

## EVALUATION OF SOLID BIOGAS FERMENTATION RESIDUE AS A SOURCE OF ORGANIC FERTILIZER ON BERSEEM CLOVER (*Trifolium alexandrinum* L.)

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Received: 08.04.2019

### ABSTRACT

The study was carried out in order to investigate the effects of 5 different solid biogas fermentation residue doses (0, 5, 10, 15, 20 t ha<sup>-1</sup>) on the yield and quality of berseem clover (*Trifolium alexandrinum* L.) in Aydın province. In the study, no chemical fertilizers were applied and the harvest was carried out at 50-100% flowering time. In the study, hay yield (t ha<sup>-1</sup>), leaf / stem ratio (%), Ca (%), Mg (%), crude ash content (%), acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %), crude protein content (%) parameters are measured. Crude protein yield (t ha<sup>-1</sup>) and relative feed value calculated after the measurements. According to the results, in 2016-2018 years average hay yield was obtained from 5.529 t ha<sup>-1</sup> of 15 t ha<sup>-1</sup> solid biogas residue application. In terms of crude protein content, the highest value in 2016-2018 years was found as 22,88% in 10 t ha<sup>-1</sup> fertilizer application. As a result of the study, it was found that solid biogas waste had positive effects on feed efficiency and quality when all parameters were evaluated. Especially 10 t ha<sup>-1</sup> and 15 t ha<sup>-1</sup> solid biogas residue fertilizer doses were determined to come to the fore in the berseem clover.

**Keywords:** Berseem clover, biogas residue, fertilizer, forage quality

### INTRODUCTION

Feeding programmes for ruminant livestock in Mediterranean basin can't rely on seasonally roughage and low quality forage resources. Some alternative forage crops should be grown as winter catch crops for complete quality fodder deficit (Caballero Garcia de Arevelo et al., 1994). One of these resources. Berseem clover (*Trifolium alexandrinum* L.) is an annual forage legume species native to western Asia. It is extensively cultivated in Mediterranean basin in irrigated and rainfed farming conditions due to its excellent feeding value (Abogedallah and Quick, 2010; Zaghلامي Khelil et al., 2015; Yucel et al., 2018). However this is not widely grown in Turkey but, it has the potential to be considered as a winter catch crops in corn and cotton cultivation areas in order to reduce the lack of quality roughage.

The number of biogas and biomass power plants has reached 82 in Turkey (Atlas of Energy, 2019). Not all of these plants have the same characteristics, while some power plants evaluate animal wastes, while some power plants evaluate herbal and garbage waste. The biogas can be used for producing heat, electric power and vehicle fuel. Moreover, the digestate can be used as a fertilizer on arable land, enabling recirculation of plant nutrients and then reducing the need for fossil fuel-dependent mineral fertilizer. (Holm-Neilsen et. al., 2009; Moller et al., 2009;

Abubaker et al., 2012; Wentzel and Joergensen, 2016a; Hupfauf et al., 2016; Risberg et al., 2017). Biogas production is increasingly popular in many countries due to climate change and environmental pollution (Islam et al., 2010; Insam et al., 2015; Rozylo et al., 2015; Risberg et al., 2017).

Biogas fermentation residue is a secondary product that is also inexpensive and organic fertilizer. It is not only rich in macronutrients and micronutrients, but also rich in amino acids, vitamins, enzymes, materials or factors inhibited the pest and diseases (Wu-Di, 2002; Islam et al., 2010; Albuquerque et al., 2012). The use of these residues as fertilizers closes nutrient cycles in organic farming systems. It provides N, P, K and improves the structure of the soil (Stinner et al., 2008; Arthurson, 2009; Fouda et al., 2013; Nkoa, 2014; Wentzel and Joergensen, 2016b). Although it is considered that there is no need to fertilize the legumes due to nitrogen binding, they are more sensitive in terms of phosphorus than grasses. Thus it is possible to use in legumes as it contains phosphorus.

This experiment was conducted to examine the possibility of using the solid biogas fermentation residue as an organic fertilizer for production of berseem clover (*T. alexandrinum* L.).

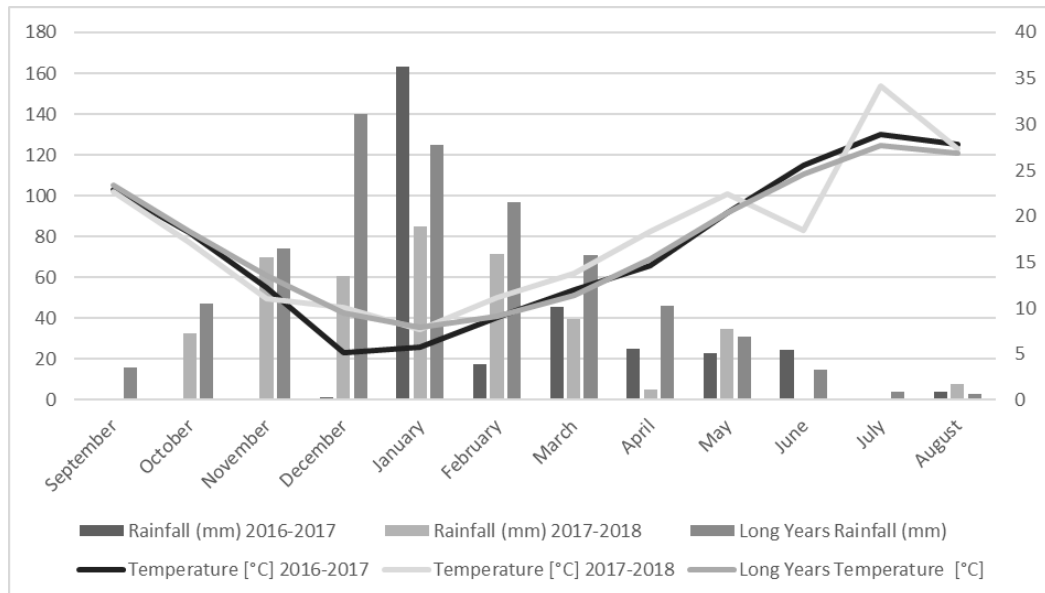
## MATERIALS AND METHODS

The research was conducted during the 2016-2018 growing seasons in Aydın Province (37°45' 44" N, 27°45'31" E; elevation 30 m), located in Aegean region of Turkey. According to soil analysis report of Soil Science Department of Faculty of Agriculture, the soil characteristics in 0-30 cm depth of the experimental area was loamy and alkaline with low organic matter. Lime content of soil is 3.82%, total saline content of 0.02%,

phosphorus (P) content of 35 ppm and available potassium of 320 ppm (Table 1). Climate data of experimental area shown in Figure 1. Total precipitation of 2016, 2017 and long years are 303.8 mm, 407.2 mm, 669 mm, respectively. Average temperature of 2016, 2017 and long years are 16.86, 17.85, 17.35, respectively. As it can be seen from the climate data, the years in which the experiment is made are dry and the amount of rainfall in the first year is less than half of the average for long years rainfall.

**Table 1.** Soil analysis report of experimental area

	Sand (%)	Silt (%)	Clay (%)	Texture	Total Salt (%)	pH	Lime (%)	Org. Matter (%)
	47,19	34,56	18,25	Tınlı	0,0189	8,10	3,82	1,10
					Saltless	Alkali	Limy	Low
P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
35	320	3218	413	240	10,62	3,71	5,24	21,80
High	High	High	Very High	High	High	Enough	Enough	Enough



**Figure 1.** The average of monthly climate values for the years of 2016-18 (obtained from the Meteorology Station of the Faculty of Agriculture of Aydın Adnan Menderes University) and for long years in the Cakmar Quarter of the Kocarlı District of Aydın province (Anonymous, 2018).

Solid biogas fermentation residues used in the experiment were obtained from Efeler Biogas Plant in Aydın. When the chemical content of biogas residue fertilizer was evaluated, it was found that the content of nitrogen was 1.42%,  $P_2O_5$  content was 2.33%,  $K_2O$  was 0.60% and organic matter was 91.18%. Also CaO content of applied material was 2.94% and MgO content was 0.90%.

The experiment was designed in randomized complete block design with 3 replications. Every plot consisted of 6 (5m x 1.2m)  $m^2$  area and sowing was carried out using 20 cm row spacing. Following the application of 5 different doses of solid biogas residue fertilizer (0, 5, 10, 15, 20 t/ha

<sup>1</sup>) to the floor, it was carried out with sowing seed drill in November of 2016 and 2017. No fertilization has been performed except for the biogas waste fertilization. Berseem clover cv. Derya was evaluated as material and 0,02 t ha<sup>-1</sup> seed was used (Acıkgoz, 2001). The mowing process was carried out from a height of 5 cm with the mower (Bcs 615 L Max Honda Gx200) when the plants reached 50-100% flowering time according to Yucel et al. (2018). Ten different plants were taken from the parcels for the measure of plant height (cm), stem diameter (mm) and leaf/stem ratio (%). One edge row from each side of plot were cut out and then rest of the plot was harvested and weighed to determine fresh forage yield. Hay yield (t ha<sup>-1</sup>) was measured by fan drying oven (Mikrotest, MST)

at 70°C until the weight was fixed (Cook and Stubbendieck, 1986). The dried samples were ground in a mill passed through a 2 mm screen. Crude ash was determined at 550 °C (Bulgurlu and Ergul, 1978). The crude protein ratio (%) of the samples taken from the experiment were measured according to the method of AOAC (2003); NDF and ADF contents (%) were measured according to Van Soest et al. (1991). The crude protein yield (t ha<sup>-1</sup>) and relative feed value were calculated by the obtained data with following procedures of Horrocks and Vallentine (1999). NIRS-FT (Brüker-MPA) method was preferred to measure Ca and Mg contents.

In order to compare the results obtained from the study, variance analysis was applied with randomized blocks trial design with the help of SAS statistical package program (SAS, 1998). Duncan multiple comparison test was used in comparison of the averages. Boxplots were made in SPSS statistical package program (SPSS, 1995)

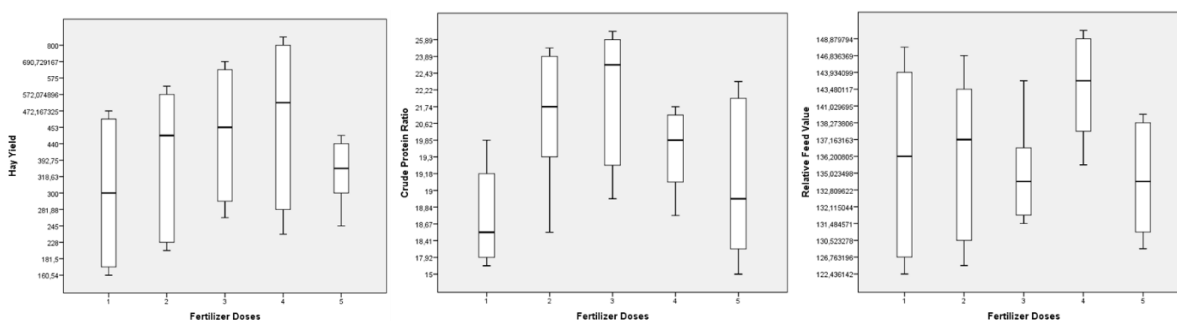
## RESULTS AND DISCUSSION

All the data obtained from the experiment was a result of first mowing. Although sprinkler irrigation was performed after mowing, no roughage of economic importance was obtained for the 2nd mowing. When the hay yield is examined, the second year average in terms of year averages is higher than the first year average with 5.76 t ha<sup>-1</sup>. When the application averages were taken into consideration, the highest value was obtained at 15 t ha<sup>-1</sup> as 5.52 t ha<sup>-1</sup>. When the interaction of the applications according to years was examined, it is observed that the yield increases due to the increase in the fertilizer dose. (Table 2. and Figure 2.) In addition, considering the difference in yield between the two production years, it is thought that the situation in the climate is different. The irregularities in rainfall and low amount of precipitation in 2016-17 had a negative effect on yield. Sullivan et al. (2006) says in their study; the application of organic fertilizer may significantly change the soil microbial communities, resulting in improvements of the soil quality. This results increase yield and quality.

**Table 2.** Hay yield (t ha<sup>-1</sup>) averages of soild biogas fermentation residue doses.

Residue Doses (t ha <sup>-1</sup> )	Hay Yield (t ha <sup>-1</sup> )		
	2016-2017	2017-2018	Average
Control (0)	1.71±1.05	4.65±1.10	3.18 <sup>D</sup>
5	2.68±0.89	5.56±0.28	4.12 <sup>BC</sup>
10	2.92±0.27	6.18±0.63	4.55 <sup>B</sup>
15	3.02±0.81	8.03±0.83	5.52 <sup>A</sup>
20	2.83±0.32	4.39±0.95	3.61 <sup>CD</sup>
<b>Average</b>	2.63 <sup>B</sup>	5.76 <sup>A</sup>	

Capital letters are important according to p<0.01. Lower case letters are important according to p<0.05, ±:standart deviation



**Figure 2.** Boxplot graphics of Hay yield (t ha<sup>-1</sup>), Crude protein ratio (%), Relative feed value

Among the nutrients, deficiency of P in soil has an adverse impact on legume production as it is required for energy transformation in nodules and enhanced N-fixation. The limited availability of P in soil leads to poor plant growth and development of legume crops. P deficiency has some negative effects on nodule formation, and photosynthetic ability in leaf and hence reduces photosynthesis (Mitran et al., 2018). Islam et al. (2010) reported from their study; the biogas residue fertilizer increase fodder maize yield. But excessive doses of fertilizer decrease forage yield and quality. Results of Koc (2013) shown the similar about this situation. He found in

his study; excessive amount of phosphorus fertilization reduced yields in legumes. Surmen et al. (2011) reported that P fertilization have positive effect on forage yield of legumes. In studies related to berseem clover, results of Hakyemez and Sancak (2005) shown similar in terms of forage yield and quality. But results of Yucel et al. (2018) shown some differences.

When the mean of plant height averages analyzed, the average of the 2nd year was determined as 93.08 cm. According to the application averages, the highest value of 5 t ha<sup>-1</sup> is 83.40 cm. When the stem diameter averages

were evaluated, the highest stem diameter was obtained with 90.40 cm in the first year. As per the application averages, the highest value was determined with 85.56 mm in 5 t ha<sup>-1</sup> application (Table 3.). It is thought that changes in plant height and stem diameter are caused by

different climates, especially between two years. Therefore, the occurrence of the rainfall seen in the first year may have affected the body diameter instead of the plant height.

**Table 3.** Plant height (cm) and stem diameter (mm) averages of soild biogas fermentation residue doses

Residue Doses (t ha <sup>-1</sup> )	Plant Height (cm)			Stem Diameter (mm)		
	2016-2017	2017-2018	Average	2016-2017	2017-2018	Average
<b>Control (0)</b>	64.50±1.21	88.53±2.13	<b>76.51<sup>B</sup></b>	92.66±1.15	70.46±6.17	<b>81.56<sup>A</sup></b>
<b>5</b>	67.13±2.51	99.66±3.05	<b>83.40<sup>A</sup></b>	101.33±5.77	69.80±7.20	<b>85.56<sup>A</sup></b>
<b>10</b>	56.93±0.41	84.26±2.02	<b>70.60<sup>C</sup></b>	70.66±5.03	59.60±13.51	<b>65.13<sup>B</sup></b>
<b>15</b>	58.60±1.31	98.60±3.83	<b>78.60<sup>B</sup></b>	87.33±2.30	65.20±11.80	<b>76.26<sup>A</sup></b>
<b>20</b>	60.33±1.60	94.33±4.04	<b>77.33<sup>B</sup></b>	100.00±0.00	73.73±9.60	<b>86.86<sup>A</sup></b>
<b>Average</b>	<b>61.50<sup>B</sup></b>	<b>93.08<sup>A</sup></b>		<b>90.40<sup>A</sup></b>	<b>67.76<sup>B</sup></b>	

Capital letters are important according to p<0.01. Lower case letters are important according to p<0.05, ±:standart deviation

The first year rate of leaf / stem ratio averages is higher with 1.008 than the second year. The highest value of the application average was determined as 0.927. Although the stem diameter of the first year is high, the plant height is shorter, increasing the leaf rate. However, as a result of this situation, hay yield was lower in the first year. When the crude ash content is examined, it is the highest with 20.57% in the first year according to the year

averages. When the application averages was analyzed, the highest value was determined with 19.96% in 15 t ha<sup>-1</sup> application. It is seen that the fertilizer application increases the amount of crude ash ratio due to the organic matter it has in fertilizer. However, the effect of large amounts of fertilizer on crude ash ratio is also negative (Table 4.).

**Table 4.** Leaf/Stem ratio (%) and crude ash content (%) averages of soild fermentation residue doses

Residue Doses (t ha <sup>-1</sup> )	Leaf/Stem Ratio			Crude Ash Content (%)		
	2016-2017	2017-2018	Average	2016-2017	2017-2018	Average
<b>Control (0)</b>	0.960±0.21	0.624±0.05	<b>0.792<sup>AB</sup></b>	22.34±2.39	12.71±2.32	<b>17.52<sup>BC</sup></b>
<b>5</b>	0.820±0.07	0.478±0.03	<b>0.649<sup>BC</sup></b>	19.27±0.54	13.27±1.80	<b>16.27<sup>C</sup></b>
<b>10</b>	1.180±0.13	0.675±0.09	<b>0.927<sup>A</sup></b>	17.11±1.23	17.17±0.20	<b>17.14<sup>BC</sup></b>
<b>15</b>	1.246±0.13	0.328±0.04	<b>0.787<sup>AB</sup></b>	21.78±0.39	18.14±2.20	<b>19.96<sup>A</sup></b>
<b>20</b>	0.833±0.11	0.415±0.06	<b>0.624<sup>C</sup></b>	22.38±1.84	15.66±0.45	<b>19.02<sup>AB</sup></b>
<b>Average</b>	<b>1.008<sup>A</sup></b>	<b>0.504<sup>B</sup></b>		<b>20.57<sup>A</sup></b>	<b>15.39<sup>B</sup></b>	

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Low Ca and Mg levels of forages have been implicated in several animal diseases of economic importance (Gross and Jung, 1981). Solid biogas applications made in terms of Ca and Mg content did not make a statistical difference, but only differences were observed between years. It is thought that these contents are not affected due to the fact that these elements in the fertilizer are not present in very high amounts and due to the study on the same variety. The highest value in terms

of Ca content was determined as 1.82% in the first year, while the highest value in Mg content was found as 0.71% in the first year (Table 5.). Yavuz et al. (2012) reported that Ca contents of their experiment for Gelemen clover was changed between 1.39-1.61%. Mg contents was found between 0.33-0.39%. Our findings were higher than these values. Different species and environmental factors are thought to affect this situation.

**Table 5.** Ca (%) and Mg (%) contents averages of soild fermentation residue doses in 2016-2018

Residue Doses (t ha <sup>-1</sup> )	Ca (%)			Mg (%)		
	2016-2017	2017-2018	Average	2016-2017	2017-2018	Average
<b>Control (0)</b>	1.81±0.09	1.20±0.14	<b>1.50</b>	0.76±0.01	0.50±0.11	<b>0.63</b>
<b>5</b>	1.82±0.05	1.20±0.09	<b>1.51</b>	0.75±0.05	0.53±0.04	<b>0.64</b>
<b>10</b>	1.70±0.17	1.31±0.12	<b>1.51</b>	0.60±0.05	0.55±0.08	<b>0.57</b>
<b>15</b>	1.98±0.08	1.07±0.13	<b>1.52</b>	0.74±0.12	0.46±0.11	<b>0.60</b>
<b>20</b>	1.79±0.25	1.36±0.17	<b>1.58</b>	0.70±0.09	0.62±0.02	<b>0.66</b>
<b>Average</b>	<b>1.82<sup>A</sup></b>	<b>1.23<sup>B</sup></b>		<b>0.71<sup>A</sup></b>	<b>0.53<sup>B</sup></b>	

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As the ADF increases, the digestibility of the forage usually decreases. The NDF value refers to the total cell wall and is composed of the ADF fraction plus hemicelluloses. As NDF percentages increase, the dry matter intake will generally decrease (Joachim and Jung, 1997). With regards to the ADF averages, the lowest value was the first year, while the value was 32.11%. The effect of fertilizer applications on ADF ratio was not found to be significant. According to NDF averages, the lowest year average was found as 41.91% in the first year and the lowest average was obtained with 41.06% in 15 t ha<sup>-1</sup> fertilizer application. (Table 5). The lower values of ADF and NDF in the first year showed itself in the relative feed value. Plant height is higher than the first year of the

second year to increase the amount of lignin in order to keep the plant upright, this situation has led to an increase in ADF and NDF rates. Results of Koc (2013), when the dose of phosphorus fertilizer increased, the rate of ADF and NDF increased. Results of Yucel et al. (2018) shown higher ADF and NDF ratio than our results. They found already higher hay yield and these results related between ADF and NDF results.

ADF and NDF contents were white clover (36.9-55.4%) and red clover (41.9-58.5%) (Stewart et al., 2008); berseem clover (22.0-32.0%) (Karlı et al., 1999); crimson clover (25.8-43.7%) (Ladyman et al., 2003). ADF and NDF contents of berseem clover were similar to other clovers.

**Table 6.** ADF (%) and NDF (%) averages of soild fermentation residue doses

Residue Doses (t ha <sup>-1</sup> )	Acid Detergent Fiber (%)			Neutral Detergent Fiber (%)		
	2016-2017	2017-2018	Average	2016-2017	2017-2018	Average
<b>Control (0)</b>	32.22±0.71	34.68±0.87	<b>33.45</b>	41.58±1.17	45.25±2.21	<b>43.41<sup>A</sup></b>
<b>5</b>	32.01±0.35	34.72±1.58	<b>33.36</b>	41.24±0.49	44.35±0.35	<b>42.80<sup>A</sup></b>
<b>10</b>	31.94±0.30	34.36±0.96	<b>33.15</b>	43.39±1.71	43.33±0.65	<b>43.36<sup>A</sup></b>
<b>15</b>	31.66±0.22	34.61±1.25	<b>33.13</b>	40.42±0.72	41.70±0.25	<b>41.06<sup>B</sup></b>
<b>20</b>	32.73±0.75	34.38±0.75	<b>33.55</b>	42.94±0.82	44.38±0.45	<b>43.66<sup>A</sup></b>
<b>Average</b>	<b>32.11<sup>B</sup></b>	<b>34.55<sup>A</sup></b>		<b>41.91<sup>B</sup></b>	<b>43.80<sup>A</sup></b>	

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When the average of the crude protein ratio was examined, the highest year average was determined as 21.26% in the 2nd year average. According to the application averages, the fertilizer amount has a positive effect and the highest value is obtained as 22.88% in 10 t ha<sup>-1</sup>. Graves et al. (1987) reported that berseem clover has 21% average crude protein ratios. Our results seen similar with this research. When the crude protein yield data were examined, the highest value in terms of year averages was 1.24 t ha<sup>-1</sup> in 2nd year average. The highest application average was found in 15 t ha<sup>-1</sup> fertilizer dose with 1.12 t ha<sup>-1</sup>. It is observed that the second year average of crude protein yield is significantly higher than the first year average. The reason for this is that the second year

average of hay yield is higher than the average of the first year. On the other hand, in terms of the relative feedvalue averages, there has been a statistical difference just between the years. The highest value was obtained with 141.89 in the first year. It is thought that this situation is due to the low amount of fiber due to the low yield in the first year (Table 7. and Figure 2.). Yavuz et al. (2012) stated that forages with an RFV value over 151, 150-125, 124-103, 102-87, 86-75, and less than 75 are categorized as prime, premium, good, fair, poor and rejected, respectively. Based on the average of the 2 years berseem clover relative feed values ranging between 133.74-142.97. Therefore, our results could be categorized as premium values.

**Table 7.** Crude protein ratio (%), crude protein yield (t ha<sup>-1</sup>) and relative feed value averages of soild fermentation residue doses

Residue Doses (t ha <sup>-1</sup> )	Crude Protein Ratio (%)			Crude Protein Yield (t ha <sup>-1</sup> )		
	2016-2017	2017-2018	Average	2016-2017	2017-2018	Average
Control (0)	19.23±0.59	17.79±0.68	18.51 <sup>C</sup>	0.32±0.10	0.82±0.21	<b>0.57<sup>B</sup></b>
5	19.52±0.99	23.83±1.58	21.67 <sup>AB</sup>	0.51±1.42	1.32±1.09	<b>0.92<sup>A</sup></b>
10	20.23±1.90	25.53±1.71	22.88 <sup>A</sup>	0.59±0.83	1.58±2.13	<b>1.08<sup>A</sup></b>
15	19.67±0.56	20.51±1.52	20.09 <sup>BC</sup>	0.59±1.45	1.65±2.88	<b>1.12<sup>A</sup></b>
20	19.74±2.27	18.66±3.51	19.20 <sup>C</sup>	0.56±1.04	0.82±1.63	<b>0.69<sup>B</sup></b>
<b>Average</b>	<b>19.68<sup>B</sup></b>	<b>21.26<sup>A</sup></b>		<b>0.51<sup>B</sup></b>	<b>1.24<sup>A</sup></b>	
Relative Feed Value						
Residue Doses (t ha <sup>-1</sup> )	2016-2017	2017-2018	Average			
Control (0)	142.76±5.11	127.35±5.23	<b>135.05<sup>B</sup></b>			
5	144.23±2.31	129.71±3.57	<b>136.97<sup>B</sup></b>			
10	137.35±5.90	133.38±3.12	<b>135.37<sup>B</sup></b>			
15	147.82±0.45	138.11±3.95	<b>142.97<sup>A</sup></b>			
20	137.31±1.99	130.18±2.44	<b>133.74<sup>B</sup></b>			
<b>Average</b>	<b>141.89<sup>A</sup></b>	<b>131.74<sup>B</sup></b>				

Capital letters are important according to p<0.01. Lower case letters are important according to p<0.05, ±:standart deviation

## CONCLUSION

Increasing production activities as well as increasing use of chemical fertilizers negatively affect the environment and constitute an intensive economic cost. In this respect, biogas residues, which are one of the organic fertilizer sources in developed countries, have been used more intensively in agricultural activities. In this study, the effects of solid biogas fermentation residues on berseem clover were investigated. In regions with Mediterranean climate, there is the potential to reduce the lack of quality roughage with increasing quality of berseem clover. It has been observed that the fertilizer source used has a positive effect on the yield and quality of the plant. However, the use of large amounts of fertilizers has shown toxic effects and reduced yield and quality. It was concluded that the best fertilizer dose would be 10 t ha<sup>-1</sup> and 15 t ha<sup>-1</sup>. In addition, the effect of different climates between in twoyears on yield and quality is evident. This situation shows us the effect of climate factors on yield and quality of forage crops.

## ACKNOWLEDGEMENTS

We are very grateful to Efeler Biogas Plant managers and Agricultural Engineer Burak Alp KANTIK who helped us to provide solid biogas fermentation residues. We would like to extend our gratitude to the Eastern Mediterranean agricultural research institute and the field crops department regarding seed supply.

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