Journal of Animal & Plant Sciences, 32(2): 2022, Page: 460-465
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2022.2.0443

EFFECT OF SOYBEAN (*GLYCINE MAX* L. MERR) INTERCROPPING INTO RHODESGRASS (*CHLORIS GAYANA* KUNTH.) ON DRY MATTER YIELD, CRUDE PROTEIN, AND SILAGE CHARACTERISTICS GROWN IN SOUTHWEST JAPAN

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ABSTRACT

This study aimed to investigate the quantify yield and silage quality response of forage soybean (*Glycine max* L. Merr) intercropping into rhodesgrass (*Chloris gayana* Kunth.) cultivation compare to monoculture rhodesgrass in warm temperate zone. Rhodesgrass and soybean were intercropped in 2017 and 2018 at Sumiyoshi Livestock Science Station, University of Miyazaki in southwest Japan. As the results, there were no differences between the height of monocultured rhodesgrass (MC) and intercropped rhodesgrass (IC). Height of soybean was less than rhodesgrass. Dry matter yield (DMY) was not significantly different between MC and IC in both years. For soybean ratio of intercropped total yield, the two-years-average was 18.3%. The lower soybean ratio of intercropped total yield in 2018 (13.3%) than those in 2017 (30.8%) seems to be caused by the larger rhodesgrass DMY. Crude protein (CP) content of IC (11.0%) was not significantly higher than that of MC (10.6%). There was no significant difference of CP yield between MC (28.3 kg/10 a) and IC (22.3 kg/10 a). All silages produced in this two-year experiment had only trace amounts of organic acids and volatile basic nitrogen regardless of the presence of soybean. In conclusion, the existence of soybean did not affect the silage quality. Early growth type soybean cultivar, appreciate harvesting time and fertilizer improvement might be increase DMY and CP with soybean intercropping.

Key words: intercropping system; rhodesgrass; silage characteristic; soybean.

Published first online August 13, 2021 Published final March 15, 2022.

INTRODUCTION

Rhodesgrass (*Chloris gayana* Kunth.) is a tropical grass native to African that produces high yield on sandy soils (Guggenheim and Waisel, 1977). In Japan, rhodesgrass is often used as an annual grass in Kyushu (warm temperate zone) and as a perennial grass in Okinawa islands (subtropical zone). Though tropical grasses including rhodesgrass exhibited higher dry matter yield (DMY) as the growth stages developed, nutrient value (crude protein, non-fibrous carbohydrates) of grass dramatically decreased as the growth stages developed (Mbwile and Uden, 1997). If such problem can be solved and high-protein forage production can be developed in a warm region of the southwest Japan, will be possible to achieved crude protein sustainability for feed livestock. The introductions or breeding the new grasses or crops that have high potential as high protein source is available solutions. In USA, some forage soybean cultivars were released (Devine *et al.* 1998 a, b). Since the 2000s in a mainly cool region (Europe, North America), interest has mounted in using soybean (*Glycine max* L. Merr) as a high-protein forage for livestock production (e.g., Acikgoz *et al.* 2007; Rogers *et al.* 2017; Peiretti *et al.* 2018). In Japan, Uchino *et al.* (2016) reported that eight soybean cultivars under Italian ryegrass sod as living mulch plant exhibited high whole-plant yield and 17-26% crude protein in the northern part of Japan, cool temperate zone. Because Munekado and Tsurusaki (1990) reported a practical success cases of forage soybean cultivation with 20% crude protein content at mid-podding stage in warm temperate zone in Japan, soybean intercropping is expected to increase crude protein content of rhodesgrass pasture. In addition, Koten *et al.* (2013) in study about sorghum and legume also recommend to introduction the plant legume in perennial pasture to improve ruminant feed sustainability. Castagnara *et al.* (2015) reported that grain soybean yield decreased with brachiaria grass intercropping in Brazil. Redfearn *et al.* (1999) reported that forage soybean yield decreased with sorghum intercropping in Iowa. Prasojo *et al.* (2019) reported yield and silage chemical compositions of soybean planted into rhodesgrass pasture after first cutting, in which rhodesgrass play a role as living mulch in warm temperate zone of Japan. However, there is no report about effect on tropical grass yield from soybean intercropping. It is necessary to clarify the effect of soybean intercropping into rhodesgrass pasture.

Because of leaf loss at harvesting, tedding, and roll baling and rainy condition at harvest time, silage is more suitable for forage soybean than hay. Touno *et al.* (2014) reported good silage quality of soybean at seed stage in northern Japan. On the other hand, Blount *et al.* (2013) guided that 'When ensiled alone, soybean silage has an unpleasant odor and emits free ammonia and butyric acid, which are characteristic of undesirable ensiling fermentation. For proper fermentation to occur with soybean silage, a source of soluble, readily fermentable carbohydrates, such as ground corn grain or molasses, should be added'. And in general, because of the low water-soluble carbohydrate content, tropical forages are not to be expected to produce lactic acid fermented silage (Kim and Uchida, 1991). There is no report about silage quality of rhodesgrass with soybean. It is necessary to clarify the effect on silage quality of rhodesgrass intercropped with soybean.

Our objectives were to clarify the effect of soybean intercropping into rhodesgrass as annual summer grass on dry matter yield, crude protein, and silage characteristics grown in warm temperate zone at southwest Japan.

MATERIALS AND METHODS

Experimental site. The field trials were carried out at the Sumiyoshi Livestock Science Station Faculty of Agriculture, University of Miyazaki, Southern Kyushu, Japan (39°59′N, 131° 28′E, the elevation of 12 m above sea level). The soil type was characterized as sandy regosols, according to Obara *et al.* (2015) about soil classification system in Japan. The climate of Miyazaki according to the Köppen classification, is Cfa that is humid subtropical climate, relatively high temperature and evenly distributed precipitation throughout the year. Precipitation and air temperature of the site during the experimental period were obtained from the database of the Geospatial Information Authority of Japan (URL: https://www.jma-net.go.jp/miyazaki/). The site, where the precipitation and air temperature were recorded, is sixteen kilometers away from the experimental site.

Experimental design. Rhodesgrass (cv. Callide, early cultivar) and soybean (cv. Tachinagaha, medium-maturing and erect type without nematode resistance) were used for this study. The randomized complete block design was applied for monoculture rhodesgrass (MC) and soybean intercropping into rhodesgrass (IC), and each plot was consisted of four replications in a 12 m^2 (3m×4m) area of plot with 1m^2 spacing between plots.

The seeds of rhodesgrasss and soybean were sown on 28 May in 2017 and 24 May in 2018. The seeding rate of rhodesgrass were 3.0 kg/10 a into all plot. Soybean was sown manually by hand at 5-6 cm depth and 50 cm row spacing at 7.35 kg/10 an into IC plot. A basal fertilizer consisting of nitrogen (13% N), double superphosphate (13% P_2O_5) and potassium chloride (13% K_2O) was applied to the plot area at a rate of 4 kg, 3.4 kg and 2.9 kg/10 a each. Manure (2.5% N, 4.0% P_2O_5 and 2.1% K_2O) was supplied at a rate of 1 t/10 a. Plant height of five randomly selected plants in all plot was measured every 15 days after sowing until harvesting at 60 DAS. Plant sampling at 10 cm above ground level were taken from 1m^2 include two soybean rows with around 20 plants of harvested soybean row in all plot on 60 days after sowing (DAS), and then dried at 60ºC for 48hr to determine dry matter yield (DMY). The value of soybean ratio of total yield for silage usage was calculated by using DMY value.

Ensiling. Rhodesgrass and soybeans samples for silage experiment were harvested at 60 days after seeding at 10 cm above ground level in one MC plot and one IC plot and then dried under sun-drying condition at field for about 30 hours and inverted manually three times during sun-drying. The sun-dried plants were chopped into approximately 3-5 cm in length using a forage cutter and 300 g chopped sample packed into a 30 cm x 15 cm sealed plastic bag with five replications. Silage bag was sealed by using a vacuum to reduce air inside, and fermented for 60 days at room temperature without sun light.

Chemical analysis. Part of 60 days fermented silages were dried at 60ºC for 48hr and then ground and passed through the sieve (1 mm) for the analysis of crude protein (CP) content. CP content was analyzed according to Association of Official Analytical Chemists (AOAC) (1990). Subsamples (50 g) were mixed with 400 mL of distilled water and stored in a refrigerator at 5°C for about 12hr and then filtrated. The filtrated extracts of silage samples were measured for pH value, volatile basic nitrogen (VBN) and volatile fatty acid (VFA). The pH value was analyzed by a glass electrode pH meter (AS800, ASONE Ltd.). VBN was analyzed by semi-micro vacuum distillation method (Cai, 2009) with modification. Lactic acid was analyzed by Barker and Summerson (1940) method. VFA concentration was analyzed by using gas chromatography system (GC-14B, Shimazu) (column: FAL-M, Shimazu; detector: FID; eluent: Helium gas, 60ml/min; temperature: 140ºC).

Statistical analysis. Data were analyzed by two-way ANOVA and t-test with a 0.05 level of significance.

Statistical analysis was carried out with R program (Version 3.1.1, R Core Team 2014).

RESULTS AND DISCUSSION

Monthly precipitation and average temperature during experiment in 2017 and 2018 are summarized in Table 1. The precipitation in 2017 were lower than the 10-year average for June, July and August. On the other hand, the precipitation in 2018 were higher than 10-year average for July and August.

Table 1. Monthly precipitation and average temperatures during experimental cultivation.

Year	Precipitation (mm)			Temperature (°C)		
	June	July	August	June	July	August
2017	363.0	124.0	189.5	22.6	28.5	29.0
2018	479.5	579.5	342.5	24.5	27.9	28.7
Avarage†	630.6	303.9	247.7	23.0	27.4	28.0

† 2008-2018 years averages.

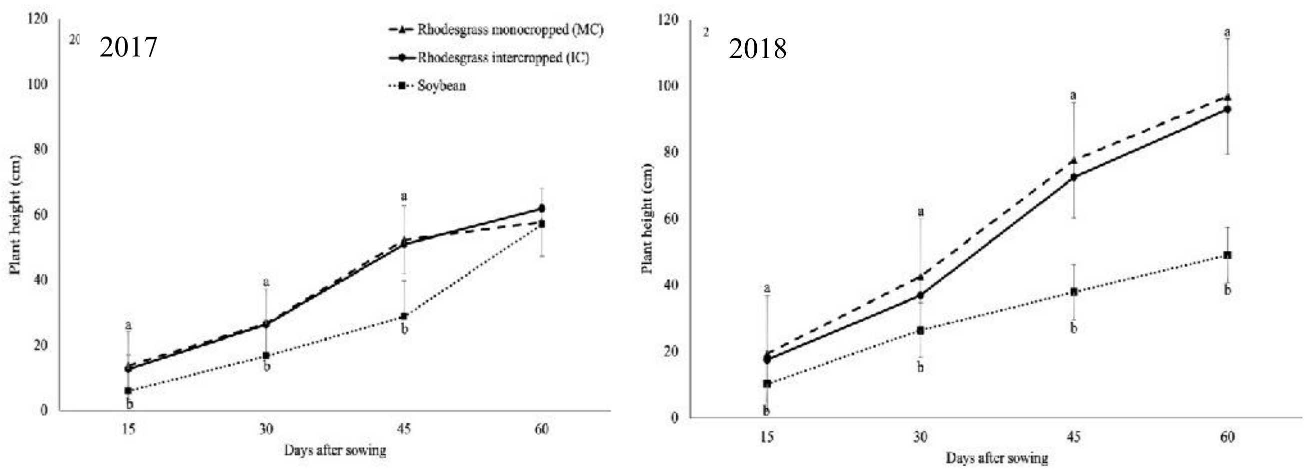

Figure 1. Change in plant height of rhodesgrass of monoculture and intercropped with soybean in two-year experiment (2017 and 2018). Plant height of rhodesgrass and soybean were measured every 15 days. Lower-case letters denote significant differences between rhodesgrass and soybean in intercropped (P < 0.05).

Changes of plant height of MC and IC are shown in Figure 1. There was no significant ($P > 0.05$) difference of rhodesgrass plant height between MC and IC in both years. This indicated that intercropped soybean did not influence rhodesgrass plant height. On the other hand, the two-years-average DMY of MC (280 kg/10 a) was higher than that of IC (209 kg/10 a). This result of DMY indicated that rhodesgrass growth per area was influenced by intercropped soybean (Table 2). As one of the reasons for this DMY decline in IC, it is suggested that a space and light competition between rhodesgrass and soybean. Borghi *et al.* (2007) reported that grass population should be kept low to avoid higher competition between the two species from the study about corn intercropped with brachiariagrass. It is not sure if the number of plants had decreased, but soybean intercropping decreased rhodesgrass density per area. In addition, except at 60 DAS in 2017, soybean plant heights were smaller than rhodesgrass. This indicated that soybean was shaded by rhodesgrass.

Soybean plant height (cv. Miyakonojo and Williams 82) with rhodesgrass aftermath living mulch were 47.7 – 57.3 cm at 90 DAS (Prasojo *et al.* 2019). This was similar to the present study data though living mulch rhodesgrass plant height by rhodesgrass shading. Though soybean plant height at 60 DAS did not differ between in 2017 (57.1 cm) and 2018 (49.1 cm), there is significant difference between rhodesgrass plant height and soybean plant height at 60 DAS only in 2018. Rhodesgrass plant height in 2017 was significantly ($P < 0.05$) higher than those in 2018 at both MC and IC. This rhodesgrass plant height declines in 2017 (Figure 1) might have been due to the lower precipitation in July and August 2017 (Table 1) and the reason for that there is no significant difference on plant height between rhodesgrass and soybean at 60 DAS in 2017. Not only plant height, rhodesgrass DMY in both MC and IC in 2017 was significantly less than those in 2018. However, soybean DMY in 2017 was not less than in 2018. For soybean ration of total yield in MC, the two-years-average was 18.3% (2017: 30.8%, 2018: 13.3%). In the present study, plant was harvested at 60 DAS when soybean maturity stage was R_1 (beginning of flower development). Because Peiretti *et al.* (2018) reported the DMY differences between harvesting stage of soybean, farther study about harvest time could lead the increase in soybean ratio of total yield thorough the increase of soybean DMY. In addition, use of more early growth soybean cultivar also available to increase soybean DMY.

Table 2. Yield traits, soybean ratio of total yield and crude protein under different cultivation system.

Factor	DMY kg/10a	Soybean ratio of total yield %	CP	CPY
			%DM	kg/10a
Year				
2017	$168.3^{b} \pm 60.2$	$30.8^{a} \pm 13.9$	$12.3^{a} \pm 0.6$	$20.6^{a} \pm 4.3$
2018	$307.4^{a} \pm 58.6$	$13.3^{b} \pm 6.1$	$9.5^{b} \pm 0.1$	$29.0^{b} \pm 4.3$
Cultivation				
Rhodesgrass	$280^{a} \pm 51$	-	10.6 ± 0.6	28.3 ± 4.3
Rhodesgrass + Soybean	$209^{b} \pm 36$	18.3 ± 3.3	11.0 ± 0.9 (14.3±0.8)†	22.3 ± 2.5
P-value				
Year (Y)	<0.01	<0.05	<0.01	<0.05
Cultivation (C)	<0.05	-	NS	<0.1
Y x C	NS	-	NS	NS

Values are means ± standard error of the mean (SEM). Means followed by different letters within the year and cultivation of a column are significantly different ($P < 0.05$). CP, crude protein; CPY, crude protein yield; DM, dry matter; DMY, dry matter yield. † The value in parentheses is crude protein of soybean only.

Table 2 shows yield traits and crude protein quality of silage in MC (rhodesgrass) and IC (rhodesgrass + soybean) as the two-years-average. The DMY of MC (280 kg/10a) was higher than that of IC (209 kg/10a). Even though there was not significant different, the two-years-average CP content of IC (11.0%) were higher than that of MC (10.6%). However, the CPY in MC (28.3 kg/10 a) tend to be larger amount than IC (22.3 kg/10 a) ($P < 0.1$). This trend was caused by DMY difference. The CP content of IC in 2017 (12.8%) was higher than that in 2018 (9.5%). There are two reasons for this year difference in CP content. One is the difference of rhodesgrass growth. Another reason for year difference in CP content is the difference of soybean ratio of total yield. Because soybean has higher CP content than rhodesgrass, higher soybean ratio in 2017 lead to higher CP content. Tobia *et al.* (2008) reported that forage soybean harvested at R_6 (full seed stage with a fully developed leaf) had average CP content 20.2% DM.

Bayorbor *et al.* (1992) reported that the monocultured rhodesgrass DMY (527 kg/10 a) at 71 DAS with 15 kg N fertilizer in southwest Japan. The monocultured rhodesgrass DMY at 60 DAS (280 kg/10 a as two years average) in the present study was less than those in Bayorbor *et al.* (1992). Acikgoz *et al.* (2007) reported that forage soybean CP content decrease among with growth from 17.9% at vegetative stage V_5 (the fifth node above the cotyledon leaf fully opened) to 12.5% at reproduction stage R_6 (full seed stage with a fully developed leaf) in the coastal area of Turkey. Munekado and Tsurusaki (1990) reported same decrease from 19.2% at bud emergence stage to 14.1% at early-pod stage. Though soybean maturity at 60 DAS harvesting was R_1 stage in the present study. CP content in the present study was lower than Acikgoz *et al.* (2007) and Munekado and Tsurusaki (1990). Though fertilizer application of Munekado and Tsurusaki (1990) was uncertain, these lower values of DMY and CP content was not enough for soybean intercropping. Fertilizer application is same between MC and IC in the present study. Initial growth of soybean requires fertilizer application. Additional fertilizer by the amount for intercropped soybeans into soybean rows may increase DMY of both rhodesgrass and soybean.

The fermentation quality of the silage is shown in Table 3. There was no significant ($P > 0.05$) difference between MC and IC in almost parameter. Acetic acid is often regarded as the primary fermentation product in tropical silages (Kim and Uchida, 1991) and result in the present study also showed acetic acid as primary fermentation production. In addition, lactic acid, acetic acid and butyric acid contents derived from silages of both cultivations were similar as forage soybean silage reported in Jahanzad *et al.* (2015). Therefore, the existence of soybean didn't affect the rhodesgrass silage fermentation. Li *et al.* (2019) reported that high concentrations of acetic acid (ranging from 0.08 to 3.9%DM) were detected in tropical forage silages with relatively low DM (ranging from 23.9% to 30.8% fresh material) content, while high DM materials (50.8%FM of white popinac) showed small concentrations of organic acid (ranging from 0.03 to 0.3%DM). Results of DM, pH and organic acid concentration in the present study was similar to results of high DM silage reported in Li *et al.* (2019). In the present study, the moisture of material decreased to about 50% might have leaded to the suppress the fermentation and generation of organic acid and VBN.

Table 3. The fermentation quality of the silage under different cultivation system.

Cultivation	DM (%)	pH	Lactic acid (%)	Acetic acid (%)	Butyric acid (%)	VBN (%)
Year						
2017	$54.7^a \pm 1.0$	$6.1^a \pm 0.1$	0.08 ± 0.01	$0.23^b \pm 0.03$	$0.01^b \pm 0.01$	0.007
2018	$36.6^b \pm 0.8$	$5.5^b \pm 0.1$	0.19 ± 0.13	$0.35^a \pm 0.04$	$0.03^a \pm 0.01$	0.009
Cultivation						
Rhodesgrass	50.3±3.5	5.8±0.1	0.19 ± 0.09	0.27 ± 0.05	0.02 ± 0.01	0.007
Rhodesgrass + Soybean	47.3±4.0	5.6±0.1	0.09 ± 0.01	0.33 ± 0.05	0.02 ± 0.01	0.008
P-value						
Year (Y)	<0.01	<0.05	NS	<0.05	<0.01	NS
Cultivation (C)	NS	NS	<0.1	NS	NS	NS
Y x C	NS	NS	NS	NS	NS	NS

Values are means ± standard error of the mean (SEM). Means followed by different letters within the year and cultivation of a column are significantly different ($P < 0.05$). DM, dry matter; VBN, volatile basic nitrogen.

Conclusions: In conclusion, though intercropped soybean did not influence rhodesgrass plant height, soybean intercropping decreased rhodesgrass density per area. For silage treatment, the existence of soybean didn't affect the rhodesgrass silage fermentation under low moisture condition. A further study of soybean cultivars, harvesting time and fertilizer could improve this intercropping system.

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