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RESEARCH ARTICLE

Performance, carcass traits, and meat attributes of steers finished on tropical pasture under increasing supplementation levels

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ABSTRACT

Concentrate supplementation during the finishing phase in tropical pastures improves the average daily gain and carcass characteristics of the animals. The aim of this study was to evaluate the effect of concentrate supplementation level (% of body weight, BW) on intake, apparent digestibility, performance, carcass traits, and meat attributes of steers finished on tropical pasture during the dry season. Forty male, uncastrated Girolando steers, weighing 439.25 ± 36.67 kg and aged 20 mo, were utilized. Treatments involved levels of concentrate supplementation: 0.2%, 0.3%, 0.4%, and 0.5% BW for steers finished on *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster 'Marandu' pasture over an 84-d period in the dry season. The DM intake from the supplement (9.00 to 7.49 kg d^{-1}) and total digestible nutrients (5.99 to 7.29 kg d^{-1}) linearly increased ($P < 0.05$) with the rising level of concentrate supplementation. The average daily gain of the steers (0.44 kg d^{-1}) remained unaffected ($P > 0.05$) by the increasing levels of concentrate supplementation. Hot carcass weight (228.62 to 257.20 kg) and yield increased linearly ($P < 0.05$) with the levels of concentrate supplement. The 0.3% BW level of supplement resulted in carcasses with a thicker ($P < 0.05$) backfat layer (4.75 mm). Protein and cholesterol contents in the steers' meat were not influenced ($P > 0.05$) by the level of concentrate supplementation. In conclusion, we recommend a concentrate supplementation level of 0.5% BW for steers finished on pasture during the dry season.

Key words: Crude protein level, dry-season finishing, early-weaned steer, supplement supply.

INTRODUCTION

The utilization of tropical forage as the primary nutrient source for beef production is highly efficient. This is mainly attributed to the high conversion rates of light energy into potentially degradable carbohydrates in the rumen observed in tropical regions (Costa et al., 2021). Nevertheless, the variability in rainfall, characteristic of tropical areas, poses challenges to maintaining livestock productivity, particularly during the dry season (Sollenberger et al., 2020). Consistent changes in forage production and composition disrupt the nutrient supply to ruminants, necessitating compensation from alternative nutrient sources like concentrate supplementation (Cardoso et al., 2020).

There appears to be a consensus that the strategies involving concentrate supplementation in finishing cattle depend on the type of season (e.g., dry and rainy seasons and transitions) (Detmann et al., 2014; Tambara et al., 2021). Specifically, in the dry season, concentrate supplementation during the finishing phase in tropical pastures improves the average daily gain and carcass characteristics of the animals (Ramos et al., 2022).

However, the optimal level of concentrate supplementation ensuring the production of heavier, better-finished carcasses during the dry season in the tropics remains unclear (Simioni et al., 2021; Vitor et al., 2022). Moreover, there are limited studies examining the relationship between the level of supplementation and the crude protein content of the supplement for steers finished on tropical pasture during the dry season (Silva et al., 2020; Manoukian et al., 2022; Barroso et al., 2024). Within this framework, elevating the supplementation level while ensuring a consistent daily provision of crude protein through supplements yields additional energy supplements, potentially improving the carcass fatness of finishing animals (Franco et al., 2021).

We hypothesize that increasing the level of supplementation, with a reduction in the supplement's crude protein content, will enhance the performance, carcass traits, and meat attributes of steers finished on tropical pasture. Therefore, the objective was to assess the effect of increasing the level of concentrate supplementation (% of body weight, BW) on intake, digestibility, performance, carcass traits, and meat attributes of steers finished on tropical pasture in the dry season.

MATERIAL AND METHODS

All experimental procedures complied with the Ethics Committee on Animal Use (license 017/2012, Ethics Committee on Animal Use/Southwestern Bahia State University, UESB, Bahia, Brazil).

Locations, animals, and treatments

The field trial was conducted in Bahia (15°26′ 46″ S, 40°44′24″ W; 800 m a.s.l.), Brazil. The region features a tropical climate with a dry season of type Aw, according to Köppen-Geiger.

Forty uncastrated male Girolando breed steers (1/2) with an average body weight of 439.25 ± 36.67 kg and an age of 20 mo were utilized. Prior to the experimental period, the cattle underwent treatment against endo- and ectoparasites. The trial lasted 84 d, encompassing the finishing phase and occurred during the dry season. The experiment was laid out in a completely randomized design, comprising four treatments with 10 replicates each. Treatments involved incremental supplementation levels (0.2%, 0.3%, 0.4%, and 0.5%) based on the animals' body weight (BW) (Table 1). The supplement was consistently provided at 10:00 h in uncovered troughs crafted from reused plastic barrels, accessible from both sides (70 linear cm per animal). Diets were formulated following NRC (2016) guidelines to meet the nutritional requirements for animals with a body weight of 450 kg, predicting gains of 0.5 kg d^{-1}.

The animals were allocated in a 14 ha area, comprising 12 paddocks of approximately 1.17 ha each, planted with *Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster 'Marandu'. These paddocks were organized into three modules, each containing four paddocks, equipped with troughs and automatic drinkers. Occupancy within each module lasted for 28 d, with relocations of treatment animals occurring within the module's paddocks every 7 d. This periodic reshuffling aimed to minimize the impact of paddocks on the steers' performance. After 28 d, a new module was occupied, and relocations within the module followed the same procedure as previously described.

Forage evaluation

The forage was evaluated at 28 d intervals in the four entry and exit paddocks of the utilized module. The comparative visual yield method was employed to determine the availability of total forage DM (Table 2).

In the entry paddocks, all collections were combined, and approximately 300 g natural forage material was sampled for manual separation into components (leaf blade, stem + sheath, and senescent material). This separation aimed to determine the availability of each component (green DM) and ascertain the leaf:stem ratio. To calculate forage allowance (kg DM 100 kg^{-1} BW d^{-1}), it was necessary to estimate residual dry biomass and daily DM accumulation rate. Residual dry biomass was estimated using the double sampling methodology, while the DM accumulation rate was obtained using the equation. Potentially digestible DM in the pasture was estimated: Potentially digestible DM = 0.98 (100 – neutral detergent fiber, %) + (neutral detergent fiber, % – insoluble neutral detergent fiber, %).

Table 1. Percentage and chemical composition of concentrate supplements and forage. [1]Provided per kilogram: 175 g Ca; 60 g P; 107 g Na; 12 g S; 5000 mg Mg; 107 mg Co; 1300 mg Cu; 70 mg I; 1000 mg Mn; 18 mg Se; 4000 mg Zn; 1400 mg Fe; 600 mg F (maximum). [2]Simulated grazing. NDFap: Neutral detergent fiber corrected for ash and protein; NFC: non-fibrous carbohydrates; iNDF: indigestible neutral detergent fiber; TDN: Estimated total digestible nutrients.

		Concentrate supplement level in the diet (%body weight)			
Ingredient (g kg^{-1})		0.2	0.3	0.4	0.5
Ground sorghum		492.2	688.6	800.6	863.3
Soybean meal		313.4	190.8	113.0	67.7
Urea		139.1	83.9	59.1	45.0
Mineral salt[1]		55.3	36.7	27.3	24.0
Component (g kg^{-1} DM)	*Urochloa brizantha* 'Marandu'[2]				
Dry matter (g kg^{-1} as fed)	348.7	865.2	880.5	879.0	889.1
Ash	71.0	104.8	73.0	55.5	46.6
Crude protein	71.0	568.5	392.9	293.9	237.4
Ether extract	22.7	23.8	22.2	21.5	24.9
NDFap	617.4	87.3	116.1	148.6	233.0
NFC	189.6	467.6	548.7	588.5	539.1
iNDF	230.0	12.1	13.6	16.9	17.5
TDN	535.4	649.6	713.6	743.9	762.0

Table 2. Characteristics of the pasture during the steer finishing period.

Variable	Value
Total DM availability, kg ha^{-1}	2.354
Digestible DM availability, kg ha^{-1}	1.956
Green DM availability, kg ha^{-1}	1.515
Forage allowance, kg DM 100 kg^{-1} BW d^{-1}	8.89
Leaf:stem ratio	1.37

Collection, processing, and analysis of food samples

Forage samples from simulated grazing (Johnson, 1978) for estimating nutrient intake and apparent digestibility coefficients. Concentrate supplement samples were collected in each period, with a composite created at the conclusion of the experiment. Both the supplement and forage samples underwent drying in a forced-air oven at 55 °C and grinding in a Wiley mill to 1 mm for subsequent chemical analyses.

The DM, ash, crude protein (CP), and ether extract (EE) contents were determined according to the AOAC (1990) methodology. Neutral (NDF) and acid detergent fibers (ADF) were determined following the methodology of Van Soest et al. (1991). Ash-and protein-free neutral detergent fiber (NDFap) was measured as described by Mertens (2002). Non-fibrous carbohydrates were determined also free of ash and protein (NFCap), by the following equation: NFCap = 100 – ash – CP – EE – NDFap. Because the supplement contained urea, its NFCap content was determined by the following equation: NFCap = 100 – ash – EE – NDFap – (CP – CPu + U), where CPu is CP in urea; and U is urea content. Total digestible nutrients (TDN) were calculated using the equation TDN% = DCP + DNDFap + DNFC + 2.25 DEE, where DCP is digestible CP, DNDFap is digestible NDFap, DNFC is digestible NFC, and DEE is digestible EE.

Nutrient intake and apparent digestibility

Between the 31st and 42nd days of the experiment, estimates of fecal output, intake, and DM digestibility and nutrients were conducted. Fecal DM output was estimated using the external marker chromic oxide (10 g animal^{-1} d^{-1}) supplied for 12 d. The first 7 d served as an adaptation period, and the final 5 d involved fecal collection at specific times: 16:00 h (1st day), 14:00 h (2nd day), 12:00 h (3rd day), 10:00 h (4th day), and 08:00 h

(5^{th} day). Fecal samples were stored in the freezer at 10 °C, pre-dried individually, and ground for subsequent chemical composition analysis. Chromic oxide quantification followed the methodology of Detmann et al. (2012), with readings performed on an atomic absorption spectrophotometer (Avanta Sigma, GBC Scientific Equipment, Perai, Malasia).

Forage DM intake was estimated using the internal marker indigestible NDF (iNDF) obtained after ruminal incubation for 288 h (Detmann et al., 2012). Bags made with non-woven fabric (TNT, 20 mg cm^{-2} grammage, 5 × 5 cm) were used for incubation, and the remaining material underwent extraction with neutral detergent to determine iNDF.

Once the fecal output and iNDF values were determined, the intake of forage DM was calculated using the formula: Forage DM intake (kg d^{-1}) = {[(Fecal output, kg d^{-1} × Concentration of marker (iNDF) in feces, %) – Quantity of marker (iNDF) in the concentrate supplement, kg]/Concentration of marker (iNDF) in the forage, kg kg^{-1}}.

Dry matter intake from the supplement was estimated using the external marker titanium dioxide (TiO_2) (15 g $animal^{-1}$ d^{-1}) mixed with the concentrate for 11 d. The marker was provided directly in the trough. Supplement DM intake was calculated through the equation: Supplement DM intake (kg d^{-1}) = [(Fecal output, kg d^{-1} × Concentration of titanium dioxide in feces, %)/Concentration of titanium dioxide in the supplement, %].

Quantification of titanium dioxide followed the methodology of Detmann et al. (2012), with readings performed on an atomic absorption spectrophotometer (Libra S22, Biochrom, Cambridge, UK).

Apparent nutrient digestibility was determined using the formula: D = [(kg nutrient intake – kg nutrient output)/kg nutrient intake] × 100.

Performance, carcass traits, and meat attribute

Average daily gain (ADG) was calculated as the difference between the final body weight and the initial body weight. To regulate the supplement supply, steers were weighed every 28 d after a 12 h fasting period. Feed conversion was computed as the ratio of feed intake (kg d^{-1}) to ADG (kg d^{-1}).

After 84 d, the steers were slaughtered following current legislation. The process involved stunning, bleeding, skinning, evisceration, decapitation, and removal of limbs to obtain the hot carcass weight. Hot carcass yield (HCY) was determined as HCY = [(Hot carcass weight/Cold carcass weight) × 100]. Subsequently, the carcasses were cooled for 24 h in a cold room at 2 °C. In the cold carcasses, a section was made on the right side between the 12^{th} and 13^{th} ribs, exposing the *Longissimus dorsi* muscle. The outline of this muscle was traced on paper using a pen, and its area was measured with a planimeter for ribeye area and a graduated ruler to calculate the ratio of ribeye area height to width. Additionally, within the area exposed by the cut between the 12^{th} and 13^{th} ribs, backfat thickness was determined using a caliper, following the methodology recommended by Gomes et al. (2021).

A section of the *Longissimus dorsi* muscle, located between the 11^{th} and 13^{th} ribs of each left half carcass, was excised, preserved in aluminum foil and plastic insulation film, and stored at a frozen temperature of -24 °C. The *Longissimus dorsi* muscle was subsequently divided into two samples for further analyses. For chemical analyses, an aliquot of the sample was thawed at room temperature, and the covering fat was removed. The muscle was then ground to determine the moisture, ash, crude protein and cholesterol contents, following the AOAC (1990) methodology. The unsaponifiable matter was analyzed using a high-performance liquid chromatograph (Shimadzu GC-2010 Plus, Shimadzu, Kyoto, Japan) equipped with a degasser (DGU-20 A5R) and two pumps (LC-20 AR) with a UV-visible detector (SPD-20 A). The analytical column used was a C18, measuring 250 mm × 4.6 mm × 5 µm. The mobile phase consisted of acetonitrile:isopropanol (85:15), at a flow rate of 2 mL min^{-1}, with an analysis time of 20 min. Chromatograms were processed at 202 nm. Cholesterol identification was conducted by comparing the retention time of the samples with the standard, and quantification was achieved through the corresponding areas of the peaks, employing internal standardization with 6-ketocholestanol as the internal standard.

Statistical analysis

The data underwent ANOVA using orthogonal polynomial contrasts for linear and quadratic adjustments of the level of concentrate supplementation (0.2%, 0.3%, 0.4%, and 0.5% BW) on the variables studied. The mathematical model used was Yij = µ + Hj + eij, where Yij is value referring to the observation of the

replicate i of the treatment j; μ is overall average; Hj is effect of treatment j (0.3%, 0.4%, and 0.5% BW) and eij is random error associated with observation. A 5% significance level ($P < 0.05$) was considered, and the statistical package used was SAEG – System of Statistical Analysis and Genetic (UFV, Viçosa, Brazil).

RESULTS

Increasing levels of concentrate supplement led to a heightened ($P < 0.05$) intake of supplement DM and reduced ($P < 0.05$) forage DM intake by the finishing steers, but had nonsignificant effect ($P > 0.05$) on total DM intake. There was no observable effect ($P > 0.05$) on the intakes of crude protein or neutral detergent fiber. In contrast, the intakes of non-fibrous carbohydrates and total digestible nutrients increased significantly ($P < 0.05$) with the level of concentrate supplement in the steers' diet (Table 3).

There was a significant increase ($P < 0.05$) in the apparent digestibility coefficient of DM in the steers' diet with the increase in the supply of concentrate supplement. However, a negative quadratic effect ($P < 0.05$) was observed on the apparent digestibility of NDFap with an increase in the offer of concentrate supplement for the finishing steers. Nonsignificant effect ($P > 0.05$) of increasing the supply of concentrate supplement was observed on the apparent digestibility of CP or NFC from the diets (Table 3).

The steers' final body weight showed an increasing linear trend ($P = 0.072$) with the increasing supply of concentrate supplement in the trough. However, average daily gain and feed conversion ratio were not influenced ($P > 0.05$) by increasing concentrate supplementation levels (Table 4).

Hot carcass weight and yield increased ($P < 0.05$) with increasing supply of concentrate supplement in the trough (Table 5). Backfat thickness increased ($P < 0.05$) up to the 0.3% supplement supply level, decreasing thereafter. Conversely, the ratio of ribeye area height to width exhibited a linear increase ($P < 0.05$) with supplement supply in the trough. Nonsignificant effect ($P > 0.05$) was observed on the moisture, ash, crude protein, or cholesterol (34.56 ± 2.21 mg 100 g^{-1}) content of the steers' meat with increasing levels of concentrate supplementation.

Table 3. Intake and apparent digestibility of nutrients by steers finished on pasture and supplemented with increasing levels of concentrate. [1]Standard deviation of the mean. [2]Probability of linear and quadratic order effects, significant at the 0.05 probability level. NDFap: Neutral detergent fiber corrected for ash and protein; NFC: non-fibrous carbohydrates; [5]TDN: total digestible nutrients.

	Concentrate supplement level in the diet (%body weight)					P-value[2]	
	0.2	0.3	0.4	0.5	SDM[1]	L	Q
Intake, kg d^{-1}							
Total DM	10.05	9.83	9.56	10.25	1.256	0.988	0.258
Forage DM	9.00	8.16	7.54	7.49	1.125	0.002	0.274
Supplement DM	0.90	1.50	2.03	2.69	0.762	0.001	0.989
Crude protein	1.20	1.16	1.13	1.17	0.203	0.892	0.818
Ether extract	0.22	0.21	0.21	0.23	0.029	0.810	0.105
NDFap	5.64	5.21	4.95	5.25	0.715	0.168	0.118
NFC	2.16	2.37	2.62	2.87	0.368	0.006	0.990
TDN	5.99	5.88	6.11	7.29	1.084	0.011	0.070
Apparent digestibility, %							
Dry matter	57.18	55.77	55.98	61.42	4.250	0.038	0.015
Crude protein	60.83	61.42	60.69	60.11	4.393	0.958	0.968
Ether extract	71.90	75.00	73.48	77.63	18.730	0.789	0.998
NDFap	47.89	43.63	43.11	50.02	7.035	0.790	0.016
NFC	86.36	88.66	89.97	91.09	6.116	0.081	0.963
TDN	59.96	59.03	58.75	59.05	2.877	0.658	0.716

Table 4. Performance of steers finished on pasture and supplemented with increasing levels of concentrate. [1]Standard deviation of the mean. [2]Probability of linear and quadratic order effects, significant at the 0.05 probability level.

	Concentrate supplement level in the diet (% body weight)					P-value[2]	
	0.2	0.3	0.4	0.5	SDM[1]	L	Q
Initial body weight	424.20	436.70	435.60	460.50	36.677	0.045	0.835
Final body weight	464.10	465.80	476.40	498.20	43.089	0.072	0.655
Average daily gain	0.475	0.346	0.486	0.449	0.141	0.963	0.311
Feed conversion	26.42	34.00	24.22	26.94	8.631	0.716	0.506

Table 5. Carcass characteristics and chemical composition of meat from steers finished on pasture and supplemented with increasing levels of concentrate. [1]Standard deviation of the mean. [2]Probability of linear and quadratic order effects, significant at the 0.05 probability level. [3]Ratio of ribeye area height to width.

	Concentrate supplement level in the diet (% body weight)					P-value[2]	
	0.2	0.3	0.4	0.5	SDM[1]	L	Q
Hot carcass weight, kg	228.62	232.68	240.06	257.20	23.485	0.008	0.516
Hot carcass yield, %	49.21	49.93	50.38	51.66	1.720	0.002	0.836
Backfat thickness, mm	2.69	4.75	3.20	2.11	1.766	0.198	0.007
Ribeye area, cm^2	69.40	70.40	73.10	72.70	12.145	0.659	0.991
Ratio[3]	67.50	75.00	82.50	84.30	16.122	0.015	0.816
Moisture, %	71.43	72.76	72.3	71.69	2.543	0.999	0.236
Mineral matter, %	1.07	1.10	1.08	1.11	0.044	0.173	0.965
Crude protein, %	27.83	27.74	26.82	27.01	1.597	0.144	0.862
Cholesterol, mg 100 g^{-1}	33.97	34.68	34.66	34.93	2.212	0.484	0.958

DISCUSSION

Increasing levels of concentrate supplement offered to the steers, as indicated by the rise in supplement DM intake, resulted in a substitution effect on forage DM intake, leading to a decrease (Lins et al., 2022). Owing to the dry season, we observed a low supply (8.89 kg DM 100 kg^{-1} BW d^{-1}) and quality (71.0 g kg^{-1} CP, 617.4 g kg^{-1} NDFap, 230.0 g kg^{-1} iNDF, and 535.4 g kg^{-1} TDN) of forage, fostering a preference for the concentrate supplement and diminishing the demand for forage (Barbero et al., 2020). The observed increases in NFC and TDN intakes by the steers can also be attributed to the heightened intake of concentrate supplement, which is richer in these fractions compared to the available forage.

The increase in DM intake from the supplement likely accounts for the observed rise in the apparent digestibility of DM from the diet provided to the steers. It is plausible that the augmented intakes of NFC and CP, facilitated by the supplement, stimulated greater microbial growth in the rumen of steers receiving higher concentrations of concentrate supplement. This, in turn, resulted in increased ruminal degradability of DM from the supplement and forage (Dettman et al., 2014). Within this context, the rise in NFC intake, primarily sourced from the sorghum grain in the supplement, may have contributed to the observed decrease in the apparent digestibility of NDFap from the steers' diet, particularly between the supplement levels of 0.2% to 0.4% BW (Chen et al., 2022). Conversely, considering a negative quadratic response, the increase in apparent digestibility of NDFap at the 0.5% BW level of concentrate supply to the steers can be elucidated by the reduction in forage DM intake. This reduction leads to an increase in the residence time of content in the rumen-reticulum, which can stimulate the rate of disappearance due to degradation (Lechartier and Peyraud, 2010). The reduction in passage rate can further enhance the rate of disappearance due to degradation, particularly in the rumen-reticulum with a higher microbial population due to supplement consumption.

Final body weight tended to increase with greater supplement supply, possibly attributed to the increased availability of protein and energy precursors for deposition in the carcass. This results from the greater energy input via the supplement (Chen et al., 2021; Franco et al., 2021). In this regard, the increase in supplementation from 0.2% to 0.5% BW resulted in a 12.5% increase in hot carcass weight and a 4.9% increase in hot carcass yield. Vitor et al. (2022) also reported no differences in the body weight at slaughter or hot carcass weight of finishing steers according to the supplementation level.

Higher supplementation levels also led to increased yields of meat cuts (see the ratio of ribeye area height to width), demonstrating greater muscularity of the carcasses with increased concentrate supply to the steers (Koh et al., 2014). Similarly, Valle et al. (2021) reported that increased supplementation to pasture-finished steers enhances the efficiency of muscle deposition, explaining the greater muscularity of the carcasses observed in this study.

The results of backfat thickness revealed that the supplementation level of 0.3% BW produced fatter carcasses meeting the minimum fat cover (± 5 mm) required by the Brazilian meatpacking industry. It is conceivable that at the 0.3% CP level of concentrate supply, the ruminal degradability of NDFap from the forage was favored (fermentable organic matter:rumen-degradable protein ratio) by the presence of the supplement (Rufino et al., 2020), leading to greater production of acetate, the main precursor of subcutaneous fat in ruminants (Smith et al., 2018). Furthermore, Pacheco et al. (2023) reported in a recent meta-analysis that carcasses with fat cover between 3-6 mm tend to have more intramuscular fat and produce softer meat, being better accepted by consumers.

The composition of the steers' meat did not differ across increasing levels of concentrate supplement, possibly due to the similarity of the physiological stage observed in the animals (Augusto et al., 2019). Even in animals consuming more concentrate (i.e., higher supplementation levels), meat cholesterol levels remained similar to those observed in meat from not supplemented cattle grazing in the dry season (Muchenje et al., 2009). Ramos et al. (2022) also found that different concentrate supplementation strategies for steers finished on tropical pasture do not influence the composition of the animals' meat. In this context, Torrecilhas et al. (2021) and Subiabre et al. (2024) reported that the quality of steer meat is modified only by the feedlotting regime, in comparison to pasture finishing with supplementation.

CONCLUSIONS

Increasing the supply of concentrate supplement from 0.2% to 0.5% of body weight for Girolando steers finished on pasture during the dry season increases energy intake, leading to greater weight, yield, and fatness in the animals' carcass. However, the reduction in forage intake does not impact the average daily gain or the characteristics of the meat produced by the steers. We advocate for a concentrate supplement supply level of 0.3% BW for steers finished on pasture during the dry season to optimize the production of better-finished carcasses. On the other hand, a concentrate supplement supply level of 0.5% of body weight is recommended for steers finished on pasture in the dry season to achieve heavier carcasses with a greater yield of meat cuts.

Author contribution

Conceptualization: D.S.B., F.F.S., G.T-J. Methodology: J.W.D.S., T.R.P. Software: F.B.L.M. Validation: J.A.G.A. Formal analysis: D.S.B., F.F.S. Investigation: D.S.B., J.W.D.S., T.R.P. Resources: R.R.S. Data curation: F.F.S. Writing-original draft: D.M.L-J. Writing-review & editing: D.M.L-J. Visualization: D.M.L-J. Supervision: R.R.S., J.A.G.A. Project administration: R.R.S., J.A.G.A., G.G.P.C. Funding acquisition: G.G.P.C., R.R.S. All co-authors reviewed the final version and approved the manuscript before submission.

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