




Article

Production of Preserved Forage for Horses under Water Scarcity Conditions: A Case Study

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Abstract: The Mediterranean region is one of the areas most affected by climate change, which influences the production of forages. This has led producers to change from one to several forage cuttings, aiming to maintain crop productivity in increasingly water-scarce conditions. This study aimed to evaluate the nutrient content and productivity of forage produced for horses when subjected to variable water availability conditions at a Lusitano stud farm located in the central region of Portugal. The soil water content was evaluated throughout the growing season, using the gravimetric method, with soil samples collected every 15 days. Forage samples were collected from three grass cuttings (two for haylage and one for hay production), harvested from the same sward during the 2018/2019 growing season. The nutrient content of the forage samples was determined by chemical analysis. The global productivity throughout the crop-growing season was 8.3 t DM·ha⁻¹, with the second harvest presenting the highest productivity (3.42 t DM·ha⁻¹), corresponding to an adequate water supply, whereas the last cut, produced under water deficit conditions, presented the lowest productivity (2.1 t DM·ha⁻¹). The estimated nutritive value by chemical composition analysis for both haylage and hay fell within the range reported in the literature for preserved forages for horses.

Keywords: hay; haylage; productivity; nutritive content; water deficit



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1. Introduction

The horse is a non-ruminant grazing herbivore adapted to eating plant-fiber or forage-based diets. As many horses are kept in the stable most of the day and do not have free access to pastures, the use of preserved forages is essential for those animals. To promote the health and welfare of stabled horses, it is recommended that their diets contain a minimum daily amount of forage of 15 g dry matter (DM)/kg body weight [1]. Thus, preserved forages such as hay, haylage, or silage are essential for horses' diets, especially in the Mediterranean climate, which is characterized by increasing water scarcity [2].

The use of preserved forage in horses' diets depends on its quality, and its capacity to provide the nutrients and energy required by the animals. However, forage quality is affected by crop management factors, such as fertilizer applications, harvest techniques, and storage conditions, among others [1,3]. Furthermore, the nutrient content of forage is greatly influenced by herbage maturity at harvest [4]. Forage conservation methods also have a high degree of influence on the chemical composition and microbiological quality of forages, although with little or no effects on equine digestion and the utilization of these feeds [5]. Forage can cover 30% to 90% of the daily nutritional requirements of a horse, depending on the type of horse and its physiological stage [6]. The same authors also reported that preserved forages can cover 20% to 95% of the protein and 25% to 85% of a

horse's energy requirements, depending on the plant species, their composition, and the type of conservation performed. It is recognized that there are some differences between the nutrient content of green forage and the corresponding hay harvested at a specific physiological stage [7,8]. For instance, with respect to the tillering stage of Italian ryegrass (*Lolium multiflorum* Lam.), the values observed on a DM basis were 15.8% and 14% for crude protein (CP), 26.6% and 28.2% for crude fiber (CF), and 54.1% and 62.0% for neutral detergent fiber (NDF), for green forage and hay, respectively [7]. Moreover, according to the same authors, the values observed for Persian clover (*Trifolium resupinatum* L.) at the first stage of development were 24.2% and 23.6% for CP, 16.1% and 17.4% for CF, and 30.0% and 30.9% for NDF for green forage and hay, respectively. These values show the importance of the harvest time on the quality of typical mixed forage produced in Mediterranean climatic conditions.

The center-south region of Portugal is one of the areas most affected by climate change, and the region has experienced an increase in the frequency and intensity of droughts and heatwaves [9,10], making it necessary to adopt forage production strategies that reduce the effects of water scarcity, such as deficit irrigation [11] since this crop presents a high tolerance to water deficit or the use of wastewater for irrigation [12,13]. Forage and pasture production is extremely dependent on weather conditions, being particularly affected by periods of drought, which have a considerable impact on productivity and quality [14]. Dumont et al. [15] reported that an increase in air temperature promotes quick crop growth, increases cell wall fractions, and, consequently, reduces forage digestibility. In contrast, and depending on plant species, higher concentrations of atmospheric CO₂ seem to promote an increase in non-structural carbohydrates and produce a smaller decrease in nitrogen content. In a drought context, when plants are subjected to water stress, the observed increase in digestibility is probably a consequence of a decrease in plant cell wall content [15]. In Portugal, a large part of forage production occurs under rainfed conditions, making it very vulnerable to climate change due to the anticipated increase in crop water deficit in rainfed crops [16,17]. Climate changes currently observed in Portugal [10] are already leading to changes in the forage production system [18]. Traditionally, only one cut of forage is performed per growing season but due to climate change, specifically the intensification of droughts, producers are increasing the number of harvests from the same crop sward. This change is due to the increase in water stress, which has led to a reduction in the productivity of the rainfed forage, forcing some producers to compensate for this yield loss by increasing productivity via an increase in the number of cuttings and/or irrigation. However, this type of change may influence both productivity and forage quality, as cuttings occur in periods with very different climatic conditions and different soil water content.

Therefore, this study aims to evaluate the productivity and chemical composition of the forage produced from three cuttings of the same sward, under different water availability conditions at a Lusitano stud farm located in a central region of Portugal.

2. Materials and Methods

2.1. Description of the Study Area

The experimental field is located in a stud farm of Lusitano horses, along the right margin of the Tagus River, Azambuja municipality, central Portugal (Figure 1). This study was performed during the crop season of 2018/2019, on a parcel equipped with a 17 ha center pivot, where only 8 ha were used to produce irrigated forage. The center pivot has a 25.6 L/s flow rate, applying an irrigation depth varying between a minimum of 2.4 mm and a maximum of 12.2 mm. The producer followed an irrigation schedule consisting of applying a fixed water depth of 3.6 mm at variable time intervals, irrigating every two days during the most demanding period, from the second half of May to the beginning of June. In the 2018/2019 season, irrigation was initiated on 28 March 2019, after the first harvest, and was finished on 2 June 2019, ten days before the last harvest for hay, with an applied seasonal irrigation depth of approximately 83 mm [18]. Accumulated crop

evapotranspiration (ETc) and precipitation during the crop growing season were 381.1 and 487.1 mm, respectively.



Figure 1. Location of the stud farm in the Azambuja region, central Portugal.

2.2. Climate

According to the Köppen-Geiger classification system, this region has a temperate climate with dry hot summers (Csa) [19]. The monthly climate averages for the period 1971–2000, registered at the Santarém weather station (39°15' N; 08°41' W; 54 m a.s.l.), belonging to the Portuguese Institute for Sea and Atmosphere (IPMA), are presented in Figure 2. Monthly average air temperatures in the 1971–2000 period present a minimum of 9.6 °C in January and a maximum of 22.7 °C in August, with an average annual air temperature of 16.0 °C. Average monthly precipitation presented a minimum value in August with 6.2 mm and a maximum in December with 104.1 mm, with an average annual precipitation of 696.5 mm.

The meteorological data recorded during the 2018/2019 crop growing season at the Barragem de Magos weather station (38°59' N; 8°42' W; 43 m a.s.l.), belonging to the Sistema Nacional de Informação de Recursos Hídricos (SNIRH), which is the nearest weather station to the experimental site, are shown in Table 1.

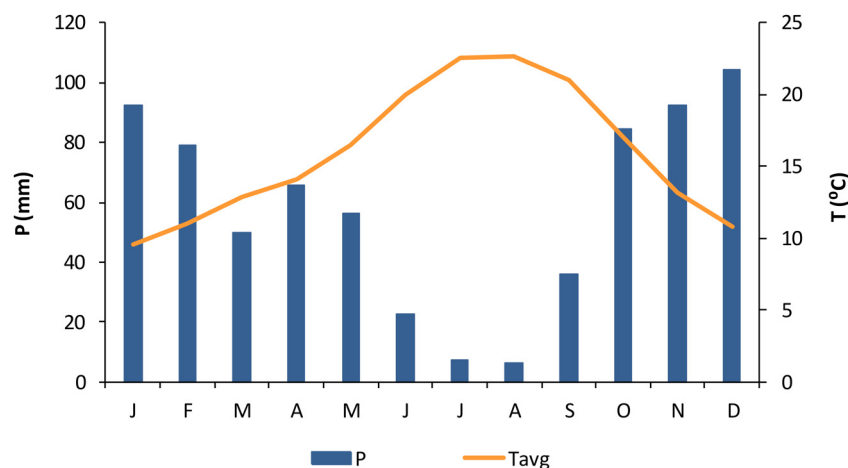


Figure 2. Monthly average air temperature and precipitation for the 1971–2000 period at the Santarém weather station.

Table 1. Monthly averages of minimum, maximum, and average temperatures, precipitation, and reference evapotranspiration (ETo), recorded during the experimental period at the Barragem de Magos weather station.

Month	Tmin (°C)	Tmax (°C)	Tavg (°C)	P (mm)	ETo (mm d ⁻¹)
October/2018	11	24.2	17.3	85.7	3.1
November/2018	8.9	17.3	12.8	143.2	1.5
December/2018	5.9	16.1	10.5	49	1.4
January/2019	3.3	14.8	8.3	29.5	1.4
February/2019	4.9	17.9	10.8	70.8	2.3
March/2019	6.6	20.6	13.3	44.1	3.4
April/20198	8.7	19.3	13.8	64.8	3.8
May/2019	12.1	26.5	19	0	5.8
June/2019	12.6	24.9	18.8	7.2	5.5

2.3. Soil

The predominant soil in the experimental parcel is solonchaks, according to the World Reference Base (WRB) classification system, which is a soil characterized by a moderate concentration of soluble salts [20,21]. This soil has a fine texture in the top 30 cm layer, with the following textural classes distribution: 8.7% of sand, 21.3% of silt, and 70% of clay [22]. The soil bulk density (1.29 g/cm³), soil water content at field capacity (0.44 m³/m³), and soil water content at wilting point (0.27 m³/m³) were retrieved from field samples, as detailed in Soares et al. [18]. The gravimetric method [23] was used to assess the evolution of the soil water content (SWC) throughout the growing season, with soil samples being collected every 15 days, at four random points within the plot, with a half-cane probe, considering a soil depth up to 20 cm, corresponding to the forage root depth [18].

2.4. Forage

The parcel studied was sown with a mixture of *Lolium multiflorum* Lam. (Italian ryegrass) and *Trifolium resupinatum* L. (Persian clover) with a ratio of 2:1. Italian ryegrass is a grass usually preferred by horses [6], which is why the proportion of this species in the seed mixture was higher. Persian clover is a legume with the ability to develop a bacterial symbiosis in the root nodules that enables satisfactory quantities of atmospheric nitrogen to be fixed [4]. Thus, protein content and some minerals (calcium, phosphorus, magnesium, copper, and cobalt) are superior in legumes than in grasses [1,24].

The sowing took place on 10 October 2018. Three harvests were made during the crop growth cycle, at a cutting height of 7 cm. The dates of each harvest were 23 March, 12 May, and 13 June 2019. It is important to mention that, after each cutting, nitrogen fertilization

(chemical composition: 27% nitrogen plus 3.5% magnesium oxide) was conducted. The first two harvests were intended for haylage production, with the bales wrapped in plastic film. The last harvest was for hay production. In the analyzed period (the 2018/2019 campaign), the farmer changed from the traditional practice of a single harvest per growing season to three, intending to increase forage production in the irrigated plot, thereby balancing the reduction in crop production in the rainfed plots after three years of drought.

2.5. Forage Sampling

Samples from the sward were collected for evaluation every 15 days (a total of eight different dates) between February and June 2019. Sampling was conducted according to the stratified random sampling methodology [25], with the parcel divided into four parts due to the heterogeneity of the parcel. Thus, four samples were collected per date, with a total of 32 samples (4 samples \times 8 dates). According to relevant literature, the area of the sampling units for forage can vary from 0.1 to 1 m² [26] and must be located randomly within these sub-areas. In this study, forage sampling (for chemical composition and productivity assessment) was performed within 1 m² and all plants inside each square were cut and collected at ground level. In addition, one sample of soil was collected in each sub parcel to evaluate SWC as referred to above.

Samples of preserved forage, hay, and haylage, produced from the plot were also collected for chemical analysis. These samples were collected from bales at the opening for feeding to preserve the forage quality for the animals. To ensure that samples were representative of the bale as much as possible, four samples were collected per bale.

2.6. Forage Productivity

The total forage yield was assessed from the yield of four spots, each 1 m², using a stratified random sampling methodology (as mentioned above). The harvested biomass was weighed, dried for 48 h at 65 °C, and then re-weighed to determine dry matter content in order to estimate the forage productivity.

2.7. Forage Chemical Analysis

To evaluate the nutrient content of the forages (green and preserved), a chemical analysis was performed. The analysis included the determination of dry matter (DM), crude protein (CP), plant structural components (neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL)), and ashes.

Dry matter (DM) content was determined in two steps. Firstly, samples were dried for 48 h at 65 °C. After air equilibration, the samples were milled through a 1 mm screen. DM content was measured by oven drying at 103 °C, for 24 h, and ash content by burning overnight at 550 °C [27]. The organic matter was calculated as the difference between DM and ashes.

Crude protein (CP) was determined using the Kjeldahl method. This method has three different steps: digestion, distillation, and titration of samples [28]. The last step allows the total nitrogen to be determined and, once multiplied by the protein conversion factor (6.25), the protein value of the sample is obtained.

Plant structural components were determined according to the methodology of Van Soest et al. [3]. Hemicellulose was calculated as the difference between NDF and ADF and cellulose as the difference between ADF and ADL. All determinations were performed in duplicate (32 samples \times 2).

2.8. Data Analysis

Descriptive statistics were used to describe chemical composition values of the green forage in the primary growth and in the following growth cycles. Data regarding nutrient content of the forage before each cutting were analyzed using the MIXED procedure of SAS (SAS 9.4 Institute Inc., Cary, NC, USA). For comparison among means, the Tukey-test was used and statistical significance was assumed when $p < 0.05$.

3. Results and Discussion

3.1. Floristic Composition

During the fieldwork, the growth cycle of the forage (mixture *Lolium multiflorum* Lam. × *Trifolium resupinatum* L.) was monitored. In addition to the sown species, plants such as *Phalaris minor* Retz (known as little seed canary grass), *Trifolium squarrosum* L. (commonly known as squarrose clover), and *Medicago italica* Mill. (known as hairy medick) were also observed in the plot. Although they are considered weeds, their presence enriches the floristic composition of the forage and those plants are often installed in forage production, given their interesting nutritive value. In addition to those species, some invasive plants such as *Chamaemelum mixtum* L. and *Ranunculus trilobus* Desf. were also observed. Although of no interest in forage production, these plants are not toxic after drying.

3.2. Soil Water Content

To relate the crop productivity and nutritive value with the soil water status, soil water content was measured throughout the crop-growing season. During the first cycle, the crop was under waterlogging conditions since the amount of water exceeded the soil storage capacity and was above the field capacity, resulting in saturation conditions, as shown in Figure 3. The drainage in this type of soil is hampered by the shallow level of the water table and the high clay content. For this reason, this parcel has previously been used for rice production.

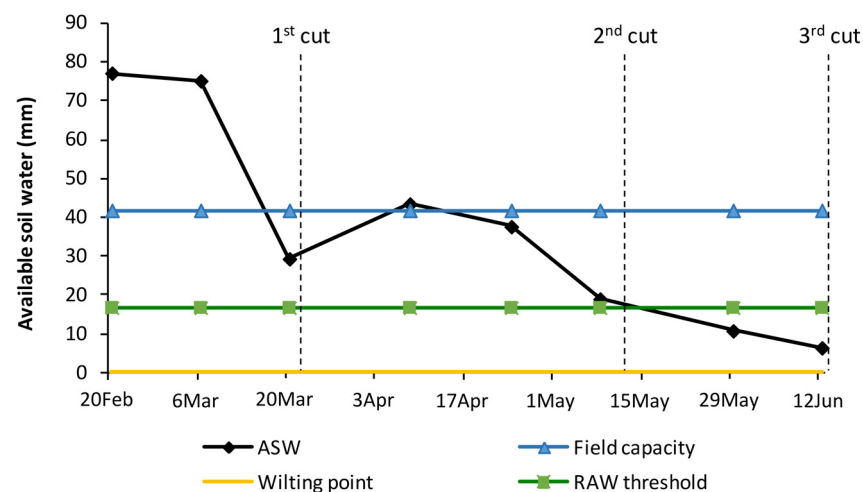


Figure 3. Soil water availability during the crop growing season.

During the second growth cycle, plants were not subjected to water stress and the available soil water (ASW) remained between field capacity and the readily available water (RAW) threshold. RAW is defined as the fraction of the total available soil water in the root zone (TAW) that a crop can extract without water stress, as estimated using the following expression $RAW = p \cdot TAW$, where p is the fraction of the readily available water [29]. Finally, in the third cycle, the crop was exposed to water stress conditions, since the soil water content was below the readily available water threshold, indicating that the farmer adopted a deficit irrigation strategy. The water stress conditions contributed to the lower productivity observed in the last cycle.

3.3. Forage Productivity

The global productivity throughout the 2018/2019 crop-growing season was $8.3 \text{ t DM} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$, which corresponds to 2.76, 3.42, and $2.1 \text{ t DM} \cdot \text{ha}^{-1}$ for the first (23 March), second (12 May), and third harvests (13 June), respectively (Figure 4). This value reflects the sum of productivity values immediately before each cut of forage. Although with

a consociation of two plant species, this result is within the values presented by Martin-Rosset [6] for the production of perennial ryegrass (from 2.5 to 13 t DM·ha⁻¹·year⁻¹) and by Lopes [30] for the production of annual ryegrass (from 6 to 12 t DM·ha⁻¹·year⁻¹), though both depended on nitrogen fertilization.

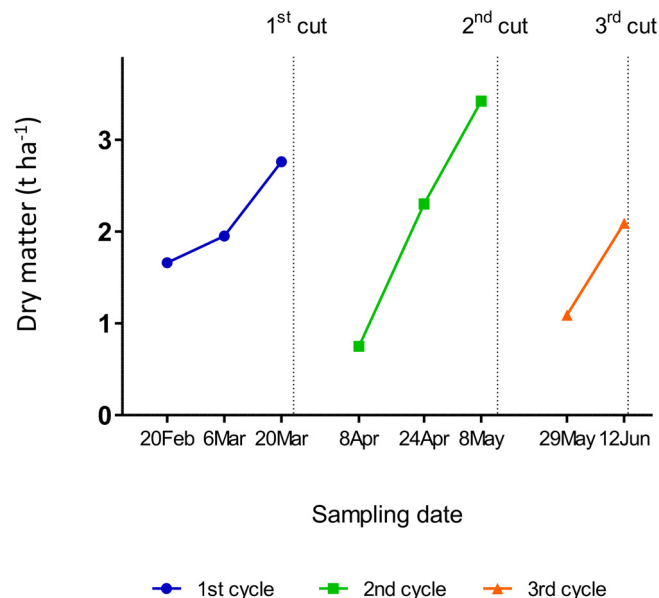


Figure 4. Forage productivity throughout the crop growing season.

Usually, during a crop-growing season, most of the production occurs in the first growth cycle (the first harvest) when soil and climatic factors are most favorable [6]. However, in this study, the first cycle of forage production was less productive than the second growth cycle, which presented the highest productivity value (Figure 4). The reason for the lower productivity of the first harvest can be attributed to the lower temperatures and lower radiation in the winter months, and the soil waterlogging, as shown in Figure 3, caused by the poor drainage conditions in this plot. The average temperatures observed during the three crop growth cycles reinforce this conclusion, with the first cycle presenting an average temperature of 11.7 °C, the second 15.0 °C, and the third of 19.3 °C. Although the plants were in a more advanced stage of development, the third cycle was less productive, probably due to the water stress suffered by the crop due to the soil water deficit conditions (Figure 3).

The productivity of the 2018/2019 season was higher than the productivity usually achieved in this parcel, according to the information provided by the farmer. This increase probably occurred due to the change in the production system, with the realization of three harvests instead of only one, and due to a dryer winter, which improved the drainage conditions.

3.4. Forage Chemical Composition

3.4.1. Green Forage

The results obtained for the chemical composition of the green forage are presented in Figure 5. The nutrient content throughout the growing period presents the usual evolution pattern [1,24,31]. As reported by McDonald et al. [24], as the plant matures, the fiber portion increases (NDF, ADF, and ADL) and protein decreases, resulting in a decline in the digestibility and hence in the total energy availability. The higher values of crude protein (CP) observed in Figure 5 regarding sampling dates 8 April and 29 May, may reflect not only the plant protein content but also the plant answer to the nitrogen fertilization, as mentioned by Bijelić et al. [32]. Generally, the protein content of green forage is associated with the

type of plants that comprise the consociation. Nevertheless, it is also important to notice that nutrient content is influenced by plant species and morphology at harvest [1,24,31].

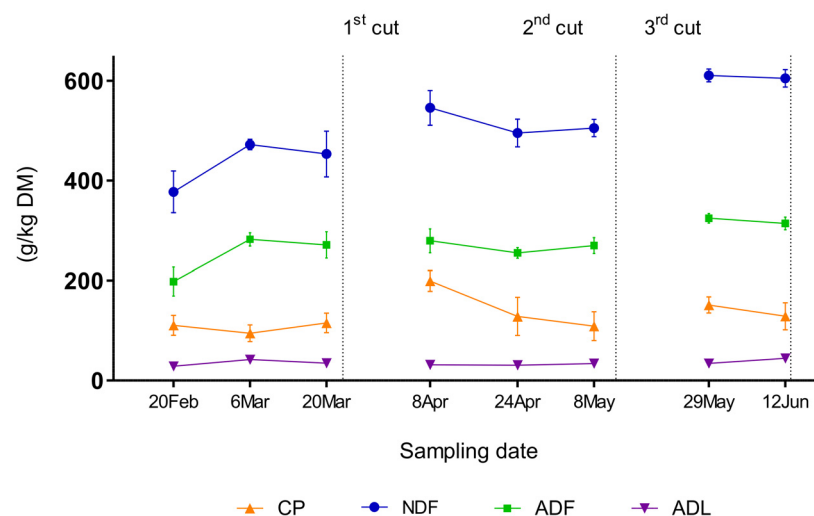


Figure 5. Changes in chemical composition during the crop growth cycle. All values are expressed as mean \pm SD on a DM basis. CP—crude protein; NDF—neutral detergent fiber; ADF—acid detergent fiber; ADL—acid detergent lignin.

The chemical composition of forage before the three cuts performed for haylage and hay production is presented in Table 2. Several differences were observed for all nutritional components, which could be associated with the development stage of the plant crop at harvest time. The higher values observed for ashes in the samples collected before the first and the second cuttings were possibly related to some soil remaining with plant material at sampling time.

Table 2. Dry matter (DM) content (g/kg) and chemical composition (g/kg DM) of forage before cutting. All values are presented as least square means.

	1st (Haylage)	2nd (Haylage)	3rd (Hay)	SEM	<i>p</i> -Value
DM	141.8 ^a	169.8 ^b	244.8 ^c	7.3	<0.0001
OM	888.6 ^a	892.3 ^a	921.1 ^b	4.8	<0.001
CP	115.6	109.3	128.8	9.0	0.316
NDF	453.4 ^a	505.5 ^b	605.1 ^c	10.6	<0.0001
ADF	271.1 ^a	270.1 ^a	314.1 ^b	6.8	<0.001
ADL	34.9 ^a	33.9 ^a	44.6 ^b	2.1	<0.01
Ash	111.4 ^a	107.8 ^a	78.9 ^b	4.8	<0.001

DM—dry-matter; OM—organic matter; CP—crude protein; NDF—neutral detergent fiber; ADF—acid detergent fiber; ADL—acid detergent lignin. Lowercase superscripts indicate significant differences.

3.4.2. Preserved Forage

The results of the chemical analysis obtained for the preserved forage, haylage (first two cuts), and hay (last cut) are presented in Figure 6. It can be observed that DM content of haylage increased from the first to the second harvest (61.3% for the first and 68.4% for the second). These values are within the 50% to 85% range advised by Harris et al. [1] for haylage. The value of 88.1% DM observed for hay (last forage harvest) is consistent with the values indicated by Martin-Rosset [6] and Harris et al. [1], where the ideal dry-matter content of hay was stated to be above 85%.

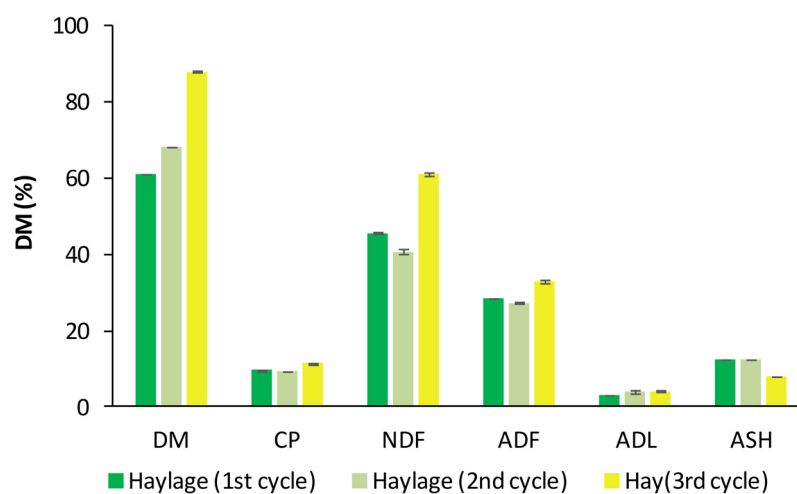


Figure 6. Chemical composition of haylage (first two cuttings) and hay (third cutting). Values are presented as mean \pm SD. DM—dry-matter; CP—crude protein; NDF—neutral detergent fiber; ADF—acid detergent fiber and ADL—acid detergent lignin.

Regarding other parameters we analyzed, the values are globally similar between the two haylages with a slightly lower NDF content in the second harvest. As expected, the highest fiber content (namely NDF and ADF) was observed in the hay, due to the advanced vegetative state of the plants, when harvested.

A comparison between the values of the fresh forage before cutting and the preserved forage shows that CP decreased and cell wall components increased, as described in the relevant literature. The increase in fiber content and the reduction in CP in the preserved forage was probably due to the losses that occurred from the moment the forage was cut (first in the field), and during the preservation and storage process [6,8,33]. However, it should be noted that these losses were relatively small, probably due to a suitable preservation process.

Direct observation of the hay confirmed the absence of mold. The development of this type of fungi was prevented by the absence of excessive moisture during the drying and storage processes. The absence of mold minimizes the risk of chronic respiratory diseases that are often observed in animals fed with low-quality forages [5].

3.4.3. Nutritional Value

The nutritional value of the preserved forages was estimated according to the French feeding system for horses [6]. Thus, the net energy value, expressed in horse feed units (UFC), and the horse digestible crude protein content (MADC) were predicted using the equations proposed by Martin-Rosset [6] (Table 3).

Table 3. Estimated energy (UFC—horse feed unit) and protein content (MADC—horse digestible crude protein) per kg DM, for haylage (1st and 2nd harvest) and hay (3rd harvest).

Preserved Forage	UFC/kg DM	MADC (g/kg DM)
Haylage 1st harvest	0.65	45.96
Haylage 2nd harvest	0.66	43.74
Hay 3rd harvest	0.57	56.65

Considering the INRA-recommended [6] allowances for an adult horse weighing 500 kg, performing very light work, with a recommended intake level of 10 kg/day of DM, the preserved forages presented in Table 3, used as a single feed in the diet, can cover, and even exceed, a horse's nutritional needs. Moreover, for broodmares in the last months of gestation (a period that usually corresponds with low pasture production and

an increase in nutritional requirements) the energy and protein values of these forages seem to be appropriate. However, in this study, the mineral content of the forages was not analyzed. This information would be important for a more detailed characterization of their nutritional value.

4. Conclusions

The effects of several cuttings, under different water availability conditions, on forage production and quality were evaluated at a Lusitano horse stud farm, located in central Portugal. In this study, the strategy adopted by the producer (three cuts from the same sward) led to an increase in productivity. In particular, forage mass obtained in the second harvest appears to have been favored by precipitation, temperature, solar radiation, and soil drainage conditions.

The nutrient content observed along the crop growth cycle and the values found in the preserved forages were in the range of values of other similar plant mixtures. Furthermore, the nutritional value estimated for energy and protein appears to be adequate to fulfill the average nutritional requirements of horses. In conclusion, the additional harvests did not seem to compromise the overall forage nutritional quality, although there was a considerable impact of water stress on the last cut productivity.

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