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Study of the Use of Chicken Manure Digestate as Organic Fertilizer in Comparison with Fresh Chicken Manure

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ABSTRACT

The aim of this study was to compare the fertilizer properties of anaerobic digestion residues (digestate) of chicken manure and fresh chicken manure with the aim of benefiting from the produced biogas and alleviating the environmental problems of fresh chicken manure. The study was conducted in a completely randomized design. In addition to the control treatment, experimental treatment groups were Fresh Chicken Manure (FCM), Chicken Manure Digestate (CMD) and Enriched Chicken manure (ECM), each at three levels and with 3 replications. A total of 30 experimental pots were used for soil treatments and wheat planting. At the end of the growing season, soil variables including nitrogen, organic carbon, absorbable phosphorus, and pH, and physical properties of wheat including dry weight of shoots, seeds and roots were measured. The results were analyzed using SPSS statistical software. CMD application relatively increased soil nitrogen, organic carbon and phosphorus, kept the soil pH neutral, and improved wheat yield components. Compared to FCM, not only has the quality of CMD as biofertilizer not decreased, but it has also improved in some respects. CMD is superior to ECM in all studied indicators. The results are also better or at least equal in comparison with FCM. Due to the environmental benefits of anaerobic digestion of chicken manure and also the production of biogas as a valuable product and proving that the quality of the resulting fertilizer is not reduced, the use of CMD as organic fertilizer has more advantages than the use of FCM and even ECM.

INTRODUCTION

Although the use of chemical fertilizers, has helped to enhance agricultural production to meet the growing global demand for food (Srivastav, 2020), but in addition to the very high cost of these fertilizers (Khandare *et al.*, 2020) have had negative impacts on soil, water and air quality. Soil salinity, accumulation of heavy metals, leakage of nitrate and phosphate into surface and groundwater and causing eutrophication and participation in air pollution with NOx emissions are some of the major effects of overuse of chemical fertilizers (Savci, 2012; Khandare *et al.*, 2020).

By replacing mineral fertilizers with organic fertilizers, excessive use of chemical fertilizers and consequent nitrate pollution and loss of soil carbon and of course the overall cost is reduced (Nkoa, 2014). Chicken manure as an organic waste is generally rich in nutrients such as nitrogen, potassium, phosphate, calcium, magnesium and sulfur (Wedwitschka *et al.*, 2020; Rajagopal *et al.*, 2021), and can be used as an organic fertilizer. It has been observed that chicken manure compared to charcoal, coffee husk, pine-bark, cattle manure, coconut fiber, sewage sludge, peat, and vermiculite has the highest nutrient concentrations, especially total N, N-NH4+, Ca, P, S and B (Higashikawa *et al.*, 2010). But its EC value and sodium content can even reach 40.6 $dS.m^{-1}$ and 4.75 $g.kg^{-1}$ respectively, which is higher than the appropriate values for growth of most crops (Higashikawa *et al.*, 2010).

One of the most efficient processes for treating organic waste is anaerobic digestion (Rajagopal *et al.*, 2021). Hereby in addition to producing biogas as a clean energy carrier, it can minimize the potential negative environmental effects that agricultural waste may cause (Chen *et al.*, 2008; Mahato *et al.*, 2020; Rajagopal *et al.*, 2021). Moreover, the remaining sludge after digestion can be used as agricultural fertilizer (Mortola *et al.*, 2019). Some researchers (Nkoa, 2014; Sürmeli *et al.*, 2018; Iocoli *et al.*, 2019; Mortola *et al.*, 2019; Busato *et al.*, 2020) have confirmed that biogas digestate, which is rich in macro and microelements (Mortola *et al.*, 2019), if properly handled and controlled (Nkoa, 2014), can be used as organic fertilizer. Although, digesting organic matter decreases its C/N ratio and increases its $NH_4^+ - N/N$ ratio, amending digestate in soil can lead to low CO2 emission and improve soil overall C balance. One of the major problems in using biogas digestate as organic fertilizer is the higher potential release of NH3 than in the use of undigested fertilizer. (Nkoa, 2014; Iocoli *et al.*, 2019). To address this problem, it is recommended to

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recirculate the liquid phase after its air stripping and also use sulfuric acid to recover N as the form of ammonium sulfate (Busato *et al.*, 2020).

The effluent stream of anaerobically digested manure consists of two phases, liquid and solid. The solid part is rich in phosphorus while the liquid part is rich in nitrogen in the form of ammonium (Liedl *et al.*, 2006). Using both liquid and solid fraction as biofertilizer was studied to produce vegetable, fruit and grassland crops (Liedl *et al.*, 2006). Despite the increase in soil phosphorus in the use of the solid part, the yield of vegetables and blueberries did not increase and even decreased. However, the use of liquid part increased the yield of grasses and vegetables more than the use of conventional nitrogen fertilizers. This study generally confirmed the effectiveness of digested effluent as part of a nutrient management program and as a solution to the problem of animal waste (Liedl *et al.*, 2006). It has already been confirmed that chicken manure digestate has a large amount of nutrients with acceptable contents of heavy metals and pathogens (Mortola *et al.*, 2019). Chicken manure digestate application, probably because of the slow nutrient release from the digestate during the crop growth, didn't increase total inorganic N and available P in the soil, but the higher the dose of digestate used, the greater the increase in the fresh weight of crop. The parameters of soil pH and EC were slightly high but within the range suitable for crop growth (Mortola *et al.*, 2019). The aim of this study was to compare the effect of biogas digestate, fresh chicken manure and enriched chicken manure on physical and chemical properties of soil and crop yield components.

MATERIALS AND METHODS

Chicken manure was obtained from a poultry farm around the city of Ahvaz, Iran. After adding water to manure and reaching the medium concentration 8%, it was digested anaerobically in 1.5 liter polyethylene bottles (Fig 1) for 35 days. The bottle cap was completely closed so that only one tube equipped with a drain valve at the top of the bottle to exit the produced gas, was the only pass into the bottle. All seams were completely sealed with glue. To keep the temperature in the range of 35-40 degrees Celsius (mesophilic range), a heating element and a thermostat were used in a hot water bath around the bottle. Throughout the experiment, the pH of the manure solution was kept in the neutral range. Three molar solution of sodium hydroxide (NaOH) was used for this adjustment. After 35 days, the resulting digestate was filtered and the solid part was used as fertilizer.

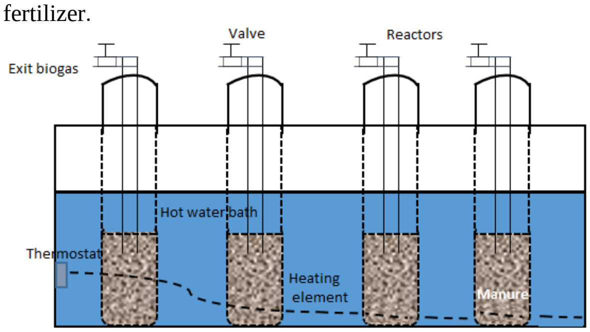

Fig 1. Reactor set for anaerobic digestion of chicken manure

Experimental design and treatments specifications

This study was conducted in a completely randomized design. In addition to the control treatment, three experimental groups including Fresh Chicken Manure (FCM), Chicken Manure Digestate (CMD) and Enriched Chicken manure (ECM), each were studied at three levels and in 3 replications. A total of 30 experimental pots were used, with a final weight of 5 kg per pot. Experimental treatments were:

- Chicken manure digestate at three levels of 15, 20 and 25 tons per hectare (D_{15}, D_{20}, D_{25})
- Fresh chicken manure at three levels of 15, 20 and 25 tons per hectare (F_{15}, F_{20}, F_{25})
- Enriched chicken manure or pelletted (20-20-20, 20%N-20%K-20%P) at three levels of 15, 20 and 25 tons per hectare (P_{15}, P_{20}, P_{25}), and
- Control (no fertilizer application)

Soil Sampling and Preparation

The required soil was collected from the farm of the Faculty of Agriculture of Shahid Chamran University of Ahvaz from a depth of 0-30 cm and after transferring to the laboratory, was air-dried. Samples were then passed through sieve No. 4 to prepare the soil for cultivation. Also, some of the primary soil was passed through sieve No. 2 to measure some of the initial physical and chemical properties. These properties included soil nitrogen, organic carbon, absorbable phosphorus, electrical conductivity (EC), pH and texture. Initially, the samples were oven-dried at 110 ° C for 24 hours. The pH of the samples was measured according to ASTM D4972 (ASTM, 2001). Soil-available phosphorous was determined according to Olsen method (Carter & Gregorich, 2007 p.267–279) and total nitrogen content by the Kjeldahl method (Bremner & Mulvaney, 1982). Soil organic carbon (SOC) was determined using the method of Walkley and Black (Walkley & Black, 1934). Soil texture was determined according to Stokes' law, hydrometer method (Faroughi & Huber, 2016) and based on this, the amount of water required to saturate the potting soil in irrigation was determined. Table 1 shows some initial properties of soil used in pots, before adding fertilizers and planting. These properties were measured again after the experiment and wheat harvest.

Table 1. Some basic properties of soil used in pots

Soil texture	Clay loam
Phosphorus content (mg/kg)	7.4
N (%)	0.07
Organic matter (%)	0.81
Organic carbon (%)	0.46
pH	7.97
EC	2.89

Preparation of fertilizer treatments

The amount of fertilizer required for each pot was calculated based on the amount of fertilizer must be applied per hectare of soil volume (1-hectare area with 30 cm depth), according to Equations (1) and (2). The amount of fertilizer calculated for each treatment was mixed with 5 kg of soil and filled the related pot.

$$\rho_b = \frac{m_s}{v_t} \quad (1)$$

$$v_t = A \times h \quad (2)$$

Where: ρ_b, m_s and v_t are the density, mass and volume of the soil, respectively. The density of the soil was measured 1400 $gr.m^{-3}$.

Preparing Pots for Planting Wheat

This section of the experiment was conducted in the research greenhouse of the Faculty of Agriculture, Shahid Chamran University of Ahvaz. Initially, 30 experimental pots were prepared. Some sand was placed on the bottom of each pot and a mesh and filter paper were placed on the sand to prevent leakage of the contents of the pots during the test. Then fertilizer treatments were measured for each pot separately and after mixing with the required soil, 5 kg of soil and fertilizer mixture were filled in each pot. Then, 12 seeds of durum wheat, Karkheh cultivar, which is compatible with the climatic conditions of Ahvaz, were planted in each pot. Germination and plant establishment in the soil are shown in Fig 2. In each pot, 6 plants that had better growth were kept and the rest were removed. Soil moisture during the test time was maintained at 70% of field capacity moisture.

Fig 2. Germination of plants in pots

Wheat Harvesting, Separation and Preparation of Potting Soil

At the end of the growing season, the aerial parts of the wheat were harvested manually (Fig 3) and transferred to the laboratory. Also, the soil of each pot was separately passed through a 2 mm sieve, at the same time the roots of the plant were separated and transferred to the laboratory for weighing and analysis. The sieved soil in each pot was air dried and stored in plastic bags for following tests.

Fig 3. Wheat harvest

Final Tests

Plant Samples

At the end of the growing season, the wheat aerial parts were cut from the soil surface. The roots isolated from the soil were then washed in fresh water, then with 0.01 N hydrochloric acid and finally with distilled water.

Plant Physical Properties

Shoot and root dry weight was measured as one of the factors indicating crop physical properties. The shoots and roots (wet) were weighed immediately after harvest and then oven-dried at 70 ° C for 72 hours. The dry weight of these organs was measured again.

Data Analyzing

Analysis of variance (ANOVA) was performed using SPSS software version 22. The means of treatments were also compared using Duncan's multiple range test. To study the effects of fertilizer application with no fertilizer application, the mean of control treatment was compared with the mean of other treatments using Dunnett test. Also, in order to investigate the effects of using different types of chicken manure on soil chemical properties, as well as crop physical properties, a group comparison between treatment groups was performed.

Checking The Collected Data

In order to analyze the variance of the data, at first, the two basic assumptions of normal data distribution and constant variance of errors were examined using Shapiro-Wilk and Leven test, respectively. Accordingly, the normality of all variables was confirmed. Also the equality of error variances of all variables was confirmed, except stem weight, available P and pH. Therefore, these variables were analyzed based on the results of Welch test.

RESULTS AND DISCUSION

Soil Chemical Properties

Table 2 shows the results of mean comparison with Duncan's multiple range test for soil chemical properties variables: organic carbon (OC), soil nitrogen (N) and soil available p (P). The treatments of D_{25}; D_{20} and D_{25}; and D_{20} and D_{25}, which are highlighted in Table 2, have had significant positive effects on soil available phosphorus, soil nitrogen and soil organic carbon content, respectively. In all three treatment groups studied, digestate group, specially D_{20} and D_{25}, increased the variables of soil chemical properties more than other treatments. It seems that the application of 25 tons of digestate per hectare leads to better results. Nitrogen (N), phosphorus (P) and potassium (K) as essential plant nutrients are more available in digestate than untreated manure (Alfa *et al.*, 2014). It is already confirmed that anaerobic digestion is a favorable option to stabilize the nitrogen in chicken manure, which is a nitrogen-rich organic waste (Busato *et al.*, 2020). Accordingly, it is clear that the higher the digestate rate used, the higher the soil nitrogen content. However, it should be noted that excessive consumption of digestate may lead to leakage of nutrients in running water and ultimately cause eutrophication. Phosphorus content of digestate are mainly in the type of $NaHCO_3$-P, H_2O-P, which are the main contributors to eutrophication and hypoxia (Li *et al.*, 2020).

Table 2. Results of mean comparison with Duncan's multiple range test for variables organic carbon (OC), soil nitrogen (N) and soil available p (P)*

OC							N							P				
Treatment	1	2	3	4	5	6	Treatment	1	2	3	4	5	6	Treatment	1	2	3	4
P15	.39						P15	.07						P15	7.5			
P20		.42					P20		.08					P20	7.67			
P25		.44					P25		.08					P25	7.7			
F15			.48				F15			.089				F15		8.1		
F20				.53			F20				.09			F20		8.13		
D15				.54			D15				.10			F25		8.16		
F25				.55	.55		F25				.10	.10		D15			8.5	
D20					.57	.57	**D20**					.11	.11	D20			8.6	
D25						.60	**D25**						.11	**D25**				8.9
Sig.	1.0	.35	1.0	.19	.07	.07	Sig.	1.0	.38	1.0	.17	.07	.07	Sig.	.08	.61	.42	1.0

*The means that are not in the same column are significantly different from each other (Alpha=0.05)

Table 3 shows the pH comparison results of the samples. As expected, the pH of digestate treatments, specially D_{25} is lower than that's of other samples and their differences with other samples are statistically significant. Usually, the pH of anaerobic reactors varies in the range of 7.5-8 and, of course, is controlled by the addition of lime or sodium hydroxide, so that the pH of the effluent does not exceed 9.5 (Guštin & Marinšek-Logar, 2011; Alfa *et al.*, 2014). The presence of bacterial and fungal species in digestate leads to N stabilization and phosphate dissolution (Alfa *et al.*, 2014).

Physical Properties of Wheat

Results of mean comparison with Duncan's multiple range test for physical properties of wheat (grain, root and stem weight) is shown in

Table 4. Results of mean comparison with Duncan's multiple range test for variables Grain weight, Root weight and stem weight*

. The treatments that are better than the others and their differences from other treatments are statistically significant are highlighted. Although in terms of grain weight, as the most important treatment studied, digestate treatments (D_{15}, D_{20}, D_{25}) have a higher mean than other treatments, but the difference between their mean compared to the average of fresh manure treatment (F_{15}, F_{20}, F_{25}) is not significant. Even if the average grain weight in digestate treatments is not higher than fresh manure treatment, since the anaerobic digestion process produces biogas a valuable energy carrier, digestate has an advantage over fresh fertilizer application.

Table 3. Results of mean comparison with Duncan's multiple range test for soil pH*

nt	pH					
Treatme	1	2	3	4	5	6
D25	7.6					
D20	7. 7	7.7				
D15	7.7	7.77	7.77			
F25		7.77	7.77	7.77		
F20			7.8	7.8		
F15				7.87	7.87	
P20					7.9	7.9
P25					7.9	7.9
P15						8.0
Sig.	.071	.071	.22	.07	.22	.22

*The means that are not in the same column are significantly different from each other (Alpha=0.05)

Table 4. Results of mean comparison with Duncan's multiple range test for variables Grain weight, Root weight and stem weight*

Grain Weight (gr)				Root Weight (gr)						Stem weight (gr)		
Treatment	1	2	3	Treatment	1	2	3	4	5	Treatment	1	2
P15	1.69			F15	.75					P15	10.05	
P20	2.40	2.40		D15	.80	.80				P20	10.38	
P25	2.43	2.43		P20	.95	.95	.95			**F15**	11.37	**11.37**
F15	2.83	2.83	**2.83**	F20	1.02	1.02	1.02			**F20**	11.55	**11.55**
F20	2.92	2.92	**2.92**	P15		1.07	1.07			**P25**	11.58	**11.58**
D15	3.03	3.03	**3.03**	D20		1.07	1.07			**D15**		**12.27**
D20		3.68	**3.68**	P25			1.12	1.12		**D20**		**12.45**
D25		3.93	**3.93**	**F25**				1.38	**1.38**	**F25**		**12.57**
F25			**4.30**	**D25**					**1.48**	**D25**		**12.58**
Sig.	.10	.07	.08	Sig.	.09	.09	.28	.06	.46	Sig.	.066	.147

*Significant effects at the 5% probability level are highlighted (Alpha=0.05)

Treatments with higher fertilizer application rates are better in terms of mean root weight compared to treatments with lower fertilizer application rates. It seems that this variable is affected by the amount of fertilizer used, more than the type of fertilizer. In this regard, D_{25} and F_{25}treatments are in a better condition than the others. Also in terms of stem weight, there is no significant difference between digestate and fresh manure treatments. It is noteworthy that in all three variables of grain, stem and root weight, the results of the application of pelletted chicken manure are disappointing. Therefore, anaerobic digestion process, in comparison with the costly method of fertilizer pelleting, in addition to producing biogas as a valuable by-product, and alleviating the potential environmental problems of chicken manure, also produces chicken manure digestate as a preferable organic fertilizer.

To study the effect of fertilizer application and non-application, Dunnett test, which compares the mean of fertilizer treatments and control treatment, was performed. The results are shown in **Error! Not a valid bookmark self-reference.**. In general, the use of chicken manure has reduced the pH of the soil. All treatments except P_{15}, have increased soil organic carbon and nitrogen. On the other hand, all treatments except the group of pelleted manure increased the soil phosphorus and decreased soil pH. These results have led to an increase in wheat stem weight in all treatments compared to this index in the control treatment. Only the use of 25 tons of fresh chicken manure and digestate per hectare has led to the development and increase of root weight. All treatments except the F_{25} hasn't significantly increased the grain weight. Although digestate treatments, especially D_{20} and D_{25}, have increased grain weight, but this increase is not significant. One reason could be that this experiment was only performed for one year and there was not enough time for treatments to improve soil quality and to increase yield significantly.

To compare the treatment groups, regardless of the amount of fertilizer application per hectare, group comparisons of treatments including digestate and control (L1), digestate and pelletted manure (L2) as well as digestate and fresh manure (L3) were performed. The results are shown in

Table 6. Results of group comparisons of treatments; L1: digestate against control, L2: digestate against pelletted chicken manure and L3: digestate against fresh manure*

and Fig 4 and show that the use of digestate in almost all variables has led to significant positive results.

In general, the effect of digestate compared to not using fertilizer (L1) in all variables has been increasing and has only been decreasing on soil pH, and in all these cases the effect of treatment is statistically significant. The trend is the same in comparison between digestate and pelletted manure, and the only difference is that the mean difference in the root weight variable, despite being positive, is not statistically significant. Compared to fresh chicken manure, digestate has increased all variables and decreased soil pH, but the differences in crop physical variables (grain, stem and root weight), despite being incremental, are not statistically significant.

Table 5. The results of Dunnett's test*

Treatment	Mean Difference	Sig.	Mean Difference	Sig.	Mean Difference	Sig.	Mean Difference	Sig.
	N		pH		OC		P	
P15	.0046	.423	.0000	1.000	.0267	.475	.1333	.899
P20	**.0102***	**.006**	-.0667	.764	**.0567***	**.014**	.3000	.191
P25	**.0125***	**.001**	-.0667	.764	**.0700***	**.002**	.3667	.074
D15	**.0319***	**.000**	**-.2667***	**.001**	**.1733***	**.000**	**1.1667***	**.000**
D20	**.0378***	**.000**	**-.3333***	**.000**	**.2067***	**.000**	**1.2667***	**.000**
D25	**.0428***	**.000**	**-.3667***	**.000**	**.2333***	**.000**	**1.5667***	**.000**
F15	**.0205***	**.000**	-.1333	.126	**.1133***	**.000**	**.7333***	**.000**
F20	**.0291***	**.000**	**-.2000***	**.009**	**.1600***	**.000**	**.7667***	**.000**
F25	**.0330***	**.000**	**-.2333***	**.002**	**.1800***	**.000**	**.8000***	**.000**
	RootWeight		**StemWeight**		**GrainWeight**			
P15	.2167	.515	**3.9000***	**.000**	-.6267	.921		
P20	.1000	.979	**4.2333***	**.000**	.0833	1.000		
P25	.2667	.301	**5.4333***	**.000**	.1167	1.000		
D15	-.0500	1.000	**6.1167***	**.000**	.7167	.857		
D20	.2167	.515	**6.3000***	**.000**	1.3667	.261		
D25	**.6333***	**.001**	**6.4333***	**.000**	1.6167	.134		
F15	-.0967	.983	**5.2167***	**.000**	.5167	.971		
F20	.1667	.766	**5.4000***	**.000**	.6000	.936		
F25	**.5333***	**.006**	**6.3667***	**.000**	**1.9833***	**.045**		

*Significant effects at the 5% probability level are highlighted

Table 6. Results of group comparisons of treatments; L1: digestate against control, L2: digestate against pelletted chicken manure and L3: digestate against fresh manure*

		Dependent Variable						
		GrainWeight	StemWeight	RootWeight	OC	P	pH	N
L1	Contrast Estimate	3.700	18.850	.800	.613	4.000	-.967	.112
	Hypothesized Value	0	0	0	0	0	0	0
	Difference (Estimate - Hypothesized)	3.700	18.850	.800	.613	4.000	-.967	.112
	Std. Error	1.619	1.688	.330	.039	.327	.132	.006
	Sig.	**.033**	**.000**	**.025**	**.000**	**.000**	**.000**	**.000**
L2	Contrast Estimate	4.127	5.283	.217	.460	3.200	-.833	.085
	Hypothesized Value	0	0	0	0	0	0	0
	Difference (Estimate - Hypothesized)	4.127	5.283	.217	.460	3.200	-.833	.085
	Std. Error	1.145	1.193	.233	.028	.231	.093	.005
	Sig.	**.002**	**.000**	.364	**.000**	**.000**	**.000**	**.000**
L3	Contrast Estimate	.600	1.867	.197	.160	1.700	-.400	.030
	Hypothesized Value	0	0	0	0	0	0	0
	Difference (Estimate - Hypothesized)	.600	1.867	.197	.160	1.700	-.400	.030
	Std. Error	1.145	1.193	.233	.028	.231	.093	.005
	Sig.	.606	.133	.410	**.000**	**.000**	**.000**	**.000**

*Significant effects at the 5% probability level are highlighted

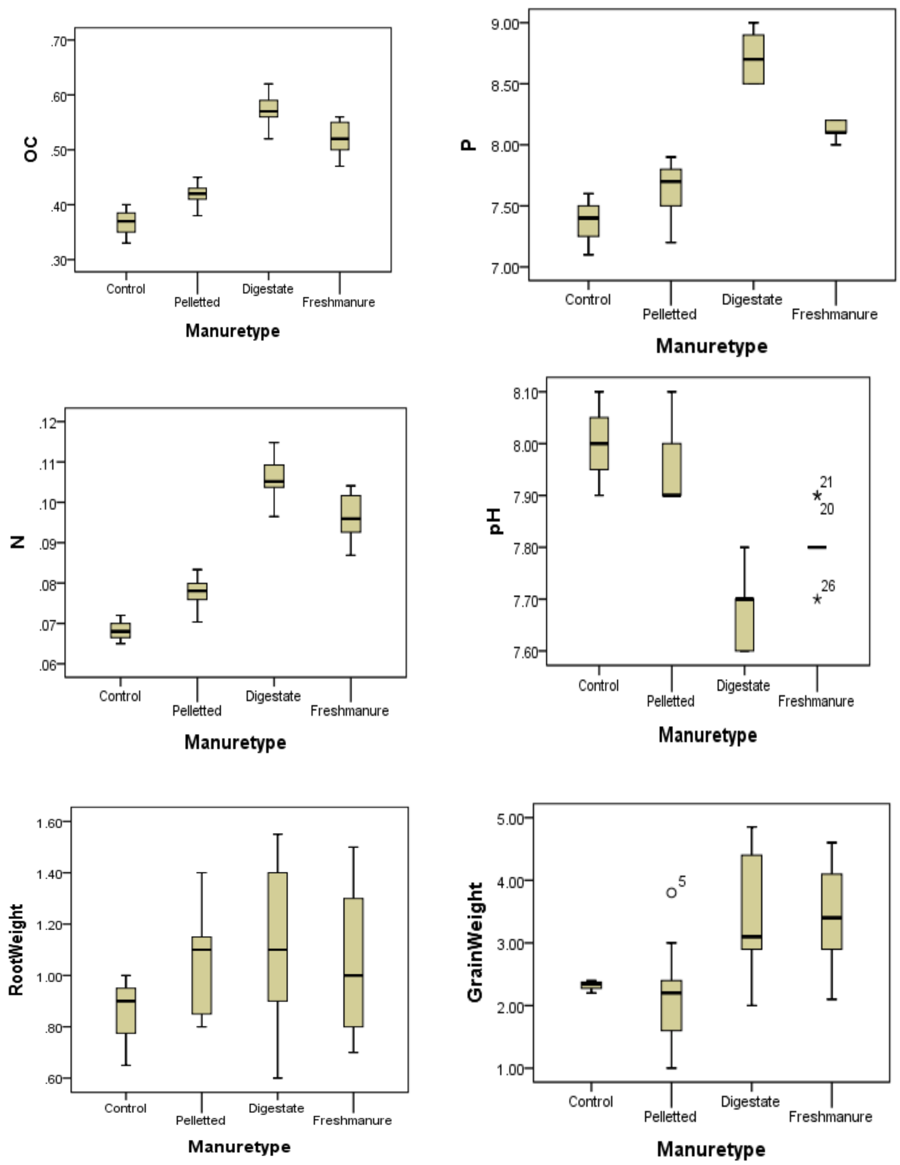

Fig 4: Box plots of treatment groups and studied variables

CONCLUSION

According to the results, the use of digestate as an organic fertilizer in comparison with pelleted chicken manure in all studied indicators, has led to positive results. The results of the studied indicators also were better or at least equal compared to the results obtained from the application of fresh chicken manure. Digestate application has relatively increased soil nitrogen, organic carbon and phosphorus, kept the pH of soil neutral, and improved the yield components of the wheat. Therefore, anaerobic digestion process, in comparison with the costly method of fertilizer pelleting, in addition to producing a valuable by-product, biogas, and alleviating the environmental problems of chicken manure, also produces chicken manure digestate as a proper organic fertilizer. Compared to fresh chicken manure, not only has the quality of chicken manure digestate not decreased, but it has also improved in some ways. Therefore, considering the environmental benefits of anaerobic digestion as well as biogas production and by proving that the quality of the resulting fertilizer does not decline, the use of digestate as an organic fertilizer has more benefits than the use of fresh chicken manure.

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