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Selenium Application in Improving Chicory *(Cichorium intybus)* Productivity and Quality

N. Umami[a,*], E. R. V. Rahayu[a], B. Suhartanto[a], A. Agus[a], & M. M. Rahman[b]
[a]Faculty of Animal Science, Universitas Gadjah Mada
Jalan Fauna No. 3 Bulaksumur, Yogyakarta 55281, Indonesia
[b]Faculty of Agro Based Industry, Universiti Malaysia Kelantan
17600 Jeli, Kelantan, Malaysia
*Corresponding author: nafiatul.umami@ugm.ac.id
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ABSTRACT

Selenium (Se) is an essential mineral element needed by livestock and human. The amount of Se intake is largely determined by the Se content in the plants (food and feed) consumed. This study aimed to analyze the effect of Se fertilizer on the morphological characters, biomass yield, nutrient content, and *in vitro* nutrient digestibility of *Cichorium intybus*. This study was conducted for four months, starting from May to August 2019, located at the Faculty of Animal Science, Universitas Gadjah Mada. The seeds of *Cichorium intybus* var. Chico was sown in 24 plots and the plots were arranged in a completely randomized block design. Three levels of Se fertilization treatments (0, 3.5, and 7.5 mg/m^2) were applied with 8 replicates. Plant defoliation on every 45 days: namely the first defoliation (from sowing to 45 days), the second defoliation (regrowth 1 up to 45 days), and the third defoliation (regrowth 2 up to 45 days). In all studied parameters, the results showed that chicory with Se fertilizer (3.5 and 7.5 mg/m^2) was higher ($p<0.05$) than chicory without Se fertilization. The chicory with Se fertilizer at the level of 7.5 mg/m^2 had higher ($p<0.05$) leaf width, biomass yield, crude protein, and Se content, and *in vitro* nutrient digestibility (dry matter, organic matter, crude protein) compared to that with Se fertilizer at the level of 3.5 mg/m^2. Chicory at the third defoliation had a higher biomass yield and Se content than at the second defoliation. In conclusion, the best combination from this study was the third defoliation, with Se fertilizer level of 7.5 mg/m^2.

Keywords: Cichorium intybus; growth; in vitro digestibility; nutrient content; selenium

INTRODUCTION

Selenium (Se) is an essential micromineral for livestock and human, which is required in small amounts but has very large effects (Chilimba *et al.*, 2014). Selenium deficiency in ruminants can cause mandibular thickening, premature tooth shedding, myopathy (white muscle disease, "stiff lamb" muscular dystrophy), weight loss, and feed-intake decrease (Flueck, 2015; Huo *et al.*, 2020). Plants play an important role in overcoming Se deficiency in ruminants (Gupta & Gupta, 2017). The amount of Se intakes in livestock and human are largely determined by the Se content in plants, both food and feed consumed. The Se content in plants is determined by the availability of Se in the soil (Boghdady *et al.*, 2017). Thus, the Se content and availability in the soil greatly determine the Se that enters a food chain (plants, livestock, and human).

Selenium is formed from the sedimentary rocks during the Carboniferous period to the Quaternary period (White *et al.*, 2004; Mechora *et al.*, 2017). Selenium exists in two forms: organic and inorganic forms. The inorganic forms of Se are selenate (SeO_4^{-2}) and selenite (SeO_3^{-2}) while the organic forms are selenomethionine (SeMet) and selenocysteine (SeCys). Most of the Se in plants are in the form of SeMet. The SeMet has good bioavailability because approximately 90% of the SeMet available in the food and feed are absorbed by the body (Mehdi *et al.*, 2013; Mangiapane *et al.*, 2014). The range of Se levels needed by plants is 0.001-2 ppm, starting to be toxic at concentrations of 10-30 ppm for livestock (De Temmerman *et al.*, 2014; Favorito *et al.*, 2021).

Cichorium intybus is a subtropical forbs group that can be developed in Indonesia as a forage source. This plant has potential in increasing the soil mineral layer because it has deep roots and is drought-tolerant (Lee *et al.*, 2015; Dhamala *et al.*, 2017). The seeds are small and light, so they are easily carried away by birds or the wind, so basically easy to scatter (Lee *et al.*, 2015). In addition, *Cichorium intybus* contains high crude protein (29.6%) and high digestibility (74%) (Nwafor *et al.*, 2017). The secondary metabolites (flavonoids, tannins, and coumarins) found in chicory have some biological activities such as antioxidant, anticancer, anti-inflammatory, antiparasitic, antihepatotoxic, with the impact of positive health effect on livestock (Das *et al.*, 2016; Peña-Espinoza *et al.*, 2018). Therefore, chicory has potential as a nutraceutical anthelmintic for parasite control in

livestock and a source of novel antiparasitic compounds (Peña-Espinoza *et al.,* 2020). Defoliation of plants affects the regrowth process. Tiller growth will increase along with plant regrowth. Meuriot *et al.* (2018) reported that when the apical meristem of a stressed plant is cut, the carbohydrate level in the remaining cutting causes more new shoots. A study on chicory plants showed that the first regrowth biomass production was 1.04 ton/ha while the second regrowth was 1.47 ton/ha (Zaini *et al.,* 2021).

Information regarding chicory as a Se carrier plant is still limited. Therefore, this study is aimed to measure and identify the effect of Se fertilizer application on the production, nutrient content, and digestibility of chicory forages. The data obtained can be used as a reference in determining the strategy of providing rations to fulfill the Se adequacy in livestock.

MATERIALS AND METHODS

Experimental Design

The experiment was performed in the Forage and Pasture Science Laboratory, Faculty of Animal Science, Universitas Gadjah Mada (a latitude of -7.769238 and a longitude of 110.386085), Indonesia, from May to August 2019. The altitude of the experimental field is 114 meters above sea level. The experimental field has a variation in the slope of 0% to 2%. Climatic conditions of the research location during the plant growth period were recorded at the Meteorology, Climatology and Geophysics Agency (BMKG) in Sleman, Yogyakarta in 2019 (Figure 1).

The soil was classified as Regosol and pH level of 6.87. Soil samples in the 0 to 30 cm depth from 5 sampling locations were collected before the development of the experiment and destined for chemical analysis (Table 1).

The ground of the area was prepared in conventional, plowed, and plotted before the seedling. The area of each plot was 2m × 1.5m with the distance between plots of 0.5 m, and then 1.5 kg/m^2 matured bovine manure mixed homogeneously, left for a week to maintain stability (Zaini *et al.,* 2021). The seeds of *Cichorium intybus* var. Chico was sown in 24 plots. The seeds were sown by scattering at a seeding rate of 0.1 gram/m^2 or 60 seeds/m^2. The seeds that had been sown were then covered with soil. The sowing was executed by putting a distance of 7 to 10 cm between the edges of the plots. Chicory is maintained until defoliation.

Three levels of Se fertilizer (0, 3.5, and 7.5 mg/m^2) were applied to each treatment on one-week-old plants. Each treatment of Se fertilizer was replicated 8 times using a completely randomized block design. The area was preserved free of weeds by hand removal. Watering was done twice, in the morning at 07 00 and in the afternoon at 16 00, on non-rainy days. Defoliations were performed at a 45 days interval, namely the first defoliation (from sowing to 45 days), the second defoliation (regrowth 1 up to 45 days), and the third defoliation (regrowth 2 up to 45 days).

Table 1. The soil analysis results of the collection area of Forage and Pasture Science, Faculty of Animal Science, Universitas Gadjah Mada in 2019

Test parameters	Values	Criteria (Score)
pH (H_2O)	6.87[a]	Neutral (±7)[c]
C-organic (%)	5.46[a]	Very high (>5.00)[c]
N-Total (%)	0.57[a]	High (0.5 to 1)[c]
P_2O_5 (ppm)	18[a]	Low (10 to 20)[c]
K (ppm)	6[a]	Low (10 to 20)[c]
Selenium (mcg/100g)	7.98[b]	Low (10^5 to 10^8)[d]
C/N	9.58[a]	Low (5 to 10)[c]

Note: [a]Results of analysis of the Agriculture Technology Research Center in Yogyakarta
[b]Results of analysis of PT. Saraswati Indo Genetect
[c]Hardjowigeno (2007)
[d]De Temmerman *et al.* (2014)

Sampling Procedure and Observed Variables

Plant-morphology measurements were carried out before defoliation. All plants for each plot were measured and yields were recorded. Plant measurements included the number of leaves, leaf width, and plant height. Plants were cut at 4 cm above the ground level (Mangwe *et al.,* 2020) and transported to the laboratory for weighing, where the biomass yield was calculated in kg/m^2, then converted into ton/ha.

Chemical Analysis

Samples of plant tissue (dried, ground, and composited) were analyzed for chemical composition, which included dry matter (DM), organic matter (OM), crude protein (CP), and crude fiber (CF) (AOAC, 2005). For DM analysis, around one gram of sample was oven-dried at the temperature of 105 °C overnight until the weight was constant. The content of DM was calculated with the following formula:

DM= [Weight after oven-dried (g) / Weight before oven-dried (g)] x 100%

For OM analysis, the dry sample was then furnaced at 600 °C for 2 h until the weight is constant. The content of OM was calculated as 100% minus ash content on which ash with the following formula:

OM= {[Weight before oven-dried (g) – Weight after furnaced 600 °C (g)] / Weight before oven-dried (g)} x 100%

The sample was digested in a Kjeldahl tube for CP analysis with the following step. Around 0.5 g of sample was added with the catalyst of one gram of $CuSO_4$ and two grams of K_2SO_4 and 20 mL of concentrated H_2SO_4 for destruction. The destruction results were diluted with 75 mL of distilled water and homogenized. Erlenmeyer 300 mL was prepared and filled with 60 mL of borax solution and indicator mix. The tube that has been filled with the destruction results and indicator mix is installed in the distillation apparatus, and the 50 mL of NaOH is flowed. The distillate was titrated with 0.1 N HCl until the color changed. The content of CP was calculated with the following formula:

CP= {[(Number of HCl sample (mL) - Number of HCl blank (mL)) x N x 0.014x 6.25] / Sample weight} x 100%

For CF analysis, around two grams of sample was put in beaker glass and added 200 mL of 1.25% H_2SO_4 and boiled for 30 minutes. The sample was filtered and then rinsed with hot distilled water. The residue was put to the beaker glass again, added with 200 mL of 1.25% NaOH and boiled for 30 minutes. The mixture was filtered with glass wool using Gooch crucible and washed with 95% ethyl alcohol. Filtered results, including glass wool, were oven-dried at the temperature of 105 ℃ overnight and the last furnaced at 600 ℃ for 2 h until the weight was constant. The CF was calculated by using the following formula:

CF= {[Weight of sample after oven 105 ℃ – Weight of ashed sample] / Weight of initial sample} x 100%

Selenium content in plant tissue was analyzed using the ICP-MS method as described by Thomas (2004). Torch position and ion lenses were first optimized daily and tested by performing a short-term stability test in the standard mode with a 1 μg 1^{-1} tuning solution to maximize ion signals, stability, and oxide levels. Then, the collision cell was flushed with collision gases (mixture of He dan H_2) for 30 minutes, and the second optimization of torch position and ion lenses was carried out. The mas-scanning data-acquisition mode was used for both optimizations. A multi-element internal calibration curve was based on the use of multiple reference standards, prepared at the levels ranging from 2 to 100 μg 1^{-1}, according to the element relative-sensitivity factors. The following mass-to-charge ratio (m*lz*) ^{80}Se were measured in CCT mode. A 5 μg 1^{-1} mixed internal standard solution of ^{70}Ga, ^{89}Y, and ^{115}In was used, because of the wide range of *mlz* ratios between 80, to allow short-term drift corrections. In standard mode, the alternative isotope of ^{82}Se was measured and a correction equation was automatically used at *mlz* 82.

For *in vitro* digestibility, which consisted of dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), and crude fiber digestibility (CFD), were measured following the method of Tilley & Terry (1963). The method is divided into 2 stages. The first stage with fermentative digestion was conducted by inserting 0.5 g of sample into the fermenter tube. The McDougall solution, as much as 40 mL and 10 mL of rumen fluid were added to the fermenter tube, and then the tube was closed and incubated for 48 h. During the incubation, the fermenter tube was shaken every 6 h. In the second stage, the residue was given 3 mL of 0.5% HCl and 1 mL pepsin solution and incubated for 48 h aerobically. The sample was filtered using Whatman paper and the residue obtained was analyzed proximately according to AOAC (2005).

For pH of the rumen fluid, the measurement followed the methods of Nahm (1992), entering the digital pH into the rumen fluid that had been incubated for 2 days.

For Ammonia (NH_3), the filtrate was centrifuged at 3000 rpm for 15 minutes and as much as 0.5 mL was used for the analysis of N-ammonia (Chaney & Marbach, 1962). The sample was then centrifuged at 10.000 rpm for 10 minutes. The supernatant as much as 20 μL was taken, added with 2.5 mL LC solution and 2.5 mL LD solution, then homogenized. The mixture was incubated at 40 ℃ for 30 minutes. Then the solution was cooled at room temperature. The last step is reading using spectrophotometry with a wavelength of 630 nm. The NH_3 was calculated by using the following formula:

Y= 0.0068X + 0.0278, where Y for absorbance and X for NH_3 content (mg/100mL)

Data Analysis

Statistical analysis was processed using Statistical Package for the Social Sciences software (SPSS) version 23.0 with a significant level of 5%. The normality of data was evaluated using the Kolmogorov-Smirnov test while the homogeneity of variance among treatments was examined using Levene's test. Comparison of means of the influence of Se fertilizer and different defoliation on plant morphology, biomass yield, chemical composition, and in vitro digestibility in chicory were tested using analysis of variance with a factorial pattern and followed by Duncan's Multiple Range Test. Moreover, the analysis of Pearson-correlation was also applied to understand the relationship among Se, nutrient content (CF and protein) on *in vitro* digestibility (DM and CP) of the chicory forage. If those parameters indicated a significant correlation, then the analysis of regression was conducted to determine the magnitude effects of Se, CF, and CP on *in vitro* digestibility (DM and CP) of the chicory forage. In this context, those parameters were evaluated in the pattern of a simple linear model.

RESULTS

The Condition of Research Area

The experiment was performed in the Forage and Pasture Science Laboratory, Faculty of Animal Science, Universitas Gadjah Mada (a latitude of -7.769238 and a longitude of 110.386085), Indonesia, from May to August 2019. The soil used in this study was regosol condition and high content pH, C-organic, and N-Total. However, P_2O_5, potassium, and C/N ratio were lower. The soil contents are presented in Table 1. Climatic conditions of the research location during the plant growth period were recorded at the Meteorology, Climatology and Geophysics Agency (BMKG) in Sleman, Yogyakarta, in 2019. The rainfall, relative humidity, temperature, and sunlight duration during the experimental period ranged from 0 mm to 33.92 mm; 75.71% to 86.33%; 23.7 °C to 27.19 °C; and 3.71 h to 9.72 h, respectively (Figure 1 and 2).

Morphological Characteristics

Measurements of plant morphology (leaf number, leaf width, and plant height) affected by different levels of Se fertilization and defoliation are shown in Table 2.

The chicory plants fertilized with Se fertilizer at a level of 7.5 mg/m^2 showed the wider leaf width ($p<0.05$) than the plants treated with Se fertilizer at the level of 3.5 mg/m^2, and the plants treated at the levels of 3.5 and 7.5 mg/m^2 showed a higher quantity of leaves and higher leaf width ($p<0.05$) than those without Se fertilization. The average number of leaves for each plant, leaf width, and plant height at the first defoliation were lower ($p<0.05$) than at the second and third defoliations. Conversely, the second and the third defoliations showed the same results ($p>0.05$). Statistical analysis showed a significant interaction ($p<0.05$) between Se levels and the defoliation affecting the number of leaves. It indicates that the level of Se fertilizer and defoliation can mutually influence the number of chicory plant leaves. Plants were treated with Se fertilizer at 3.5 and 7.5 mg/m^2. The second and the third defoliations showed a higher number of leaves plant than the other treatments and control (0 mg/m^2 Se) in the first defoliation ($p<0.05$). Chicory plants fertilized with Se at the level of 3.5 mg/m^2 harvested at the second and the third defoliations tended to be more efficient than those fertilized with Se at the level of 7.5 mg/m^2 harvested at the second and the third defoliations, although they showed the non-significant results.

Biomass Yield

The biomass yield of chicory forage was affected by the application of different Se levels and defoliations and is shown in Table 3. Results showed that biomass yield tended to increase ($p<0.05$) with the increasing level of Se application. Plants treated with Se fertilizer at the level of 7.5 mg/m^2 showed the highest biomass yield ($p<0.05$), followed by plants fertilized with Se at doses of 3.5 and 0 mg/m^2. Chicory forages harvested

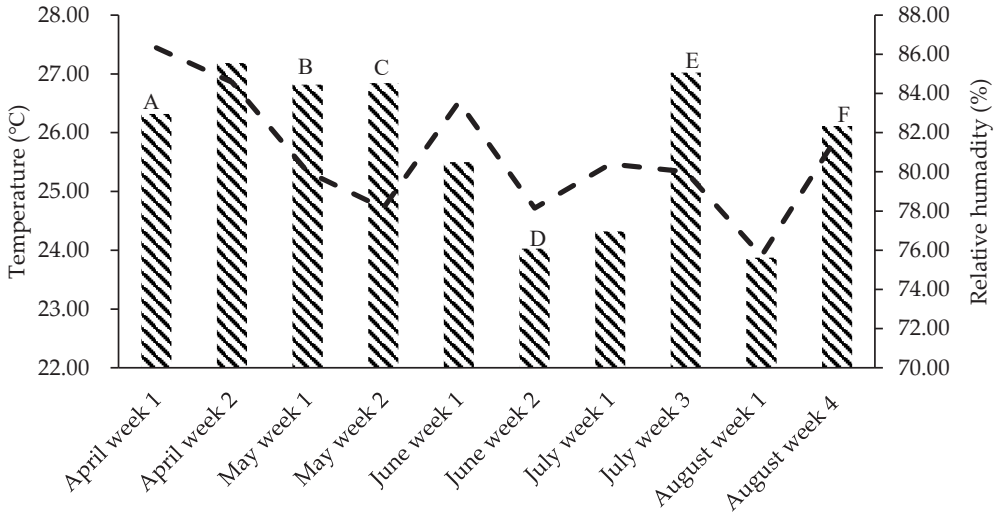

Figure 1. The temperature (N) and relative humidity conditions (- -) of the research location were based on the Meteorology, Climatology and Geophysics Agency (BMKG) in Sleman, Yogyakarta in 2019, namely land preparation (A), planting (B), fertilization (C), first defoliation (D), second defoliation (E), third defoliation (F).

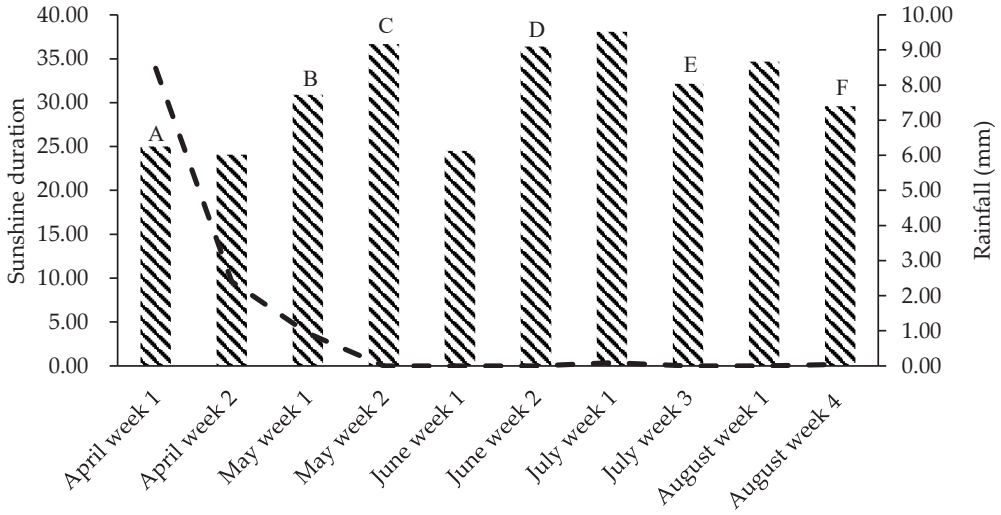

Figure 2. The sunshine duration (N) and rainfall conditions (- -) of the research location were based on the Meteorology, Climatology and Geophysics Agency (BMKG) in Sleman, Yogyakarta in 2019, namely land preparation (A), planting (B), fertilization (C), the first defoliation (D), the second defoliation (E), the third defoliation (F).

Table 2. Morphological characteristics (mean ± SD) of chicory plants fertilized with different levels of selenium at different defoliations

Variables	Defoliation	Selenium level (mg/m²)			Average
		0	3.5	7.5	
Number of leaves per plant	1	8.7±0.58^{ax}	10.3±0.58^{bx}	10.3±0.58^{bx}	9.8±0.97^{x}
	2	12.3±2.08^{y}	16.3±0.58^{y}	18.3±0.58^{y}	15.7±2.87^{y}
	3	11.3±1.53^{y}	17.0±1.00^{y}	17.7±1.53^{y}	15.3±3.24^{y}
	Average	10.8±2.10^{a}	14.6±3.24^{b}	15.4±3.94^{b}	
Leaf width (cm)	1	4.4±0.21	5.3±0.58	5.6±1.01	5.1±0.79^{x}
	2	5.7±0.46	7.2±0.74	8.1±0.30	7.0±1.14^{y}
	3	6.2±0.62	7.1±0.61	8.6±0.17	7.3±1.14^{y}
	Average	5.4±0.88^{a}	6.5±1.08^{b}	7.4±1.49^{c}	
Plant height (cm)	1	25.5±0.25	27.4±1.33	28.0±1.36	27.0±1.50^{x}
	2	35.7±0.45	31.0±26.87	48.1±0.65	38.3±15.47^{y}
	3	37.8±2.99	47.2±0.45	48.9±0.25	44.6±5.40^{y}
	Average	33.0±5.91	35.2±16.25	41.7±10.28	

Note: ^{abc} Means within a row for respective variable with different superscripts differ significantly ($p<0.05$); ^{xyz} Means within a column for respective variable with different superscripts differ significantly ($p<0.05$). SD= standard deviation.

Table 3. Fresh biomass yield (g/m²) (mean ± SD) of chicory forage fertilized with different levels of selenium at different defoliations

Defoliation	Selenium level (mg/m²)			Average
	0	3.5	7.5	
1	2146.7±32.1^{x}	2246.7±20.8^{x}	2360.0±65.0^{x}	2251.1±99.8^{x}
2	4373.3±68.1^{ay}	4536.7±136.5^{ay}	6523.3± 0.8^{by}	5144.4±1039.4^{y}
3	5601.7±27.5^{az}	6720.0±156.0^{bz}	7555.0±294.5^{cz}	6625.6±865.1^{z}
Average	4040.6±1517.3^{a}	4501.1±1940.0^{b}	5479.4±2386.6^{c}	

Note: ^{abc} Means within a row for respective variable with different superscripts differ significantly ($p<0.05$); ^{xyz} Means within a column for respective variable with different superscripts differ significantly ($p<0.05$). SD= standard deviation.

at the first defoliation showed lower biomass yield ($p<0.05$) compared to those harvested at the second and the third defoliations. The third defoliation showed a higher ($p<0.05$) biomass yield than the second defoliation. Statistical analysis showed a significant interaction between different Se levels and defoliations ($p<0.05$) affecting biomass yield. The best combination in this study was the Se fertilization level of 7.5 mg/m² with the third defoliation. It indicates that chicory forages fertilized with Se at the level of 7.5 mg/m² and harvested at the third defoliation can exhibit higher biomass yield when compared to the other treatments.

Nutrient Content of Chicory Forages

The chemical compositions of chicory forages were affected by applying different Se levels and defoliations and are shown in Table 4. Chicory forage treated with Se fertilizer at the levels of 3.5 and 7.5 mg/m² showed higher ($p<0.05$) DM, OM, CP, and Se contents than the control forage without Se fertilization. Chicory forage treated with Se fertilization at the level of 7.5 mg/m² showed higher ($p<0.05$) CP and Se contents than those plants treated with Se fertilizer at the level of 3.5 mg/m² while forage treated with Se fertilizer at the level of 3.5 mg/m² showed higher ($p<0.05$) DM and OM contents than those treated with Se fertilizer at the level of 7.5 mg/m². Plant tissue at the third defoliation contained lower DM and CF contents but higher Se content than those plants harvested at the second defoliation ($p<0.05$). The results showed a significant ($p<0.05$) interaction between Se levels and different defoliations affecting DM, OM, CF, and Se contents of chicory plants. In increasing DM, OM, and CF contents, the best combination was Se fertilizer at the level of 3.5 mg/m² harvested at the second defoliation. However, the highest Se content was found in plants treated with Se fertilizer at the level of 7.5 mg/m² and harvested at the third defoliation.

In Vitro Nutrient Digestibility of Chicory Forage

The average *in vitro* nutrient digestibility (DM, OM, CP, and CF) of chicory forage treated with different levels of Se fertilizer and harvested at a different stage of defoliation are shown in Table 5. Plants treated with Se fertilizer at the level of 7.5 mg/m² showed significantly ($p<0.05$) higher DMD, OMD, and CPD followed by those plants treated with Se fertilizer at the levels of 3.5 and 0 mg/m². Similarly, plants treated with Se fertilizer at the level of 3.5 mg/m² showed significantly ($p<0.05$) higher CFD than those control plants without Se fertilizer, but no difference was observed between chicory forages treated with Se fertilization at the levels of 0 and 7.5 mg/m². Chicory forages harvested at the first defoliation showed lower ($p<0.05$) DMD and OMD than those harvested at the second and the third defoliations. Chicory forages harvested at the first defoliation showed significantly ($p<0.05$) lower CPD than those harvested at the second defoliation, but no difference ($p>0.05$) was observed between control forage without Se fertilizer

Table 4. Nutrient content (mean ± SD) of chicory forage fertilized with different levels of selenium at different defoliations

Nutrient content	Defoliation	Selenium level (mg/m²)			Average
		0	3.5	7.5	
Dry matter (%)	1	6.3±0.34^{ax}	8.9±0.13^{cy}	7.4±0.00^{bx}	7.5±1.12^{x}
	2	7.5±0.26^{ay}	10.4±0.08^{cz}	8.7±0.41^{by}	8.8±1.28^{y}
	3	7.9±0.07^{bz}	7.8±0.03^{bx}	7.4±0.08^{ax}	7.7±0.21^{x}
	Average	7.2±0.73^{a}	9.0±1.10^{c}	7.9±0.65^{b}	
Organic matter (%)	1	77.9±0.72^{ax}	83.2±0.09^{b}	82.9±0.50^{bxy}	81.3±2.64
	2	78.9±0.51^{axy}	83.5±0.32^{c}	81.8±0.63^{bx}	81.4±2.05
	3	79.3±0.52^{ay}	83.1±0.67^{c}	82.0±1.15^{bx}	81.5±1.83
	Average	78.7±0.83^{a}	83.3±0.40^{c}	82.3±0.87^{b}	
Crude fiber (%)	1	16.6±0.57^{x}	16.1±0.22^{y}	15.6±0.20	16.1±0.54^{z}
	2	15.4±0.92^{y}	15.3±0.47^{xy}	15.7±0.23	15.5±0.55^{y}
	3	13.7±1.01^{az}	14.9±0.40^{bx}	15.3±0.08^{b}	14.6±0.88^{x}
	Average	15.2±1.44	15.4±0.62	15.5±0.23	
Crude protein (%)	1	18.6±0.58	21.8±0.82	22.3±1.09	20.9±1.88^{x}
	2	19.1±0.78	21.7±1.14	22.6±0.69	21.1±1.75^{xy}
	3	19.8±0.20	22.3±0.49	23.5±1.06	21.8±1.75^{y}
	Average	19.1±0.70^{a}	21.9±0.79^{b}	22.8±0.98^{c}	
Selenium (mcg/100g)	1	27.0±3.18^{a}	46.5±14.66^{by}	47.0±3.87^{bx}	40.2±12.55^{x}
	2	28.8±2.35^{a}	39.6±5.13^{axy}	53.4±8.34^{bx}	40.6±11.80^{x}
	3	28.4±6.88^{a}	54.9±8.65^{bz}	101.4±3.15^{cy}	61.5±32.52^{y}
	Average	28.0±4.04^{a}	47.0±11.08^{b}	67.2±26.20^{c}	

Note: ^{abc} Means within a row for respective variable with different superscripts differ significantly (p<0.05); ^{xyz} Means within a column for respective variable with different superscripts differ significantly (p<0.05). SD= standard deviation.

Table 5. *In vitro* nutrient digestibility (mean ± SD) of chicory forages fertilized with different levels of selenium at different defoliations

In vitro digestibility (%)	Defoliation	Selenium level (mg/m²)			Average
		0	3.5	7.5	
Dry matter	1	78.6±0.11^{ax}	82.9±0.03^{bx}	85.6±0.24^{c}	82.3±3.07^{x}
	2	80.9±0.11^{az}	83.6±0.04^{bz}	85.5±0.17^{c}	83.3±2.00^{y}
	3	80.5±0.06^{ay}	83.1±0.12^{by}	85.7±0.11^{c}	83.1±2.24^{y}
	Average	80.0±1.09^{a}	83.2±0.31^{b}	85.6±0.17^{c}	
Organic matter	1	76.1±0.42ax	82.4±0.07bx	84.7±0.16c	81.1±3.86^{x}
	2	78.1±0.13ay	82.7±0.39bxy	84.6±0.14c	81.8±2.90^{y}
	3	78.5±0.05ay	83.1±0.55by	85.0±0.24c	82.2±2.90^{z}
	Average	77.6±1.14^{a}	82.8±0.46^{b}	84.8±0.23^{c}	
Crude fiber	1	61.6±0.15	62.5±0.09	62.6±0.06	62.2±0.49
	2	62.2±0.40	62.5±0.16	62.0±0.63	62.2±0.42
	3	62.1±0.46	62.4±0.16	62.1±0.91	62.2±0.53
	Average	61.9±0.41^{a}	62.4±0.12^{b}	62.2±0.61^{ab}	
Crude protein	1	69.3±0.31	72.2±0.06	74.7±0.50	72.1±2.38^{x}
	2	69.5±0.46	72.6±0.89	75.7±0.92	72.6±2.7^{y}
	3	69.4±0.41	72.5±0.11	75.7±0.01	72.5±2.69^{xy}
	Average	69.4±0.35^{a}	72.4±0.49^{b}	75.4±0.71^{c}	

Note: ^{abc} Means within a row for respective variable with different superscripts differ significantly (p<0.05); ^{xyz} Means within a column for respective variable with different superscripts differ significantly (p<0.05). SD= standard deviation.

treatment and chicory forages fertilized with Se at the level of 7.5 mg/m². In addition, there was no difference in CFD of plant tissue among the application of Se fertilizer at different levels. A significant interaction was observed between the level of Se fertilizer and stages of defoliation (p<0.05) in affecting DMD and OMD of chicory forages. The best combination for DMD and OMD was observed with a Se fertilizer at the level of 7.5 mg/m² harvested at the third defoliation.

The pH and NH_3 content in chicory forages affected by the application of different levels of Se fertilization and defoliation are shown in Table 6. Chicory forages treated with Se fertilizer at the level of 3.5 mg/m² showed significantly (p<0.05) higher pH than those treated with Se fertilization at the levels of 0 and 7.5 mg/m². The NH_3 content in chicory forage decreased (p<0.05) with the increasing level of Se application. Chicory forages without Se fertilization showed the highest NH_3 content, while chicory forages treated with

Se fertilizer at the level of 7.5 mg/m^2 showed the lowest NH_3 content. However, there was no difference observed on pH and NH_3 contents among the plant tissues of chicory forages treated with Se fertilizer at the levels 0, 3.5, and 7.5 mg/m^2.

Correlation Coefficient Between Selenium with Nutrient Contents and *In Vitro* Digestibility

Correlation coefficient values, regression line equation, and determination values between Se and nutrient contents (CF and CP) and *in vitro* digestibility (DM and CP) of plants are shown in Table 7. The correlation value describes the degree of relationship with the correlation coefficient interpretation. The coefficient of determination (R^2) shows the magnitude of the influence of the value of the measured variables (Se, nutrient content, and nutrient digestibility) of chicory forages. Results showed that CP content correlated with CP digestibility with the highest regression equation value. The regression analysis showed a constant of 1.037 which means that the digestibility coefficient of CP was 1.037. The regression coefficient for CP content was 1.427. This value illustrated that every 1% increase in CP content would increase DM digestibility by 1.427%.

DISCUSSION

The Condition of Research Area

The soil used in this study was regosol with the contents as presented in Table 1. Delgado-Moreno *et al.* (2010) stated that regosol soil is a newly formed soil type characterized by a large amount of stone and gravel content that has not been completely weathered. Regosol soil is a very young soil that is at the initial stage of development (Martí *et al.*, 2011). The treatment at the land preparation stage is aimed to optimize soil conditions to maximize plant productivity. Plant productivity is influenced by soil fertility, namely the completeness and adequacy of soil nutrients. Levels of pH do no affect Se uptake by plants (Száková *et al.*, 2015). The environment influences Chicory plant growth, including sunlight, air temperature, and relative humidity. Fluctuating environmental conditions occurred every day during the study, both in the morning, afternoon, and evening. In general, the sunlight intensity during the day is stronger than in the morning and evening. Also, the average air temperature during the day is generally higher than the air temperature in the morning and evening. However, the relative humidity during the day is lower than in the morning and evening.

Plant Morphological Characteristics

The heights of chicory forages at the second and the third defoliations were higher than at the first defoliation. Zaini *et al.* (2021) reported that the chicory forages in the first defoliation showed fewer leaves than at the second defoliation, with 8 and 9 leaves per plant, respectively. The results also showed that the height of chicory forages at the first defoliation was 36.5 cm, and in the second defoliation was 39.6 cm, meaning that the result of planting chicory with the second defoliation was higher than the first defoliation. It was further observed that chicory forages in the first defoliation

Table 6. The pH and NH_3 content (mean ± SD) of chicory plants fertilized with different levels of selenium at different defoliations

Variables	Defoliation	Selenium level (mg/m^2)			Average
		0	3.5	7.5	
pH	1	7.2± .06	7.3±0.05	7.2±0.10	7.2±0.06
	2	7.2±0.05	7.3±0.02	7.2±0.14	7.2±0.10
	3	7.2±0.07	7.3±0.06	7.2±0.06	7.2±0.07
	Average	7.2±0.05^a	7.3±0.04^b	7.2±0.09^a	
NH_3	1	22.7±0.49	20.4±0.23	19.0±0.33	20.7±1.64
	2	22.6±0.37	20.3±0.16	18.9±0.48	20.6±1.66
	3	22.9±0.62	20.3±0.17	19.3±0.44	20.8±1.65
	Average	22.7±0.45^c	20.3±0.16^b	19.0±0.40^a	

Note: ^{abc} Means within a row for respective variable with different superscripts differ significantly ($p<0.05$); SD= standard deviation.

Table 7. Correlation coefficient values, regression line equation, and determination coefficient values between selenium, nutrient content (crude fiber and protein) on *in vitro* digestibility (dry matter and crude protein) of the chicory forage fertilized with different levels of Se at different defoliations

Variables	R	R^2	Regression equation
Selenium-DMD	0.720	0.518	Y_1=79.158+0.079X_1
CF-DMD	0.844	0.713	Y_1=16.454+4.143X_2
CP-DMD	0.941	0.886	Y_1=53.307+1.391X_3
Selenium-CPD	0.773	0.597	Y_2=68.236+0.088X_1
CF-CPD	0.930	0.865	Y_2=-3.091+4.706X_2
CP-CPD	0.936	0.876	Y_2=42.037+1.427X_3

Note: DMD= Dry matter digestibility, CPD= crude protein digestibility, CF= crude fiber, CP= crude protein, Y_1= dry matter digestibility, Y_2= crude protein digestibility, X_1= selenium, X_2= crude fiber, X_3= crude protein, R^2= coefficient of determination, R= Correlation coefficient.

showed lower plant height than in the second defoliation, which was 37.8 cm and 38.8 cm, respectively. The reason for the second and the third defoliations showing the greater height can be attributed to the fact that in the first defoliation, the plants were cut, which leads to plant stress. Post defoliation N remained the major source for leaves regrowing (Meuriot *et al.*, 2018). When the cut plant regrowth, meristem conditions will stimulate faster growth, and this event being a genetic response. Although encouraging the development of the secondary shoot, this defoliation process suppresses the development of the primary shoots.

Selenium is chemically similar to sulfur (S) (El Mehdawi & Pilon-Smits, 2012). Selenium in the soil will be transported by plant roots and then converted into organic Se through the S metabolic pathway (Kolbert *et al.*, 2019). Selenium that enters plants is then translocated to leaves and metabolized in plastids through assimilation pathways (Trippe III & Pilon-Smits, 2021). This causes the higher Se levels in chicory plants to increase plant growth. Selenium has a positive effect on plant growth and development. In addition, the number and width of chicory leaves are affected by their nutrient adequacy. Selenium also helps in the plant's photosynthesis process, which is correlated with elemental S. Photosynthesis will run perfectly if its requirements are met (Boghdady *et al.*, 2017). This requirement can be supplied from the Se administration to chicory plants. In photosynthesis, the main targets are the thylakoid membrane, PS II, photosynthetic pigments, and carbon fixation reactions (Hawrylak-Nowak *et al.*, 2018). Selenium could not significantly affect the height of chicory plants due to the high Se content in the soil is not being fully absorbed. Selenium content and dietary sources vary from plant to plant. The factors that influence this are the Se uptake, the accumulating capacity of the plant, the Se content in the soil, and the presence of other elements in the soil that affect plant height (Winkel *et al.*, 2015; Gupta & Gupta, 2017).

Biomass Yield

The application of Se in chicory forages can increase biomass yield and increase plant metabolic activity, especially antioxidant activity, leading to increase plant tolerance under unfavorable growing conditions (Feng *et al.*, 2013; Cranston *et al.*, 2016). Chicory forages fertilized with Se at the levels of 3.5 and 7.5 mg/m^2 showed higher biomass yields ($p<0.05$) than chicory without Se fertilization (control), probably because Se can prevent the decrease in chicory forages production. The Se is correlated with the percentage of water content in the upper part of the plant; it contributes to the increasing level of the hydration of leaf tissues and prevents the excessive water loss of the leaves from plant growth. In their study, Hawrylak-Nowak *et al.* (2018) reported that control plants (without Se fertilization) grown under excessive heat showed a significant decrease in fresh production of 47% and dry weight of 29%. Since the application of Se in chicory forages (as reported by Feng *et al.*, 2013) increases plant tolerance, it would be better to add Se to the plants mentioned above grown under excessive heat to improve their conditions. Chicory forages at the second and the third defoliations showed a higher biomass yield ($p<0.05$) than at the first defoliation. The results of this study are in line with the findings of Zaini *et al.* (2021), where the second defoliation of chicory with a yield of 5.93 tons/ha was taller than the first defoliation with a yield of 4.92 tons/ha. The Se fertilization at 7.5 mg/m^2 and harvest at the third defoliation gave the best biomass yield. Fresh weight can provide a measure of the quality of plant growth and crop yield and is associated with photosynthesis as well as nutrient assimilation (Mudhita *et al.*, 2016).

Nutrient Content of Chicory Forage

Averages DM, OM, CP, and Se contents of chicory forage treated with Se fertilizer at the levels of 3.5 and 7.5 mg/m^2 were higher ($p<0.05$) than that chicory forages without Se fertilizer. This result is due to the physiological conditions and Se uptake being effectively accumulated by the chicory plants treated with Se fertilizer. The addition of Se can also cause changes in chemical quality, including DM. When Se fertilizer is given at a level of 3.5 mg/m^2, the DM content will be maximized. However, if the level of Se fertilizer exceeds 3.5 mg/m^2, the DM content will decrease. The limited movement of ions in the soil allows the exchange of Se ion with nitrogen (N) ion in the roots. The Se ion that has entered the roots will be accumulated throughout the plant body tissues. The distribution of ions throughout the tissues is highly dependent on the plant physiological conditions. The DM content decreased with the addition of Se fertilizer at 7.5 mg/m^2 but could still be found in the roots. Schiavon *et al.* (2017) stated that Se in high doses could interfere with nitrogen assimilation, which causes a decrease in nitrogen which negatively affects the structural functions of the plant. After all, nitrogen is an essential nutrient needed by plants to increase the growth of tillers and leaves, especially in the phase of vegetative growth. Differentiated Se doses in the soil cause an increase in Se content in the plant DM (White, 2016).

The interaction of Se with several metabolic pathways in the plants has considerable effects on chicory forage growth; Se can increase the DM in chicory. The administration of Se in biofortificated *Valerianella locusta* L. can increase crop yields and nutrient content (Hawrylak-Nowak *et al.*, 2018). Plants given Se accumulate more phenolic compounds that are important for plant health than plants without Se supplementation. Al-Tameemi *et al.* (2018) stated that Se administration at the increased dose from 0 to 40 gm h^{-1} in the soil could increase the dry weight of maize from 16.91 to 18.01 gm pot^{-1} with a percentage increase of 2.90 to 6.51%. Gupta & Gupta (2017) explained that the Se entering the plants would be distributed to all parts of the plant organs in the form of protein-conjugated compounds (selenoproteins) such as SeCys, SeMet, and other Se-proteins. These selenoproteins have an impact on increasing the CP content in the plants. Also, based on the study results, the application of Se fertilizer at doses of 3.5 and 7.5 mg/m^2 specifically increased the CP content in the chicory forages. The increase of Se levels

in the soils is always accompanied by an increase in the accumulation of Se in chicory leaves (Germ *et al.,* 2020). Phytofortification with Se markedly increases the Se content of plants and thus can help avoid Se deficiency (Gupta & Gupta, 2017; Newman *et al.,* 2019). Selenium application in chicory increases thermo-tolerance in plants through antioxidant enzymes such as guaiacol peroxidase (GPOX), catalase (CAT), and glutathione reductase (GSH) (Hawrylak-Nowak *et al.,* 2018). In livestock, the minimum requirement of Se is 0.05 to 0.10 mg/kg DM, while the concentration of toxic Se in feed is 2 to 5 mg/kg DM.

In Vitro Nutrient Digestibility of Chicory Forages

Average DMD, OMD, CPD, and CFD of plants treated with Se fertilization at the levels of 3.5 and 7.5 mg/m^2 were higher ($p<0.05$) than that chicory forages without Se supplementation. This increase was influenced by the increase in the DM after Se application (Table 4). Selenium affects plant chlorophyll, which increases the plants' biosynthesis process (Saffaryazdi *et al.,* 2012). The process of biosynthesis in plants causes an increase in the nutrient content of the plants. The nutrient content is positively correlated with digestibility. The higher the soil Se level, the higher the accumulation of Se in the plants. Mazej *et al.* (2008) stated that in their research in which chicory forages with Se supplementation at the dose of 7 mg/L, organic Se content was increased at the harvest age of 41 days (SeMet 4.2%-8.4%).

Correlation Coefficient Between Selenium and Nutrient Contents on *In Vitro* Digestibility of Chicory Biomass

The correlation value (R) of Se and the DM digestibility was 0.720. From the output, the coefficient of determination (R^2) of 0.518 implied that the effect of Se on DM digestibility was 51.8%. The constant 79.158 showed that the digestibility coefficient value of DM was 79.158. The Se regression coefficient of 0.079 showed that for every addition of 1 unit of Se, the DM digestibility increased by 0.079. The correlation coefficient between CF and DM digestibility was 0.844, with a perfect positive correlation degree (between 0.81 to 1.00), in line with McDonal *et al.* (2002), who stated that the CF content in feed ingredients would affect the digestibility or degradation of DM. High CF can reduce digestive performance (Mayulu *et al.,* 2013). The higher the CF content, the lower the DM degradation. One factor affecting digestibility is the availability of nutrients as food for the growth of rumen microbes. Nasrollahi *et al.* (2015) stated that feed digestibility depends on the activities of rumen microbes which play a role in the fermentation process. Energy availability is an essential factor in accelerating the growth and proliferation of rumen microbes to degrade organic components and increase feed ingredients' digestibility (Kung *et al.,* 2018). The coefficient of determination on the content of CF and digestibility of DM was 0.713. Thus, the effect of CF content on the digestibility of DM was 71.3%. The constant 16.454 showed that the digestibility coefficient value of DM was 16.454. The regression coefficient for CF content was 4.143. This value illustrated that every 1% increase in CF content would impact decreasing DM digestibility by 4.143%. High CP content will also increase CP digestibility. The higher the CP level in the ration, the higher the palatability of livestock and digestibility, so it can be interpreted that with high CP levels, digestibility is high (Astuti *et al.,* 2012).

CONCLUSION

For almost all of observed parameters, plants treated with Se showed higher and better values than those plants without Se supplementation. Plants at the third defoliation showed the best results, followed by the second and the first defoliations. It was observed that the Se content in chicory plants is directly proportional to the amount of Se administered. The best combination of adding Se to chicory forages was the Se level of 7.5 mg/m^2 at the third defoliation; it gave the most improved growth parameters, biomass yield, CP, *in vitro* nutrient digestibility, and Se content.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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