**R. Bras. Zootec., 52:e20210051, 2023** https://doi.org/10.37496/rbz5220210051

> **Ruminants** Full-length research article



Brazilian Journal of Animal Science e-ISSN 1806-9290 www.rbz.org.br

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Received: March 26, 2021 Accepted: February 3, 2023

How to cite: Schmitz, G. R.; Paris, W.; Kuss, F.; Nörnberg, J. L.; Costa, O. A. D.; Souza, S. S. and Menezes, L. F. G. 2023. Legume inclusion or nitrogen fertilization on Aruana grass overseeded with temperate grasses: Performance, carcass characteristics, and fatty acid profile of the meat of beef steers. Revista Brasileira de Zootecnia 52:e20210051. https://doi.org/10.37496/rbz5220210051

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Legume inclusion or nitrogen fertilization on Aruana grass overseeded with temperate grasses: Performance, carcass characteristics, and fatty acid profile of the meat of beef steers

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ABSTRACT - The objective of our study was to evaluate the effect of the use of legume (Arachis pintoi) or nitrogen fertilization on animal performance, characteristics of carcass and meat, and fatty acids profile of crossbred steers on Megathyrsus maximus cv. Aruana pasture, overseeded with temperate grasses. The experimental design was completely randomized, with three treatments and three replicates. The experiment was carried out from June to October (127 d). The treatments were: Low-N: 100 kg of N/ha; Medium-N: 200 kg of N/ha; and Legume: Arachis pintoi + 100 kg of N/ha. The pasture with higher nitrogen fertilization (N200) showed a more significant forage mass yield. The mixed grass with legumes presented a higher concentration of saturated fatty acids and saturated:unsaturated ratio in the meat. However, the grass pastures resulted in a higher content of unsaturated fatty acids in the meat. The other pasture variables, and characteristics of carcass and meat were not influenced by the treatments. The increase in nitrogen fertilization, from 100 to 200 kg/ha, and Arachis pintoi mixed with Aruana grass pasture overseeded with black oat and ryegrass does not affect the daily weight gain and the carcass and meat characteristics of the steers. The grass-legume mixture decreases the total concentration of unsaturated fatty acids in meat without influencing the concentration of polyunsaturated fatty acids.

Keywords: animal production, annual grassland, *Arachis pintoi*, fatty acid, mixed pasture

### **1. Introduction**

Nitrogen fertilization on forage production brings good results (Beck et al., 2017), influencing animal productivity like the carrying capacity (Pontes et al., 2018). However, there is some pressure to reduce the use of nitrogen fertilizers due to economic and environmental issues (Beck et al., 2017). The replacement of nitrogen fertilizers by nitrogen fixation of legumes can be a potential alternative to substitute part of the nitrogen fertilization.

In tropical regions, the grass-legume mixture is rare, and single crops are more common (Muir et al., 2011). For the subtropical and warm-temperate latitudes, a wide range of persistent warm-season perennial grasses is available. They commonly occur as near-monoculture grass pastures. In this way,

the use of legumes in pasture can promote some increases in animal production by improving the forage nutritional value (Beck et al., 2017). This high nutritional value results from high levels of crude protein (CP) and high digestibility of forage legumes.

The most studied temperate legume in the world is white clover. However, under subtropical climate conditions, the persistence of this species is compromised by the hot and dry summers and its slow growth (Muir et al., 2011). An option, especially in grazing areas, is forage peanut (*Arachis pintoi* Krapovickas and Gregory). This legume represents a potentially viable alternative for mixed cropping systems in subtropical and tropical regions because of its high nutritional value, persistence, soil cover, shading and grazing tolerance, and fast growth (Barcellos et al., 2008; Costa et al., 2020).

Finishing on pasture leads to a more significant deposition of n-3, CLA fatty acids, and a lower n-6:n-3 ratio in the meat than grain-finishing (Aldai et al., 2011; Patino et al., 2015). A higher concentration of linolenic acid facilitates the deposition of n-3 PUFA (polyunsaturated fatty acids) in the muscle and is often found in pasture. Although higher levels of PUFA are associated with higher lipid oxidation (Mello et al., 2012), pasture is a natural source of antioxidants (Lindqvist et al., 2012), which lowers lipid oxidation rates that are usually observed in grass-finished beef. However, studies evaluating carcass characteristics and fatty acid profile of beef cattle finished on pasture of temperate grasses overseeded in tropical pasture with or without legumes are scarce in the literature. Thus, the hypothesis of this research is to determine whether the inclusion of a perennial tropical legume changes the carcass characteristics, quality, and fatty acid profile of the meat of finished cattle, exclusively on pasture.

# 2. Material and Methods

## 2.1. Location, area, and soil fertility management

The experimental area was in Dois Vizinhos, Paraná state, Brazil (elevation of 520 m above sea level, 25°44' South and 53°04' West). The soil is classified as Dystroferric Red Nitosol. The climate is humid subtropical mesothermal (Cfa) according to the Köppen criteria. The total rainfall of the experimental period (June – September) was 650 mm, with average temperatures between 14 and 21 °C.

In September 2013, the *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs cv. Aruana, was seeded in nine paddocks using a seeder with 17 cm of row spacing (5 kg of viable seeds/ha). The legume (*Arachis pintoi* Krapovickas and Gregory cv. Amarillo) was distributed in 3 m wide (10 kg of seeds/ha) in three paddocks, which was 30% of the experimental area. During the summer, the cattle used to graze in all paddocks, until the temperate grasses was established.

Soil analysis at the beginning of the experiment showed the following results (0-20 cm): pH (CaCl<sub>2</sub>) = 5.0; MO = 29 g/dm<sup>3</sup>; carbon = 20.1 g/dm<sup>3</sup>; argil = 64%; P-Mehlich = 3.7 mg/dm<sup>3</sup>; K = 0.2 cmolc/dm<sup>3</sup>; Ca KCl = 4.5 cmolc/dm<sup>3</sup>; Mg = 2.5 cmolc/dm<sup>3</sup>; H+Al = 5.8 cmolc/dm<sup>3</sup>; base sum = 7.3 cmolc/dm<sup>3</sup>; CTC effective = 13 cmolc/dm<sup>3</sup>; saturation Al = 1.4%; base saturation = 56%. Based on the soil analysis results and recommendations of the CQFS RS/SC (2004), for grass-legume mixture, the base fertilization happened at the seeding of the temperate grasses; for that, 80 kg/ha of P<sub>2</sub>O<sub>5</sub> and 100 kg/ha of K<sub>2</sub>O were applied together with the first nitrogen fertilization. Ryegrass (*Lolium multiflorum* Lam. 'Fepagro São Gabriel' - 30 kg of seed/ha) and black oat (*Avena strigosa* Schreb 'IAPAR 61' - 60 kg/ha of pure and viable seeds) were seeded in line with 17 cm spacing, in April 2016. The overseeding was carried out with the Aruana pasture at 15 cm.

## 2.2. Experimental design and treatments

The experimental design was in completely randomized design with three replicates. The experiment was carried out during winter and spring 2016, from June to October, with 18 days for adaptation and 109 days for evaluations. The experimental area had nine paddocks, 0.7 ha each, with a total area of 6.3 ha. Treatments were: 100 kg N/ha (Low-N), 200 kg N/ha (Medium-N) and, 100 kg/ha of N with the *Arachis pintoi*-Aruana grass mixture (Legume). The topdressing of urea (45% of N) had

five applications according to each treatment, on the following dates: 06/01/2016, 06/25/2016, 07/21/2016, 08/19/2016, and 09/03/2016.

### 2.3. Pasture management and evaluation

The pasture was managed under a continuous stocking rate, with the put-and-take adjustment method (Mott and Lucas, 1952). Two tester animals were used per paddock. Forage mass (FM) was estimated for each period of 21 days through the double sampling technique, with 20 visual estimates and five clips per paddock (Wilm et al., 1944). Simultaneously, pasture height was measured using a ruler in 20 spots. Forage accumulation rate was measured by two exclusion cages per paddock for each period of 21 days. *Arachis pintoi* mass contribution was estimated after botanical and sample separation; the value was 18% (total dry matter [DM] yield) throughout the experiment. Forage mass and structural composition of the pasture are presented in Schmitz et al. (2019).

Forage allowance (FA) was calculated as described by Sollenberger et al. (2005), according to the equation:  $FA = (FM_{mean})/(kg LW/ha)$ , in which FM = forage mass, calculated as  $((FM_{cut 1} + FM_{cut 2})/2)$ , and LW = live weight. Stocking rate (SR) was calculated as the sum of the tester animals' live weight, corrected to the area size, and added to the weight of the grazing-height regulator animals, taking into account the number of days that the regulators remained in each paddock. The management criterion for adjusting the SR was the estimated FA of 8% body weight (BW) during the entire grazing period.

The samples for the chemical analysis (Table 1) of the pasture were obtained, for each period of 21 days, through the hand-plucking method (Moore and Sollenberger, 1997). Samples were dried in a forced-air oven at 55 °C for 72 h to obtain the partially dried samples, and ground in a Wiley-type mill<sup>m</sup> (Thomas Scientific<sup>®</sup>) fitted with a 1-mm-sieve. The DM, ash, organic matter (OM), and CP analyses were performed according to AOAC (2012). The neutral detergent insoluble fiber was determined by the method proposed by Van Soest et al. (1991), adapted for the ANKOM2000 apparatus (ANKOM A2000 Fiber Analyzer, ANKOM Technology Corporation, Fairport, NY, USA) using filter bags. The *in vitro* DM digestibility (IVDMD) was estimated with filter bags (Tilley and Terry, 1963; Komarek, 1993) through a TECNAL<sup>®</sup> TE-150 *in vitro* incubator and a detergent solution treatment (Goering and Van Soest, 1970) using the ANKOM<sup>®</sup> Fiber Analyzer A2000. Total digestible nutrients (TDN) were calculated with the following equation: TDN = %OM \* [(26.8 + 0.595) \* (IVOMD))/100] (Kunkle and Bates, 1998), in which IVOMD = *in vitro* organic matter digestibility.

		Treatment <sup>1</sup>	
Chemical composition -	Medium-N	Legume	Low-N
Dry matter (DM, %)	19.24	20.82	21.52
Ash (% DM)	12.48	12.47	12.39
Crude protein (% DM)	28.07	28.15	27.63
Neutral detergent insoluble fiber (% DM)	46.30	46.67	47.80
Acid detergent insoluble fiber (% DM)	26.19	25.92	27.19
Dry matter digestibility (% DM)	75.71	76.50	75.01
Total digestible nutrients (% DM)	62.18	62.87	62.13

**Table 1** - Chemical composition of Aruana grass overseeded with winter grasses subjected to nitrogen fertilization

 or grass-legume mixture obtained through simulated grazing

<sup>1</sup> Medium-N = 200 kg N/ha; Legume = 100 kg N/ha + forage peanut (*Arachis pintoi*); Low-N = 100 kg N/ha. The means were tested through analysis of variance, but they did not show any differences.

### 2.4. Animal, carcass and meat evaluations

The steers began grazing on 06/13/2016, when the pasture reached an average sward height of  $23\pm1.5$  cm and forage mass of  $1940\pm232$  kg DM/ha. Twenty-four crossbred steers (1/4 Marchegiana,

1/4 Aberdeen Angus, and 2/4 Nelore), at 21±2 months old and an initial live weight of 412±8.2 kg, were used. The animals had free access to water and mineral salt.

We assessed the average daily gain (ADG, kg/animal) through two weightings of each tester animal during the grazing, which was at the beginning and the end of each experimental period with a fasting of 14 h before the weighing. All animals were slaughtered when the average subcutaneous fat thickness in all treatments reached a minimum of 3.0 mm. We took this measure during weighing through *in vivo* ultrasonography performed between the 12th and 13th ribs. Animals were slaughtered in a slaughterhouse, according to the laws of humane slaughter. The carcass characteristics were measured using the methodology described by Müller (1987). The two half carcasses were weighed to obtain the hot carcass weight (HCW) and the hot dressing percentage (HCP = HCW/SLW × 100), in which SWL = slaughter live weight. The cold carcass weight (CCW) and cold dressing percentage (CDP = CCW/SLW × 100) were obtained after cooling in a chill room at 0 °C for 24 h.

In the right half cold carcass, we made the cut of the HH section (Hankins and Howe, 1946) modified by Müller (1987) with the cut between the 11th and 12th ribs. The physical separation of tissues in muscles, fat, and bone was carried out to determine the total quantity and cold carcass percentage from that cut. In that same section, at the height of the 12th rib, the surface of the *longissimus dorsi* muscle was exposed. After at least 30 min of exposure to air, we performed evaluations of color, texture, and marbling of the meat, assigning scores from 1 to 5. The CIE Lab System was implemented, and color measurements were recorded for the L\*(lightness), a\* (redness), and b\* (yellowness) values. For this, we used a portable spectrophotometer CM-600D (Konica Minolta Sensing Inc, Osaka, Japan) equipped with illuminant A, 8 mm aperture, and a 10° standard observer. Average color values were recorded at three random points on the meat surface, keeping a distance of 1 cm from the edge of the piece.

Samples of the *longissimus dorsi* were taken from the cuts, identified, vacuum packed, wrapped with brown paper, and immediately frozen at -18 °C. From the samples still frozen, three cuts of 2.5 cm thickness were taken. The cut "A" was weighed to determine the loss during thawing and then cooked at an internal meat temperature of 70 °C for 15 min to assess the possible losses in the cooking. After cooking the cut "B", three samples of 1 cm<sup>2</sup> were taken in the perpendicular direction to the muscle fibers, and in each one, two readings were performed by a Warner Bratzler Shear device to determine the fatty acids methyl esters, following the adapted method described by Giotto et al. (2020). The determination was made in a gas chromatograph equipped with a flame ionization detector and Supelco SP2340 capillary column (60 m × 0.25 mm × 0.2 µm). Detector and injector temperatures were 260 and 240 °C, respectively. The column heating schedule was started at 140 °C for 5 min, gradually increased 4 °C per minute to the final temperature of 240 °C, and finally maintained for 5 min. The entrainment gas flow (H<sub>2</sub>) was 17 mL/min. The injection volume was 0.5 µL with a split ratio of 1:100. Peak identification, as well as quantification, were done by comparing the retention times and peak area of the samples with those of fatty acid methyl esters (Supelco 37 components FAMEs Mix, ref. 47885-U).

### 2.5. Statistical analysis

Data were analyzed in a completely randomized design, with three replicates. The N doses and legume were considered fixed effects. For the pasture characteristics and animal performance, the animal, period, interaction period × treatment, and the residual error were used in the model as random variables. For the post-mortem variables, we did not use period and interaction effects. For the forage evaluation, the paddock was the experimental unit. For animal performance, carcass, meat evaluation, and fatty acids profile, each animal was considered as an experimental unit, being a random effect f according to the model:

$$Y_{ijk} = \mu + \alpha_i + p_j + a_k + (\alpha^* p)_{ij} + \varepsilon_{ijk},$$

in which  $Y_{ijk}$  is the observation concerning the *i*-th N and legume used ( $\alpha_i$ ) in the *j*-th grazing period ( $p_j$ ) and *k*-th animal ( $a_k$ ). Data were submitted to analysis of variance with the Glimmix procedure of SAS (Statistical Analysis System, 2013) using generalized linear mixed models. Then, the variables were

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compared through the Tukey-Kramer test (P = 0.05) only when the effect of treatments was significant. The marbling score was analyzed by the Npar1way procedure of SAS in a Dwass-Steel-Critchlow-Fligner (DSCF) multiple comparison analysis.

## 3. Results

There was a difference between the concentrations of fatty acids in the grazing simulation samples (Table 2). The pasture with legumes showed a higher concentration of saturated fatty acids (SFA) and a higher SFA to unsaturated fatty acids (UFA) ratio. Single grass pastures had a higher content of monounsaturated fatty acids (MUFA) and PUFA than the mixed pastures. The N fertilization without legume presented a higher sward height (Table 3). Still, the N increase (Medium-N) resulted in higher forage mass yield. Forage allowance, SR, and ADG did not show any difference.

There was no treatment effect on the carcass and meat characteristics (Table 4). In general, we observed high hot dressing percentage (56.48%). However, the rib fat thickness (+ 2.74 mm) and marbling score ( $\mu$  = 3.4 points; median = 3.0) were low. The UFA profile showed a difference between treatments, with higher values for pasture without legume (Table 5). Still, the SFA:UFA ratio showed lower values for this treatment (no legume).

Fatty acids (% fat)		Treatment <sup>1</sup>			
	Medium-N	Legume	Low-N	SEM	P-value
Lauric (C12:0)	0.41	0.00	0.65	0.20	0.5519
Myristic (C14:0)	1.56	1.15	1.44	0.25	0.5448
Palmitic (C16:0)	23.24	22.45	23.57	0.90	0.7141
Margaric (C17:0)	0.77	0.56	1.02	0.20	0.4307
Stearic (C18:0)	8.82	6.95	8.53	1.12	0.5216
SFA	51.09b	59.65a	54.82ab	1.61	0.0183
Palmitoleic (C16:1)	1.77	1.54	1.89	0.29	0.7293
Oleic (C18:1)	22.70	16.19	18.07	4.15	0.5779
MUFA	41.01a	31.18b	37.13ab	1.35	0.0121
Linoleic (C18:2)	6.72	8.04	7.15	0.60	0.3569
Linolenic (C18:3)	0.52	0.41	0.73	0.23	0.6433
PUFA	7.89	9.17	8.05	0.65	0.4233
UFA	48.90a	40.34b	45.18ab	1.24	0.0149
SFA/UFA	1.05b	1.48a	1.22ab	0.06	0.0167

# **Table 2** - Fatty acids profile of Aruana grass overseeded with winter grasses subjected to nitrogen fertilization or grass-legume mixture obtained through simulated grazing

SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; UFA - unsaturated fatty acids; SEM - standard error of mean.

<sup>1</sup> Medium-N = 200 kg N/ha; Legume = 100 kg N/ha + forage peanut (*Arachis pintoi*); Low-N = 100 kg N/ha. a-b - Different letters in a row indicate significant differences (P<0.05) between the means.

**Table 3 -** Minimum mean squares of animal daily gain and pasture characteristics of Aruana grass overseeded with winter grasses subjected to nitrogen fertilization or grass-legume mixture

	Treatment <sup>1</sup>			CEM	
	Medium-N	Legume	Low-N	SEM	P-value
Sward height (cm)	12.36a	8.95b	10.40ab	0.61	0.0322
Forage mass (kg/ha)	1467.29a	1301.73b	1376.02b	18.46	0.0033
Forage allowance (kg/ha)	1.089	0.977	1.034	0.057	0.1477
Stocking rate (kg/ha)	1343.10	1340.35	1327.34	64.30	0.9693
Average daily gain (kg/day)	0.864	0.809	0.892	0.161	0.2283

SEM - standard error of mean.

<sup>1</sup> Medium-N = 200 kg N/ha; Legume = 100 kg N/ha + forage peanut (*Arachis pintoi*); Low-N = 100 kg N/ha.

a-b - Different letters in a row indicate significant differences (P<0.05) between the means.

	Treatment <sup>1</sup>			CEM	
	Medium-N	Legume	Low-N	SEM	P-value
Slaughter weight (kg)	517.00	515.50	531.83	17.09	0.7646
Hot carcass weight (kg)	291.05	290.70	302.50	11.52	0.7214
Cold carcass weight (kg)	286.90	286.80	297.58	11.35	0.7501
Cooling loss (%)	1.43	1.34	1.62	0.07	0.0531
Hot dressing (%)	56.27	56.39	56.79	0.80	0.8943
Cold dressing (%)	55.47	55.64	55.87	0.77	0.9358
Rib fat thickness (mm)	2.33	2.17	2.67	0.37	0.6376
Marbling (scores) <sup>2</sup>	2.60	3.40	3.0	0.93	0.8847
	C	arcass compositio	n		
Carcass lean (%)	68.61	64.55	68.03	1.26	0.0705
Carcass fat (%)	12.37	14.08	12.58	1.32	0.6205
Carcass bone (%)	19.03	21.39	19.03	0.68	0.0754
Cooking loss (%)	20.77	22.61	19.99	1.39	0.4157
Shear force (kgF)	8.43	7.21	8.31	0.69	0.3957
Lean lightness (L*)	36.48	36.42	37.62	1.28	0.7661
Lean redness (a*)	17.97	19.26	19.76	0.88	0.3482
Lean yellowness (b*)	8.76	8.65	10.29	0.77	0.3162

### Table 4 - Minimum mean squares of carcass and meat characteristics of finished steers on Aruana grass overseeded with winter grasses subjected to nitrogen fertilization or grass-legume mixture

SEM - standard error of mean. <sup>1</sup> Medium-N = 200 kg N/ha; Legume = 100 kg N/ha + forage peanut (*Arachis pintoi*); Low-N = 100 kg N/ha.

<sup>2</sup> Dwass-Steel-Critchlow-Fligner Method.

The means were tested through analysis of variance, but they did not show any differences.

Fatty acid (% fat)	Treatment <sup>1</sup>			0.514	
	Medium-N	Legume	Low-N	SEM	P-value
Lauric (C12:0)	0.59	0.34	0.79	0.27	0.4477
Myristic (C14:0)	54.82ab	51.09b	59.65a	2.06	0.0297
Palmitic (C16:0)	22.89	23.65	22.49	0.18	0.4967
Margaric (C17:0)	0.94	1.03	0.94	0.03	0.6403
Stearic (C18:0)	17.21	19.06	17.00	0.40	0.2562
Arachidic (20:0)	0.55	0.36	0.19	0.18	0.2574
Lignoceric (24:0)	1.03	1.42	1.14	0.14	0.2265
SFA	47.20	50.02	47.23	0.86	0.0540
Palmitoleic (C16:1)	2.15	1.83	1.86	0.18	0.4453
Oleic (C18:1)	33.50	28.89	31.62	1.44	0.1334
Elaidic (18:1)	2.09	2.53	2.19	0.94	0.7118
Gondoic (20:1)	2.37	2.79	3.04	0.43	0.5280
Nervonic (24:1)	0.85	0.30	0.26	0.15	0.0594
MUFA	39.19	34.51	36.99	2.01	0.2818
Linoleic (18:2n6)	6.88	7.67	8.59	0.05	0.6942
Linoleic (18:2n6 t9t12)	0.27	0.19	0.30	0.02	0.2444
Linoleic (18:2 <i>cis</i> -9 <i>trans</i> -11) <sup>2</sup>	0.50	0.49	0.52	0.06	0.9447
γ-Linolenic (18:3)	0.22	0.12	0.38	0.45	0.5021
α-Linolenic (18:3)	0.13	0.27	0.12	0.47	0.0599
Arachidonic (20:4)	2.58	3.33	2.70	0.17	0.4969
PUFA	11.79	13.28	14.16	2.00	0.6972
UFA	50.98a	47.79b	51.15a	0.79	0.0116
SFA/UFA	0.93b	1.05a	0.92b	0.03	0.0110

### Table 5 - Minimum mean squares of the main fatty acids (%) found in the longissimus muscle of steers on Aruana grass overseeded with winter grasses subjected to nitrogen fertilization or grass-legume mixture

SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; UFA - unsaturated fatty acids; SEM - standard error of mean. <sup>1</sup> Medium-N = 200 kg N/ha; Legume = 100 kg N/ha + forage peanut (*Arachis pintoi*); Low-N = 100 kg N/ha.

<sup>2</sup> CLA - conjugated linoleic fatty acids.

a-b - Different letters in a row indicate significant differences (P<0.05) between the means.

# 4. Discussion

The higher content of SFA in the grass-legume pastures than in single grasses can be explained by the fact that growing grasses mixed with legumes may decrease the sugar content of fresh forages compared with single grasses (Brown et al., 2018). Besides, plants need to synthesize PUFA to ensure fluidity of their membranes at low temperatures (Beltrão and Oliveira, 2007). As black oat and ryegrass are more adapted to low temperatures, they may have higher UFA levels.

Forage mass and sward height differences were not sufficient to affect the animal performance as FA was similar in all treatments (Table 3). Forage mass remained above 1,200 kg DM/ha, which allowed the animals to choose a diet with a high nutritional value in all treatments. We worked around these differences by adjusting the SR, as the pasture allowance was the same for all treatments. *Arachis pintoi* accounted for 18% of the FM (+ 245 kg DM). These forage amounts may have contributed to the increase of species diversity and the beginning of growth before the spring.

We did not observe any increase in animal performance in the treatment with *Arachis pintoi* due to the similar nutritional values (Table 1) observed in the samples obtained by the grazing simulation technique. According to Barcellos et al. (2008), there is a lower animal preference for legumes in tropical regions than grasses and, consequently, a trend for choosing grasses. Similar results (Lazzarotto et al., 2019; Lisbinski et al., 2019) were found in other studies, and the authors verified similar gains between animals on oat + ryegrass pastures mixed with vetch and those that only grazed grasses.

Diet quality is one of the main factors influencing carcass and meat quality (Duckett et al., 2013). In our study, we did not observe any difference between the treatments on the carcass characteristics due to the similar diet nutritional quality between treatments and the similarity in animal performance (Table 4). Likewise, Moloney et al. (2018) observed that dressing percentage was similar for cattle that grazed a grass-legume pasture and those that grazed fertilized grass, highlighting the potential cost reduction due to the replacement of inorganic N fertilizer by *Arachis pintoi*.

The slaughtering point was achieved when the average fat thickness of all treatments reached 3 mm, measured through an ultrasound. However, the measurement of the ultrasound device is not 100% reliable (Suguisawa et al., 2006), explaining why the actual thickness was lower than the one observed before slaughter. Also, during the hide removal of the animal, the adipose tissue may adhere to the hide walls, which adversely affects the fat thickness. Another circumstance that negatively affected the fat deposition in the carcasses was the pasture energy level (Table 1). The cattle gain becomes predominantly adipose tissue in the finishing phase, with a lower protein requirement and a high energy requirement for fat deposition and marbling. In this context, Ducket et al. (2013) reported that in the presence of legumes, animals tend to decrease the energy intake, which affects the deposition of subcutaneous fat in carcasses. The increasing degradability of dietary protein leads to ruminal ammonia production, resulting in a higher N loss through urine and feces (Agle et al., 2010). Consequently, the net energy storage for maintenance, growth, and fat reserves is lower due to excess dietary protein, resulting in lower animal performance (Wright et al., 2015).

Several studies demonstrated the lack of effects of legumes on carcass and meat quality. Jaturasitha et al. (2009) did not find any effect of a tropical legume (*Stylosanthes guianensis*) on the carcass and meat quality of the steers, while Moloney et al. (2018) obtained similar results when they included white clover in ryegrass pasture. Some authors point out the need for further studies on legumes influencing meat sensory analysis (Mapiye et al., 2011). Animals deposit a lower iron content in the meat (Jaturasitha et al., 2009) in legume pastures, resulting in light-colored muscles. However, Mapiye et al. (2011) reported that cattle fed sweet thorn (*Acacia karroo*) presented red meat, mainly related to the high intake of dietary iron.

Legumes in the pasture can alter the rumen environment, mainly due to the type of fiber of these plants, which can affect the type of fatty acid synthesized by the ruminal microbiota. Still, even with the lowest

concentration of UFA in the meat of beef steers from the grass-legume mixture (Table 5), there was no difference in PUFA, being a response to treatment with *Arachis pintoi* in which the pasture presented a high concentration of SFA, low UFA, and no change in the proportion of PUFA (Table 2). Also, phenolic compounds, such as lignin, can affect the biohydrogenation of PUFA (Kalač and Samková, 2010).

The literature reports improvements in the fatty acids profile in the meat of animals that consumed legumes, particularly in relation to PUFA. The red and, especially, white clover were clearly beneficial by decreasing the n-6:n-3 fatty acid ratio in milk even though the clover lipids are not necessarily superior to the grass in that respect (Dewhurst et al., 2006; Van Dorland et al., 2008). Scollan et al. (2006) found that increasing levels of red clover substituting ryegrass may result in a significant decline in the n6:n3 ratio of beef. This response is mainly owing to the higher concentration of C18:3 in these forages, which was not observed in the present study. Fruet et al. (2018) evaluated finishing cattle on legume-grass pasture compared to animals fed only with grains. They observed that the finishing system did not affect CP, SFA, PUFA, and PUFA:SFA ratio. However, the animals without roughage presented lower C18:0 and higher concentrations of C18:1n7, C18:1*trans*, and MUFA than steers finished on legume-grass pasture. Beef from steers fed diets with roughage had a low n-6:n-3 ratio and high values of C18:2*cis*-9 *trans*-11, C18:3n3, and total n-3.

However, there is little information on the effect of tropical legumes on the fatty acid profile of beef. Jaturasitha et al. (2009) did not observe any effect when they included *Stylosanthes* in the pasture. In the present study, *Arachis pintoi* decreased the percentage of UFA and increased the SFA:UFA ratio of pasture and meat of the animals when they were finished with mixed forages of high nutritional value.

# **5.** Conclusions

The inclusion of *Arachis pintoi* or an increase in nitrogen fertilizer in Aruana grass pasture overseeded with black oat and ryegrass does not change the daily weight gain and carcass and meat characteristics of steers in the finishing phase. Including *Arachis pintoi* in grass pastures decreases the concentration of unsaturated fatty acids and increases the saturated:unsaturated fatty acids ratio in the pasture and meat without affecting the concentration of polyunsaturated fatty acids.

# **Conflict of Interest**

The authors declare no conflict of interest.

# **Author Contributions**

Conceptualization: W. Paris, F. Kuss and L.F.G. Menezes. Data curation: G.R. Schmitz and O.A.D. Costa. Formal analysis: O.A.D. Costa and L.F.G. Menezes. Funding acquisition: W. Paris. Investigation: G.R. Schmitz, W. Paris and S.S. Souza. Methodology: G.R. Schmitz, O.A.D. Costa and S.S. Souza. Project administration: G.R. Schmitz, W. Paris, S.S. Souza and L.F.G. Menezes. Resources: F. Kuss and J.L. Nörnberg. Software: O.A.D. Costa and L.F.G. Menezes. Supervision: W. Paris. Validation: G.R. Schmitz and J.L. Nörnberg. Visualization: W. Paris. Writing – original draft: G.R. Schmitz, W. Paris and O.A.D. Costa. Writing – review & editing: G.R. Schmitz, W. Paris, F. Kuss, J.L. Nörnberg and L.F.G. Menezes.

## Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil (CAPES; Finance Code 001), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil – Process 477966/2013-6. The authors would like to thank the Bromatologia Animal Multiuser Laboratory from the Universidade Tecnológica Federal do Paraná, Campus Dois Vizinhos, for the performed analyses.

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