

Plant Growth and Morphological Characters of *Sonchus arvensis* L. from Different Chicken Manure Rates and Harvest Intervals with Ratooning Practices

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Abstract

Sonchus arvensis L., commonly known as perennial sow thistle, field milk thistle, among other names, is a weedy species that has medicinal values. Belongs to the Daisy family (Asteraceae), *S. arvensis* has a rosette form and it can be harvested more than once. The determination of harvest interval for ratoon of *S. arvensis* is necessary because ratooning can save time to produce the consecutive plant biomass, but the second harvest must be conducted at the correct time as it will determine the quantity and quality of leaves. As source of herbal medicine, cultivation of *S. arvensis* with organic fertilizer is preferred, and determination of organic fertilizer rates is also important for ratooning. The purpose of the study was to evaluate the plant growth response and yield with different chicken manure rates and different harvest intervals. The field experiment was conducted at IPB experimental station in Cikarawang, Darmaga Bogor, Indonesia. The study used a randomized complete block design with different manure rates (0, 7, 14, 21 ton.ha⁻¹) and various harvest intervals (6, 8, and 10 weeks after the first harvest) as treatments. The results showed that for the first harvest, the effect of chicken manure rates was linear almost on all variables. For the ratoon (second harvest), manure rate of 14 ton.ha⁻¹ (for first harvest) added with 4 ton.ha⁻¹ (after first harvest) was enough for leaf production. The results indicate that ratoon should be harvested at 10 weeks after the first harvest to obtain the highest yield. The interaction between manure rates and harvest interval was not significant for growth variables of the second harvest.

Keywords: axillary shoot, chlorophyll, flavonoid, leaf nutrient.

Introduction

Sonchus arvensis L. (family Asteraceae) also known as perennial sow thistle, and locally known in Indonesia as *tempuyung*, *jombang*, *lalakina*, *galibug*, *lempung* is a medicinal species known for its anthelmintic, antibacterial (Wadekar et al., 2012), and antioxidant properties (Khan, 2012). The leaves of *S. arvensis* are also commonly used as diuretic, lithotropic, and antiurolithic source (Dhianawaty et al., 2004). Several chemical compounds have been identified in the extract of *S. arvensis* including flavonoids (Khan, 2012) and triterpenoids (Putra et al., 2013) for antioxidant activity, and triterpenoids for antibacterial effects (Rumondang et al., 2013).

Sonchus arvensis contains a root rosette and it can be harvested once or more after ratooning. Ratooning is a process of recovery and regrowth of new shoots following harvests of the above-ground portion of the crops, leaving the roots and growing shoot apices intact. Ratooning practice will produce a fresh crop in the following season. The success of ratooning can save the time to produce the next plant biomass. Ratooning has been practiced for cultivation of rice (Kailou Liu, 2012; Mareza et al., 2016; Shiraki et al., 2020), sugar cane (Hassan et al., 2017; Sakaigaichi et al., 2017; Komala et al., 2018), sorghum (Ardiyanti et al., 2019), and amaranthus (Samuel et al., 2019). This vegetable harvest practice is also called multiple harvest, continuous harvest, or 'cut and regrow'. The proper cultivation technique, namely the amount of fertilizer and time of harvest, will determine the success of ratooning of *S. arvensis* to obtain high yield and good characteristics of leaves.

As source of herbal medicine, cultivation of *S. arvensis* without synthetic chemicals input is preferred. The nutrient content of organic fertilizer is inconsistent, particularly depending on type of animals. Chicken

(from laying hen) manure is commonly used as organic fertilizer because it decomposes easily and contains high P nutrient. One analysis shows the level of N, P, and K of laying hen manure are 1.43, 2.49, and 1.07% (Kurniawati et al., 2017). There is no yet consistent recommendation of fertilizer rates for *S. arvensis*. The use of inorganic fertilizer 20 t ha⁻¹ manure + 125 kg.ha⁻¹ urea + 125 kg.ha⁻¹ SP36 + 125 kg.ha⁻¹ KCl was found to be the fertilizer rates for highest leaf yield of *S. arvensis* (Nurhayati, 2016). With goat manure, the highest leaf yield was obtained from 20 ton manure ha⁻¹ (P > 0.05).

Growth phases of *S. arvensis* have been identified, they are vegetative stage (before stem begins to grow); early generative stage (flower buds begin to form); and maximum generative stage (full flowering stage) (Hasan et al., 2017a). The period of every phase can be determined by soil nutrient supply to the plant.

Sonchus arvensis leaves are mostly harvested once. As such, data on harvest time for ratoon of *S. arvensis* is lacking, making it necessary to conduct a study to determine optimum harvest time. The interval of harvest time between the first and second harvest will be evaluated based on the plant growth phase that determines the leaf yield and flavonoid content. Leaf production and flavonoid content were high at plant phase of early flowering (Hasan et al., 2017a, 2017b). The objective of this study was to evaluate the response of *S. arvensis* plants and plant yield using different chicken manure rates and harvest intervals. Consequently, by doing this study, chicken manure rates that support yield of *S. arvensis* ratoon was determined.

Material and Methods

The field experiment was conducted on August to December 2015 on clay-loam latosol soil at IPB experimental station, Cikarawang, Bogor, Indonesia (\pm 250 m asl, 6°30'-6°45' S, 106°30'-106°45' E). The analysis of flavonoid was performed at Biopharmaca Tropical Research Center, Bogor Agricultural University, Indonesia.

In this study factorial treatment in a completely randomized block design with 3 replicates was used. Two factors were considered, namely rates of chicken manure treatment (0, 7, 14, 21 ton.ha⁻¹) and harvest intervals (6, 8, and 10 weeks after the first harvest). Additional manure of 0 (control), 2, 4, and 7 ton.ha⁻¹ (approximately one third of the first rates), respectively, was provided after the first harvest to ensure that plants were still adequately supplied with soil nutrients needed.

Sampling and Harvesting

Seeds of *S. arvensis* collected from wild plants in IPB campus, were sown on trays filled with mixture of soil, coco pit, and biochar from rice hull (1:1:1 v/v/v). The taxonomy identity of *S. arvensis* was confirmed based on the similarity of described plant morphology (Wahyuni et al., 2019). Two-week-old sprouts were transplanted into seedling trays filled with mixture of manure and biochar from rice hull (1:1 v/v). Eight-week-old seedlings were finally transplanted into plots of 1.8 m x 2.6 m size, limed with dolomite of as much as 1 ton.ha⁻¹ and laid with manure two weeks prior to transplanting. Planting distance of 40 cm x 30 cm was established for the 24 plants that were placed on each plant bed/plot.

The first harvest for all treatments was done at 7 weeks after transplanting (WAT). The succeeding harvests were carried out according to the harvest interval treatments (6, 8, or 10 weeks after the first harvest). Leaves, including the ones near the ground, and stem were collected, by using pruning shears. Part of the stem (2 cm from soil surface) was retained. All harvested parts of the plants were washed, separated, and weighed to obtain the fresh weight of the samples. The samples were air dried for 2 days to reduce water content, then oven dried for 48 hours at 50°C. Leaf NPK contents were analyzed from dried samples. Nitrogen content was determined using Kjeldahl method, while phosphorus and potassium contents were determined using HNO₃/UV-VIS Spectrophotometer and HNO₃/AAS, respectively. Average of three plants were used to determine fresh and dry weight of individual plant biomass, but all plants in the plot were used to measure biomass yield per plot. Data were recorded on the leaf size, leaf number, leaf nutrients, leaf yield, leaf chlorophyll content, and flavonoid content.

Analysis of Bioactive Compounds

Dried leaf samples were used to measure the content of bioactive compounds. Leaf extraction (Makkar et al., 1988) with slight modifications was conducted. Dried leaves were added with 50% methanol (1:3; v/v), then ground using mortar. The extracts were incubated for 24 hours at 4°C, then centrifuged for 10 minutes at a speed of 10.000 rpm. The supernatant was analyzed for total flavonoid content and antioxidant activity.

Total flavonoid was determined according to the methods described in Lamaison and Carnet (1990), i.e. 50 μ L extract was dissolved in 250 μ L methanol, then added with 1200 μ L distilled water and 90 μ L 5% NaNO₂, the solution was then incubated for 5 minutes. After 5 minutes, the mixture was added with

90 μL 10% AlCl_3 , then incubated for 6 minutes. The resulting solution was added with 600 μL 1 N NaOH and 840 μL distilled water. The absorbance was measured at wavelength of 415 nm with quercetin as standard.

Statistical Analysis

Data were processed using analysis of variance (ANOVA). Mean separations were performed through the Duncan multiple range test, with reference to 0.05 probability level, using SAS version 9.2. Data of variables obtained until the first harvest were analyzed as single factor namely, rates of manure.

Results

Different chicken manure treatment and varying the interval of harvest affected *S. arvensis* plants in terms of growth phases (Figure 1). Although the experiment was designed with two factors of treatment, the plant growth from planting until the first harvest were only affected by manure treatment. Manure rates and harvest internal treatments started to affect plant growth after the first harvest, i.e. at 7 weeks after treatment (WAT); plants applied with 21 ton manure.

ha^{-1} had started the generative phase as indicated by the emergence of flower buds.

At the second harvest, different harvest intervals resulted in different growth stages of the plant (Figure 1). The plants with 6-week harvest interval were still at vegetative phase, while plants with 8-week harvest interval started bolting indicating the start of generative stage. The ones with 10-week harvest interval had started flowering during second harvest (Figure 1).

At the first harvest, most plants did not form axillary shoot. However, the first harvest had induced the formation of new axillary shoots and the plants were no longer as individual basal-rosette plant. At the 3rd day after the first harvest, the axillary shoots started to emerge from the remaining plant base (Figure 2a). New rosette plants with growing leaves could be seen at 14 days after first harvest (Figure 2b).

The application of chicken manure improved plant growth and performances before the first harvest. The values of plant morphological characters increased, particularly with the application of 21 ton manure ha^{-1} ; they increased by 29-219% compared to those of without manure (control) (Table 1).

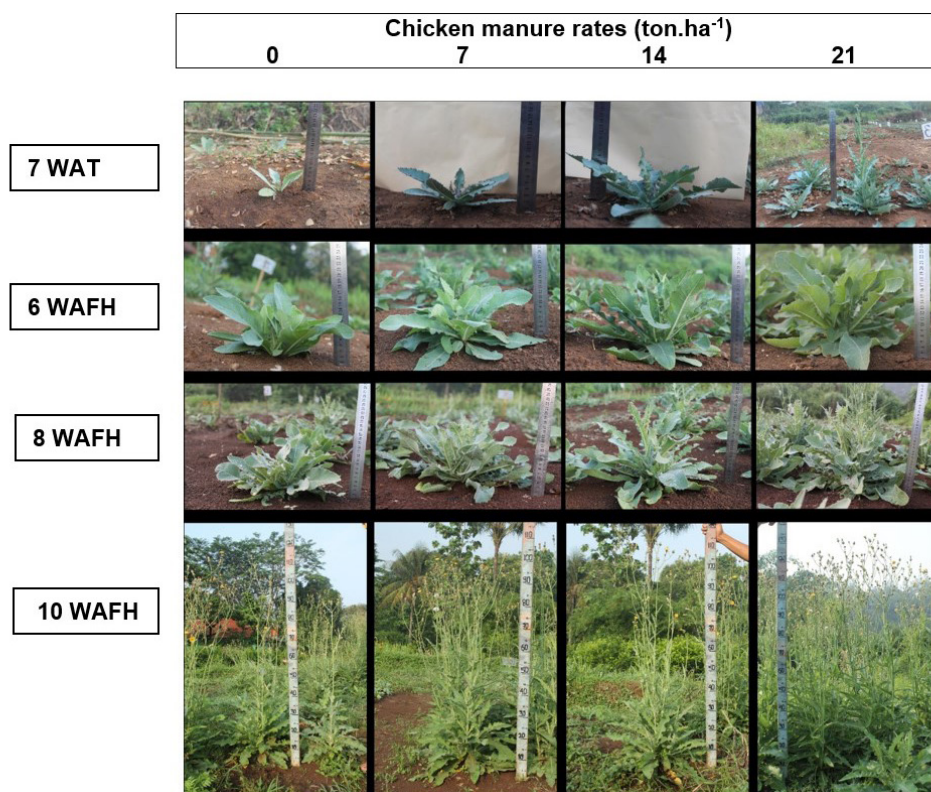


Figure 1. Growth and morphology of *Sonchus arvensis* plants treated with different chicken manure rates (0, 7, 14, and 21 $\text{ton} \cdot \text{ha}^{-1}$) and harvested at different intervals (6, 8, and 10 weeks after the first harvest). WAT = Weeks after transplanting, WAFH = weeks after first harvest.

Plant growth was shown particularly by increasing leaf numbers per plant where leaf numbers increased by 134-210%, from 2 to 6 weeks after transplanting, which in turn increased plant height by 90-443% (Table 1). At the same period, leaf sizes only increased by 16-40%. For the first harvest, there was no optimum rates of manure that resulted in the maximum values of plant morphological characters (Table 1).

The application of chicken manure also significantly increased the values of plant morphological characters after the first harvest. The first application

of 14 ton manure.ha⁻¹ plus 4 ton.ha⁻¹ after the first harvest increased the growth by 9.4-38.8 %; whereas the application of 21+7 ton manure.ha⁻¹ increased it by 14.5-54.3% compared to control (Table 2 and 3).

The first harvest had resulted in the formation of axillary shoots that became new rosette plants but they were still attached to each other. The application of 14 (+4) ton manure.ha⁻¹ increased the number of axillary shoots by 50% compared to those of control (Table 2). Each new rosette plant has 4-5 single leaves, therefore plants after the first harvest had

Table 1. Morphology of *S. arvensis* treated with different chicken manure rates before the first harvest.

Chicken manure rates (ton. ha ⁻¹)	Times of observation (weeks after transplanting)					
	2		4		6	
Leaf number						
0	6.1 ± 0.9	c	11.3 ± 5.0	b	15.8 ± 7.1	b
7	6.7 ± 0.8	bc	13.2 ± 5.0	b	16.9 ± 6.4	b
14	7.3 ± 0.5	ab	13.7 ± 2.7	b	17.2 ± 3.7	b
21	8.1 ± 1.2	a	18.4 ± 5.8	a	25.2 ± 7.9	a
Significance	**		**		**	
Regression test	L**		L**		L**	
Length of the largest leaf (cm)						
0	9.42 ± 1.75	b	11.81 ± 2.51	b	13.14 ± 2.65	b
7	10.82 ± 2.16	b	13.37 ± 3.05	b	13.95 ± 3.30	b
14	11.29 ± 1.35	b	14.10 ± 1.10	b	14.44 ± 1.40	b
21	14.78 ± 3.33	a	16.60 ± 3.81	a	18.33 ± 3.10	a
Significance	**		**		**	
Regression test	L**		L**		L**	
Width of the largest leaf (cm)						
0	3.74 ± 0.57	b	4.22 ± 0.91	b	4.60 ± 0.93	b
7	3.95 ± 0.67	b	4.50 ± 1.04	b	4.70 ± 0.94	b
14	4.23 ± 0.57	b	4.56 ± 0.34	b	4.89 ± 0.25	b
21	5.12 ± 1.02	a	5.69 ± 0.97	a	5.96 ± 0.83	a
Significance	**		**		**	
Reg test	L**		L**		L**	
Canopy diameter (cm)						
0	16.47 ± 3.66	b	20.99 ± 5.60	b	22.80 ± 7.19	b
7	18.83 ± 4.69	b	23.16 ± 5.78	b	25.04 ± 8.01	b
14	19.71 ± 2.49	b	23.97 ± 2.63	b	26.00 ± 2.69	b
21	26.67 ± 7.54	a	32.33 ± 8.10	a	32.73 ± 6.23	a
Significance	**		**		**	
Regression test	L**		L**		L**	

Notes: Numbers with the same letters in the same column for each variable were not significantly different based on DMRT at α=5%. [†] Transformation with $\sqrt{N} \times 0.5$, s.d. = standard deviations (n=9). Numbers following ± indicate standard deviations of n=3

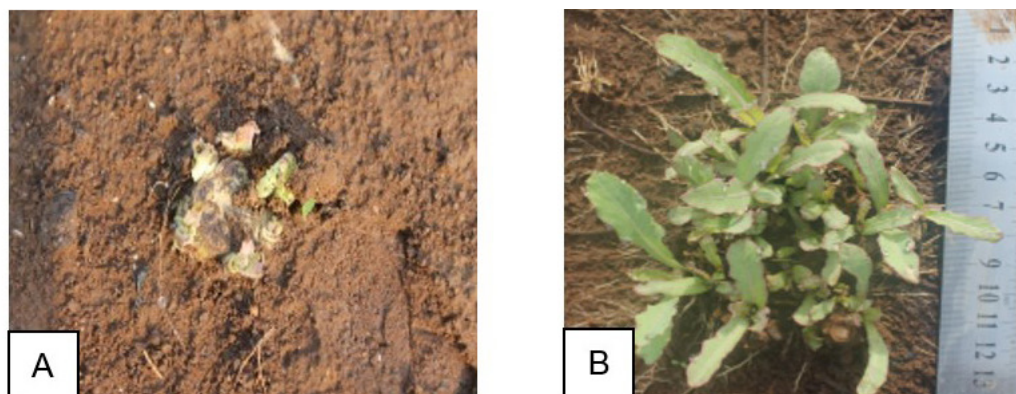


Figure 2. Axillary shoot formation at 3 days (a) and growing leaves at 14 days after the first harvest (b)

Table 2. Plant growth performance during the second harvest with different chicken manure rates and harvest intervals

Chicken manure rates (ton.ha ⁻¹)	Shoot number	Plant height (cm)	Canopy diameter (cm)
0	10.50 ± 5.70 b	48.10 ± 37.65 bc	40.47 ± 11.34 b
9	9.60 ± 5.80 b	37.12 ± 27.65 c	40.34 ± 13.85 b
18	15.10 ± 7.80 a	61.75 ± 41.80 ab	47.48 ± 11.36 a
28	16.20 ± 9.00 a	72.81 ± 49.01 a	49.48 ± 12.14 a
Significance	**	**	*
Regression test	ns	ns	ns
Harvest intervals (weeks after first harvest)			
6	6.00 ± 1.10 c	11.39 ± 4.63 c	30.31 ± 6.33 c
8	13.10 ± 5.50 b	55.38 ± 24.47 b	47.42 ± 6.59 b
10	18.70 ± 7.30 a	93.36 ± 27.39 a	54.37 ± 7.68 a
Significance	**	**	**
Interaction	ns	ns	ns

Notes: **= significant at $P < 0.01$, *= significant at $P < 0.05$, ns = not significant. Values with the same letters in the same column for each variable were not significantly different based on DMRT at $\alpha=5\%$.

higher number of leaves (51.6-79.1) (Table 3) than those before the first harvest (15.8-25.2) (Table 1).

Leaf Yield

At the first harvest, fresh and dry weight of leaves with 21 ton manure.ha⁻¹ were not significantly different from those of control. However, at the second harvest, the increased manure rates to 14 (plus 4 ton after first harvest) and to 21 (plus 7 ton after first harvest) ton.ha⁻¹ significantly increased fresh and dry weight of *S. arvensis* leaves by 38-50% and 39-60%, respectively (Table 4).

Longer harvest intervals increased *S. arvensis* yield. Delaying harvest by 2 weeks increased fresh weight and dry weight of leaves by 210 and 99%, respectively, compared to those from 6-week harvest after the first harvest. Second harvest at 10 weeks after the first harvest had increased fresh weight and

dry weight of leaves by 399 and 330%, respectively, compared to those from 6-week harvest after the first harvest (Table 4).

Leaf Nutrient, Chlorophyll, and Flavonoid Content

During the first and second harvests, there were no significant differences of N, P, K in leaves among different manure rate treatments. The influence of harvest intervals showed that longer interval lowered the N but increased the K contents of leaves (Table 5). Relationship between leaf nutrient with leaf yield of first and second harvest shows that high leaf weight was reached when N was approximately 3.2%, when P was approximately at 0.3%, and K at 4.70% (Figure 3). There were no significant differences of chlorophyll and flavonoid contents of leaves among manure rates and harvest interval (Table 6).

Table 3. Leaf number and size during the second harvest based on different chicken manure rates and harvest intervals

Chicken manure rates (ton.ha ⁻¹)	Leaf number	Leaf length (cm)	Leaf width (cm)
0	51.60 ± 26.10 b	21.77 ± 5.06 b	6.55 ± 1.21 bc
9	48.40 ± 24.50 b	21.06 ± 6.46 b	6.19 ± 1.35 c
18	71.60 ± 31.80 a	24.45 ± 5.06 ab	7.16 ± 0.94 ab
28	79.10 ± 36.00 a	28.43 ± 9.33 a	7.50 ± 0.93 a
Significance	**	**	**
Regression test	L*	L*	ns
Harvest intervals (weeks after first harvest)			
6	34.30 ± 7.00 c	16.55 ± 3.03 c	5.63 ± 0.98 c
8	59.30 ± 20.40 b	24.76 ± 2.94 b	7.11 ± 0.71 b
10	91.60 ± 28.40 a	29.64 ± 6.13 a	7.70 ± 0.65 a
Significance	**	**	**
Interaction	ns	ns	ns

Notes: **= significant at P < 0.01; *= significant at P < 0.05; ns = not significant. Values with the same letters in the same column for each variable were not significantly different based on DMRT at α=5%

Table 4. Fresh and dry weight of leaves during first and second harvest with different chicken manure rates and harvest intervals.

Chicken manure rates (ton.ha ⁻¹)	First harvest		Second harvest	
	Leaf FW	Leaf DW	Leaf FW	Leaf DW
g per plant				
0 (+ 0)	14.67 ± 7.65	3.00 ± 1.58	122.62 ± 91.92 b	14.82 ± 12.05 b
7 (+ 2)	15.96 ± 12.03	2.68 ± 1.92	117.11 ± 75.42 b	14.16 ± 9.42 b
14 (+ 4)	16.09 ± 7.01	2.69 ± 1.12	169.49 ± 93.76 a	20.62 ± 12.34 a
21 (+ 7)	23.71 ± 9.95	3.70 ± 1.42	183.50 ± 107.55 a	23.72 ± 13.36 a
Significance	ns	ns	**	**
Regression	ns	ns	ns	ns
Harvest intervals (weeks after first harvest)				
6			48.87 ± 27.80 c	7.54 ± 4.98 c
8			151.72 ± 51.81 b	15.00 ± 5.30 b
10			243.94 ± 59.75 a	32.44 ± 7.34 a
Significance			**	**
Interaction			ns	ns

Notes: **= significant at P < 0.01; *= significant at P < 0.05; ns = not significant. Values in bracket of treatments show additional application of manure after the first harvest

Comparing between variables of *S. arvensis* ratoon with plant from the first harvest, the t-test results show that the differences of value were significant from 8-week interval period, except for the leaf size, canopy diameter, and leaf K content (Table 7). Addition of interval period to 10 weeks after first harvest had resulted in highly significant differences in leaf numbers, therefore leaf dry weight was 12 times that of the first harvest.

The leaf N content of ratoon was lower than those of first harvest, and even further lower compared to those with longer harvest interval. A similar trend was found in terms of chlorophyll content. In contrast, P content was found to be higher in plant from ratoon compared to those in the first harvest.

Table 5. Leaf nutrients during first and second harvest using different chicken manure rates and harvest intervals.

Chicken manure rates (ton.ha ⁻¹)	First harvest (7 WAT)			Second harvest (at 13, 15, or 17 WAT)		
	N	P	K	N	P	K
	%					
0 (+ 0)	3.54 ± 0.10	0.24 ± 0.02	4.59 ± 0.74	3.15 ± 0.43	0.31 ± 0.07	4.57 ± 0.64
7 (+ 2)	3.56 ± 0.03	0.25 ± 0.02	4.49 ± 0.18	3.24 ± 0.43	0.29 ± 0.06	4.63 ± 0.61
14 (+ 4)	3.64 ± 0.03	0.24 ± 0.03	5.10 ± 0.71	3.17 ± 0.51	0.29 ± 0.07	4.60 ± 0.48
21 (+ 7)	3.49 ± 0.11	0.27 ± 0.02	4.72 ± 0.32	3.19 ± 0.39	0.30 ± 0.05	4.70 ± 0.48
Significance	ns	ns	ns	ns	ns	ns
Regression test	ns	ns	ns	ns	ns	ns
Harvest intervals (weeks after first harvest)						
6				3.52 ± 0.17 a	0.26 ± 0.05 b	4.24 ± 0.51 b
8				3.37 ± 0.20 a	0.34 ± 0.06 a	4.79 ± 0.51 a
10				2.68 ± 0.24 b	0.28 ± 0.05 b	4.85 ± 0.38 a
Significance				**	**	**
Interaction				ns	ns	ns

Notes: **= significant at P < 0.01; *= significant at P < 0.05; ns = not significant. Values in the same column followed by same letters means are not significantly different between harvest intervals according to DMRT at α= 5%

Table 6. Chlorophyll and flavonoid contents during first and second harvest with different manure rates and harvest intervals.

Treatments	First harvest (7 WAT)		Second harvest (at 13, 15, or 17 WAT)	
	Total chlorophyll		Total chlorophyll	Flavonoid content
	mg.g ⁻¹ wet basis		mg.g ⁻¹ wet	mg.100g ⁻¹ QE/dry weight
Manure rates (ton ha ⁻¹)				
0 (+ 0)	0.921 ± 0.042		0.727 ± 0.067	0.162 ± 0.024
7 (+ 2)	0.900 ± 0.071		0.784 ± 0.093	0.163 ± 0.019
14 (+ 4)	0.901 ± 0.011		0.829 ± 0.130	0.151 ± 0.019
21 (+ 7)	0.808 ± 0.090		0.771 ± 0.128	0.160 ± 0.023
Significance	ns		ns	ns
Regression test	ns		ns	ns
Harvest intervals (weeks after first harvest)				
6			0.811 ± 0.102	0.149 ± 0.017
8			0.786 ± 0.090	0.163 ± 0.025
10			0.733 ± 0.129	0.165 ± 0.019
Significance			ns	ns
Interaction			ns	ns

Note: ns = not significant. Values in the same column followed by same letters are not significantly different between harvest intervals according to DMRT at α= 5%; QE: quercetin equivalent.

Discussion

The Effect of Different Chicken Manure Rates

Our study demonstrated that nutrients from organic fertilizer only, can support *S. arvensis* growth. Assuming that chicken manure contains 1.43% N, 2.49% P, and 1.07% K, based on the study of

Kurniawati et al. (2017) a 21 ton manure potentially adds 300, 523, and 225 kg N, P, and K, respectively. Although organic manure contains low level of nutrient, its application can improve soil physical-chemical properties by increasing microorganism population. Microorganisms living in the soil play important role in decomposing, mineralizing, and recycling organic matters (Nguyen et al., 2016).

Table 7. Comparison of plant morphology among different samples of *S. arvensis* collected from first and second harvests.

Variables	First	Second harvest					
	harvest	Harvest interval					
	(7 WAP)	6 WAFH	t test	8 WAFH	t test	10 WAFH	t test
Leaf number	19.00	34.00	ns	59.00	*	125.00	**
Plant height (cm)	4.84	11.39	ns	55.37	*	93.36	*
Leaf length (cm)	15.00	16.55	ns	24.76	ns	29.64	ns
Leaf width (cm)	5.04	5.62	ns	6.24	ns	7.70	ns
Canopy diameter (cm)	26.67	29.73	ns	27.42	ns	54.36	ns
Leaf fresh weight (g/plant)	20.33	48.87	ns	151.72	*	243.95	*
Leaf dry weight (g/plant)	3.12	7.54	**	15.00	**	32.44	**
Leaf N content (%)	3.56	3.52	ns	3.37	**	2.68	**
Leaf P content (%)	0.25	0.26	ns	0.34	**	0.28	*
Leaf K content (%)	4.73	4.24	*	4.79	ns	4.85	ns
Chlorophyll content	0.88	0.81	*	0.78	**	0.73	**

Note: **= significant at P < 0.01; *= significant at P < 0.05; ns = not significant; WAP = weeks after planting; WAFH = weeks after first harvest

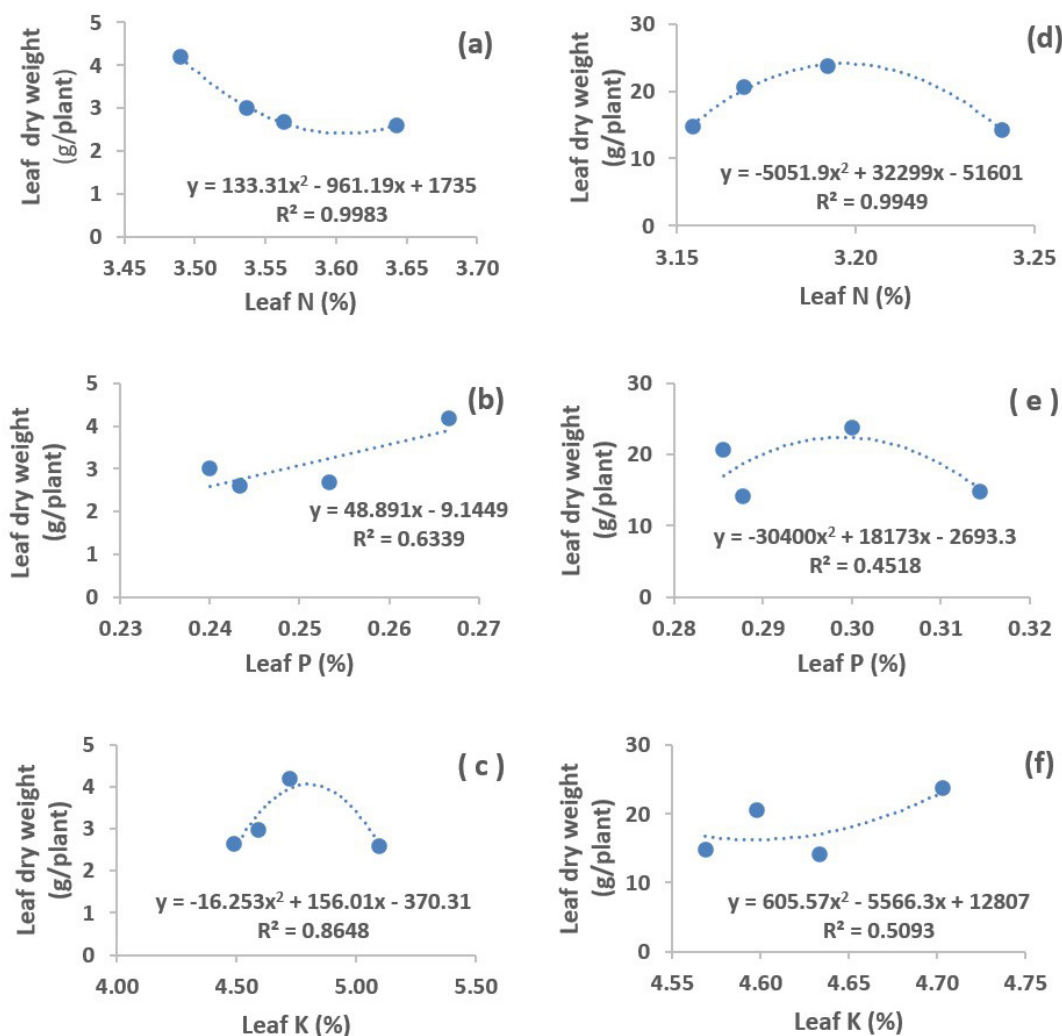


Figure 3. Relationship between leaf N, P, K with leaf yield at first harvest (a, b, c) and second harvest (d, e, f)

Microbes induce the production of phytohormones such as gibberellin and auxin in plant roots grown in fertile soil with rich organic manures which stimulate plant growth (Albiach et al., 2000).

The effects of increasing manure rates were likely to be more pronounced after the first harvest due to better nutrient released from manure with higher rainfall intensity (above 50-100 mm/week) compared to that before first harvest below 50 mm/week. Therefore, to produce the highest yield at the first harvest seemed to require the highest rate of manure (21 ton manure.ha⁻¹), while yield for the second harvest require less amount of manure (14 + 4 ton manure.ha⁻¹). The mineralization of manure is influenced by, among other things, temperature, soil moisture, soil properties (Wienhold and Gilley, 2002).

The Effect of Harvest Intervals

Longer harvest intervals increased *S. arvensis* yield. Six weeks were not enough to maximally regrow the leaves of *S. arvensis*. The first harvest was conducted by cutting the stem and there was no leaf left causing the regrowth of new leaves to rely solely on the reserve of carbohydrates in the roots. The axillary shoot started to emerge 3 days after harvest (Figure 2a) and the full expansion of leaves with photosynthetic function could occur several days after shoot emergence. Therefore postponing the second harvest to 2 and 4 weeks later could increase dry weight of leaves by 99 and 330%, respectively (Table 4). The increase in leaf yield with longer harvest interval is directly related to the increasing number and size of *S. arvensis* leaves (Table 3). The larger the leaf size, the higher the area of photosynthetic organs which in turn results in more leaves numbers and greater weight (yield).

Different N, P, and K contents in leaf were significantly affected by different harvest intervals (Table 5). Nitrogen content was higher in leaf from 6-week interval than that from 10-week interval because at 6 weeks after the first harvest, plants were still at vegetative phase while 4 weeks later, plants were already at the end of generative phase. These data could be attributed to different leaf ages, where leaves in younger plant accumulate higher N than leaves in mature plants. Different plants have different accumulation patterns of nutrient. There could be less N in young plants (Garbin and Dillenburg, 2008) but there could be higher N in young leaves (Hikosaka et al., 1994). Unlike nitrogen, P and K nutrients were accumulated more in leaf of mature plants than those in young plant as indicated by higher level of those nutrients in plant of 8 and 10 weeks interval (Table 5).

The nitrogen and chlorophyll contents in *S. arvensis* are directly related as shown in Table 5 and 6. The higher the N content, the higher the chlorophyll content. The values of nitrogen in second harvest were lower than those in first harvest, so did the values of chlorophyll content. The trend was also shown between values of nitrogen and chlorophyll content with different harvest intervals, where the nitrogen content and chlorophyll content decreased with longer harvest interval (although differences in chlorophyll content were not significant). Direct relationship between nitrogen and chlorophyll has been reported in some studies, for example, in maize (Hokmalipour and Darbandi, 2012).

Conclusion

Our study demonstrated that the application of poultry manure at 14 ton.ha⁻¹ added with 4 ton.ha⁻¹ is sufficient to produce the optimum dry weight of *S. arvensis* leaf for the first harvest and its ratoon. Additionally, ratoon *S. arvensis* should be harvested 10 weeks after the first harvest in order to obtain the highest leaf yield with the growth stage that fulfil the criteria of high flavonoid contents. There is no significant effect of interaction between manure rates and harvest interval in affecting leaf variables. The study has shown that ratooning practices can be done to produce leaves of *S. arvensis* with the benefit of saving time in growing the plant for multiple harvests.

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