agronomy

MDPI

Article

Comparison of a Conventional Harvesting Technique in Alfalfa and Red Clover with a Leaf Stripping Technique Regarding Dry Matter Yield, Total Leaf Mass, Leaf Portion, Crude Protein and Amino Acid Contents

Peter Liebhardt [1,2,*], Jan Maxa [2], Heinz Bernhardt [1], Karen Aulrich [3] and Stefan Thurner [2,*]

[1] Agricultural System Engineering, Technische Universität München, Dürnast 10, 85354 Freising, Germany; heinz.bernhardt@wzw.tum.de
[2] Bavarian State Research Centre for Agriculture, Institute for Agricultural Engineering and Animal Husbandry, Voettinger Strasse 36, 85354 Freising, Germany; jan.maxa@lfl.bayern.de
[3] Institute of Organic Farming, Johann Heinrich von Thuenen Institute, Trenthorst 32, 23847 Westerau, Germany; karen.aulrich@thuenen.de
* Correspondence: peter.liebhardt@mytum.de or peter.liebhardt@lfl.bayern.de (P.L.); stefan.thurner@lfl.bayern.de (S.T.)

Abstract: In this study, we compared an innovative stripping technique with a conventional whole plant harvesting technique for alfalfa and red clover to develop an approach to regional production of proteins and amino acids. While the stems were harvested separately, the leaves were fractionated by stripping them from the stems using a prototype machine, which resulted in higher crude protein (CP) and amino acid yield for alfalfa (CP 26.53% of dry matter (DM), in g/kg DM, Lys 16.29, Met 4.45, Cys 3.42, Thr 12.38, Tyr 8.90) and red clover (CP 26.88% of DM, in g/kg DM, Cys 2.65, Met 4.33, Lys 16.09, Thr 12.99, Tyr 9.84). The stripped fraction contained approximately 73% and 82% of the leaf portion for alfalfa and red clover, respectively. In the conventional plant harvesting technique, the crop wilted in the field to approximately 30% of DM resulting in following CP and amino acid contents in the harvested material of alfalfa (CP 20.44% of DM, in g/kg DM, Cys 2.56, Met 3.12, Lys 11.72, Thr 8.98, Tyr 6.24) and red clover(CP 20.31% of DM, in g/kg DM, Cys 2.05, Met 2.98, Lys 11.41, Thr 9.04, Tyr 6.96).

Keywords: fractionation; ingredients; leaf; stem

Citation: Liebhardt, P.; Maxa, J.; Bernhardt, H.; Aulrich, K.; Thurner, S. Comparison of a Conventional Harvesting Technique in Alfalfa and Red Clover with a Leaf Stripping Technique Regarding Dry Matter Yield, Total Leaf Mass, Leaf Portion, Crude Protein and Amino Acid Contents. *Agronomy* **2022**, *12*, 1408. https://doi.org/10.3390/agronomy12061408

Academic Editor: Jerome H. Cherney

Received: 18 May 2022
Accepted: 9 June 2022
Published: 11 June 2022

1. Introduction

There is an increased demand for home-grown protein and amino acids based on EU guidelines [1–3]. In general, forage legumes such as alfalfa and red clover have a high potential to fix nitrogen and have great potential to increase sustainability [4]. Although alfalfa and red clover are common legumes used for ruminant feeding systems [5], recent studies have shown that these legumes can be a potential source of crude proteins (CP) with concentrations up to 30% and amino acids such as lysine (Lys) up to 18 g per 1000 g DM and methionine (Met) up to 5 g per 1000 g DM for monogastric animals [6–8]. The leaves of these legumes have high protein content, hence innovative harvesting technologies are needed to tap this potential and achieve high protein yield [9]. Presently, soybean is the most commonly used protein feed [10]. Additionally, in the EU, a shortage of protein for livestock rations has been of concern for many years [11].

In 2022, the EU Agricultural Council approved new organic regulations focusing on protein extraction from regional sources. In the case of pigs and poultry, at least 20% of the feed must come from the farm unit itself and if this is not feasible, it should be produced in the same region in cooperation with other organic farms or feed business operators [3]. Based on this, Sommer and Sundrum [7] suggested a fractionation technique for the stems and leaves of legumes to achieve higher protein and amino acid concentrations.

Several previous studies have shown that it is possible to separate the leaf fraction from the stem fraction using a machine [12–16] and the stripped material can be conserved by drying or ensiling the fresh or wilted material [17–19]. According to Weltin et al. [8], alfalfa leaves can provide crude protein yield up to 30% of leaf dry matter (DM).

The digestibility of alfalfa stems decreases as the plant matures due to increasing concentration of cell walls and lignin, while the digestibility of the leaves changes only slightly. Moreover, the CP content in alfalfa leaves is two to three times higher than in stems, while fibre and lignin concentration in stems is two to three times higher than in leaves [20]. Similarly, higher CP contents can be obtained from red clover during the early growth stages [21].

Another way of harvesting legumes is via the production of hay, where the whole plant is cut, tedded and turned several times on the field to increase the DM content. Then, the hay is swathed and harvested by machines such as a loader wagon that picks up the sun cured plants. The goal of haymaking machines is to keep the nutritive quality of the hay as high as possible [22,23]. The legumes can also be conserved by hot air drying. The harvested plants (fresh or wilted) are dried in a drum and after harvesting, processes such as pelleting in pellets or sieving are needed to produce feed for animal nutrition [23].

The stem proportion increases when alfalfa matures. The highest DM yield of leaves with high CP content is achieved at the early developmental stages of alfalfa and as the plant matures, the yield decreases due to senescence and leaf loss from the lower, shaded parts of the plant [20]. Hence, the trials were conducted before the plants bloomed.

Therefore, understanding how harvesting practices influence the quantity and quality of the harvested material is necessary. Using the right machine at the right time and in the right manner can save a significant amount of the original forage feed value. The aim of this study was to investigate and compare two harvesting techniques for alfalfa and red clover, an innovative leaf stripping technique versus conventional whole plant harvesting. The dry mater (DM) yield, leaf portion, leaf yield, content of crude protein (CP) and the content of important amino acids from the harvested material were also described and evaluated in this study.

2. Materials and Methods

2.1. Harvesting Trials

Samples were obtained from an organically managed plot of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) in the north of Freising, Bavaria, Germany (48°27′32.4″ N 11°39′56.2″ E). In August 2016, alfalfa and red clover cultivars, 'Plato' and 'Titus', were sown on a total of about 4.4 hectares and later used for the harvesting trials of the third cut in 2017 and the first and third cut in 2018. The first cut harvesting trials in 2019 were carried out on an adjacent plot with more than 2.0 hectares with alfalfa and red clover cultivars, 'Fleetwood' and 'Taifun', which were sown in August 2017. The alfalfa varieties 'Plato' and 'Fleetwood' were approved in Germany in 1990 and 2012, respectively. The tetraploid red clover varieties 'Titus' and 'Taifun' were approved in Germany in 1994 and 2004, respectively [24]. The soil was tested and brought to an adequate level of fertility before sowing. Two different harvesting techniques (variants) were applied during the four cuts. Harvesting trials (randomised block design) were conducted on 200 m^2 sized plots with four repetitions for each harvesting technique during four cuts from 2017 to 2019. The growth stage of alfalfa and red clover described with the BBCH code and plant height are presented in Table 1.

Before starting the trial, samples were collected from seven spots next to the trials to determine the leaf portion of alfalfa and red clover. The first (stripping) technique harvested the leaves and stems separately. For this method, a special prototype machine MRF, (French origin: machine de récolte fractionnée) with a roller transverse to the direction of motion and multiple metal tines actuated by a tractor, was used to strip the leaves from the stems. The working height was 15 cm above the ground and leaves were plucked from the stem at this height. The harvested leaves were collected on a conveyer belt, thrown on a loader wagon by an accelerator and transported to a dumpsite for weighing and assigned to the variants labelled A1L and R1L for alfalfa and red clover leaves, respectively. This prototype was not configured to cut the stems while stripping. Therefore, the stems were cut afterwards, tedded, windrowed, harvested and further processed to obtain 60% DM content. The variants were termed A1S and R1S for alfalfa and red clover stems, respectively. The leaf and stem yields were determined and calculated separately, added together (variant A1 and R1 for alfalfa and red clover, respectively) and then extrapolated per hectare (kg ha^{-1}).

Table 1. Details of the investigated harvesting trials (four cuts) of alfalfa and red clover.

Year	Stripping/ Cutting Date	Cut	Growth Stage Alfalfa/Red Clover (BBCH Code According to Meier [25])	Plant Height Alfalfa/Red Clover (cm)
2017	21 September	3	65/64	51/45
2018	4 May	1	55/55	44/26
	11 July	3	57/63	45/24
2019	8 May	1	52/52	44/26

Unlike A1 and R1, the conventional technique (A2 and R2 for alfalfa and red clover, respectively) harvested the whole plants. Both harvesting techniques were applied at the same time. After cutting the whole plants in A2 and R2, they were tedded on the cutting day and windrowed the next day to achieve target DM content between 30% and 35% and then harvested with a loader wagon. Therefore, several steps such as tedding, turning (only stems) and windrowing of the material need to be completed within two days before the material was ready for harvesting with a loader wagon. Each harvesting technique was replicated four times using a complete randomised block design. The equipment used in the trials are listed in Appendix A Table A1. The average DM yield was calculated for each technique per hectare (kg ha^{-1}). DM was determined at 105 °C by taking forty single harvested samples, mixing them and then separating them into three pooled samples [26]. In the third cut for A2 and R2 in 2017, the DM content of the windrow was used to determine the content at harvest because the data for the DM content of the harvested material were not available for this variant. For the first cut in 2018, the DM was determined from a pooled sample per variant.

2.2. Chemical Analysis

The samples from the harvesting trials were sent to the Laboratory of the Thuenen Institute of Organic Farming (Trenthorst, Germany). The samples were milled through a 1 mm and 0.5 mm sieve to analyse the CP and the amino acid content, respectively. The analysis of DM and CP was carried out according to Commission Regulation No 152/2009 [27]. The amino acid content was determined using HPLC according to the commission regulation EC no. 152/2009 [27] regarding sample preparation by oxidation for methionine and cysteine and hydrolysis. The derivatisation and chromatography methods were adapted and carried out according to Cohen and Michaud [28]. The analytical method adopted was recently published in detail by Witten et al. [29]. All results were given on DM basis.

2.3. Determination of Leaf Portion and Leaf Yield

To determine the leaf portion and yield of every cut and plot for each harvesting technique, a pooled sample was air separated to fractionate the leaves and stems. First, the plants were roughly chopped by hand. Subsequently, the sample was gradually added to the air separator. For separating the leaves from the stems, an air separator, Saatmeister (Kurt Pelz Saatreinigungsmaschinen, Saatzucht-Laborgeräte, Bad Godesberg) was used. The sample was roughly chopped by hand and then air separated for four times. Afterwards, the relative weights of the leaves and stems were used to obtain the leaf proportion. For calculating the leaf yield per hectare, the DM yield per plot was multiplied with the leaf portion per plot.

2.4. Statistical Analysis

Statistical analysis was carried out for the harvested materials to compare both the harvesting techniques and the achieved key data such as DM yield, leaf portion, leaf yield and nutritional content of leaves (A1L and R1L), stems (A1S and R1S) and the whole plant (A1, A2, R1 and R2) for alfalfa and red clover. To calculate the difference in DM and leaf yield for A1 and R1, the yields of the leaves and stems were summed up per culture. Additionally, the leaf portion and yield were calculated. Statistical analyses were carried out using R Studio Software (Version 2021.09.1 + 372). We compared the DM yield, leaf portion, leaf yield and analysed the parameters (CP, all amino acids) per culture and pairwise. Regarding the leaf portion, all variants of red clover and alfalfa were compared.

The statistical model to determine the differences of the initial plant state leaf portion applied was as follows:

$$\gamma_{ij} = \mu + \alpha_i + \gamma_j + \varepsilon_{ij}$$

where γ_{ij} is the dependent variable, μ the overall mean, α_i the fixed effect of the culture (i = 1, 2), γ_j the fixed effect of the trial (j = 1, 2, 3, 4) and ε_{ij} is the residual error.

The statistical model used to determine the differences in the DM yield, leaf portion and leaf yield was as follows:

$$\gamma_{ijk} = \mu + \alpha_i + \gamma_j + \beta_k + \varepsilon_{ijk}$$

where γ_{ijk} is the dependent variable, μ the overall mean, α_i the fixed effect of the variant (i = 1, 2), γ_j the fixed effect of the trial (j = 1, 2, 3, 4), β_k the fixed effect of the repetition (k = 1, 2, 3, 4) and ε_{ijk} is the residual error.

The statistical model used to determine the differences in the CP content and amino acids was as follows:

$$\gamma_{ij} = \mu + \alpha_i + \gamma_j + \varepsilon_{ij}$$

where γ_{ij} is the dependent variable, μ the overall mean, α_i the fixed effect of the variant (i = 1, 2), γ_j the fixed effect of the trial (j = 1, 2, 3, 4) and ε_{ij} is the residual error.

For comparing two dependent variables, such as the leaves and stems, we used Student's *t*-test.

3. Results

3.1. DM Yield, Leaf Yield and Leaf Portion

Table 2 displays the DM yield and leaf yield for both harvesting techniques. The highest DM yields were achieved for the variants R2 and A2 for red clover and alfalfa, respectively, using the conventional technique. The A1 and R1 variants, derived using the stripping technique, had significantly lower DM yields than the variants A2 and R2, respectively. There was no significant difference between the DM yields of separately harvested leaves and stems of alfalfa and red clover by the stripping technique (Table 3).

Regarding the leaf portion of the initial plant state, there were no significant differences between the leaf portion of red clover and alfalfa (Table 4).

The stripping technique fractionated primarily leaves from stems. In red clover and alfalfa, respectively, there were significant differences between the stripped leaf fraction and the stem fraction compared to the whole plant harvesting technique (Table 5). The leaf fraction in red clover and alfalfa had a significantly higher leaf portion compared to the whole plant harvesting technique 2. The stem fraction in red clover and alfalfa had a significantly lower leaf portion. The weighted mean in red clover and alfalfa achieved a significantly higher leaf portion than in variant 2. To evaluate the separation accuracy of the stripping technique in both cultures, the leaf portion was tested across alfalfa and red clover. The leaf portion of the leaf and stem fraction of red clover was significantly higher compared to leaf and stem fraction in alfalfa, respectively.

Table 2. DM yield of the variants 1 and 2 for alfalfa and red clover per cut.

	Var	DM yield [kg ha^{-1}]
Alfalfa	A1	2253.17 (±196.22) ^{a}
	A2	3396.69 (±284.56) ^{b}
	Var	**DM yield [kg ha^{-1}]**
Red clover	R1	2096.35 (±267.37) ^{a}
	R2	3286.05 (±361.28) ^{b}

Abbreviations: DM, dry matter; A1, alfalfa leaves and stems of the stripping technique; A2, alfalfa leaves and stems of the conventional technique; R1, red clover leaves and stems of the stripping harvesting technique; R2, red clover leaves and stems of the conventional harvesting technique. Means (standard error) followed by different superscript letters are significantly different within analysed variable ($p \leq 0.05$). The alfalfa and red clover cultivars, 'Plato' and 'Titus', were harvested for the trials in 2017 and 2018. For the trials in 2019, the alfalfa and red clover cultivars, 'Fleetwood' and 'Taifun', were harvested.

Table 3. DM yield in kg ha^{-1} of the variants 1L and 1S for alfalfa and red clover per cut.

	Var	DM yield [kg ha^{-1}]
Alfalfa	A1L	1180.52 (±111.11) ^{a}
	A1S	1072.64 (±114.63) ^{a}
	Var	**DM yield [kg ha^{-1}]**
Red clover	R1L	991.49 (±103.34) ^{a}
	R1S	1104.85 (±193.54) ^{a}

Abbreviations: DM, dry matter; A1L, alfalfa leaves; A1S, alfalfa stems; R1L, red clover leaves; R1S, red clover stems; Means (standard error) followed by different superscript letters are significantly different within the analysed variable ($p \leq 0.05$). Uppercase letters in parenthesis by leaf portion correspond to the evaluation of the stripping technique in red clover and alfalfa; The alfalfa and red clover cultivars, 'Plato' and 'Titus', were harvested for the trials in 2017 and 2018. For the trials in 2019, the alfalfa and red clover cultivars, 'Fleetwood' and 'Taifun', were harvested.

Table 4. Leaf portion in percent of the whole plant before cutting/stripping of red clover and alfalfa.

Culture	Leaf Portion
Alfalfa	42.61% (±2.08%) ^{a}
Red clover	45.58% (±2.14%) ^{a}

Means (standard error) followed by different superscript letters are significantly different within the analysed variable ($p \leq 0.05$). The alfalfa and red clover cultivars, 'Plato' and 'Titus', were harvested for the trials in 2017 and 2018. For the trials in 2019, the alfalfa and red clover cultivars, 'Fleetwood' and 'Taifun', were harvested.

Table 5. Pairwise comparison of leaf portion in percent of the different harvesting techniques' overall trials.

	Var	Leaf portion
Alfalfa	A1L	73.34% (±2.51%) ^{a}
	A2	41.31% (±1.69%) ^{b}
	A1S	21.02% (±4.24%) ^{a}
	A2	41.31% (±1.69%) ^{b}
	A1	47.97% (±3.70%) ^{a}
	A2	41.31% (±1.69%) ^{b}

Table 5. *Cont.*

	Var	Leaf portion
Red Clover	R1L	82.04% (±1.71%) [a]
	R2	47.31% (±3.01%) [b]
	R1S	27.27% (±3.50%) [a]
	R2	47.31% (±3.01%) [b]
	R1	54.95% (±3.85%) [a]
	R2	47.31% (±3.01%) [b]
	A1L	73.34% (±2.51%) [a]
	R1L	82.04% (±1.71%) [b]
	A1S	21.02% (±4.24%) [a]
	R1S	27.27% (±3.50%) [b]

Abbreviations: A1L, alfalfa leaves; A1S, alfalfa stems; A1, alfalfa leaves and stems from the stripping technique; A2, alfalfa leaves and stems of the conventional technique; R1L, red clover leaves; R1S, red clover stems; R1, red clover leaves and stems from the stripping technique; R2, red clover leaves and stems of the conventional technique. Means (standard error) followed by different superscript letters are significantly different within the analysed variable ($p \leq 0.05$). The alfalfa and red clover cultivars, 'Plato' and 'Titus', were harvested for the trials in 2017 and 2018. For the trials in 2019, the alfalfa and red clover cultivars, 'Fleetwood' and 'Taifun', were harvested.

We obtained higher leaf yields via the conventional technique than the stripping technique. Comparing the A1L vs. A2 and R1L vs. R2 leaf yields (Table 6), 62% of leaf yield was obtained for A1L compared to A2 the whole plants. For red clover, the variant R1L harvested 56% of the leaves compared to R2.

Table 6. Pairwise comparison of leaf yield in kg ha^{-1} of the different harvesting techniques' overall trials.

	Var	Leaf yield [kg ha^{-1}]
Alfalfa	A1L	839.43 (±60.59) [a]
	A2	1349.80 (±105.82) [b]
	A1	1009.53 (±66.01) [a]
	A2	1349.80 (±105.82) [b]
	Var	**Leaf yield [kg ha^{-1}]**
Red Clover	R1L	796.55 (±74.77) [a]
	R2	1413.06 (±121.99) [b]
	R1	1032.27 (±96.39) [a]
	R2	1413.06 (±121.99) [b]

The alfalfa and red clover cultivars, 'Plato' and 'Titus', were harvested for the trials in 2017 and 2018. For the trials in 2019, the alfalfa and red clover cultivars, 'Fleetwood' and 'Taifun', were harvested. The leaf yield was calculated from DM yield and leaf portion per cut overall trials. Means (standard error) followed by different uppercase letters are significantly different within the analysed variable ($p \leq 0.05$).

3.2. Comparison of the CP Content of the Two Harvesting Techniques

The conventional harvesting technique was compared to the weighted mean of the CP content depending on DM yields and CP contents of the stripped fraction and the harvested stems of red clover and alfalfa (Table 7). There were no significant differences in CP content observed between the stripping and the conventional technique in alfalfa and red clover.

3.3. CP Content and Amino Acid Concentrations Based on Harvesting Technique

A comparison of CP content and important amino acids, Cysteine (Cys), Methionine (Met), Lysine (Lys), Threonine (Thr) and Tyrosine (Tyr), between all harvested products is presented in Table 8. Harvested leaf fraction of the stripping technique resulted in a significantly higher concentration of CP content for A1L versus A2 and amino acids contents compared to the stem fraction and the conventional technique. Details on further analysed amino acids are shown in Appendix B Table A2.

Table 7. Comparison of CP content of the conventional harvesting technique and the weighted means of the CP content of the stripping technique.

	Var	CP content [% of DM]
Alfalfa	A1	21.09 (±1.70) [a]
	A2	20.45 (±1.43) [a]
	Var	**CP content [% of DM]**
Red Clover	R1	21.72 (±1.53) [a]
	R2	20.31 (±1.10) [a]

The alfalfa and red clover cultivars, 'Plato' and 'Titus', were harvested for the trials in 2017 and 2018. For the trials in 2019, the alfalfa and red clover cultivars, 'Fleetwood' and 'Taifun', were harvested. Abbreviations: Var, variant; CP, crude protein; A1, alfalfa leaves and stems of the stripping harvesting technique; A2, alfalfa leaves and stems of the conventional harvesting technique; R1, red clover leaves and stems of the stripping harvesting technique; R2, red clover leaves and stems of the conventional harvesting technique. Means (standard error) followed by different superscript letters are significantly different within the analysed variable ($p \leq 0.05$).

Table 8. CP and amino acid concentrations of the different variants of harvesting techniques for alfalfa and red clover.

	CP [% DM]	Cys [g kg^{-1} DM]	Met [g kg^{-1} DM]	Lys [g kg^{-1} DM]	Thr [g kg^{-1} DM]	Tyr [g kg^{-1} DM]
A2	20.44 (±1.43) [a]	2.56 (±0.11) [a]	3.12 (±0.27) [a]	11.72 (±0.89) [a]	8.98 (±0.62) [a]	6.24 (±0.40) [a]
A1L	26.53 (±1.57) [b]	3.42 (±0.15) [b]	4.45 (±0.35) [b]	16.29 (±1.02) [b]	12.38 (±0.83) [b]	8.90 (±0.59) [b]
A2	20.44 (±1.43) [a]	2.56 (±0.11) [a]	3.12 (±0.27) [a]	11.72 (±0.89) [a]	8.98 (±0.62) [a]	6.24 (±0.40) [a]
A1S	15.09 (±2.10) [b]	1.87 (±0.23) [b]	2.16 (±0.36) [b]	8.28 (±1.16) [b]	6.37 (±0.98) [b]	4.44 (±0.62) [b]
A1L	26.53 (±1.57) [a]	3.42 (±0.15) [a]	4.45 (±0.35) [a]	16.29 (±1.02) [a]	12.38 (±0.83) [a]	8.90 (±0.59) [a]
A1S	15.09 (±2.10) [b]	1.87 (±0.23) [b]	2.16 (±0.36) [b]	8.28 (±1.16) [b]	6.37 (±0.98) [b]	4.44 (±0.62) [b]
	CP [% DM]	**Cys [g kg^{-1} DM]**	**Met [g kg^{-1} DM]**	**Lys [g kg^{-1} DM]**	**Thr [g kg^{-1} DM]**	**Tyr [g kg^{-1} DM]**
R2	20.31 (±1.10) [a]	2.05 (±0.13) [a]	2.98 (±0.19) [a]	11.41 (±0.65) [a]	9.04 (±0.46) [a]	6.69 (±0.36) [a]
R1L	26.88 (±1.30) [b]	2.65 (±0.11) [b]	4.33 (±0.29) [b]	16.09 (±0.61) [b]	12.99 (±0.89) [b]	9.84 (±0.62) [b]
R2	20.31 (±1.10) [a]	2.05 (±0.13) [a]	2.98 (±0.19) [a]	11.41 (±0.65) [a]	9.04 (±0.46) [a]	6.69 (±0.36) [a]
R1S	16.49 (±1.76) [b]	1.63 (±0.14) [b]	2.28 (±0.22) [b]	8.88 (±0.98) [b]	7.07 (±0.74) [b]	5.25 (±0.58) [b]
R1L	26.88 (±1.30) [a]	2.65 (±0.11) [a]	4.33 (±0.29) [a]	16.09 (±0.61) [a]	12.99 (±0.89) [a]	9.84 (±0.62) [a]
R1S	16.49 (±1.76) [b]	1.63 (±0.14) [b]	2.28 (±0.22) [b]	8.88 (±0.98) [b]	7.07 (±0.74) [b]	5.25 (±0.58) [b]

Abbreviations: CP, crude protein; Cys, Cysteine; Met, Methionine; Lys, Lysine; Thr, Threonine (Thr); Tyr, Tyrosine; A1L, alfalfa leaves; A1S, alfalfa stems; A2, alfalfa leaves and stems of the conventional harvesting technique; R1L, red clover leaves; R1S, red clover stems; R2, red clover leaves and stems of the conventional harvesting technique. Means (standard error) followed by different superscript letters are significantly different within the analysed variable ($p \leq 0.05$). The alfalfa and red clover cultivars, 'Plato' and 'Titus', were harvested for the trials in 2017 and 2018. For the trials in 2019, the alfalfa and red clover cultivars, 'Fleetwood' and 'Taifun', were harvested.

4. Discussion

We aimed to compare the commonly used whole plant harvesting technique with a new stripping technique by fractionating the leaves from the stems. Additionally, we further investigated by applying the stripping techniques to alfalfa and red clover.

4.1. Comparison of Harvesting Techniques Based on DM Yield, Leaf Portion and Leaf Yield

As previous studies mostly focused on separately harvesting the alfalfa leaves, there are no current studies on separate harvesting of red clover leaves. Using the stripping machine prototype, we could harvest red clover leaves and obtain high concentrations of harvested materials (Table 2). Currence et al. [13], Shinners et al. [15] and Andrzejewksa et al. [12] used a modified stripper for alfalfa and obtained higher DM yields. The reason we obtained lower DM yields for alfalfa is due to the additional working steps such as the accelerator blowing the leaves onto the loader wagon, resulting in losses. Currence et al. [13] used a leaf stripper for alfalfa and obtained a DM yield of approximately 750–1200 pounds per acre for stripped leaves, which is consistent with our DM yields of approximately 840–1345 kg per hectare. To improve the stripping machine prototype used in our study and reduce DM yield loss, stripping and mowing should be combined as one working step as carried out by Shinners et al. [15].

We compared both the harvesting techniques using the DM yield as sum of the two harvested fractions, A1 for alfalfa and R1 for red clover. This showed lower DM yields compared to A2 and R2, respectively. Comparing the leaf yields between red clover and alfalfa, we obtained 62% of alfalfa leaves via the stripping technique compared to the whole plant harvesting technique. For red clover, this was approximately 56%. The quantity of harvested leaves was reduced in the stripping technique due to a higher number of working steps compared to the conventional technique. Additionally, the MRF accelerator caused losses by blowing the leaves onto the loader wagon. Further losses occurred due to separate harvesting of leaves and stems. The prototype stripped the leaves from the plants while the stems were cut in a second step and harvested after wilting at a DM content of approximately 60%. These working steps cause further losses while harvesting plants with higher DM as shown by Manns et al. [30]. During stripping, the leaves were thrown onto a loader wagon driven next to the prototype, so the stems might have been knocked over by the tyres of the two machines, causing further loss. The leaf portion as shown by Andrzejewksa et al. [12] using a leaf stripper with metal bars varied from 56% to 82% whereas our results ranged between 73% and 82% for alfalfa and red clover, respectively.

There was no significant difference in the leaf portion in the initial plant state between both techniques (Table 4). However, regarding the fractionation, there were significant differences between the harvested material after harvesting alfalfa and red clover. The leaf portion in red clover and alfalfa was up to 82.04% and 73.34%, respectively. The concentration for the leaf material was achieved in the stripped faction while that for the steam fraction was from the lower leaf portion.

4.2. CP Content and Amino Acid Values Based on Harvesting Technique

There was no difference between the analysed CP content for variant 2 and the calculated weighted mean of variant 1. Hartfield et al. harvested alfalfa across the cuts, which was not tedded with a CP content of 21.7% [26]. The CP content of wilted alfalfa (30–40% DM) ranged from approximately 22–27% [31,32]. The CP content of wilted red clover with a DM of approximately 40–46% yielded approximately 20% CP [33,34].

Regarding the CP content, Shinners et al. [15] achieved similar values as our results, in contrast to Andrzejewska et al. [12] who investigated the CP contents within different growth stages. In this study, the CP content was lower than in our values at the growth stage. Pleger et al. [35] investigated the digestibility of alfalfa and red clover pellets harvested with the MRF stripper at the third cut in 2017 that we used. Pleger et al. [35] showed lower CP contents of 21.9% and 26.2% for alfalfa and red clover, respectively, probably because the processing of the pellets resulted in CP loss. Higher CP content with at least 32% from stripped alfalfa leaves was achieved by Grev et al. [36] due to hand separation of the stripped leaves. Additionally, higher CP contents of 34% in alfalfa leaves were obtained by Popovic et al. [37], where the leaves were manually separated from stems by hand.

Another way of legume processing by using screw press to fractionate the plant material into a green juice and fibrous pulp. Using this method, the CP contents in the pulp fraction were 13.3% and 20.5% for alfalfa and red clover, respectively [38]. Lower CP contents were obtained for harvested alfalfa leaves by Ritteser [39] similar to the results in studies where alfalfa and red clover were fractionated [6]. Blume et al. [17] also fractionated alfalfa by wilting and sieving the plants. These pellets had lower CP content of 20.57% compared to our study due to further processing steps such as drying, sieving and producing pellets. The Grub feeding table, which determined the CP contents of alfalfa pellets in Bavaria, Germany, investigated lower CP content ranging from approximately 18–21% due to post-harvest processing losses and various field locations across Bavaria [40]. The results of the amino acids were comparable to those shown by Hoischen-Taubner [6].

5. Conclusions

In this study, an innovative harvesting technique (stripping technique) was investigated and compared with the conventional whole plant harvesting for alfalfa and red clover. During a three-year field trial period, the data on DM yield, leaf portion, leaf yield together with nutritive values (CP content and amino acids concentrations) were collected. The stripping technique resulted in higher concentration of important amino acids and CP especially for red clover and leaf fraction content from the harvested material. However, the stripping technique resulted in comparatively lower DM and leaf yields from the harvested material. Fractionation of alfalfa and red clover using this novel harvesting method can help to meet the demands for feeding the monogastric animals, resulting from new EU organic regulations. Nevertheless, these techniques need to be further improved and optimised before practical use. Further investigations should be carried out to describe the DM losses of the stripping technique compared to other harvesting techniques. The same holds true for harvested stems.

In addition, the economic value of the stripping technique needs to be evaluated, in particular the economic impact depending on parameters such as working time, fuel, machine costs, etc.

Additionally, further investigations should be carried out for post-harvesting processing. The harvested leaves need to be processed for conservation by ensiling or drying. Wilted plants such as in variant 2 also could be sieved after drying to obtain a higher concentration of ingredients.

Author Contributions: Conceptualisation, S.T., J.M. and K.A.; methodology, S.T. and J.M.; formal analysis, P.L., J.M. and S.T.; investigation, P.L., J.M., S.T. and K.A.; resources, S.T.; data curation, P.L.; writing—original draft preparation, P.L. and J.M.; writing—review and editing, P.L., J.M., H.B., K.A. and S.T.; visualisation, P.L. and J.M.; supervision, S.T., J.M. and H.B.; project administration, S.T. and J.M.; funding acquisition, S.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Federal Ministry of Food and Agriculture (BMEL, Bonn, Germany), grant number 2815OE039.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to Gerhard Bellof and Peter Weindl (University of Applied Sciences Weihenstephan), members of the Institute of Bavarian State Research Center for Agriculture, students, feed drying Lamerdingen, the farmer, contractors and supporting TUM statistician.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

Table A1. Equipment used in the different harvesting techniques for alfalfa and red clover.

Trial	Working Step	Type
3rd cut 2018	Stripping prototype	John Deere 6830 with MRF
	Cutting	John Deere 6400, Kuhn GMD 802
	Tedding	John Deere 6400, Claas W 640 SL
	Ranking	John Deere 6400, Deutz-Fahr Swatmaster 4622
	Harvesting with loader wagon	John Deere 6400, Pöttinger BOSS 2 Supermatic

Table A1. *Cont.*

Trial	Working Step	Type
1st cut 2018	Stripping prototype	John Deere 6830 with MRF
	Cutting	New Holland TL 80, Vicon CM 247
	Tedding	John Deere 6830, Deutz-Fahr KH 3.60 Hydro Super
	Ranking	John Deere 6830, Kuhn GA 6632
	Harvesting with loader wagon	John Deere 6400, Mengele LW 310 Quadro
3rd cut 2018	Stripping prototype	John Deere 6830 with MRF
	Cutting	Fendt Vario 716, Kuhn GMD 802
	Tedding	Valtra N 101 H, Claas W 640 SL
	Ranking	Valtra N 101 H, Deutz-Fahr Swatmaster 4622
	Harvesting with loader wagon	Valtra N 101 H, Pöttinger BOSS 2 Supermatic
1st cut 2019	Stripping prototype	John Deere 6830 with MRF
	Cutting	New Holland TL 80, Vicon CM 247
	Tedding	John Deere 6400, Deutz-Fahr KH 3.60 Hydro Super
	Ranking	John Deere 6830, Kuhn GA 6632
	Harvesting with loader wagon	John Deere 6400, Mengele LW 310 Quadro

Appendix B

Table A2. Amino acid concentrations obtained using different harvesting techniques for alfalfa and red clover.

Amino Acid	Asp [g kg^{-1} DM]	Glu [g kg^{-1} DM]	Ser [g kg^{-1} DM]	His [g kg^{-1} DM]	Gly [g kg^{-1} DM]	Arg [g kg^{-1} DM]
A2	28.31 (±3.09) ^{a}	20.16 (±1.45) ^{a}	8.77 (±0.52) ^{a}	4.61 (±0.32) ^{a}	9.15 (±0.55) ^{a}	9.08 (±0.89) ^{a}
A1L	32.63 (±1.81) ^{a}	28.41 (±1.97) ^{b}	11.42 (±0.53) ^{b}	6.37 (±0.45) ^{b}	13.01 (±0.82) ^{b}	13.62 (±1.18) ^{b}
A2	28.31 (±3.09) ^{a}	20.16 (±1.45) ^{a}	8.77 (±0.52) ^{a}	4.61 (±0.32) ^{a}	9.15 (±0.55) ^{a}	9.08 (±0.89) ^{a}
A1S	23.25 (±3.21) ^{b}	13.99 (±2.32) ^{b}	6.66 (±0.94) ^{b}	3.21 (±0.47) ^{b}	6.42 (±0.94) ^{b}	6.05 (±1.18) ^{b}
A1L	32.63 (±1.81) ^{a}	28.41 (±1.97) ^{a}	11.42 (±0.53) ^{a}	6.37 (±0.45) ^{a}	13.01 (±0.82) ^{a}	13.62 (±1.18) ^{a}
A1S	23.25 (±3.21) ^{b}	13.99 (±2.32) ^{b}	6.66 (±0.94) ^{b}	3.21 (±0.47) ^{b}	6.42 (±0.94) ^{b}	6.05 (±1.18) ^{b}
Amino acid	**Asp [g kg^{-1} DM]**	**Glu [g kg^{-1} DM]**	**Ser [g kg^{-1} DM]**	**His [g kg^{-1} DM]**	**Gly [g kg^{-1} DM]**	**Arg [g kg^{-1} DM]**
R2	27.08 (±2.78) ^{a}	20.20 (±1.00) ^{a}	8.82 (±0.38) ^{a}	4.64 (±0.27) ^{a}	9.43 (±0.44) ^{a}	9.42 (±0.68) ^{a}
R1L	29.68 (±1.27) ^{a}	29.55 (±1.63) ^{b}	12.12 (±0.80) ^{b}	6.58 (±0.40) ^{b}	14.08 (±0.97) ^{b}	14.48 (±1.06) ^{b}
R2	27.08 (±2.78) ^{a}	20.20 (±1.00) ^{a}	8.82 (±0.38) ^{a}	4.64 (±0.27) ^{a}	9.43 (±0.44) ^{a}	9.42 (±0.68) ^{a}
R1S	24.63 (±3.81) ^{a}	16.02 (±1.69) ^{b}	7.27 (±0.72) ^{b}	3.56 (±0.40) ^{b}	7.40 (±0.78) ^{b}	7.16 (±0.97) ^{b}
R1L	29.68 (±1.27) ^{a}	29.55 (±1.63) ^{a}	12.12 (±0.80) ^{a}	6.58 (±0.40) ^{a}	14.08 (±0.97) ^{a}	14.48 (±1.06) ^{a}
R1S	24.63 (±3.81) ^{a}	16.02 (±1.69) ^{b}	7.27 (±0.72) ^{b}	3.56 (±0.40) ^{b}	7.40 (±0.78) ^{b}	7.16 (±0.97) ^{b}
Amino acid	**Ala [g kg^{-1} DM]**	**Pro [g kg^{-1} DM]**	**Val [g kg^{-1} DM]**	**Ile [g kg^{-1} DM]**	**Leu [g kg^{-1} DM]**	**Phe [g kg^{-1} DM]**
A2	10.47 (±0.87) ^{a}	14.43 (±1.97) ^{a}	10.34 (±0.84) ^{a}	7.84 (±0.66) ^{a}	13.99 (±1.15) ^{a}	9.29 (±0.80) ^{a}
A1L	15.02 (±1.21) ^{b}	15.23 (±0.86) ^{a}	14.07 (±0.95) ^{b}	11.00 (±0.81) ^{b}	20.35 (±1.59) ^{b}	13.54 (±1.07) ^{b}
A2	10.47 (±0.87) ^{a}	14.43 (±1.97) ^{a}	10.34 (±0.84) ^{a}	7.84 (±0.66) ^{a}	13.99 (±1.15) ^{a}	9.29 (±0.80) ^{a}
A1S	7.11 (±1.22) ^{b}	12.54 (±2.27) ^{a}	7.45 (±1.21) ^{b}	5.52 (±0.93) ^{b}	9.50 (±1.63) ^{b}	6.29 (±1.11) ^{b}
A1L	15.02 (±1.21) ^{a}	15.23 (±0.86) ^{a}	14.07 (±0.95) ^{a}	11.00 (±0.81) ^{a}	20.35 (±1.59) ^{a}	13.54 (±1.07) ^{a}
A1S	7.11 (±1.22) ^{b}	12.54 (±2.27) ^{a}	7.45 (±1.21) ^{b}	5.52 (±0.93) ^{b}	9.50 (±1.63) ^{b}	6.29 (±1.11) ^{b}

Table A2. *Cont.*

Amino acid	Ala [g kg^{-1} DM]	Pro [g kg^{-1} DM]	Val [g kg^{-1} DM]	Ile [g kg^{-1} DM]	Leu [g kg^{-1} DM]	Phe [g kg^{-1} DM]
R2	10.69 (±0.54) [a]	15.22 (±1.78) [a]	10.85 (±0.59) [a]	8.09 (±0.41) [a]	14.86 (±0.84) [a]	9.85 (±0.61) [a]
R1L	15.87 (±0.91) [b]	14.34 (±0.92) [a]	15.42 (±1.09) [b]	11.71 (±0.80) [b]	22.53 (±1.55) [b]	15.03 (±1.19) [b]
R2	10.69 (±0.54) [a]	15.22 (±1.78) [a]	10.85 (±0.59) [a]	8.09 (±0.41) [a]	14.86 (±0.84) [a]	9.85 (±0.61) [a]
R1S	8.31 (±0.88) [b]	12.23 (±1.62) [a]	8.56 (±0.95) [b]	6.36 (±0.69) [b]	11.35 (±1.28) [b]	7.37 (±0.87) [b]
R1L	15.87 (±0.91) [a]	14.34 (±0.92) [a]	15.42 (±1.09) [a]	11.71 (±0.80) [a]	22.53 (±1.55) [a]	15.03 (±1.19) [a]
R1S	8.31 (±0.88) [b]	12.23 (±1.62) [a]	8.56 (±0.95) [b]	6.36 (±0.69) [b]	11.35 (±1.28) [b]	7.37 (±0.87) [b]

Abbreviations: Asp, Aspartic acid; Glu, Glutamic acid; Ser, Serine; His, Histidine; Gly, Glycine; Arg, Arginine; Ala, Alanine; Pro, Proline; Val, Valine; Ile, Isoleucine; Leu, Leucine; Phe, Phenylalanine; A1L, alfalfa leaves; A1S, alfalfa stems; A2, alfalfa leaves and stems of the conventional harvesting technique; R1L, red clover leaves; R1S, red clover stems; R2, red clover leaves and stems of the conventional harvesting technique. Means (standard error) followed by different superscript letters are significantly different within the analysed variable ($p \leq 0.05$). The alfalfa and red clover cultivars, 'Plato' and 'Titus', were harvested for the trials in 2017 and 2018. For the trials in 2019, the alfalfa and red clover cultivars, 'Fleetwood' and 'Taifun', were harvested.

References

1. EU. *Council Regulation (EC) No. 834/2007 of 28 June 2007 on Organic Production and Labelling of Organic Products and Repealing Regulation (EEC) No. 2092/91*; EU: Brussels, Belgium, 2007.
2. EU. *Commission Regulation (EC) No. 889/2008 of 5 September 2008 Laying down Detailed Rules for the Implementation of Council Regulation (EC) No. 834/2007 on Organic Production and Labelling of Organic Products with Regard to Organic Production, Labelling and Control*; EU: Brussels, Belgium, 2008.
3. EU. *Commission Implementing Regulation (EU) No. 505/2012 of 14 June 2012 Amending and Correcting Regulation (EC) No. 889/2008 Laying down Detailed Rules for the Implementation of Council Regulation (EC) No. 834/2007 on Organic Production and Labelling of Organic Products with Regard to Organic Production, Labelling and Control*; EU: Brussels, Belgium, 2012.
4. Carlsson, G.; Huss-Danell, K. Nitrogen fixation in perennial forage legumes in the field. *Plant Soil* **2003**, *2*, 353–372. [CrossRef]
5. Sousa, D.O.; Hansen, H.H.; Hallin, O.; Nussio, L.G.; Nadeau, E. A two-year comparison on nutritive value and yield of eight lucerne cultivars and one red clover cultivar. *Grass Forage Sci.* **2020**, *1*, 76–85. [CrossRef]
6. Hoischen-Taubner, S.; Sundrum, A. Determining the Feeding Value and Digestibility of the Leaf Mass of Alfalfa (Medicago sativa) and Various Types of Clover. Ermittlung des Futterwertes und der Verdaulichkeiten der Blattmassen von Luzerne und Perserklee. 2016. Available online: https://orgprints.org/id/eprint/30426/ (accessed on 24 July 2021).
7. Sommer, H.; Sundrum, A. (Eds.) *Ganzpflanze und Blattmasse Verschiedener Grünleguminosen als Eiweißquelle in der Schweinefütterung. Am Mut Hängt der Erfolg—Rückblicke und Ausblicke auf die Ökologische Landbewirtschaftung*; Köster: Berlin, Germany, 2015.
8. Weltin, J.; Carrasco Alarcon, L.S.; Berger, U.; Bellof, G. Alfalfa-Silage Silage after Suitable Preparation in Organic Poultry and Pig Feeding. 2014. Available online: https://orgprints.org/26279/ (accessed on 7 May 2020).
9. de Visser, C.L.M.; Schreuder, R.; Stoddard, F. The EU's dependency on soya bean import for the animal feed industry and potential for EU produced alternatives. *OCL* **2014**, *21*, D407. [CrossRef]
10. Wang, J.P.; Hong, S.M.; Yan, L.; Cho, J.H.; Lee, H.S.; Kim, I.H. The evaluation of soybean meals from 3 major soybean-producing countries on productive performance and feeding value of pig diets. *J. Anim. Sci.* **2011**, *9*, 2768–2773. [CrossRef]
11. EU. *Report from the Commission to the Council and the European Parliament on the Development of Plant Proteins in the European Union*; EU: Brussels, Belgium, 2018.
12. Andrzejewska, J.; Ignaczak, S.; Albrecht, K.A. Nutritive Value of Alfalfa Harvested with a Modified Flail Chopper. *Agronomy* **2020**, *5*, 690. [CrossRef]
13. Currence, H.D.; Buchele, W.F. Leaf-strip harvester for alfalfa. *Agric. Eng.* **1967**, *1*, 20.
14. Ignaczak, S.; Andrzejewska, J.; Sadowska, K.; Albrecht, K.A. Fractional Harvest of Fodder Galega for Improved Herbage Nutritive Value. *Agronomy* **2021**, *3*, 480. [CrossRef]
15. Shinners, K.J.; Herzmann, M.E.; Binversie, B.N.; Digman, M.F. Harvest Fractionation of Alfalfa. *Trans. ASABE* **2007**, *3*, 713–718. [CrossRef]
16. Liebhardt, P.; Maxa, J.; Bernhardt, H.; Thurner, S. Harvesting techniques for legumes (especially leaves) as protein feed for monogastric animals. *Grassl. Sci. Eur.* **2019**, *24*, 69–71.
17. Blume, L.; Hoischen-Taubner, S.; Sundrum, A. Alfalfa—A regional protein source for all farm animals. *Landbauforschung* **2021**, *1*, 1–13.
18. Digman, M.F.; Runge, T.M.; Shinners, K.J.; Hatfield, R.D. Wet Fractionation for Improved Utilization of Alfalfa Leaves. *Biol. Eng.* **2013**, *1*, 29–42.
19. Sikora, M.C.; Hatfield, R.D.; Kalscheur, K.F. Fermentation and chemical composition of high-moisture lucerne leaf and stem silages harvested at different stages of development using a leaf stripper. *Grass Forage Sci.* **2019**, *2*, 254–263. [CrossRef]
20. Albrecht, K.A.; Wedin, W.F. Buxton Cell-Wall Composition and Digestibility of Alfalfa Stems and Leaves 1. *Crop Sci.* **1987**, *4*, 735–741. [CrossRef]

21. Wiersma, D.W.; Smith, R.R.; Sharpee, D.K.; Mlynarek, M.J.; Rand, R.E.; Undersander, D.J. Harvest management effects on red clover forage yield, quality, and persistence. *J. Prod. Agric.* **1998**, *3*, 309–313. [CrossRef]
22. Hopkins, A. (Ed.) *Grass, Its Production and Utilization*; Blackwell Science: Malden, MA, USA, 2000.
23. Schön, H.; Auernhammer, H.; Bauer, R.; Boxberger, J.; Demmel, M.; Estler, M.; Gronauer, A.; Haidn, B.; Meyer, J.; Pirkelmann, H.; et al. *Landtechnik-Bauwesen//Landtechnik Bauwesen. Verfahrenstechnik-Arbeit-Gebäude-Umwelt//Verfahrenstechniken-Arbeit-Gebäude-Umwelt*; BLV Verlagsgesellschaft mbH; BLV-Verlagsges: München, Germany, 1998.
24. Bundessortenamt Beschreibende Sortenliste Futtergräser. Esparsette, Klee, Luzerne. 2020. Available online: https://www.bundessortenamt.de/bsa/media/Files/BSL/bsl_futtergraeser_2020.pdf (accessed on 3 June 2022).
25. Meier, U. *Growth Stages of Mono- and Dicotyledonous Plants: BBCH Monograph*; Open Agrar Repositorium: Berlin/Braunschweig, Germany, 2018.
26. Hartfield, L.M.; Digman, M.F. The Impact of Tedding on Alfalfa Nutritive Value in a Silage Production system. *Appl. Eng. Agric.* **2021**, *37*, 1115–1118. [CrossRef]
27. EU. *Regulations Commission Regulation (EC) No. 152/2009 of 27 January 2009 Laying down the Methods of Sampling and Analysis for the Official Control of Feed (Text with EEA Relevance)*; EU: Brussels, Belgium, 2009.
28. Cohen, S.A.; Michaud, D.P. Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. *Anal. Biochem.* **1993**, *2*, 279–287. [CrossRef]
29. Witten, S.; Böhm, H.; Aulrich, K. Effect of variety and environment on the contents of crude nutrients and amino acids in organically produced cereal and legume grains. *Org. Agric.* **2020**, *2*, 199–219. [CrossRef]
30. Manns, C.; Hensel, O. Bestimmung der Bröckelverluste bei der Luzernebergung unter Prüfstandbedingungen. *Landtechnik* **2009**, *64*, 360–362.
31. Hashemzadeh-Cigari, F.; Khorvash, M.; Ghorbani, G.R.; Ghasemi, E.; Taghizadeh, A.; Kargar, S.; Yang, W.Z. Interactive effects of molasses by homofermentative and heterofermentative inoculants on fermentation quality, nitrogen fractionation, nutritive value and aerobic stability of wilted alfalfa (*Medicago sativa* L.) silage. *J. Anim. Physiol. Anim. Nutr.* **2014**, *2*, 290–299. [CrossRef]
32. Li, P.; Ji, S.; Hou, C.; Tang, H.; Wang, Q.; Shen, Y. Effects of chemical additives on the fermentation quality and N distribution of alfalfa silage in south of China. *Anim. Sci. J.* **2016**, *12*, 1472–1479. [CrossRef]
33. Makoni, N.F.; Shelford, J.A.; Nakai, S.; Fisher, L.J. Changes in lipids, chlorophyll pigments, hot water insoluble N and pH of alfalfa during ensiling. *J. Sci. Food Agric.* **1993**, *3*, 273–280. [CrossRef]
34. Udén, P. Fresh and ensiled forage plants-total composition, silage losses and the prediction of silage composition from the crop. *Grass Forage Sci.* **2018**, *2*, 420–431. [CrossRef]
35. Pleger, L.; Weindl, P.N.; Weindl, P.A.; Carrasco, L.S.; Leitao, C.; Zhao, M.; Aulrich, K.; Bellof, G. Precaecal digestibility of crude protein and amino acids from alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*) leaves and silages in broilers. *Anim. Feed. Sci. Technol.* **2021**, *275*, 114856. [CrossRef]
36. Grev, A.M.; Wells, M.S.; Catalano, D.N.; Martinson, K.L.; Jungers, J.M.; Sheaffer, C.C. Stem and leaf forage nutritive value and morphology of reduced lignin alfalfa. *Agron. J.* **2020**, *1*, 406–417. [CrossRef]
37. Popovic, S.; Stjepanovic, M.; Grljusic, S.; Cupic, T.; Tucak, M. Protein and fiber contents in alfalfa leaves and stems. *Options Méditerranéennes Série A Séminaires Méditerranéens* **2001**, *45*, 215–218.
38. Stødkilde, L.; Damborg, V.K.; Jørgensen, H.; Lærke, H.N.; Jensen, S.K. Digestibility of fractionated green biomass as protein source for monogastric animals. *Anim. Int. J. Anim. Biosci.* **2019**, *9*, 1817–1825. [CrossRef] [PubMed]
39. Ritteser Estimation of Ileal Nutrient Digestibility of Native Energy and Protein Feeding Stuffs for Organic Broilers. Bestimmung Präcecaler Verdaulichkeitskoeffizienten für Heimische Energiefuttermittel für die Hühnermast. 2015. Available online: https://www.orgprints.org/id/eprint/29363/ (accessed on 24 July 2021).
40. Bayerische Landesanstalt für Landwirtschaft (LfL) Gruber Tabelle zur Fütterung der Milchkühe Zuchtrinder Schafe Ziegen. 46. unveränderte Auflage/Stand 2020, Bayerische Landesanstalt für Landwirtschaft (LfL) Vöttinger Straße 38, 85354 Freising-Weihenstephan. 2020. Available online: https://www.lfl.bayern.de/mam/cms07/publikationen/daten/informationen/gruber_tabelle_fuetterung_milchkuehe_zuchtrinder_schafe_ziegen_lfl-information.pdf (accessed on 29 August 2021).