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ORIGINAL PAPER

The effect of growth stage and cutting height of Virginia fanpetals (*Sida hermaphrodita*) on quality and nutrition value of herbage and silage*

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Abstract

The aim of this study was to determine the effect of growth stage and cutting height on the quality and nutritional value of Virginia fanpetals (*Sida hermaphrodita* R.) herbage and silage. Virginia fanpetals herbage was harvested on three dates (11, 18, 25 June), at four cutting heights (15, 25, 35, 45 cm). The first harvest was carried out in the early bud stage, the second – bud stage, and the third – early bloom stage. The chemical composition, carbohydrate and protein fractions, indicators of ensiling suitability and fermentation parameters were analyzed. Growth stage had a significant effect on the content of dry matter (DM), neutral detergent fiber (NDF). Cutting height induced significant differences in the content of crude protein (CP), NDF, and acid detergent lignin (ADL), and selected indicators of ensiling suitability of herbage. Herbage harvested on 11 June and cut at a height of 45 cm was characterized by the highest nutritional value, and it was most suitable for ensiling. The pattern of lactic acid fermentation was most desirable when herbage was harvested on 18 June and cut at a height of 45 cm. Silage made from herbage harvested on 11 June and cut at a height of 45 cm had a significantly more favorable CP composition. These types of silage were characterized by the smallest extent of proteolysis, and by the highest content of fractions B1 and B2. The results of the study suggest that high-quality silage can be made from herbage harvested at the beginning of bud development (11 June) and cut and a height of 45 cm.

Keywords: Virginia fanpetals, growth stage, cutting height, protein fractions, silage

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INTRODUCTION

Virginia fanpetals (*Sida hermaphrodita* R.) is one of the alternative forage crops that can be fed to ruminants (Purwin et al. 2020). It blooms from July to the first frost. The mature plant forms up to 30-40 stiff, round-cross-sectioned stems, 5-30 mm in diameter and up to 4.40 m high at the end of vegetation. Leaf blades are 300-360 mm wide and 240-280 mm long, and leaf petioles reach a length of 150-180 mm (Tarkowski 2006, Nahm, Morhart 2018).

Virginia fanpetals can be used as a phytoremediation plant, honey plant, textile raw material or herb (Tarkowski 2006). Interest in its cultivation in Europe has been growing in recent years due to the search for sources of biomass energy. It is primarily cultivated for the purpose of obtaining dry stems for combustion as wood chips or pellets, and also the green biomass can be processed by methane fermentation (Nahm, Morhart 2018).

Previous research has shown that the chemical composition of Virginia fanpetals may vary widely depending on growth stage (harvest date). Herbage cut on 6 May contained 278 g crude protein (CP) and 64 g lignin, and herbage harvested on 25 June contained 128 g CP and 99 g lignin kg^{-1} dry matter (DM) – Tarkowski (2006). Harvest date is a key determinant of the quality and quantity of the harvested biomass. The efficiency of silage utilization by animals is largely determined by the maturity stage (Purwin et al. 2020).

The chemical composition and feed value of Virginia fanpetals may also vary depending on the leaf/stem ratio, which is affected by harvest date and cutting height (Tarkowski 2006). In general, the proportions of different morphological plant parts are determined by cutting height. Numerous studies investigating silage production from tall crops such as maize have shown that cutting height has a significant influence on the chemical composition and feed value of biomass (Lynch et al. 2012).

Previous research has focused on the efficiency of Virginia fanpetals in animal feeding, for example dairy cows (Purwin et al. 2020) or sheep (Purwin et al. 2022). The nutritional value of herbage and silage has also been examined (Starczewski et al. 2020, Borsuk et al. 2021). However, the effect of growth stage and cutting height on the quality and feed value of silage remains insufficiently investigated. The aim of this study was to determine the effect of growth stage and cutting height on the proximate chemical composition, carbohydrate fractions, protein fractions, ensiling suitability, selected fermentation parameters, and feed value of Virginia fanpetals herbage and silage.

MATERIALS AND METHODS

The experimental material was Virginia fanpetals herbage harvested on a commercial plantation in Poland (53°01′04″N 21°11′55″E). The following fertilizers were applied: 100 kg N, 50 kg K_2O, 80 kg P_2O_5 ha^{-1}. Herbage was harvested manually in 2017, on three dates (11, 18, 25 June), at four cutting heights (15, 25, 35, 45 cm), between 12:00 and 2:00 p.m. The first harvest was carried out in the early bud stage, the second – the bud stage, and the third – the early bloom stage. Plant height was around 130 cm. After harvest, whole plants were chopped to a chaff length of 10 mm. Chaff structure was damaged mechanically with the use of a Polytron PT-MR 2100 laboratory homogenizer (Kinematica AG, Werkstrasse, Switzerland). Fresh chaff was packaged in 330 x 400 mm polyamide/polyethylene (PA/PE) vacuum bags (thickness – 100 µm) using a vacuum-packaging machine (Vacutronic 2000, PP 5.4, ZTP Tepro, Poland), and ensiled. Each bag contained 600 g of fresh chaff with a natural moisture content. Silages were made in triplicate for each growth stage and cutting height.

In each treatment, herbage samples were collected before ensiling. Silage samples were collected after 120 days of ensiling. Part of the samples were dried at a temperature of 60°C for 48 h in the Binder FED 115 dryer (Binder, GmbH, Tuttlingen, Germany), and were ground in a mill for fibrous materials (ZM 200, Retsch, Haan, Germany) to a 1 mm particle size. The remaining fresh samples were frozen at a temp. of -25°C. Chemical analyses were performed for each replicate for herbage and silage.

Samples were analyzed to determine the content of DM and CP, neutral-detergent fiber (NDF), acid-detergent fiber (ADF), acid-detergent lignin (ADL) by methods described by Purwin et al. (2022), water soluble carbohydrates (WSC) – Thomas (1977) and buffering capacity (BC) – Playne and McDonald (1966).

Samples were analyzed to determine true protein (TP), neutral-detergent insoluble nitrogen (NDIN) and acid-detergent insoluble nitrogen (ADIN) – Licitra et al. (1996). The content of buffer-soluble nitrogen (BSN), including buffer-soluble protein nitrogen (BSPN) and non-protein buffer-soluble nitrogen (NPBSN), was determined using McDougall's buffer (Hedqvist, Uden 2006).

The pH of silage was measured with the use of an HI 8314 pH-meter (Hanna Instruments, Woonsocket, Rhode Island, USA). The content of ammonia-nitrogen ($N\text{-}NH_3$) and the concentrations of lactic acid (LA), acetic acid (AA) and butyric acid (BA) were determined as described by Purwin et al. (2022).

The WSC/BC ratio was calculated. The minimum DM content (DM_{min}), was determined using the following equation: DM_{min} (%) = 45 – 8 WSC/BC. The fermentability coefficient (FC) was calculated using the following equation: FC = DM (%) + 8 WSC/BC (Weissbach, Auerbach 2013).

The CP of samples was divided into fractions according to the Cornell Net Carbohydrate and Protein System (CNCPS). Fraction A was calculated as the difference between total nitrogen (TN) and TP. Fraction B1 is BSN; B2 was calculated as the difference between buffer-insoluble protein (IP) and NDIN; B3 was calculated as the difference between NDIN and ADIN. Fraction C consists of ADIN (Shniffen et al. 1992).

All results obtained from three repetitions for each experimental variant were used for statistical analysis. The effect of experimental factors was determined by analysis of variance (ANOVA). The significance of differences between mean values was estimated by the Tukey's test at a significance level of p=0.05. All statistical calculations were performed using Statistica software (Statsoft version 13.1, TIBCO Software Inc., Palo Alto, California, USA).

RESULTS

Growth stage had a significant effect on the content of DM (p=0.002) and NDF (p=0.045), and cutting height affected CP (p=0.037), NDF (p=0.038) and ADL (p=0.033) in herbage (Table 1). The content of DM and NDF increased

Table 1

Chemical composition (g kg^{-1} DM) and ensiling suitability of herbage

Item	DM	CP	NDF	ADF	ADL	WSC	BC	WSC/ BC	DM_{min}	FC
Growth stage										
Early bud	201.0^{a}	175.0	417.0^{a}	312.0	31.20	131.0	72.10^{b}	1.820^{a}	30.50^{b}	34.70
Bud	231.0^{b}	170.0	432.0^{ab}	322.0	31.90	118.0	82.70^{a}	1.430^{b}	33.60^{a}	36.70
Early bloom	252.0^{b}	169.0	462.0^{b}	339.0	33.50	133.0	84.70^{a}	1.580^{ab}	32.40^{ab}	36.70
Cutting height (cm)										
15	226.0	139.0^{b}	477.0^{a}	340.0	38.90^{a}	133.0	86.20^{a}	1.550^{ab}	32.60^{a}	35.00
25	223.0	179.0^{ab}	458.0^{ab}	338.0	36.70^{a}	130.0	81.90^{a}	1.600^{a}	32.20^{ab}	35.10
35	233.0	184.0^{a}	454.0^{ab}	322.0	34.40^{ab}	114.0	77.40^{ab}	1.490^{b}	33.10^{a}	35.20
45	230.0	192.0^{a}	441.0^{b}	311.0	31.90^{b}	132.0	73.90^{b}	1.800^{a}	30.60^{b}	37.40
SEM										
Growth stage	0.728	0.567	0.567	0.347	0.098	0.349	2.211	0.063	0.506	0.566
Cutting height	0.745	0.046	0.356	0.321	0.109	0.377	2.322	0.058	0.464	0.502
P-value										
Growth stage	0.002	0.076	0.045	0.736	0.234	0.181	0.022	0.019	0.020	0.389
Cutting height	0.976	0.037	0.038	0.353	0.033	0.197	0.027	0.038	0.039	0.416

* DM – dry matter (g kg^{-1} fresh matter), CP – crude protein, NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin, WSC – water-soluble carbohydrates, BC – buffering capacity (g lactic acid kg^{-1} DM), WSC/BC – ratio of WSC to BC, DM_{min} – minimum dry matter content, FC – fermentability coefficient. SEM – standard error of the mean. Values followed by the same superscript letters (*a* – *b*) are not significantly different at $P \leq 0.05$

at successive growth stages. Increasing cutting height induced a proportional decrease in the concentrations of NDF and ADL and it was also accompanied by increasing CP content. The values of BC increased at successive growth stages (*p*=0.002), and decreased with increasing cutting height (*p*=0.027).

Growth stage had a significant effect on NDF concentration (*p*=0.038), and cutting height affected the content of DM (*p*=0.029), CP (*p*=0.031), NDF (*p*=0.044), ADL (*p*=0.040), and WSC (*p*=0.007) in silage (Table 2). Silage made from herbage cut at a height of 45 cm had the highest concentrations

Table 2

Chemical composition and fermentation pattern (g kg^{-1} DM) of silage

Item	DM	CP	NDF	ADF	ADL	WSC	pH	LA	AA	BA	N-NH$_3$
Growth stage											
Early bud	190.0	182.0	447.0^b	335.0	33.00	15.80	4.71	49.40^b	11.60	1.980^a	108.0^a
Bud	215.0	180.0	471.0^a	338.0	33.30	15.90	4.40	80.70^a	12.10	0.030^b	62.70^b
Early bloom	217.0	171.0	480.0^a	343.0	36.20	17.90	4.34	58.80^b	15.60	0.010^b	67.50^b
Cutting height (cm)											
15	206.0^{ab}	140.0^b	488.0^a	352.0	40.60^a	10.70^b	4.34	65.00	12.90	0.000^b	69.90^{ab}
25	180.0^b	190.0^a	472.0^a	338.0	39.10^{ab}	16.80^{ab}	4.53	58.80	16.40	0.820^{ab}	96.20^a
35	218.0^{ab}	196.0^a	458.0^{ab}	333.0	36.80^b	16.40^{ab}	4.70	59.70	22.00	1.850^a	97.50^a
45	225.0^a	204.0^a	447.0^b	331.0	34.20^b	22.10^a	4.35	68.60	21.90	0.000^b	53.80^b
SEM											
Growth stage	0.714	0.900	0.776	0.436	0.104	0.152	0.083	0.606	0.012	0.048	0.966
Cutting height	0.867	1.101	0.772	0.399	0.121	0.202	0.080	0.659	0.022	0.380	0.892
P-value											
Growth stage	0.248	0.891	0.038	0.769	0.434	0.837	0.146	0.039	0.218	0.041	0.029
Cutting height	0.029	0.031	0.044	0.381	0.040	0.007	0.422	0.951	0.304	0.039	0.036

* DM – dry matter (g kg^{-1} fresh matter), CP – crude protein, NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin, WSC – water-soluble carbohydrates, LA – lactic acid, AA – acetic acid, BA – butyric acid, N-NH$_3$ – ammonia nitrogen (g kg^{-1} total N), SEM – standard error of the mean. Values followed by the same superscript letters (*a* – *b*) are not significantly different at $P \leq 0.05$.

of DM, CP and residual WSC, the lowest concentrations of NDF and ADL. Silage made from herbage harvested at different dates was characterized by different patterns of LA fermentation (p=0.039). Delayed harvest had a significant effect on the concentrations of BA (*p*=0.041) and N-NH$_3$ (*p*=0.029).

The quality of CP was evaluated based on the CNCPS (Table 3). The proportions of fractions B1 (*p*=0.046), B2 (*p*=0.025) and C (*p*=0.017) decreased significantly in herbage, whereas the proportion of B3 (*p*=0.009) increased at successive growth stages. Cutting height had a significant influence on the proportion of fraction B1 (*p*=0.031). The proportion

Table 3

Crude protein fractions in herbage and silage (g kg^{-1} CP)

Item	Herbage					Silage				
	A	B1	B2	B3	C	A	B1	B2	B3	C
Growth stage										
Early bud	216.0	62.00^{a}	630.0^{a}	14.00^{b}	78.00^{a}	624.0^{b}	81.00^{a}	214.0^{a}	12.00	69.00
Bud	242.0	54.00^{b}	612.0^{ab}	21.00^{ab}	71.00^{ab}	646.0^{ab}	73.00^{ab}	199.0^{ab}	12.00	70.00
Early bloom	245.0	52.00^{b}	599.0^{b}	38.00^{a}	66.00^{b}	689.0^{a}	64.00^{b}	159.0^{b}	17.00	71.00
Cutting height (cm)										
15	237.0	51.00^{a}	596.0^{b}	42.00^{a}	74.00^{a}	705.0^{a}	44.00^{b}	157.0^{b}	23.00	71.00^{a}
25	245.0	22.00^{b}	628.0^{a}	35.00^{ab}	70.00^{a}	665.0^{ab}	56.00^{ab}	190.0^{ab}	21.00	68.00^{ab}
35	248.0	23.00^{b}	641.0^{a}	33.00^{ab}	55.00^{b}	636.0^{ab}	79.00^{a}	210.0^{a}	18.00	57.00^{b}
45	226.0	41.00^{ab}	659.0^{a}	22.00^{b}	52.00^{b}	615.0^{b}	86.00^{a}	216.0^{a}	22.00	61.00^{ab}
SEM										
Growth stage	7.518	2.494	7.338	5.818	2.841	16.21	1.656	3.953	1.186	0.720
Cutting height	4.257	6.138	11.51	3.588	4.709	17.81	3.518	6.088	0.650	1.816
P-value										
Growth stage	0.543	0.046	0.025	0.009	0.017	0.021	0.028	0.039	0.534	0.098
Cutting height	0.487	0.031	0.038	0.024	0.015	0.030	0.042	0.047	0.432	0.019

* CP – crude protein, A – non-protein nitrogen, B1 – buffer-soluble nitrogen, B2 – neutral-detergent soluble nitrogen, B3 – acid-detergent soluble nitrogen, C – neutral-detergent insoluble nitrogen, SEM – standard error of the mean. Values followed by the same superscript letters (*a* – *b*) are not significantly different at $P \le 0.05$

of fraction B2 increased (p=0.038), and the proportions of B3 (p=0.024) and C (p=0.015) decreased with increasing cutting height.

The proportions of CP fractions in silage varied depending on growth stage: A (p=0.021), B1 (p=0.028) and B2 (p=0.039). Increasing cutting height induced a decrease in the content of A (p=0.030) and C (p=0.019), and an increase in the content of B1 (p=0.042) and B2 (p=0.047). The ensiling process caused changes in CP composition. The proportion of faction A increased, whereas the proportions of B1, B2 and B3 decreased.

DISCUSSION

Numerous studies have been conducted in recent years to determine the impact of forage quality management on the nutritional value of feed and animal performance. In general, late-harvested plants have a higher content of DM, NDF and ADF, and lower CP concentration (Guo et al. 2019), which was also observed in the present study with regard to DM and NDF. The observed increase could have resulted from a decrease in the water content of plant cells, changes in the leaf/stem ratio during the growing season, and an increase in the proportion of cell walls in stems (Yari et al. 2012). Only Tarkowski (2006) analyzed changes in the nutrient content of Virginia fanpetals herbage throughout the growing season, which, according to the cited author, was the key factor affecting the content of CP and crude fiber (CF).

In the study by Tarkowski (2006), Virginia fanpetals herbage harvested under similar environmental conditions and in a similar growth stage had a similar CP concentration of 176 g kg^{-1} DM, which decreased at successive growth stages, to 165.8 g kg^{-1} DM after 4 days and to 146.6 g kg^{-1} DM after 10 days. Fijałkowska et al. (2017) harvested Virginia fanpetals herbage in the same growth stage and reported higher CP content (199 g kg^{-1} DM), lower concentrations of NDF (403 g kg^{-1} DM) and ADF (308 g kg^{-1} DM), and higher ADL content (38 g kg^{-1} DM), compared with the results of this study.

A comparison of the present findings with the average values determined by Weissbach and Auerbach (2013), who analyzed the major silage crops in Europe, revealed that Virginia fanpetals herbage was characterized by medium ensilability because biomass with FC below 35 is considered difficult to ensile. The ensiling suitability of Virginia fanpetals herbage harvested on 11 June was also examined by Fijałkowska et al. (2017), who reported a lower concentration of WSC (73.0 g kg^{-1} DM), and a lower value of BC (69.8 g LA kg^{-1} DM). In alfalfa, delayed harvest contributes to a decrease in BC and an increase in WSC content, which promotes fermentation despite an increase in NDF content (Guo et al. 2019). Such relationships were not observed in Virginia fanpetals herbage harvested at successive dates. Lynch et al. (2012) analyzed the ensilage characteristics of whole-crop maize and found that BC may be higher in stems than in other vegetative organs.

Growth stage and cutting height have a greater influence on fermentation quality and on the nutritional value of silage than herbage does (Guo et al. 2019). In the present study, the NDF content increased with advancing maturity of Virginia fanpetals, and similar observations were made by Sikora et al. (2019), who analyzed alfalfa silage. This is due to the fact that DM content increases and the leaf/stem ratio decreases with maturation, and low-digestible structural carbohydrates are accumulated after the development of a polysaccharide network in the cell walls of plants (Yari et al. 2012, Guo et al. 2019). Tall plants harvested at high cutting heights usually have higher DM content (Aoki et al. 2013), which was also observed in the current study for silage cut at a height of 45 cm. Aoki et al. (2013) demonstrated that cutting height had a considerable influence on the content of NDF, ADF and ADL in maize silage. In the present study, the concentrations of NDF and ADL decreased with increasing cutting height. The decrease in the content of fibrous components of Virginia fanpetals, observed at higher cutting heights, resulted from the elimination of the lower parts of stems which contain higher concentrations of low-digestible structural carbohydrates (Aoki et al. 2013).

Kung et al. (2008) found that increasing cutting height induced an increase in CP content, similarly to the present experiment. This could be due to the vertical distribution of nutrients, where the bottom segment of the stem is usually richer in structural carbohydrates and poorer in CP than the upper segment, which is more flexible and provides support to leaves (Aoki et al. 2013, Guo et al. 2019).

Two types of silage fermentation (out of the types described by Huhtanen et al. 2002) were observed in the present study, i.e. organic fermentation with low LA content and a considerable concentration of residual WSC, and extensive LA fermentation with relatively low content of N-NH_3. The pH of Virginia fanpetals silage noted in this study, irrespective of growth stage and cutting height, can be considered high relative to previous findings of Fijałkowska et al. (2017) – 4.08, Starczewski et al. (2020) – 4.01 - 4.10, and Purwin et al. (2022) – 4.30. In earlier studies, the concentrations of LA (86.0 - 125 g kg^{-1} DM) and BA (3.40 - 10.0 g kg^{-1} DM) were considerably higher (Fijałkowska et al. 2017, Starczewski et al. 2020).

Unlike in the work of Neylon and Kung (2003), the LA content of Virginia fanpetals silage was significantly affected by growth stage. This factor influenced DM content, which could affect the pattern and quality of fermentation, resulting from the composition of epiphytic microflora (Aoki et al. 2013, Guo et al. 2019). At a high cutting height (45 cm), the fermentation process was satisfactory, despite low LA content (Kung et al. 2018), which corroborates the findings of Aoki et al. (2013), who reported that high cutting of maize plants, regardless of maturity stage, had no adverse effect on fermentation quality.

In the present study, increasing cutting height induced a significant decrease in the N-NH_3 content of silage which, according to Harad et al. (1996), could result from uneven vertical distribution of different N forms in the stem. This is an important consideration in Virginia fanpetals, which is a tall plant.

The results of this study are comparable with those noted previously, where extensive proteolysis was also observed in ensiled Virginia fanpetals, and the proportion of fraction A increased from 274 to 683 g kg^{-1} CP (Borsuk et al. 2021). The extent of proteolysis in Virginia fanpetals was lower than the maximum values reported in the literature for alfalfa (870 g fraction A kg^{-1} CP), and considerably higher than those recommended for red clover silage (70 - 400 g fraction A kg^{-1} CP) (Fijałkowska et al. 2015). In the study of Purwin et al. (2020), the content of fraction A in Virginia fanpetals silage was even higher (713.4 g kg^{-1} CP).

Fermentation induced changes in the composition of CP fractions, noted in this study, are consistent with those described in alfalfa (Fijałkowska et al. 2015). The proportion of fraction A increases at the expense of valuable fractions B1 and B2. The content of fraction B1, determined in this study, is comparable with the values reported in alfalfa silage (53 - 66 g kg^{-1} CP) and red clover silage (45 - 46 g kg^{-1} CP), and the content of fraction B2 is similar to that noted in alfalfa silage (198 - 313 g kg^{-1} CP) – Grabber, Coblentz (2009).

The proportion of the unavailable fraction C, which consists of ADIN, was considerably higher in Virginia fanpetals silage than in alfalfa silage (33 - 34 g kg^{-1} CP) and red clover silage (31 - 35 g kg^{-1} CP) evaluated by Grabber and Coblentz (2009). The proportion of fraction C is related to the extent of heat damage to plants during fermentation. A substantial increase in the content of fraction C, resulting from the accumulation of heat produced during fermentation, is usually observed in legumes. The values noted in this study are not indicative of the low quality of experimental silages because other authors reported even higher proportions of fraction C in alfalfa silage (65 - 105 g kg^{-1} CP) – Fijałkowska et al. (2015).

The extent of proteolysis increased at successive growth stages, and decreased with increasing cutting height, which could be indirectly linked with different concentrations of nutrients and plant enzymes in stems and leaves (Yari et al. 2012, Guo et al. 2019). Plants are usually characterized by uneven distribution of different N forms in vegetative organs. Competition for light between plants in dense stands leads to morphological changes (leaf/stem ratio), which explains the differences in N concentration, observed in this study. Hakl et al. (2015) demonstrated that plant morphology was an important factor responsible for around 75% of variation in CP fractions in alfalfa, which could explain changes in the composition of CP fractions in Virginia fanpetals in response to different growth stages and cutting heights. These factors affected the age of harvested plants and the leaf/stem ratio.

CONCLUSIONS

Growth stage and cutting height affected the content of DM and CP, the composition of structural carbohydrates, and the ensiling suitability of herbage. Harvest on the first date (11 June) and the highest cutting height (45 cm) were most favorable. However, the pattern of fermentation was more desirable when herbage was harvested on 18 June due to faster LA fermentation and a lower concentration of N-NH_3. Optimal fermentation parameters were achieved when herbage was cut at a height of 45 cm. Silage made from herbage harvested on 11 June and cut at a height of 45 cm had a favorable CP composition, the smallest extent of proteolysis, and the highest content of valuable fractions B1 and B2. The results of this study indicate that Virginia fanpetals is suitable for ensiling, and the produced silage is characterized by a highly desirable chemical composition, comparable with that of alfalfa, red clover and grass silage.

Author contributions

Conceptualization – C.P., M.B-S., M.B.; methodology – C.P., M.B., M.B-S.; software – M.B-S., B.S.; validation – C.P., M.B-S.; formal analysis – M.B., M.B-S.; investigation – M.B., M.B-S., B.S.; resources – C.P., M.B., M.B-S.; data curation – M.B-S., M.B.; writing original draft preparation and editing – M.B-S., C.P., M.B., B.S.; visualization – M.B-S., B.S.; supervision – B.S.; project administration – C.P.; funding acquisition – C.P. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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