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# Potential use of Tifton 85 cultivated in VFCW for animal feed

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## Abstract

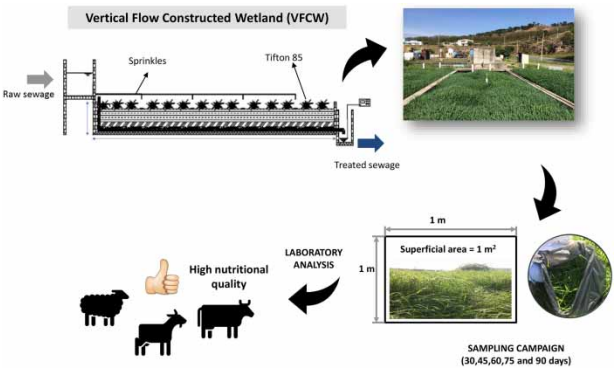
The objective of this study was to evaluate the nutritional value of Tifton 85 grass (*Cynodon dactylon* (L) Pers) cultivated in a vertical flow constructed wetland (VFCW) system treating raw sewage. The VFCW operated according to the French system, but with only the first stage and two units in parallel, using a small footprint of 0.6 $m^2$ per inhabitant. Wastewater feeding and resting periods were of seven days each. To evaluate the yield and nutritional value of the Tifton 85 cultivated, samples were collected after 30, 45, 60, 75 and 90 days of growth. They showed yields of 16, 28, 36, 36 and 38 $t \cdot ha^{-1}$ of dry matter (DM), respectively. The crude protein content decreased sharply during 60 days, however, with values of 29, 23, 15, 15 and 15% DM for the same days, respectively. The results indicate that Tifton 85 could be used for hay production and, to obtain better yield and quality, when used to treat raw wastewater the forage should be cut after between 45 and 60 days of growth.

**Key words:** animal feeding, *cynodon dactylon*, sewage treatment, Tifton 85 bermudagrass, vertical flow wetland

## Highlights

- The study evaluated the nutritional value of Tifton 85 grass cultivated in a vertical flow constructed wetland system treating raw sewage.
- The nutritional values of Tifton 85 were considered suitable for animal feeding.
- The recommended Tifton 85 harvest time period was between 45 and 60 days of growth, because crude protein content decreased after this.

## Graphical Abstract



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## INTRODUCTION

Vertical flow constructed wetlands (VFCWs) are natural and decentralised treatment systems that can be used for wastewater treatment. They represent a promising technology for developing countries due to their simplicity and low operating costs. Due to the presence of the filter medium and the characteristics of the wastewater used (raw sewage), a significant proportion of the solids and organic matter, etc, are retained in the medium's top layer, remaining susceptible to stabilisation and mineralisation. According to Stefanakis & Tsihrintzis (2011), this surface deposit layer (0 to 50 cm) provides physical and nutritional support for plant development, and enhances biomass productivity. Water losses by drainage can also be facilitated by systematic plant stem movement, arising from wind action, which causes cracks in the porous bed's surface layer (Nielsen & Cooper 2011).

Raw sewage is a complex matrix composed mostly of organic matter with high contents of nutrients. The ideal species for cultivation in VFCWs should be able to absorb as much of the macro- and micronutrients as possible from the liquid, thereby reducing their concentrations in the percolate, and have some commercial value. Grasses of the genus *Cynodon* are often recommended as feed for cattle and horses because they adapt well to both tropical and subtropical climates, and the Tifton group are among the species of this genus. The hybrid variety Tifton 85 bermudagrass (Tifton 85) is a perennial, stoloniferous, rhizome-possessing plant, with subterranean stems responsible for maintaining its carbohydrate and nutrient reserves. These characteristics make the plant relatively tall and highly productive, with high resistance to pest damage, long stems and broad leaves. The forage is readily digestible, and has high crude protein (CP) content and a good calcium: phosphorus ratio compared to other grasses of the genus *Cynodon*, resulting in its recommendation for use as animal feed (Hancock *et al.* 2013).

Tifton 85's yield can be increased if it is irrigated with wastewater with high organic content – for example, whey and dairy industry wastewaters (da Fonseca *et al.* 2007; Schwantes *et al.* 2017). In terms of dry matter (DM) yield and nutritional quality for animal feed, it is crucial to consider the best time to cut the plants. Forage tissue growth is neither random nor uniform. Growth in plants is defined as an irreversible increase in volume and can be measured in terms of change in fresh weight (Taiz *et al.* 2015). Cell walls are commonly classified into two major types: primary and secondary, differing in structural carbohydrate composition. Primary cells are formed by growing cells and their matrix is composed of two major groups of structural carbohydrates (hemicelluloses and pectins), plus structural protein. Secondary walls are those that form after cell growth has ceased, and are strengthened by cellulose and lignin. In ecosystems, animals consume plants as major sources of carbohydrates to produce meat and milk, and forage digestibility is linked to the type and content of structural carbohydrate present in forage cell walls (Wu 2018).

Tifton 85 cultivation in constructed wetlands has been increasing in Brazil, but little information is available in the literature regarding the productivity and nutritional quality of animal fodder produced. Pathogen identification and the sanitary implications of use as fodder were not the objective of this study, but it is necessary to guarantee a safe, healthy product for sewage-irrigated Tifton 85 final consumers, especially young animals. Bevilacqua *et al.* (2014) showed that forage irrigated with treated sewage had no significant impact on the health of goats or calves. The aim of this study was to evaluate the productivity and bromatological characteristics of the aerial part of Tifton 85 bermudagrass cultivated in VFCWs treating domestic sewage.

## METHODS

The study was conducted in the VFCW at the Centre for Research and Training in Sanitation UFMG/COPASA, at Arrudas Wastewater Treatment Plant, Belo Horizonte, Brazil (19°53′42″ S, 43°52′42″ W). The region's climate, according to the Köppen classification, is Cwa – dry winter, humid, sub-tropical.

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The average annual temperature and precipitation are 22.1 °C and 1,540 mm. The VFCW has operated since 2009 and was designed as the first stage of the French raw sewage treatment system after screening and grit removal, for a population equivalent of 100. It has been modified since commissioning, and operates using only two units and a small footprint of 0.6 $m^2$/inhabitant. Both units are operated in cycles, with alternating feeding and resting periods. During this study, the feeding and resting periods were of seven days each.

To evaluate the yield and nutritive value of the Tifton 85 cultivated in the wetland units, samples of the sub-aerial stems were collected after 30, 45, 60, 75 and 90 days of growth in demarcated 1.0 $m^2$ areas. The resulting database comprises agricultural analyses in terms of chemical composition covering a three-month period (November 2017 to January 2018), which corresponds to the rainy season in south-eastern Brazil. At the start of the study, therefore, sludge had been accumulating on top of the filter medium for approximately eight years.

DM was quantified by oven drying at 105 °C, and the extent of mineralisation was determined by calcination in a muffle furnace at 600 °C. CP was determined by the Kjeldahl method, and ether extract (EE), calcium (Ca) and phosphorus (P) according to AOAC (1995). The fibre fractions were quantified as neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose (HEM), cellulose (CEL) and lignin (LIG) (Van Soest *et al.* 1991). The yields of FM and DM, and of CP, were determined from the dry matter results at 105 °C.

## RESULTS AND DISCUSSION

### Tifton 85 productivity

Table 1 shows the productivity, chemical composition and nutritional results for Tifton 85 used in raw sewage treatment.

**Table 1** | Effects of raw sewage application in VFCW on the nutrition value of Tifton 85 at different ages in one growth cycle

| | Age of plants in single growth cycle(days) | | | | |
|---|---|---|---|---|---|
| **Variable** | **30** | **45** | **60** | **75** | **90** |
| FM (t $ha^{-1}$) | 16 | 28 | 36 | 36 | 38 |
| DM (t $ha^{-1}$) | 2 | 6 | 9 | 9 | 11 |
| MC (%) | 85 | 77 | 76 | 74 | 72 |
| CP (%) | 29 | 23 | 15 | 15 | 15 |
| LIG (%) | 2 | 1 | 4 | 4 | 5 |
| CEL (%) | 21 | 29 | 28 | 29 | 26 |
| HEM (%) | 21 | 26 | 27 | 26 | 28 |
| EE (%) | 4 | 4 | 3 | 3 | 3 |
| NDF (%) | 48 | 58 | 59 | 61 | 61 |
| ADF (%) | 27 | 32 | 33 | 35 | 33 |
| Ca (%) | 0.5 | 0.2 | 0.4 | 0.2 | 0.3 |
| Mg (%) | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| P (%) | 0.6 | 0.4 | 0.4 | 0.4 | 0.4 |
| K (%) | 3.5 | 3.2 | 3.3 | 3.0 | 2.8 |
| S (%) | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 |

FM – fresh matter, DM – dry matter, MC – moisture content, CP – crude protein, LIG – lignin, CEL cellulose, HEM – hemicellulose, EE – ether extract, NDF – neutral detergent fibre, ADF – acid detergent fibre, plus standard chemical symbols.

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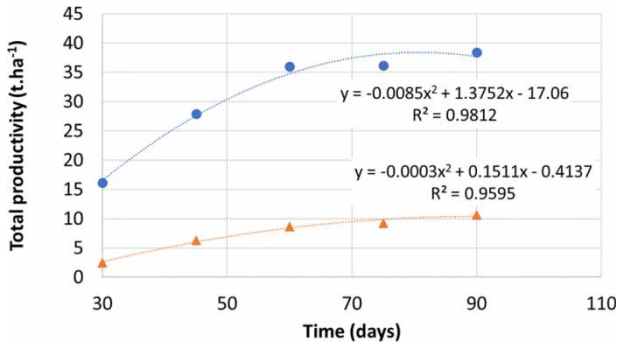


**Figure 1** | Age effects of sewage irrigation on the FM (●) and DM (▲) yields of Tifton 85 cultivated in VFCW.

In a general analysis, productivity can be evaluated by verifying the yields of both FM and DM. The FM increased with time, although with an apparent tendency to reach maximum productivity values in 90 days. Figure 1 is a plot of the observed values and equations for the productivity variables of sub-aerial Tifton 85 cultivated in VFCW as a function of age. Quadratic fitting is widely used for crops due to seasonality in forage production leading to output and nutritive value instability (Andrade *et al.* 2018). During raw sewage application in VFCW in this study, DM production increased from 2 (day 30) to 11 t·ha$^{-1}$ (day 90). Gonçalves *et al.* (2008) reported Tifton 85 DM yields ranging from 2 to 6 t·ha$^{-1}$ at 21 to 63 days of regrowth without fertilization. In this study, up to 30 days since the first harvest, the plants grew heterogeneously on the VFCW, due to the diversity of nutrient contribution provided by the sewage supply points. After 30 days' cultivation, growth became more homogeneous across the VFCW. The quadratic fitting enabled comparison between studies and the equation derived equation shows that the estimated yield of 17 t.ha$^{-1}$ per semester is similar to the results obtained by others with treated sewage, with, for instance, yields of 18 to 16 t·ha$^{-1}$ per semester (Nogueira *et al.* 2013) or 20 t·ha$^{-1}$ per year (da Fonseca *et al.* 2007). Studies have been made evaluating the impact of mineral N fertiliser on Tifton 85 biomass production and showed similar yields to treated sewage, with N doses between 100 and 300 kg-N·ha$^{-1}$ (Carvalho 2012; Andrade *et al.* 2018). This reinforces the fact that sewage could be an alternative and sustainable source of nitrogen for Tifton 85 development.

### Tifton 85 fibre development in VFCW

The digestibility of forage is closely related to its chemical composition, and the fibre fraction has the most significant influence. Both the quantity and quality of fibre are important in animal nutrition (McDonald *et al.* 2011). NDF content is a measure of cell wall content and refers to the amount of cellulose, hemicellulose, and lignin (Wu 2018). ADF, however, represents the crude lignin and cellulose fraction of the plant material, and includes silica. Determination of these parameters is particularly useful for forage, because forage cell wall digestibility is much more variable and depends on the degree of lignification.

The NDF (Figure 2(a)) and ADF (Figure 2(b)) contents of Tifton 85 fertilized with domestic sewage increased during the study. NDF content ranged from 48 (day 30) to 61% (day 90) and ADF from 27 to 33%, respectively. These results are better than those reported by Andrade *et al.* (2018). They evaluated Tifton 85 fertilized with mineral nitrogen and obtained NDF contents ranging from 72 (day 28) to 73% DM (day 49), and ADF 31 and 33% DM, respectively. Castro *et al.* (2010) evaluated Tifton 85 in a temperate climate and obtained 76% DM for NDF and 36% DM for ADF. Comparing the results of this study with those in which mineral N was used as fertiliser, the regrowth of Tifton 85 in VFCW produced leaves with more digestible NDF and ADF contents and thus better for animal feed.

According to McDonald *et al.* (2011), tropical grasses are generally less digestible than their temperate counterparts because their leaves contain more vascular bundles, and hence more lignin.

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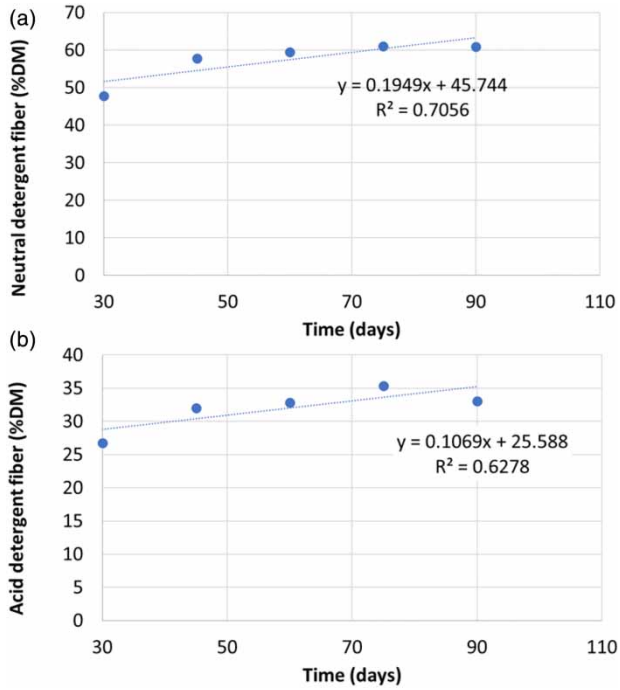


**Figure 2** | Age effects of sewage irrigation on NDF (a) and ADF (b) on Tifton 85, in the function of the age cultivated in VFCW.

Lignin is a phenolic compound that confers structure to plants. It is indigestible, therefore limiting the fodder's nutritional use, and is negatively correlated with the *in vitro* digestibility of the dry matter.

Crude fibre comprises more resistant plant structures that confer higher stiffness, and the lignin, cellulose and hemicellulose contents vary with plant age. Figure 3 presents the amounts of lignin, cellulose and hemicellulose with increasing Tifton 85 age. As can be seen, the lignin content ranged from 2 (day 30) to 5% DM (day 90). These results are similar to those from other studies under the same edaphoclimatic conditions. Carvalho (2012) evaluated Tifton 85's nutritional characteristics at different ages and observed a linear increase in lignin content from 2 (day 27) to 5% DM (day 84). Andrade *et al.* (2018), evaluating mineral N mineral fertiliser with Tifton 85, obtained higher lignin contents than found in this study, ranging from 4 (day 28) to 7% DM (day 49).

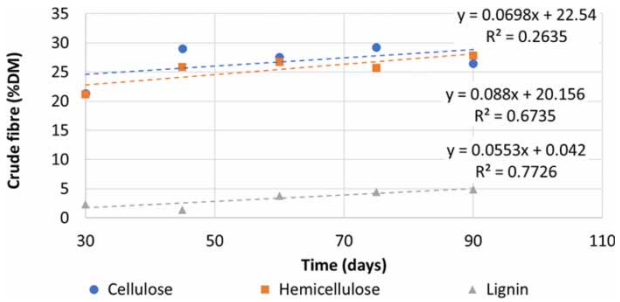


**Figure 3** | Age-related crude fibre fraction of Tifton 85 according to different ages receiving sewage on VFCW.

In this study, Tifton 85's cellulose and hemicellulose contents ranged from 21 to 26% DM and 21 to 28% DM, respectively. These results can be compared with those from Carvalho (2012), who reported cellulose content ranging from 27 to 33% and hemicellulose from 40 to 44% in Tifton 85 cultivated in soil. The comparison demonstrates that Tifton 85 developed in VFCW produces less crude fibre, which can improve animal production because of the higher digestible portion in the forage.

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### Evaluation of CP in Tifton 85 cultivated in VFCW

Concentrates with high CP and mineral content, and low energy concentration, could be used in small quantities to balance tropical pasture diets (Teixeira *et al.* 2019). The CP content is calculated from the nitrogen content of the food by sulphuric acid digestion, which converts to ammonia all nitrogen present in the sample, except that in the form of nitrate and nitrite (McDonald *et al.* 2011).

In this study, Tifton 85's CP content (Figure 4) was higher near the start – for example, 29% (day 30) – then decreased to 15% (day 60), after which it stabilised. These results showed higher CP content in Tifton 85 than reported in mineral nitrogen fertiliser studies. Different studies with mineral N fertiliser found CP contents in Tifton 85 ranging from 12 (Andrade *et al.* 2018) to 26% (Sanches *et al.* 2017) in DM with 28 days of regrowth.

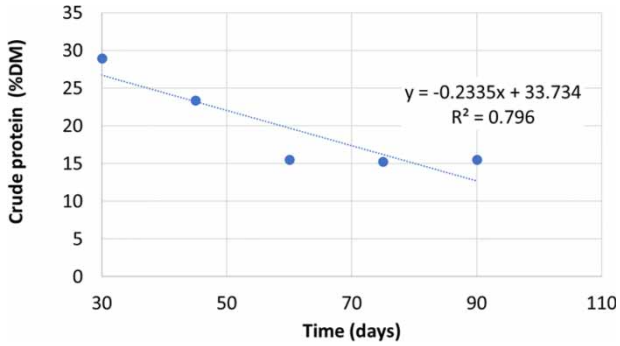


**Figure 4** | CP of Tifton 85 cultivated in VFCW used for sewage treatment.

The amount of protein an animal needs in its feed depends on issues including its size, growth, milk production and stage of pregnancy (Wu 2018). Feeding protein in animal diets increases the amino acid flux to the intestine and may improve lactation performance, especially in dairy cattle. According to the National Research Council (NRC 2001), increasing dietary CP from 14 to 18% would result in an increase of 2.1 L milk·day$^{-1}$. This study demonstrated the potential use of Tifton 85 cultivated in VFCW as an alternative CP source for farm animals.

### Major elements in Tifton 85

The term 'essential mineral element' is restricted to those proven to have a metabolic role in the body (McDonald *et al.* 2011). Ca, P, K, Mg and S were evaluated in this study.

The concentrations of mineral elements often change with plant age, tending to increase, although the maximum can sometimes occur at an intermediate maturation stage. The P and K concentrations in sub-aerial Tifton 85 found in this study are higher than those reported from studies involving mineral fertilisation (Carvalho 2012). At the same time, the Ca concentrations are lower and those of Mg are close to those reported for the same grass cultivated in soil. Carvalho (2012) also verified a tendency for P concentration reduction in the sub-aerial part of Tifton 85, as observed in this study, with the plants' harvesting age. However, Carvalho (2012) also found similar decreases in K and Mg, which differs from what was observed in this study. It is suggested that the content of these nutrients in the different cultivation situations (VFCW and soil) provided by different matrix irrigation (mineral and sewage) affect the major element concentrations in Tifton 85.

## CONCLUSIONS

This study has shown that the nutrient composition of Tifton 85 cultivated in VFCW for sewage treatment produces forage suitable for animal nutrition and productivity. Nutritional analysis showed high

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levels of CP and low levels of NDF, especially lignin, improving the material's digestibility. However, the study's results suggest that an ideal cutting period is between 45 and 60 days because the material becomes less digestible with age. The complex composition of domestic sewage appears to have enhanced Tifton 85's characteristics compared to studies in which N-mineral fertiliser was used. These results could indicate a sustainable use of Tifton 85 produced in VFCW, potentially leading to savings for farmers.

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