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Determination of the appropriate level of manure fertilisation for *Moringa oleifera* grown for animal feed

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Abstract:

The purpose of this study is to determine the appropriate level of chicken manure for the green fodder *M. oleifera* grown for leaf meal production as a supplement into poultry diet to improve poultry product quality (i.e., meat and egg). The experiment was conducted at Thai Nguyen University of Agriculture and Forestry, Vietnam, for two years from 2018 to 2019. The experiment consisted of four treatments (NT) represented by four different levels of chicken manure, namely, 0 tons (NT1), 10 tons (NT2), 20 tons (NT3) and 30 tons/ha/yr (NT4). Each treatment was carried out over an area of 24 m² with 5 replicates. The experiment was the complete randomised block design. Other factors such as plantation density, nitrogen, phosphate, potassium fertiliser levels, cutting height, and cutting intervals, etc., were similar among treatments. The results showed that the leaf dry matter yield of NT1 through NT4 was 6.919, 8.131, 8.975, and 9.494 tons/ha/yr, respectively. That of the leaf crude protein was 2.244, 2.694, 3.073, and 3.357 tons/ha/yr, respectively. Increasing manure levels from 0 to 30 tons/ha/yr decreased the dry matter content in the leaves by 1.43%, increased the crude protein in leaf dry matter basic by 2.93%, and decreased crude fibre in the leaf dry matter basic by 2.24%. Based on these results and data from statistical analysis, the most appropriate level of chicken manure application for *M. oleifera* was at 20 tons/ha/yr.

Keywords: **animal feed, level of manure fertilization, *Moringa oleifera*.**

Classification number: **3.1**

Introduction

The yield and quality of green fodders are influenced by several factors such as the varieties, seasons (rainy or dry seasons), soil fertility, and fertilisers, etc. Chemical fertilisers, especially nitrogen fertiliser, has a significant effect on green fodder yield. However, the prolonged utilization of chemical fertiliser will have negative effects on the soil such as soil acidification (decrease of pH), deficiency of organic matter in the soil, and decrease of soil microbial that leads to soil degeneration and solidification. The application of manure in combination with chemical fertilisers is believed to be the better solution to eliminate these negative effects. This is because, manure provides organic matter and other nutrients to the soil, improves soil chemo-physical characteristics, soil fertility, and fluff. Manure provides effective microbials as well as a suitable environment for the development of microbials, therefore, the decomposition of nutrients increases [1-3]. Several studies have reported that manure application improved green fodder yield and quality [4-6]. Therefore, the application of organic fertiliser, namely, manure, for green fodder plantation should be taken into account.

Materials and methods

The subject of this study, the green fodder *M. oleifera* was planted for leaf meal production for a more complete poultry diets in order to improve meat and egg quality. The experiment was conducted at Thai Nguyen University of Agriculture and Forestry, Thai Nguyen province, which is located in a mountainous area of Vietnam for a period of 2 years from 2018 to 2019.

The experiment consisted of four treatments (NT) corresponding to four different levels of chicken manure application, namely, NT1 for a 0 tons/ha/yr application, NT2 for 10 tons/ha/yr, NT3 for 20 tons/ha/yr, and NT4 for 30 tons/ha/yr. Each treatment was tested on an area of 24

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m^2 with 5 replicates (each treatment consisted of 5 lots and, each lot was 24 m^2). The experiment was carried out under the complete randomised block design. Other factors such as plantation density, chemical (N, P, K) application levels, harvesting intervals, etc., were similar between treatments.

The monitoring parameters included productivities and yields of biomass, fresh leaves, dry matter, and chemical composition of the leaves. The productivities and yields were estimated following the method described by [7].

Productivities of biomass, fresh leaves, and dry matter were expressed as the weight of the biomass, fresh leaves, and dry matters obtained per ha per harvest, i.e. kg/ha/harvest. Biomass productivity was estimated by harvesting all five lots of a single treatment and, the average biomass productivity was calculated from the total biomass productivity of five lots. Fresh leaf productivity was estimated by randomly sampling 10 kg of biomass from each lot (10 kg×5 lots=50 kg) then the leaves and stems were separated and weighed to obtain the ratio of leaves per biomass. Fresh leaf productivity was defined to equal the biomass productivity multiplied by the fresh leaf and biomass ratio. Dry matter productivity was estimated by sampling five fresh leaf samples from five treatments, then oven drying the fresh leaves and calculating the dry matter per fresh leaf ratio. Dry matter productivity was defined to equal the fresh leaf productivity multiplied by the dry matter per fresh leaf ratio.

Yields of biomass, fresh leaves, and dry matter were calculated by adding productivities of harvests in a year or multiplying the average productivity per harvest by the number of harvests per year, which is expressed as ton/ha/yr. These two calculation methods differed by 0-5‰ due to rounding the average productivity of a harvest. Crude protein yield was obtained by multiplying dry matter yield with crude protein content in dry matter.

Chemical analysis was performed as described by [8] and the data obtained was statistically analysed following the method described by [9].

Results and discussion

Effect of different manure application levels on productivity of M. oleifera

Effect on biomass productivity: biomass productivity data is the basic input data to calculate the fresh leaf and dry matter productivities and the biomass, fresh leaf, and dry matter yields. Thus, Table 1 provides the complete biomass productivity of every harvest. The total number of harvests over the 2 - year span was 11, of which five occurred in the first year and six in the second.

Table 1. Biomass productivity of treatments (kg/ha/harvest).

Harvest	NT1	NT2	NT3	NT4	SEM	p
	0 ton	*10 tons*	*20 tons*	*30 tons*		
1	26,870	32,583	37,629	40,323		
2	25,626	30,625	35,266	37,840		
3	20,100	24,470	27,612	29,748		
4	8,548	10,300	11,526	12,242		
5	6,116	7,536	8,414	8,952		
$\overline{X}_1$	17,452^c	21,103^b	24,089^a	25 821^a	1,079	0.000
6	9,800	11,290	13,358	15,246		
7	18,328	22,098	24,837	26,576		
8	19,854	22,386	25,040	26,342		
9	10,447	13,052	14,716	15,640		
10	6,554	8,019	9,139	9,796		
11	3,925	4,840	5,430	5,832		
$\overline{X}_2$	11,485^c	13,614^b	15,420^a	16,572^a	776	0.000
$\overline{X}$	14,197^c	17,018^b	19,361^a	20,776^a	913	0.000

Note: $\overline{X}=[(\overline{X}_1x5)+(\overline{X}_2x6)]:11$; Duncan's multiple range test: similar character in the column (a, or b, c, d or e) means no significant difference among the average data.

The data in Table 1 showed that the development of biomass production for a period of two years was similar among treatments. Biomass productivity is illustrated in Fig. 1. The productivity of harvest during the first half of year one was high and gradually lowered during the second half of the year. This can be explained by the annual, application of manure, phosphate, and potassium occurring at the beginning of year, thus, during this period soil fertility is highly enriched and promotes productivity. After that, soil fertility gradually declined due to exploitation by the plants, which caused the productivity of later harvests to decrease. On the other hand, the first harvests were during the rainy season and thus the temperature and humidity were favourable for plant development. The other harvests were during the dry season, which is not favourable for plant development.

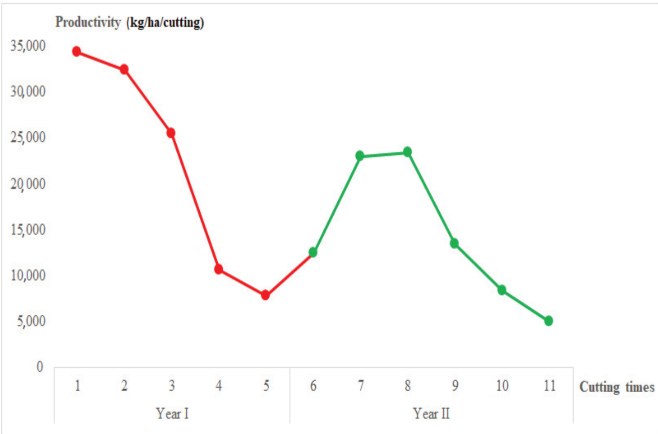

Fig. 1. Biomass productivity of harvests for 2 years.

Average productivity per harvest was higher during the first year and was lower during the second year. For example, if one considers the average productivity of biomass per harvest during the first year of NT1 as 100%, then the average productivity of harvests during the second year compared to the first would be 65%. This comparison was similar among the other treatments, which can be explained by the following two points. During the first year, plants were adequately provided with available nutrients from the soil and from supplied fertiliser; however, during the second year, soil fertility reduced gradually and nutrients were only supplied from fertiliser. Second as the age of the plants and number of cuttings increases, the regeneration capacity of the plants decrease.

The average biomass productivity per harvest for the first and second year and the average of 2 years increased when manure application levels increased. If one considers the average biomass productivity of the 2-year production of NT1 as 100%, then that of NT2 was 119.9%, NT3 was 136.4% and NT4 was 146.3%. Statistical analysis revealed that the average biomass productivity per harvest of the first year, second year, and the 2-year average of the treatments with manure application was significantly higher than that of the treatment without manure application ($p<0.001$). Among treatments with manure application, the NT3 and NT4 treatments showed significantly higher biomass productivity compared to that of NT2 ($p<0.001$), but not significant in NT3 and NT4 ($p>0.05$). These findings prove that the chicken manure application level for *M. oleifera* at 20 tons/ha/yr or above are suitable.

Effect on fresh leaf productivity: fresh leaf productivity was affected by biomass productivity and the fresh leaves per biomass ratio. Based on the biomass productivity presented in Table 1 and the ratio of fresh leaves per biomass of 38.68%, one can easily determine the fresh leaf productivity of every harvest during the 2-year spans, therefore, Table 2 does not present fresh leaf productivity of every harvest, but illustrates the average fresh leaf productivity per harvest during the first year, second year, and the overall average of the 2-year treatments.

Table 2. Fresh leaf productivity of treatments (kg/ha/harvest).

Mean/year	NT1	NT2	NT3	NT4	SEM	p
$\overline{X}_1$	6,750^c	8,163^b	9,318^a	9,988^a	417	0.000
$\overline{X}_2$	4,442^c	5,266^b	5,964^a	6,410^a	300	0.000
$\overline{X}$	5,491^c	6,583^b	7,489^a	8,036^a	353	0.000

The data presented in Table 2 shows that average fresh leaf productivity per harvest increases accordingly with manure application levels. The average fresh leaf productivity per harvest for the first year increased from 6,750 kg (NT1) to 9,988 kg (NT4). That of the second year was lower, ranging from 4,442 kg to 6,410 kg, and the average over 2-year period ranged from 5,491 kg to 8,036 kg. If one considers the average fresh leaf productivity per harvest over 2-year period of NT1 as 100%, then that of NT2 was 119.9%, NT3 was 136.4%, and NT4 was 146.3%. Average fresh leaf productivity per harvest during the first year, second year, and over 2-year period for all treatments with manure application were significantly different compared to that without manure application ($p<0.001$). Among treatments with manure application, the average fresh leaf productivity per harvest of NT3 and NT4 were significantly higher than that of NT2 ($p<0.001$) but not between NT3 and NT4 ($p>0.05$).

Effect on dry matter productivity: dry matter productivity is dependent on fresh leaf productivity and its dry matter content (i.e., dry matter productivity is equal to fresh leaf productivity multiplied by dry matter content in fresh leaves). Dry matter content in fresh leaves was different among treatments. It decreased with the increase of manure application levels as follows: NT1 was 22.91%, NT2 was 22.46%, NT3 was 21.79%, and NT4 was 21.48%. Based on the fresh leaf productivity data in Table 2 and the dry matter content in the fresh leaves, dry matter productivity is presented in Table 3.

Table 3. Dry matter productivity of treatments (kg/ha/harvest).

Mean/year	NT1	NT2	NT3	NT4	SEM	p
$\overline{X}_1$	1,546^b	1,833^b	2,030^a	2,145^a	92	0.000
$\overline{X}_2$	1,018^c	1,183^b	1,300^{ab}	1,377^a	66	0.000
$\overline{X}$	1,258^c	1,479^b	1,632^a	1,726^a	78	0.000

The data presented in Table 3 showed that dry matter productivity increased accordingly with the increase of manure application levels similar to the trends of biomass and fresh leaf productivities. However, the difference between the dry matter productivity of the treatments was not significant compared to the difference between biomass and fresh leaf productivity of the treatments. This is thought to occur because the increase in manure application resulted in the decrease of dry matter content in fresh leaves because dry matter productivity is equal to fresh leaf productivity multiplied by the dry matter content in fresh leaves. For example, the fresh leaf productivity of the overall average of 2 years of NT4 treatment was higher than that of NT1 by 46.3% but the average dry matter productivity of the overall of 2 years in NT4 was just higher than that of NT1 by 37.2%. Nevertheless, the average dry matter productivity/harvest over 2-year period in all treatments with manure application were significantly higher than that of the control (p<0.001). Among treatments with manure application, the dry matter productivity of NT3 and NT4 were significantly higher than that of NT2 (p<0.001), but was not significant between NT3 and NT4 (p>0.05).

Other studies on manure application for legumes [10, 11], *M. oleifera* [4], *Trifolium alexandrium* and *Lolium multiflorum* [5], and *Trichanthera gigantea* [6] had reported that manure application increased dry matter yield and our findings are supported by these studies.

Effect of different manure application levels on M. oleifera yield

Product yield was calculated by multiplying the average productivity/harvest by the number of harvests during the production year. There were five harvests during the first year and six harvests during second year. Based on data of mean productivity (biomass, fresh leaves, dry matter)/harvest of the first year and second year presented in Tables 1, 2, and 3, one can easily estimate the yields of biomass, fresh leaves, and dry matter from the first and second year. Therefore, Table 4 does not present yield data of the biomass, fresh leaves, and dry matter of each year but presents the mean of the overall yield of the 2 years. Crude protein yield was calculated by multiplying the dry matter yield by the crude protein content in the dry matter. This content for the NT1, NT2, NT3, and NT4 treatments was 32.43, 33.13, 34.24, and 35.36%, respectively.

Table 4. Average yields per year of treatments (ton/ha/year).

Categories	NT1	NT2	NT3	NT4	SEM	p
Biomass	78.084^c	93.599^b	106.485^a	114.268^a	5.020	0.000
Fresh leaves	30.203^c	36.204^b	41.188^a	44.199^a	1.942	0.000
Dry matter	6.919^c	8.131^b	8.975^a	9.494^a	0.429	0.000
Crude protein	2.244^d	2.694^c	3.073^b	3.357^a	0.145	0.000

The data presented in Table 4 shows that the biomass yield of *M. oleifera* increased from 78.084 tons/ha/yr (NT1) to 114.268 tons/ha/yr (NT4) and that of fresh leaves increased from 30.203 tons/ha/yr (NT1) to 44.199 tons/ha/yr (NT4). Biomass and fresh leaf yields in treatments with manure increased significantly compared to that of the control (p<0.001). Among treatments with manure, the yields of NT3 and NT4 were significantly different compared to NT2 (p<0.001) but this difference was not significant between NT3 and NT4 (p>0.05).

For the green fodders intended for leaf meal production, dry matter and crude protein yields are the most essential parameters to evaluate production capacity. Dry matter and crude protein yields were increased accordingly with the increase of manure application. The overall mean of the dry matter yield for the 2 years of *M. oleifera* harvest increased from 6.919 tons (NT1) to 9.494 tons/ha/yr (NT4). Crude protein yield increased from 2.244 to 3.357 tons/ha/yr. If one considers the dry matter yield of NT1 to be 100%, then that of NT2 was 117.5%, NT3 was 129.7%, and NT4 was 137.2%. Similarly, crude protein yield of all treatments was 100, 120.1, 136.9, and 149.6%, respectively. The overall mean of crude protein and dry matter yields of the 2 years of NT2, NT3, and NT4 treatments were higher than that of NT1. The yields of NT3 and NT4 were significantly higher than that of NT2 (p<0.001). However, the dry matter yields of NT3 and NT4 were not significantly different (p>0.05), therefore, the chicken manure application level for *M. oleifera* at 20 tons/ha/yr is the most suitable.

The average dry matter and crude protein yields of the first 2 years from several green fodders for leaf meal production such as cassava for foliage production were 7.0 tons/ha/yr and 1.7 tons/ha/yr [12], *Leucaena* were 6.0 tons and 1.7 tons/ha/yr [13], respectively; *Stylosanthes* were 7.8 tons/ha/yr and 1.4 tons/ha/yr [14], respectively; and *T. gigantea* were 10-12 tons/ha/yr and 2.4-3.2 tons/ha/yr [15], respectively. The average dry matter and crude protein yields of *M. oleifera* during the first two years in this experiment reached 6.919 to 9.494 tons/ha/yr and that of crude protein ranged between 2.244 tons to 3.357 tons/ha/yr. This proves that *M. oleifera* is a potential green fodder for leaf meal production.

The production efficiency of dry matter and crude protein from manure

The production efficiency production of dry matter and crude protein from manure can be estimated by subtracting dry matter and crude protein yields of treatments NT2, NT3, and NT4 by that of NT1, then divide by the total amount of manure applied for each ha in a year. The obtained data presented in Table 5.

Table 5. Production efficiency of manure for dry matter and crude protein.

Categories	Unit	NT2	NT3	NT4	SEM	p
DM increased	kg/ha/year	1,212^c	2,056^b	2,575^a	43.3	0.000
CP increased	kg CP/ha/year	450^c	829^b	1,113^a	21.6	0.000
M	ton/ha/year	10	20	30		
DM/M	kg DM/ton M	121.2^c	102.8^b	85.8^a	3.4	0.000
CP/M	kg CP/ton M	45.0^c	41.4^b	37.1^a	1.4	0.000

DM: dry matter; CP: crude protein; M: manure.

The data in Table 5 shows that an increase in manure application levels increased dry matter weight compared to NT1 (without manure) from 1,212 kg (NT2) to 2,575 kg/ha/yr (NT4). Crude protein increased from 450 kg to 1,113 kg/ha/yr. The increase of dry matter and crude protein over all treatments were significant (p<0.001).

When the manure application level increased, the dry matter and crude protein also increased but the production efficiency of 1 ton of manure decreased. Specifically, dry matter decreased from 121.2 kg/ton (NT2) to 85.8 kg/ton of manure (NT4), equivalent to 571 kg fresh leaves (DM/fresh leaf ratio of NT2 was 22.46%) and 425 kg fresh leaves (DM/fresh leaf ratio of NT4 was 21.48%), respectively. Additionally, crude protein decreased from 45.0 kg to 37.1 kg/ton of manure. The production efficiency of 1 ton of manure for all treatments was significant (p<0.001). Therefore, when applying manure fertiliser, it is important to consider the production efficiency as it decreases with an increase in manure levels.

Table 6. Comparison of production cost of different treatments (%).

Expenditure	NT1	NT2	NT3	NT4
	0 ton	*10 tons*	*20 tons*	*30 tons*
Expenditure/ha/2 yr	100	114.7	128.5	142.3
Leaf meal yield/ha/2 yr	100	117.5	129.7	137.2
Production cost/1 kg leaf meal	100	97.6	99.1	103.7

The expenditure for the nursery plants and chemical fertiliser were similar among the four treatments. However, there is a difference in the expenditure for manure fertiliser when levels of manure application increased. Although this resulted in an increase of yield, production labour harvesting, processing increased as well as the costs of the labour itself. The data presented in Table 6 reveals that, compared to NT1 (without manure), the total expenditure for 1 ha over the 2-yr time for NT2, NT3, and NT4 increased by 14.7, 28.5, and 42.3%, respectively. The leaf meal yield of NT2, NT3, and NT4 also increased by 17.5, 29.7, and 37.2%, respectively, compared to NT1. The manure treatments had a larger increase of leaf meal yield compared to the increase of production cost, which means the cost of production per every kg leaf meal would be lower and vice versa. Therefore, the cost to produce 1 kg leaf meal under the NT2, NT3, and NT4 schemes compared to NT1 was 97.6, 99.1, and 103.7%, respectively. Thus, an appropriate level of manure application should be chosen to reduce production costs.

Effect of manure levels on M. oleifera leaf quality

The quality of green fodders is usually evaluated by their chemical composition, thus, several main components such as dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), and ash were analysed. Along with the nitrogen free extract (NFE)=DM-(CP+EE+CF+Ash), the main component of *M. oleifera* presented in Table 7.

Table 7. Chemical composition of *M. oleifera* leaves.

NT	DM (% FL)	% DM					GE (kcal)
		CP	*EE*	*CF*	*Ash*	*NFE*	
NT1 (0)	22.91^a	32.43^c	6.63^b	9.78^a	9.04^a	42.12^a	4,644^a
NT2 (10)	22.46^{ab}	33.13^c	6.81^b	9.08^b	9.17^a	41.81^a	4,657^a
NT3 (20)	21.79^{ab}	34.24^b	7.07^a	7.94^c	9.41^a	41.34^a	4,667^a
NT4 (30)	21.48^b	35.36^a	7.17^a	7.54^c	9.45^a	40.48^a	4,679^a
SEM	0.649	0.577	0.143	0.377	0.612	1.708	54.972
p	0.013	0.000	0.000	0.000	0.681	0.475	0.779

Note: leaf sample at 50 days of cutting; FL: fresh leaves; GE: gross energy.

The data presented in Table 7 showed that when manure application levels increased, a decrease in leaf dry matter (decreased 1.43% from NT1 to NT4) was observed and the water content in the leaves increased. Therefore, the leaves of the plants with manure application were softer than those without manure application. However, the dry matter content in fresh leaves was only significantly different between NT4 and NT1 (p<0.05). The crude protein content in the dry matter increased with an increase of manure application level (increase of 2.93% from NT1 to NT4). The crude protein content in the dry matter of NT3 and NT4 were significantly higher than that of NT1 and NT2. This can be explained by the large amount of nitrogen contained within chicken manure, which is a source for crude protein synthesis. In contrast with crude protein content, crude fibre content in dry matter decreased with an increase of manure level (decreased by 2.24% from NT1 to NT4). The crude fibre content in the dry matter of NT3 and NT4 was significantly lower compared to that in NT1 and NT2. Crude protein and crude fibre are the main criteria that determine the quality of the green fodders; therefore, the application of manure significantly improved the quality of *M. oleifera*. The best improvement was seen at the level of 20 tons/ha/yr than at the level of 10 tons/ha/yr.

Results from studies on the application of manure fertiliser for *Stylo guianensis* [10, 11], *Trifolium alexandrium* and *Lolium multiflorum* [5], and *Trichanthere gigantea* [6] all showed that manure fertiliser improved green fodder qualities such as increased crude protein content and decreased crude fibre content in the leaf dry matter. The findings are also supported by the works mentioned above.

Conclusions

Increasing chicken manure from 0 to 30 tons/ha/yr increased the average leaf dry matter yield over 2 year from 6.919 to 9.494 tons/ha/yr, increased the crude protein yield of leaves from 2.244 to 3.357 tons/ha/yr, increased crude protein content in leaf dry matter by 2.93%, and decreased crude fibre content in leaf dry matter by 2.24%. Based on dry matter yield, crude protein yield, leaf chemical composition, and statistical analysis of these parameters, it was concluded that chicken manure should be applied to *M. oleifera* at 20 tons/ha/yr.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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