward height. The treatment areas were cut at five points with scissors at ground level. After the stolons, soil and roots were manually removed, the samples were dried in an oven for 72 h at 60°C.

Sward height and sward botanical, morphological and nutritive value compositions were determined in the last three paddocks occupied for each grazing cycle. For DEG in monoculture and intercropped pastures, the average height in each paddock was obtained from 30 measurements taken before and after grazing using a graduated scale and considering the height of the tallest leaf. The botanical and morphological compositions were determined in samples representative of the area that were obtained by cutting two samples of 1 m2 at 5 cm above ground level per paddock. For the peanut pastures in monoculture, twenty handfuls of herbage (approximately 800 g fresh) selected at random were cut with scissors close to the ground. The cut forage was separated into DEG (leaf blades and stems plus sheaths), peanut (stolons and petioles plus folioles), dead material, and other species. The different fractions were dried in an oven with forced ventilation at 60°C for 72 h.

The n-alkane concentration and nutritive value composition of the forage intake were determined from hand-plucked samples selected to represent the portion of the canopy being grazed. These samples were obtained from a sample comprising collections acquired by two samplers, two times per day, over the period of animal occupation of the paddocks. After collection, the samples were dried in a forced-air ventilation oven, ground into a 1-mm sieve and stored for later analysis.

Animal measurements

In the final 11 days of each grazing cycle, the steers were dosed twice daily (at 0800 and 1600 h) with a cellulose stopper (Carl Roth, GmbH, Karlsruhe, Germany) containing 248 mg of C32. During the final 5 days of each grazing cycle, fecal grab samples were collected from each steer twice daily (at 0800 and 1600 h). Feces were oven-dried at 60°C for at least 72 h, subsampled by period and by steer and then ground to pass a 1-mm screen for subsequent chemical analysis. Hand-plucked forage samples were collected on d 7 to 11 as described in the section above.

The individual herbage intake was measured by the n-alkane technique (Mayes et al., 1986), using tritriacontane (C33) as the internal marker and dotriacontane (C32) as the external marker according to the following equation:

*HI* = *D*j /*F*j/*F*i × *H*i -*H*j,

where *HI* represents daily herbage intake (kg DM); *F*i and*H*i represent the concentration (mg/kg DM) of internal alkane (C33) in feces and herbage, respectively; *F*j and*H*j represent the concentration (mg/kg DM) of external alkane (C32) in feces and herbage, respectively; and *D*j represents the oral dose (mg) of alkane C32. For the DEG+P treatment, the *HI* value was calculated from the individual concentrations of C33 in each forage species (DEG and peanut) and the proportions of each forage in the consumed herbage, which was calculated from the amount of herbage of each species removed from the paddocks daily. This procedure was necessary because the relationship between the internal markers of the different forage species was not adequate for identifying the proportions of pastures in the selected diet.

The DM digestibility of the herbage consumed was measured using tritriacontane (C33) as the internal marker according to the following equation:

*DM digestibility* = *F*i – *H*i / *F*i,

where *F*i and*H*i represent the concentration (mg/kg DM) of internal alkane (C33) in feces and herbage, respectively.

Daily CH4 emission was measured in the final two grazing cycles using the SF6 tracer technique described by Johnson *et al.* (1994). The SF6 permeation tubes used in the experiment had an average permeation rate (PR) of 4.93 ± 0.36 mg/day at the time of their deployment in the reticulum, and breath samples were collected after 10 day of tube deployment. The air sampling system used stainless steel cylinders (0.5 l volume) as the sample collection device, and sample flow was regulated by a brass ball bearing (Gere and Gratton, 2010). The cylinders were cleaned with high-purity nitrogen gas (N2) and pre-evacuated (< 0.5 mb) before each sample collection. The flow regulators were calibrated to allow a remaining vacuum in the canister of approximately 500 mb at the end of the sample collection period (5 days). The inflow restrictor was located just above the animal’s nostrils and protected against water and dust by means of a double filter. In addition, three background air samples were collected with a sampling system placed approximately 1 m above ground level. The periods of gas collection were selected to match the evaluation period for herbage DM intake as closely as possible. After the collection period, the pressure in each tube was measured, and each sample was diluted with N2 and then measured again to determine the final pressure in the tube. The breath and background samples were both analyzed for concentrations of methane (ppm, parts per million by volume) and SF6 (ppt, parts per trillion by volume) by gas chromatography (Shimadzu 2010, Japan) using flame ionization (250°C) and electron capture (350°C) detectors, respectively. Three standards containing methane and SF6 mixtures were used to calibrate the gas chromatograph and track its performance over a range of 5-20 ppm and 30-1000 ppt for CH4 and SF6, respectively. Estimates of CH4 emissions using the tracer technique over the sample collection period were calculated from the specific permeation rate (PR) of SF6 and the CH4/SF6 ratio for mixed ratios (v/v) in breath samples after correction for the background gas concentrations (Johnson *et al*., 1994). For this purpose, the PR of SF6 was expressed per day; consequently, the emission estimates corresponded to daily emissions.

To measure ADG, the animals were weighed before and after each grazing cycle after fasting from solids and liquids for 12 h. The daily patterns of grazing and ruminating times were measured by visual observation every 5 min from 0700 to 1900 h and every 10 min from 1900 to 0700 h on days 10 and 12.

Chemical analyses**.**

For analysis, all samples were pooled by animal period. DM concentration was determined after drying at 105°C for 24 h. Ash was determined by combustion in a muffle furnace at 550°C for 4 h, and OM was determined from the mass difference. Total N was assayed by the Kjeldahl method (Method 984.13; AOAC, 1997). Neutral detergent fiber analysis (assayed with heat- stable α-amylase, without sodium sulfite and expressed with residual ash) was performed according to Mertens (2002), except that the samples were weighed into filter bags and treated with neutral detergent using ANKOM equipment (ANKOM Technology, Macedon, NY, USA). Concentrations of ADF (expressed with residual ash) and ADL were analyzed according to Method 973.18 of AOAC (AOAC 1997). n-Alkanes were determined according to Dove and Mayes (2006). Total condensed tannins content was measured by the colorimetric HCl-butanol method (Graber et al., 2013).

Statistical analyses

All the animal data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) with grazing cycles considered as repeated measures using the following model:

*Yijkl = µ + αi + βj +γk* + *Ɛijkl*,

where *µ* is the overall mean; *αi* and*βj* are random effects of animals and grazing cycles, respectively; *γk* represents the fixed effects of legume access; and *Ɛijkl* is the residual error.

The forage data for DEG alone and DEG+P, averaged per treatment and period, were also analyzed using repeated measurements and the following model:

*Yijkl = µ + αi + βj +γk* + *Ɛijkl*,

where *µ* is the overall mean; *αi* and*βj* are random effects of paddock; *γk* represents the fixed effects of pasture type (grass alone or grass mixed with peanut); and*Ɛijkl* is the residual error. The peanut-only pasture data were not subjected to statistical analyses because this pasture was not available to animals in the DEG treatment.

The means were estimated with the LSMEANS procedure, and differences between means were determined by the probability of difference (PDIFF) method using Student’s t-test at the 5% significance level.

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