



Reusing livestock farming wastewater for Tifton 85 irrigation: productivity, morphological, and bromatological indicators

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ABSTRACT

The utilization of wastewater from livestock farming (WLF) represents a common agricultural practice, but the absence of research on its agronomic impacts hinders its sustainable implementation. This study assesses the reutilization of WLF combined with nitrogen fertilization on the morphological, bromatological, and productivity attributes of a Tifton 85 pasture during the winter season. The study involved the application of different types of water via sprinkler: (I) stream water; (II) mixture of WLF and stream water at a 1:1 ratio; and (III) undiluted WLF combined with mineral nitrogen fertilization (0 and 45 kg N ha⁻¹) administered at the commencement of the regrowth period. Irrigation was applied on a weekly basis, totaling 2,440 m³ ha⁻¹. At 106 days post-regrowth, parameters including forage productivity, height, leaf-stem ratio, leaf area index, nitrogen nutrition index, crude protein content, acid detergent fiber, neutral detergent fiber, and mineral matter content were determined. Mineral fertilization at the prescribed dosage (45 kg N ha⁻¹) did not have any discernible impact on the parameters under investigation. On average, the reuse of WLF raised the productivity from 2.50 to 5.12 t ha⁻¹ and the Crude Protein from 12.35% to 14.58% compared to stream water. The reuse of WLF demonstrated superior outcomes ($p < 0.01$) in terms of pasture productivity, nutritional quality, and nitrogen accumulation compared to irrigation solely with stream water. The reuse of diluted WLF (1:1) without fertilization proved adequate to sustain Tifton 85 pastures during the winter season in the Brazilian Cerrado region.

Keywords: *Cynodon dactylon*, forage, irrigated pasture.

Reúso da água residuária de bovinocultura na irrigação do Tifton 85: indicadores de produtividade, morfológicos e bromatológicos

RESUMO

A aplicação de águas residuárias da bovinocultura (ARB) é uma prática usual, entretanto, carece de investigação acerca dos seus efeitos agrônômicos para subsidiar a sua utilização sustentável. O objetivo deste estudo foi avaliar o reúso da ARB e da adubação mineral nitrogenada sobre os atributos morfológicos, bromatológicos e de produtividade da pastagem de Tifton 85 durante o inverno. Foram avaliados diferentes tipos de água aplicados por aspersão (I - água de córrego, II - ARB e água de córrego em proporção 1:1, e III - ARB) e dois níveis



de adubação nitrogenada mineral (0 e 45 kg N ha⁻¹) aplicados no início da rebrota. A irrigação foi semanal e totalizou 2.440 m³ ha⁻¹. Aos 106 dias após a rebrota foram medidas a produtividade da forrageira, altura, razão folha/colmo, índice de área foliar, índice de nutrição de nitrogênio, proteína bruta, fibras insolúveis em detergente ácido, fibras insolúveis em detergente neutro e matéria mineral. A adubação mineral na dose de 45 kg N ha⁻¹ não influenciou as variáveis avaliadas. Em média, o reúso da ARB elevou a produtividade de 2,50 para 5,12 t ha⁻¹ e a proteína bruta de 12,35% para 14,58% comparado com a água de córrego. O reúso da ARB com ou sem diluição proporcionou produtividade, qualidade nutricional e acúmulo de nitrogênio pela pastagem superiores à irrigação com apenas água de córrego ($p < 0,01$), sendo o reúso com ARB diluída (1:1) e sem adubação é suficiente para sustentar a pastagem de Tifton 85 durante o inverno no Cerrado.

Palavras-chave: *Cynodon dactylon*, forrageira, pastagem irrigada.

1. INTRODUCTION

In the milking and animal handling processes of dairy cattle, a substantial byproduct known as wastewater from livestock farming (WLF) is generated during the cleaning of milking facilities. The amount from 4.4 to 10 L of water is usually consumed for each liter of milk produced a day and, considering the 3,03 billion L of milk produced in Goiás and the Federal District region in the year 2022 (IBGE, 2022), it is possible to estimate the average amount of 21.816 billion L of WLF generated (Carvalho *et al.*, 2011; Palhares *et al.*, 2018). The application of WLF to pastures is a common global practice, adopted as an alternative means of disposing of raw WLF instead of releasing it into water bodies. Recent attention has been devoted to studying its environmental consequences (Zhang *et al.*, 2017; Cameron and Di, 2019).

Among the forage species employed in intensive ruminant farming, Tifton 85 (*Cynodon dactylon* L. Pers) stands out as a highly advantageous grass for animal feed. It exhibits high productivity rates and exceptional nutritional quality (Marchesan *et al.*, 2013; Monção *et al.*, 2016). Regarding wastewater reuse, this species demonstrates an above-average ability to extract nitrogen and sodium from the soil and exhibits tolerance to cultivation in contaminated soils (Zhang *et al.*, 2017; Spearman *et al.*, 2020; Teixeira *et al.*, 2021). These characteristics are particularly suitable for reuse during the winter season in the Goiás region, situated within the Cerrado Biome, in which the drought, short photoperiod and absence of irrigation and fertilization, collectively render it impossible to attain sufficient forage production due to the limitations imposed by the C4 metabolism (Sanches *et al.*, 2016). The low photosynthetic yield of C4 plants in winter compared to summer is an important issue when choosing forage species and improving this performance increases its viability.

In addition to its direct influence on soil and water quality and conservation, the reuse of Tifton 85 results in significant changes in animal nutrition, including heightened crude protein content and digestibility. These attributes are pivotal in ameliorating periods when the availability of roughage is limited (Spearman *et al.*, 2020). The use of practices and technologies that improve the quality of WLF makes it possible to reduce the water footprint of dairy farming, such as cleaner production techniques, reuse of materials and environmentally friendly procedures, without negative economic impacts on the activity (Palhares *et al.*, 2018).

Despite concerns about its environmental impact, especially on the safety of the surface waters, the sheer volume and frequency of WLF generation constitute the primary rationale for its reuse, especially in the production of bulky foods like cereals for silage and fodder for hay and pasture. Consequently, this study concentrates on the sustainability aspect of forage irrigation, leveraging the copious volume of WLF generated, the imperative for bulky animal

feed during periods of water scarcity, and the escalating costs associated with fertilizers.

Assessing various formulations of reuse is pivotal to optimizing the overall efficiency of this practice. This entails considering water and nutritional requirements of plants, operational expenditures, and environmental sustainability. Hence, the objective of this study is to evaluate the productivity, morphological attributes, and bromatological indicators of Tifton 85 cultivated under different dosages of WLF and nitrogen fertilization during the winter season.

2. MATERIAL AND METHODS

2.1. Description of the experimental site

The experiment was carried out on a dairy farm with a herd of 900 lactating cows, under field conditions from May 29th to September 12th, 2020 (winter) in Gameleira de Goiás, GO, located at coordinates 16°24'41.9" S and 48°47'53, 9" W, with an average altitude of 980 m. The climate at this site is classified as tropical, with dry winter, according to the Köppen-Geiger classification (Alvares *et al.*, 2013), characterized by a mean annual temperature of 24.5°C and an annual precipitation of 1,611 mm (INMET, 2022). Figure 1 presents the location of the experiment.

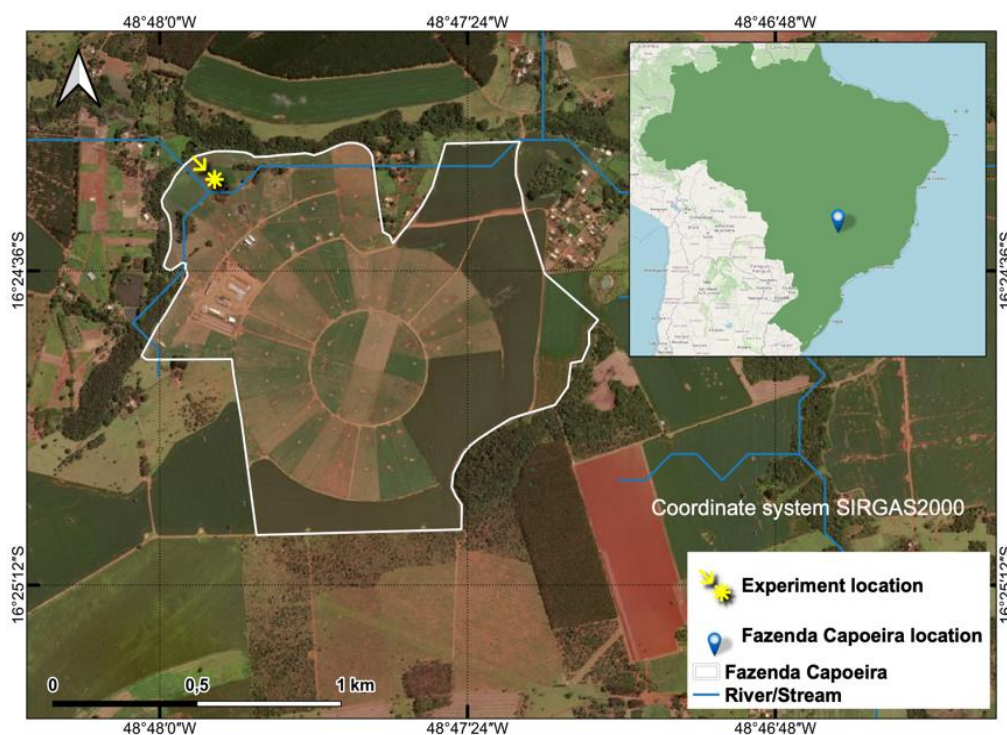


Figure 1. Experiment location.

The soil in this area is classified as Oxisol (United States, 2014) with medium texture and a smooth-undulating relief. Soil characteristics, as determined according to Teixeira *et al.* (2017), are as follows: pH 5.6, P 9.2 g kg⁻¹, K 0.1 cmolc dm⁻³, Ca 126,3 mg dm⁻³, Mg 291.6 mg dm⁻³, Fe 152.0 ppm, Mn 115.0 ppm, Zn 6.4 ppm, S 0.1 ppm dm⁻³, CEC 8.8 cmolc dm⁻³, organic matter 6.6%, base saturation 61%, clay content 51%, sand content 43%, and silt content 6%.

There were no recorded precipitation events during the experimental period. The average daily temperature fluctuated between 19 and 22°C, with a maximum of 32°C and a minimum of 10°C, as measured by a thermohygrometer installed at the experimental site. Evapotranspiration was determined using the Penman-Monteith equation, yielding maximum, mean, and minimum daily values of 9.8 mm, 5.2 mm, and 2.9 mm, respectively.

2.2. Experiment description

The experimental design consisted of randomized blocks with four replications, arranged in a 3x2 factorial scheme, distributed across 24 plots, each measuring 36 m² (6 × 6 meters). The sources of variation were the type of water used and mineral nitrogen fertilization. The types of water employed were: (I) stream water only; (II) a mixture of stream water and WLF in a 1:1 ratio; and (III) WLF only. Nitrogen mineral fertilization levels included (0) no supplementary fertilization and (45) 45 kg N ha⁻¹ in the form of urea.

The Tifton-85 pasture was initially established in 2015, managed under rainfed conditions, and subjected to an intensive rotation system until December 2019. Until April 14, 2019, the area did not receive any additional fertilizers or soil amendments. In May 2020, after mowing the pasture to achieve a uniform height of 5 cm above the soil, 45 kg N ha⁻¹ was applied according to each respective treatment.

The WLF was generated within the facilities associated with the milking parlor and comprised water (sourced from an artesian well), feces, urine, milk, secretions, and detergents. The WLF treatment system included an initial stage for separating coarse solids, followed by a system of stabilization ponds that exhibited an average removal efficiency of 86% for biochemical oxygen demand (BOD_{5,20}). The WLF was collected from the third and final pond within this system and transported via gravity to a 15-meter reservoir, waterproofed with plastic film. Stream water, on the other hand, was transported by gravity to a second excavated reservoir.

Both the WLF and stream water underwent physical and chemical analysis according to the APHA methodology (2017) on June 02, June 25, July 08, August 04, and August 27, 2020, and the average composition is shown on Table 1.

Table 1. Average characteristics of the wastewater of livestock farming (WLF) and stream water (SW).

	pH	EC ($\mu\text{S cm}^{-1}$)	TS (g L^{-1})	COD ($\text{mg O}_2 \text{ L}^{-1}$)	BOD	Turbidity (NTU)	SAR $\text{mmol}_c^{1/2}$
SW	6.8	19.0	0.1	15.3	0.0	6.0	0.0
WLF	7.4	5,375.0	4.2	1,495.8	322.7	771.2	2.6
	P_2O_5^- total	Fe^{3+} total	NO_2^-	NO_3^-	Ca^{2+}	Mg^{2+}	
(mg L ⁻¹)							
SW	0.0	0.1	0.0	0.4	0.0	1.1	
WLF	70.9	21.5	2.4	103.5	77.1	143.8	
	Hardness	Total Alkalinity	<i>E. coli</i>	Total coliforms	Na^+	K^+	
(mg CaCO ₃ L ⁻¹)		(MPN 100 mL ⁻¹)		(ppm)			
SW	4.6	4.1	10.1	307.6	0.0	0.5	
WLF	792.0	3,966.7	262,500	109,500	38.0	290.9	

EC – electrical conductivity; TS – total solid; COD – chemical oxygen demand; BOD – biochemical oxygen demand; SAR – Sodium adsorption ratio.

For irrigation, a sprinkler system with an application efficiency of 80% was employed. Irrigation was carried out on a weekly basis, delivering between 5.4 mm and 21.5 mm per event, with a cumulative total of 244 mm. This equates to approximately 42% of the estimated potential evapotranspiration for the crop during this period. Initially, the intent was to supply 100% of the water demand for Tifton 85, but adjustments were necessary due to various constraints encountered during the experiment, including limitations in the availability and quality of WLF (specifically, solid content permitting pumping and sprinkling) and the

availability of human resources in the field.

To monitor soil water content, pairs of tensiometers were installed at depths of 0-0.2 m and 0.2-0.4 m within each experimental block. Soil water content was estimated using the retention curve provided below (Equation 1):

$$\theta = 0.1102 + \frac{0.4179}{[1 + (68.684 \cdot |\Psi_m|)^{1.0941}]^{0.086}} \quad (1)$$

Soil water tension measurements were taken prior to each irrigation event. Throughout the experiment, the average soil water tension ranged from 27 to 69 kPa, corresponding to 37% and 28% of the available water capacity for the 0-0.4 m soil layer (CAD₄₀). Following each irrigation, the sum of the applied depth and the previously recorded tension accounted for 43% to 95% of the CAD₄₀.

Irrigation ceased when 70% of the plots exhibited pre-emission of panicles, which occurred 106 days after the initiation of the experiment. At the 113-day mark, a morphological evaluation of the pasture was conducted under field conditions, and forage samples were collected for subsequent laboratory analyses.

The quantity of nutrients applied was estimated by multiplying the volume applied in each irrigation event (in mm or L m⁻²) by the corresponding nutrient concentrations recorded for the analyzed period (in mg L⁻¹). Estimated nutrient load applications are presented in Table 2.

Table 2. Total nutrient load (kg ha⁻¹) applied to each water type.

Water type	NO ₃ ⁻ (N)	P _{org.}	K ⁺	Ca ²⁺	Mg ²⁺	Na ⁺	Fe ³⁺	TS
I	0.9 (0)	0.1	0.5	0.0	2.6	0.0	0.4	281
II	113 (26)	87.7	399	94.9	161	39	24	4,271
III	225 (51)	176.0	797	190.0	322	78	49	8,543

I – Stream water only; II – WLF diluted in stream water at a 1:1 ratio;
III – WLF only.

2.3. Sampling and evaluation of forage

Within the defined area of each plot, three distinct sampling sites were delineated using a template measuring 0.5 m x 0.5 m (0.25 m²). At each site, the height of the forage was measured. The forage was then harvested at 0.20 m above the soil, replicating a grazing height. The individual samples from each site were combined to create a composite sample, which was subsequently weighed to determine the fresh mass productivity of the respective plot. Approximately 400 g of this composite sample was weighed and subjected to drying in an air-circulating oven at 55°C for 48 hours. The water content of the plants was determined by calculating the ratio of water content to dry matter, thereby yielding dry mass productivity.

For morphological characterization, the dried forage was segregated into leaves, stems, and panicles, with the mass of each component being determined. The plant parts were collected for bromatological characterization, ground using a Willey knife mill (SL31, Solab Científico) equipped with a 0.5 mm sieve, and homogenized.

The following parameters were assessed: dry matter yield (Y_{DM}, ton ha⁻¹), forage height (H, m), leaf-stem ratio (LSR, g g⁻¹), leaf area index (LAI, m² m⁻²), nitrogen nutrition index (NNI, dimensionless – Lemaire *et al.*, 2008), crude protein content (CP, %_{DM}), mineral matter content (MM, %_{DM}), neutral detergent fiber (NDF, %_{DM}), and acid detergent fiber (ADF, %_{DM}). The leaf area index was estimated indirectly for Tifton 85 using a conversion factor of 1.46 dm² g⁻¹ (Rodrigues *et al.*, 2006).

Bromatological attribute determinations were carried out following the methodology outlined by Detmann *et al.* (2012). MM content was determined by incinerating organic material in a muffle furnace at 500°C. CP content was calculated by multiplying the nitrogen content, obtained using the Kjeldahl method adapted for food products, by a factor of 6.25. NDF and ADF contents were determined through autoclave-assisted procedures under pressure of 0.32 Kgf cm⁻² and temperature ≤ 105°C for 60 minutes.

2.4. Statistical analyses

The purpose of this experiment was to ascertain whether water compositions containing WLF could enhance the productivity and bromatological quality of a Tifton 85 pasture within the Cerrado Biome during the winter. Consequently, the data were initially subjected to analysis of variance and Tukey tests ($p \leq 0.05$) using the SpeedStat 2.5 software (Carvalho *et al.*, 2020).

Subsequently, principal component analysis (PCA) was employed to reduce the data dimensionality while preserving the essential covariance relationships. To accomplish this, key productivity variables (YDM and LAI), bromatological quality variables (MM, CP, NDF, and ADF), and NNI were selected. Hierarchical clustering was also conducted using Euclidean Distance and Ward's method for the agglomerative linkage process. These analyses were executed using XLStat 2022.3.2 software (Addinsoft, 2022).

3. RESULTS AND DISCUSSION

Table 3 shows the results of the ANOVA. A significant interaction ($p \leq 0.01$) between water types and nitrogen fertilization was observed only for crude protein. Differences were noted among the types of water utilized for productivity (YDM), height (H), leaf area index (LAI), neutral detergent insoluble fiber (NDF), and nitrogen nutrition index (NNI). Significant differences attributed to nitrogen fertilization were observed solely for mineral matter (MM).

Table 3. Summary of analysis of variance for productivity, morphology and bromatology indicators of Tifton 85 irrigated with wastewater from livestock farming.

Source of variation	LSR	YDM	H	LAI	MM	ADF	NDF	CP	NNI
Block	0.401 ^{ns}	0.643 ^{ns}	0.019*	0.204 ^{ns}	0.054 ^{ns}	0.120 ^{ns}	0.888 ^{ns}	0.076 ^{ns}	0.332 ^{ns}
WT	0.357 ^{ns}	< 0.001**	0.000**	0.000**	0.154 ^{ns}	0.377 ^{ns}	0.049*	< 0.000**	< 0.000**
N	0.284 ^{ns}	0.501 ^{ns}	0.560 ^{ns}	0.519 ^{ns}	0.023*	0.845 ^{ns}	0.901 ^{ns}	0.188 ^{ns}	0.220 ^{ns}
WT*N	0.628 ^{ns}	0.880 ^{ns}	0.391 ^{ns}	0.649 ^{ns}	0.672 ^{ns}	0.370 ^{ns}	0.108 ^{ns}	0.039*	0.362 ^{ns}
CV (%)	18.0	24.4	14.7	23.2	10.3	7.9	31.4	5.1	9.5

WT: water type; N: nitrogen fertilization; CV: coefficient of variation; ns: no significance; * and **: 5% and 1% error probability, respectively. LSR – leaf-stem ratio; YDM – productivity (dry mass); H – plant height; LAI – leaf area index; MM – mineral matter; ADF – acid detergent fibers; NDF – neutral detergent fibers; CP – crude protein; NNI – nitrogen nutrition index.

Contrary to expectations, mineral fertilization did not exert a discernible influence on productivity indicators (LSR, YDM, H, and LAI), even in plots receiving only stream water. Similar outcomes were observed for NDF and NNI. Since the application of 45 kg N ha⁻¹ is the standard procedure in this pasture field, it is likely that this was not a significant input. The influence of mineral nitrogen fertilization usually is not neglected, the Tifton 85 responses on forage production may just be delayed when under low nitrogen doses such as 30 to 60 kg N ha⁻¹ and can be perceived in the accumulated production values throughout the cuts (Alonso and Kaffka, 2016).

Conversely, the presence of WLF in the irrigation water engendered alterations in the attributes of YDM, H, LAI, NDF, and NNI, as delineated in Table 4.

Table 4. Productivity indicators and bromatological quality of a Tifton 85 pasture irrigated with different compositions of irrigation water and nitrogen fertilization.

Variable	Water type		
	I	II	III
Y _{DM} (t ha ⁻¹)	2.50 b	4.92 a	5.31 a
H (cm)	20.18 b	32.85 a	33.75 a
LAI (m ² m ⁻²)	2.61 b	4.95 a	4.85 a
NDF (% _{DM})	73.9 a	72.0 ab	69.5 b
NNI (dimensionless)	0.74 b	1.13 a	1.10 a
Crude protein (% _{DM})			
Fertilization			
0 kg N ha ⁻¹	12.4 bA	14.2 aB	14.3 aA
45 kg N ha ⁻¹	12.3 cA	15.8 aA	14.0 bA

I – Only stream water; II – Stream water and wastewater at the proportion 1:1; III – Wastewater only; YDM – Dry mass yield; H – Height; LAI – Leaf area index; NDF – Neutral detergent fiber; NNI – Nitrogen nutrition index. Means followed by the same lowercase letter in the row and uppercase letter in the column are the same by Tukey test at $p \leq 0.05$.

According to Alvim *et al.* (2000), Tifton 85 exhibits a linear response to nitrogen fertilization. The authors experimented with doses of up to 66 kg N ha⁻¹, 53.3 kg K₂O ha⁻¹ (equivalent to 44 kg K ha⁻¹) per cutting, and 80 kg P₂O₅ ha⁻¹ (equivalent to 35 kg P ha⁻¹) annually. However, they underscored that increasing forage productivity during the winter through fertilization exceeding 44 kg N ha⁻¹ per cut is implausible without irrigation.

The leaf-stem ratio (LSR = 4.1) remained unaffected by the type of water or nitrogen fertilization. This outcome aligns with expectations, as LSR is predominantly influenced by variations in soil water availability (Sanchez *et al.*, 2017). LSR serves as an indicator of nutritional quality, expressing the concentration of soluble carbohydrates in the plant. The obtained data fall within the range of average values for the upper extract of the Tifton 85 canopy (Poczynek *et al.*, 2016).

Greater masses of leaves and panicles were observed in treatments involving WLF application (data not presented). Likewise, WLF promoted heightened growth and accelerated physiological maturity compared to treatments without WLF. In concordance with these findings, WLF application yielded greater YDM, H, and LAI (Table 4).

YDM for Tifton 85 increased with the application of stream water, 50% diluted WLF, and pure WLF at rates of 23.6, 46.2, and 50.0 kg ha⁻¹ day⁻¹, respectively. According to Cameron and Di (2019), disparities in pasture productivity are anticipated when subjected to applications of pure wastewater versus regular water due to the nutrient content inherent to wastewater.

Marcelino *et al.* (2003) assert that the morphological variables of Tifton 85 are susceptible to variations driven by the natural reduction of plant metabolism within the C₄ cycle, triggered by environmental factors such as shortened photoperiods, cooler air temperatures, and water restrictions. It is estimated that during winter the productivity of Tifton 85, under conventional fertilization and without irrigation, can drop to as much as 3.5 times lower than in summer (Sanchez *et al.*, 2016).

Notwithstanding that the irrigation conducted during the experiment did not meet the entirety of the pasture's water requirements, soil water tensions observed prior to each application did not exceed 69 kPa. According to Marcelino *et al.* (2003), this range of soil water availability does not detrimentally affect dry mass gains in response to the available nitrogen in the soil. Indeed, as Amaral *et al.* (2019) suggest, during winter, soil water availability can decrease by up to 20% without compromising the anticipated quality and quantity of Tifton 85

forage within this specific soil-climate context.

WLF resulted in pasture heights that were 67% greater than those receiving only stream water (Table 4). Additionally, the LAI of Tifton 85 cultivated with WLF was 80% higher than that of plants irrigated solely with stream water. This was greater than the values reported by Rodrigues *et al.* (2006) (LAI ranging from 3.4 to 4.4 m² m⁻²) but lower than those obtained by Poczynek *et al.* (2016) (LAI = 5.6 m² m⁻² – evaluated over 204 days without irrigation).

As anticipated, the presence of sodium in the WLF composition did not adversely affect Tifton 85. The treatments in this study supplied between 0 and 78 kg Na ha⁻¹ to the forage. Comparable studies employing alternative wastewater sources demonstrate that even in the presence of substantial sodium loads (ranging from 300 to 1,200 kg Na ha⁻¹ year⁻¹), the species can exhibit yields and chemical quality akin to those achieved through conventional cultivation, if soil water remains available (Sousa *et al.*, 2009). Nevertheless, although WLF minimally influenced dry matter production, applications of effluents with sodium content exceeding 900 kg ha⁻¹ year⁻¹ can restrict nutrient concentrations in the forage biomass, including those of N, P, Ca, and Mg (Oliveira *et al.*, 2019).

For forage composition, applying WLF did not alter the mineral matter (MM) content. However, nitrogen fertilization led to an increase in MM. It's worth noting that both MM values obtained were lower than those reported for Tifton 85 under conventional management and cutting conditions within 49 days (MM = 7.74%) (Andrade *et al.*, 2018) or after 100 days of growth (7.0% for stems and 8.9% for leaves) (Serafim *et al.*, 2021). MM represents the residual inorganic material obtained from burning phytomass, which is converted into CO₂, H₂O, and NO₂ and eliminated along with volatile substances decomposed by heat (Souza *et al.*, 2017).

The types of water used did not affect the acid detergent fiber (ADF) content. The ADF content obtained, 30.0%, was lower than the values reported by Sanches *et al.* (2016) at 34.5%, Monção *et al.* (2016) at 32%, and Poczynek *et al.* (2016) at 32.5%. This indicates better forage digestibility compared to what the authors observed, since ADF content is inversely proportional to forage maturity. Moreover, the ADF content obtained in this experiment was even lower than the 31.7% observed by Marchesan *et al.* (2013) in Tifton 85 leaves cultivated during the same time of year (Jun-Sept).

Nitrogen fertilization did not impact neutral detergent fiber (NDF) levels. However, forage irrigated with undiluted WLF showed lower NDF (69.5%) due to its higher proportion of young tissues, mainly panicles and leaves, compared to forage irrigated solely with stream water (73.9%). NDF content is known to have an inverse relationship with voluntary forage consumption. Values between 65% and 75% are typical in young and nearly mature tissues (Gonçalves *et al.*, 2018). The impact of winter, characterized by an average air temperature of 21.6°C, a minimum temperature of 13.5°C, and drought, on Tifton 85 metabolism is evident since the pasture did not reach sufficient maturity to accumulate undesirable fibers for grazing, even with a regrowth period of 106 days.

Forage irrigated solely with stream water accumulated less crude protein (CP) regardless of nitrogen fertilization. Treatments involving WLF and fertilization achieved higher CP content. In their study with corn fertilized with urea and irrigated with sewage effluent, Guo *et al.* (2016) observed a booster effect of the effluent on nitrogen plant uptake and yield when compared with ground water irrigation. They concluded by tracking ¹⁵N labeled urea that the reuse raises the percentage of utilization of fertilizer nitrogen. For instance, CP content for Tifton 85 irrigated solely with stream water averaged 12.4%, while those receiving WLF maintained levels above 14.0% (Table 4). Forage that received diluted WLF and nitrogen fertilization achieved a CP content of 15.8%, surpassing all other fertilized treatments, as observed by Monção *et al.* (2016) for Tifton 85 cut at 79 days.

In the context of reuse, the CP values obtained in this experiment were lower than the range of 18.2% to 21.2% reported by Spearman *et al.* (2020) when reusing wastewater from swine

farming. Nevertheless, the CP content remained higher than the desirable threshold (10%) for ruminant diets and within the range of 14.0% to 16.7% expected for the environmental and soil moisture conditions in the experiment (Sanches *et al.*, 2016; Gonçalves *et al.*, 2018).

It is crucial to note that crude protein contents in Tifton 85 can significantly vary depending on management practices (fertility, irrigation and reuse), the season of the year, and the grazing or cutting cycle, with values ranging from 4.6% to 22.4%. The species growth is more responsive to nitrogen fertilization during the summer and autumn (Alvim *et al.*, 2000; Sanches *et al.*, 2016; Gonçalves *et al.*, 2018). This occurs mainly because the nutrient uptake of Tifton 85 depends on its quick recovery between cuts (Oliveira *et al.*, 2019), which enables great protein accumulation. Consequently, the observed contribution of WLF to the formation of higher protein content under these environmental conditions underscores the significance of reuse for enhancing the nutritional quality of Tifton 85. According to Wang *et al.* (2017) findings regarding reuse with three forage species including Coastal bermudagrass, to optimize the forage nutritional value and yield some management strategies can be performed, such as choosing the species by its ability to remove nutrients from soil suited for the reuse inputs, harvest during the peak growth, and prevent the forage from reaching senescence and the consequent reduction in the rate of nutrient uptake.

Mineral fertilization and irrigation with diluted WLF (Treatment II-45), which collectively contributed 70 kg N ha⁻¹, yielded the highest CP content for Tifton 85 compared to treatment with the same type of water but without fertilization (totaling 26 kg N ha⁻¹). The CP content of Treatment II-45 even surpassed that of Treatment III-45 (totaling 96 kg N ha⁻¹). This outcome demonstrates that the quantity of nitrogen supplied to Tifton 85 alone is not the only factor influencing protein production and biomass.

It is noteworthy that fertilization of 45 kg N ha⁻¹ did not guarantee a linear increase in Tifton 85 biomass. Among the types of water, the nitrogen nutrition index (NNI) of Tifton 85 irrigated solely with stream water averaged 0.74, lower than the average of treatments involving WLF application (NNI = 1.1). This index ranged from 1.06 to 1.20 with the use of WLF. As per this index, due to the application of WLF, the grass utilized the nitrogen provided through either route (WLF or fertilization) for dry matter production, tending luxury consumption (NNI > 1). Irrigation with stream water resulted in plant development with a nutritional deficit (NNI < 1) (Lemaire *et al.*, 2008). In the context of WLF reuse, luxury consumption is a positive outcome as it signifies the absorption of nutrients introduced to the soil through wastewater, thereby facilitating its recycling. Moreover, enhanced nitrogen absorption indicates the formation of higher-quality roughage.

Andrade *et al.* (2018) highlight that the response of Tifton 85 productivity to soil-available nitrogen depends on the availability of other nutrients. Therefore, the improved forage development observed when irrigated with WLF can be attributed to the likely presence of these nutrients in its composition. Considering the NNI value, adequate Tifton 85 development in this experiment was achieved through the application of diluted WLF without nitrogen fertilization (II-0), providing 26 kg N ha⁻¹, 87.7 kg P ha⁻¹, and 400 kg K ha⁻¹ (Table 2). It is crucial to emphasize that Tifton 85 has higher nutritional requirements than other forages commonly cultivated in Brazilian pastures (Moção *et al.*, 2016; Poczynek *et al.*, 2016). Nonetheless, even with these requirements, the C4 metabolism of Tifton 85 in the climatic conditions of this study ensured that the water and nutritional contributions of diluted WLF sufficed to meet the demands of the forage.

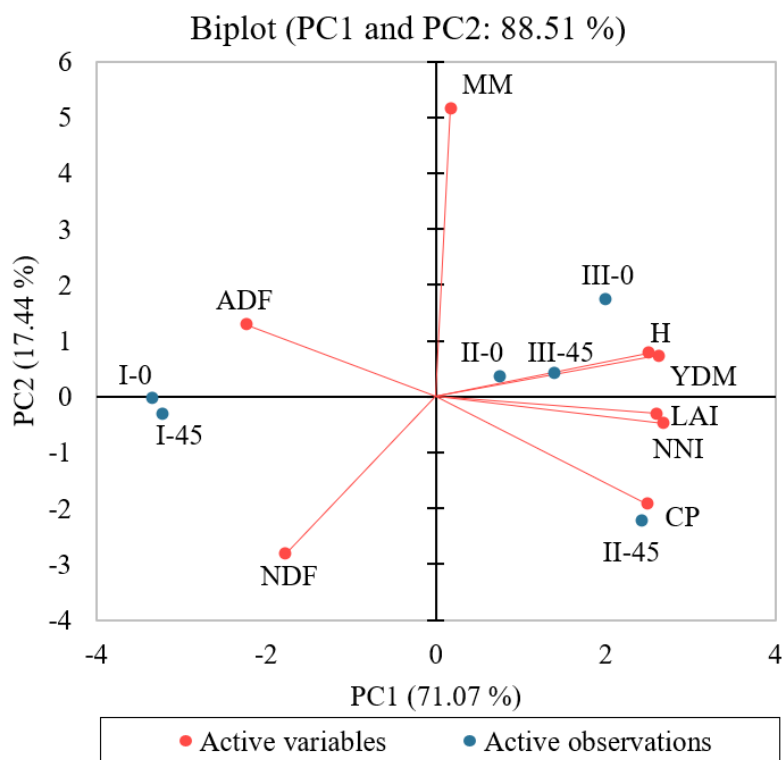
Pearson's correlation analysis revealed that Tifton 85 exhibited a moderate and positive response to the applied nitrogen (N_{load}) in terms of forage dry matter (Y_{DM}, H, and LAI) and nitrogen nutrition status (CP and NNI) (Table 5). This demonstrates that phytomass production paralleled variations in N_{load} with similar intensity as the set of bromatological quality attributes.

Table 5. Summary of linear correlation between Tifton 85 indicators of yield and bromatological quality.

	YDM	H	LAI	MM	NDF	ADF	CP	NNI	N-load
YDM	1.00								
H	0.93**	1.00							
LAI	0.95**	0.97**	1.00						
MM	0.09	-0.21	0.02	1.00					
NDF	-0.73*	-0.50*	-0.49	-0.18	1.00				
ADF	-0.69*	-0.65*	-0.71*	-0.23	0.46	1.00			
CP	0.82**	0.79**	0.88**	0.32	-0.43	-0.90**	1.00		
NNI	0.96**	0.91**	0.96**	0.20	-0.62*	-0.82**	0.94**	1.00	
N-load	0.71*	0.52*	0.66*	0.43	-0.49	-0.47	0.54*	0.67*	1.00

** Strong correlation; * Moderate correlation; LSR – Leaf/stem ratio; YDM – Dry mass yield; H – Height; LAI – Leaf area index; MM – Mineral matter; ADF – Acid detergent fiber; NDF – Neutral detergent fiber; CP – Crude protein; NNI – Nitrogen nutrition index; N-load - Nitrogen load from the type of water applied (CWW and/or W) and mineral fertilization.

Hierarchical clustering analysis highlighted two distinct groups: 1st – forage subjected only to stream water (I), and 2nd – forage subjected to some dose of WLF (II and III), irrespective of the applied nitrogen fertilization. This underscores the evidence of distinct plant responses to water types that provided different soil fertility conditions. Combining the physiological and bromatological parameters of Tifton 85, a first principal component (PC1) explained 71.1% of the data variability, while a second principal component (PC2) accounted for 17.4%. Together, these components elucidate 88.5% of the variability in results (Figure 2). All variables associated with productivity exhibited strong and positive correlations with PC1.

**Figure 2.** Principal component analysis (PCA) of pooled samples and Tifton 85 variables according to treatments.

The separation of treatment groups illustrates that WLF interactions within the soil-plant system engender different reactions in Tifton 85 compared to stream water alone. The PCA analysis emphasizes that 45 kg ha⁻¹ of mineral nitrogen fertilization by itself is irrelevant for managing Tifton 85 pasture when irrigated with WLF during the winter. This is likely due to the supply of other nutrients besides nitrogen, which are equally crucial for pasture development, such as potassium and phosphorus.

CP exhibited a positive response to PC1 and a negative response to PC2, while MM responded positively to PC2 exclusively. ADF demonstrated a negative response to PC1 and a positive response to PC2, while NDF exhibited negative responses to both principal components.

The distribution of variables and treatments along the PC1 axis suggests that forage quality, productivity, and nutrient absorption align inversely with variables associated with maturity (ADF and NDF). This aligns with the observations made by Marchesan *et al.* (2013). Treatments involving WLF clustered closely with the variables YDM, NNI, CP, H, and LAI, illustrating the contribution of these water types to these variables. These findings underscore that, in the winter conditions of the Cerrado Biome, it is more advantageous to irrigate with WLF for pasture maintenance than with stream water.

For the application of stream water alone, the absence of nutrients and consequently, the absence of additional stimuli primarily promoted cell elongation and maturation, resulting in forage with lower quality and moderate quantity. In such cases, it is well-established that biomass production, nutrient absorption, and immobilization by Tifton 85 depend on environmental conditions. These processes are less efficient during months with shorter photoperiods (Zhang *et al.*, 2017; Teixeira *et al.*, 2021). Therefore, during these periods, the plant primarily accumulates fibers (mainly NDF) and hemicellulose (Marchesan *et al.*, 2013; Sanches *et al.*, 2016).

Considering the above-mentioned insights and the fact that the three types of water examined contribute to the composition of distinct soil solutions, the impact of WLF on Tifton 85 at the physiological level substantiates the observed enhancements in productivity and nutritional values. As per Teixeira *et al.* (2021), nutrient availability in the soil solution directly influences the extraction rate of Tifton 85, facilitates nutrient accumulation in tissues, and indirectly optimizes photosynthetic activity.

MM was the only variable that exclusively responded to PC2, and this independence was also evident in its weak correlations with the other attributes. By analyzing the correlations between PC2, MM, ADF, and NDF, it is plausible to infer that PC2 is associated with pasture aging. Over time, there is a gradual reduction in digestible fiber (NDF), an increase in mature fiber (ADF), and the incorporation of minerals in biomass (MM).

The correlations established in this study indicate that the reuse of WLF in pasture promotes increased productivity and imparts chemical quality to Tifton 85. In contrast, irrigation with stream water primarily stimulates plant growth but does not contribute to nutritional quality to the extent that WLF does.

4. CONCLUSIONS

I. The reuse of WLF for irrigating Tifton 85 pasture during one regrowth cycle in winter results in a greater biomass accumulation and improved nutritional attributes compared to irrigation with stream water alone.

II. Irrigating with WLF during a regrowth cycle in the winter is sufficient for Tifton 85 to attain a nutritional quality considered adequate for this species, unlike irrigation with stream water.

III. Nitrogen fertilization (45 kg N ha⁻¹) is insufficient to enhance both the biomass production and nutritional quality of Tifton 85.

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