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CULTIVATION AND ULTILIZATION OF MORINGA (*MORINGA OLEIFERA*) FODDER IN COLOURED FEATHER CHICKEN PRODUCTION

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PUBLISHED SCIENTIFIC WORKS RELATED TO THE THESIS

1. Hoang Thi Hong Nhung, Tu Trung Kien, Tran Thi Bich Ngoc, Pham Tuan Hiep, Tu Quang Hien. 2020. A Study on plant density of *Moringa oleifera* for animal feed at the Thai Nguyen province, Vietnam Journal of agriculture and rural development, No 394 october 2020, 29 – 35.

2. Hoang Thi Hong Nhung, Tu Trung Kien, Tran Thi Bich Ngoc, Tu Quang Hien. 2021. Replacement of soybean meal by *Moringa oleifera* leaf meal in Luong Phuong layer parents stock diet. TNU Journal of science and Technology, 226 (01) 2021, 99 – 105.

3. TU Q. HIEN; TRAN T. HOAN; MAI A. KHOA; TU T. KIEN; PHAN T. HUONG; HOANG T. H. NHUNG. 2017. Nutrient digestibility determination of cassava, leucaena, stylosanthes, moringa and trichanthera leaf meals in chickens. *Bulgarian Journal of Agricultural Science, 23 (No 3) 2017, 476–480.*

4. Tu Quang Hien, Hoang Thi Hong Nhung, Tu Quang Trung and Mai Anh Khoa. 2021. Replacement of soybean meal by *Moringa oleifera* leaf meal in broiler diet. *Bulgarian Journal of Agricultural Science, 27 (No 4) 2021,* 769 – 775.

1. Introduction

Currently, animal feed price has been increasing rapidly due to climate change and high grain deman for humans. According to the Department of Livestock Production, Ministry of Agricuture and Rural Development, during the first eight months of 2021, Vietnam imported 5.09 million tons of protein-rich feed, cost 2.27 billion USD (increasing 28% in comparsion to the similar period of 2020); of which soybean meal increased by 35,5% comparing to 2020. Therefore, local alternative protein-rich ingredient which could replace to imported one is very important and necessary to enhance animal feed production security in Vietnam.

Moringa oleifera (*M. oleifera*) has been demonstrated to ultilize as rich-protein ingredient in animal feed. *M. oleifera* leaves contain 32,07 – 35,19% crude protein in dry matter with high digestibility protein (Fahey et al., 2001; Tu Quang Hien, 2019) Nutrient compositions of *M. oleifera* leaves quitely similar with those of soybean meal, such as essential amino acids pofile, crude fiber (5,9%), ash (12%), crude fat (7,09%) with 57% fatty acids in leaves are unsaturated fatty acids (Bin Su and Xiaoyang Chen, 2020). *M. oleifera* leaves and fruit are rich in carotene, vitamin C and a balance of amino acids profile (Makkar and Becker, 1996). *M. oleifera* has been considered as good source of food for humans, livestock and poultry (Afuang et al., 2003).

However, the studies on *M. oleifera* cultivation techniques mainly focus on green vegetables and medicinal herbs production for human, while the study on green fodder production to replace imported ingredients in poultry diet has been still limited. Therefore, this study aim to fill the gaps in the study of *M. oleifera* as animal feed.

2. Objectives of the project

The project aims to investigate:

The effects of planting density, cutting intervals, and the appropriate level of nitrogen fertilization on growth performance and nutrient composition of *M. oleifera* plants.

The nutrients digestibility (protein, lipid, fiber, nitrogen free extracr, derivatives) and energy value of *M. oleifera* leaf meal in chickens.

The effects of *M. oleifera* leaf meal replacing ration with soybean meal as protein resources on the performance of Luong Phuong broilers and breeder.

**** Scientific significance***

The results of study determined the effects of cultivation density, cutting interval, and nitrogen fertilization on the *M. oleifera* fodder growth performance; the nutrients digestibility and energy value of *M. oleifera* leaf meal in chickens; the effects of replacing soybean meal protein with *M. oleifera* leaf meal protein in the diets on performance of Luong Phuong broilers and breeder. These results can be used as material for education and scientific research in the field of animal feed and nutrition.

**** Practical significance***

Animal production could apply the results of the project to improve the yield and quality of *M. oleifera* leaf meal, improve livestock production, product quality, while it could reduce the cost of chicken feed.

The results of nutrient digestibility and metabolizable energy of *M. oleifera* leaf meal studies are the scientific evidence for fomulate chickens diets containig *M. oleifera* leaf meal.

**** New contributions of the dissertation***

This project studies on a number of cultivative techniques including planting density, cutting interval and nitrogen fertilization rate for *M. oleifera* cultivation as fodder for animal feed production.

This project studies on the potential of replacing soybean meal with *M. oleifera* leaf meal in the feed of coloured feathers broilers and breeder.

The results of this dissertation open a direction to exploit and use effectively and sustainably *M. oleifera* (Moringa) as raw materials for animal feed in Vietnam.

Chapter 1
DOCUMENTARY OVERVEW

1.1. Scientific evidence of research problem

The dissertation showed the biological characteristics; fertility, regeneration and propagation of *M. oleifera;* factors affecting the growth and yield of *M. oleifera* (especially climatic factors, soil, cultivation techniques: varieties, planting density, fertilizers, harvesting techniques); the chemical composition and use value of the plant (especially the leaves of *M. oleifera*). Accordingly, the results of references were considered as scientific evidences for this study.

1.2. Situation of study and use of *M. oleifera* in chicken production

M. oleifera is supplied in chicken diets with different purposes, such as improving growth and feed utilization, disease resistance, enhancing product quality of chickens, reducing feed costs, and improving chicken performance, increasing efficiency of chicken production. For these purposes, *M. oleifera* has been ultilized in the broiler and layer diet as a rich-protein supplement, feed ingredient, biologically active substances or pharmaceutical drug. The recommended levels of *M. oleifera* leaf meal in chicken diets of all references were considered as the scientific evidence for the studies of the dissertation.

Chapter 2
CONTENTS AND METHODOLOGY OF STUDY

2.1. Materials, places and time period of study

2.1.1. Material of study: *M. oleifera* (Moringa) fodder; *M. oleifera* dried leaf meal; Luong Phuong broiler and breeder.

2.1.2. Research location

M. oleifera (Moringa) and experimental chicken were conducted on the Poultry Farm, Faculty of Animal science and veterinary Medicine, Thai Nguyen University of Agriculture and Forestry, Thai Nguyen City, Thai Nguyen Province.

Soil samples, leaf meal, feed, chicken meat, eggs were analysed at the Institute of Life Sciences, Thai Nguyen University.

***2.1.3. Time period* of study** was From 2017 to 2021.

2.2. Contents of study

Determine the cultivation density, nitrogen fertilization level and interval cutting for *M. oleifera* fodder.

Determination of nutrient digestibility and metabolizable energy value of *M. oleifera* leaf meal in Luong Phuong broiler.

Determination of the possibility replacing soybean meal with *M. oleifera* leaf meal in the Luong Phuong broilers and breeder diets.

2.3. Methodology of study

2.3.1. Meteorology and soil chemical composition of the experimental area

The data of temperature, humidity and rainfall were collected from the hydro-meteorological station of Thai Nguyen province.

The chemical composition of the soil in the experimental area included: pH, total nitrogen, total P_20_5, easily digestible P_20_5, K_20_5, total metabolic K_20_5 was analyzed at the Institute of Life Sciences, Thai Nguyen University following to the conventional method.

2.3.2. Experiment 1: Study on planting appropriate density of M. oleifera plants

Table 2.1. Experimental design to determine the *M. oleifera* cultivation density

Item	Treatment 1 (NT1)	Treatment 2 (NT2)	Treatment 3 (NT3)	Treatment 4 (NT4)
Planting density (plant/hectare)	125.000	100.000	83.500	71.500
Intervals for planting (m)	0,2 m x 0,4 m	0,2m x 0,5 m	0,2m x 0,6 m	0,2m x 0,7 m
Manure (kg/hectare/year)	- Chicken manure 20 tons, phosphorus: 40 kg P_2O_5, potassium: 80 kg K_2O (calculated for 1 hectare/year). - Nitrogen 60 kgN/hectare 1 month after planting			
Area (m^2)	24 m^2 x 5			

The first harvest was carried out 4 monhts after sowing seeds in pot (or 3 months after planting seedlings), after the first harvest, the interval harvest was 9 days in January and February. The plant was firstly harvested at 45 – 50 cm of height above the ground, while the interval cutting were 10 cm higher than the first cutting point.

* Observation parameters: Total biomass, fresh leaves yeild and dry matter of each cutting time (kg/ha/interval cutting); Annual total biomass fresh leaves yeild, dry matter, crude protein (tons/hectare/year); Production costs per hectare/2 years and 1 kg of leaf meal.

* Observation methods: Monitoring the production and yield according to Tu Quang Hien et al., (2002).

2.3.3. Experiment 2: Determination of effective nitrogen fertilization level for M. oleifera cultivation

Table 2.2. Experimental design for determination of effective nitrogen ferrtilizer level

Item	**Treatment 1(NT1)**	**Treatment 2 (NT2)**	**Treatment 3 (NT3)**	**Treatment 4 (NT4)**	**Treatment 5(NT5)**
Nitrogen ferrtilizer level(kgN/hectare /interval cutting)	0	20	40	60	80
Fetilizer (kg/hectare/year)	Chicken manure 20 tons, phosphorus: 40 kg P_2O_5, potassium: 80 kg K_2O (calculated for 1 hectare/year).				
Planting density(plant/hectare)	83.500				
Area (m^2)	24 m^2 x 5				

The methodology of harvesting, and productivity and yield calculation were the same as Experiment 1.

* Observation were the same as those of experiment 1. In addition, the below observation was added:

Efficiency of dry matter and crude protein production of nitrogenous fertilizers; chemical composition of leaves, including: dry matter, crude protein, crude lipid, crude fiber, non nitrogen-extraction, crude ash and amino acids profile in leaf protein.

* Observation methods

Observation of productivity and yield was the same those of experiment 1.

Sampling and analysis of dry matter, proteins, lipids, fibers, Non-protein nitrogen compounds, total minerals, were carried out according to Vietnamese standards (TCVN) of, animal feed 2001 and 2007.

2.3.4. Experiment 3: Determination of effective cutting interval of M. oleifera plant

Table 2.3. Experimental design for effective cutting interval

Item	Treatment1 (NT1)	Treatment 2 (NT2)	Treatment 3 (NT3)	Treatment 4 (NT4)	Treatment 5 (NT5)
Interval cutting period (day/ interval cutting)	30	40	50	60	70
fertilizer	Chicken manure 20 ton, Phosphate fertilizer 40 kg P_2O_5, Potassium: 80 kg K_2O, (calculated for 1 hectare/year), nitrogenous fertilizer 60kg N/ ha/ cutting time.				
Planting density(plants/hectare)	83.500				
Area (m^2)	24 m^2 x 5				

* Observations and methodology were the same as those of experiment 1as those of experiments 1 and 2, excepted amino acids.

2.3.5. *Experiment 4: Determination of nutrient digestibility and metabolic energy value of M. oleifera leaf meal*

2.3.5.1. Experiment 4a: Determination of nutrient digestibility of M. oleifera leaf meal

The experiment included 36 Luong Phuong broiler chicken at the age of 43 – 50 days old. The chicken were equally divided into 2 groups, which contained 3 sub groups with 6 chickens /sub group (3 males + 3 females).

The experiment chicken were fed 2 diets: Group I was fed with the control diet without *M. oleifera* leaf meal; Group II was fed with experimental diet consisting of 80% control ration + 20% *M. oleifera* leaf meal (w/w). Exactly 1,5% Celite (SiO_2) [acid Insoluble Ash (AIA)] was added for each ration. The nutrients digestibility of *M. oleifera* leaf meal was calculated based on the nutrients digestibility of control and experimental diets.

The chemical composition of diets were analyzed before experiment conducting and calculated base on dry matter. The observation parameters included dry matter (DM), crude protein (CP), crude fat (EE), crude fiber (CF), Ash, nitrogen-free extract (NFE) and acid insoluble minerals (AIA). *M. oleifera* leaf meal was also analyzed for the above parameters, except for AIA.

2.3.5.2. Experiment 4b: Determination of metabolic energy value of leaf meal of M. oleifera

* Methodology:

Metabolic energy of *M. oleifera* leaf meal was determinated by using the method: "Determination of metabolic energy of feed corrected with the amount of nitrogen of feed accumulated in the body of chickens".

* Experimental design:

The experiment included 36 Luong Phuong broiler chickens at the age of 43 – 50 days old. The chicken were equally divided into 2 groups, which contained 5 sub groups with 4 chickens /sub group (2 males + 2 females).

Group1 was fed with the control diet while group 2 was fed with the experimental diet consisting of 80% control diet + 20% *M. oleifera* leaf meal. These diets were supplemented with exactly 1,5% Celite (SiO2), the acid-insoluble mineral (AIA).

2.3.6. Experiment 5: Partial replacement of soybean meal with M. oleifera leaf meal in broiler diets

Table 2.4. Design Diagram 5

Item	**Treatment1** **NT1** (0%)	**Treatment2** **NT2** (20%)	**Treatment3** **NT3** (30%)	**Treatment4** **NT4** (40%)	**Treatment5** **NT5** (50%)
Breed	Broiler Luong Phuong chicken				
Number	10 chickens/ repeated time (balanced roosters and hens)				
Repeated times (n =9)	9 times x 10 chickens = 90 chickens'/treatment				
Experimental	15 – 70 days of age				

time period 56 days					
Housing	Housing in semi-confinement type				
Feed(15–70 days of age)	KP NT1	KP NT2	KP NT3	KP NT4	KP NT5

Note: Treatment1 sign NT

* Experimental chicken feed:

Experimental feed was formulated from corn meal, rice bran, fish meal (60% protein), soybean meal extract (44% protein), vegetable oil, *M. oleifera* leaf meal and other feed additive.

The protein of soybean meal (PKD) was partly replaced by of *M. oleifera* leaf meal protein (PBL) in the diet, following: 100% soybean meal + 0% *M. oleifera* leaf meal (Treatment1); 80% soybean meal + 20% *M. oleifera* leaf meal (Treatment2), 70% soybean meal + 30% *M. oleifera* leaf meal (Treatment3); 60% soybean meal + 40% *M.oleifera* leaf meal (Treatment4), 50% soybean meal + 50% *M. oleifera* leaf meal (Treatment5). In totally, *M. oleifera* leaf meal consisted with 0%; 5,30%; 8,0%; 10,7% and 13,4% in the chicken diet. These ratios were applied to both 15 – 42 and 43 – 70 days old period diets.

Formula and nutritional value of concentration feed met the nutrient requirements for coloured broilers.

* Observation parameters:

Survival rate (%); body weight (kg), average daily gain (g/chick/day); feed intake (g) and feed conversion ratio/kg weight gain (kg feed/kg weight gain); Economic efficiency Index, PI and EN.

Carcass quality: carcass ration, thigh-drumstick ratio, breast meat ratio, abdominal fat; the chicken skin colour; dry matter, protein, lipid content of breast meat and thigh-drumstick meat; water loss of meat after storage and processing.

* Observation methods:

Grow performance, feed utilization and economic efficience were calculated following the guideline of Bui Huu Doan et al., 2011; Tran Thanh Van et al., (2015). Slaughtering method and carcass quality were carried out following Bui Quang Tien (1993).

Feed sampling and analysis of dry matter, protein, lipid of meat were followed the guideline of Vietnamese Standards (TCVN), animal feed 2001 and 2007 (analysis at Institute of Life Sciences – Thai Nguyen University of Agriculture and Forestry).

2.3.7. Experiment 6: Partial replacement of soybean meal with *M. oleifera* leaf meal in laying hens diets

Table 2.7. Diagram of experimen

Item	**Treatment1** NT1 (0%)	**Treatment2** NT2 (30%)	**Treatment3** NT3 (40%)	**Treatment4** NT4 (50%)
Breeding	Luong Phuong Breeder			
Number	30 hens + 4 cocks/ repeated time			
Repeated time (n = 3)	3 times x (30 hens + 4 cocks)/ treatment			
Experimental tine period (16 weeks)	35 – 50 weeks of age			
Housing method	Confinement in open sided poultry house			
Experimental feed	KP NT1	KP NT2	KP NT3	KP NT4

Note: Treatment 1 sign NT

* Experimental chicken feed

Experimental feed was formulated from corn meal, rice bran, fish meal (58,5% protein), soybean meal (43,6% protein), vegetable oil, *M. oleifera* leaf meal and other feed additive.

All experimental diets had the same crude protein ratio of 17% and metabolic energy 2750 kcal/1kg of feed.

The protein of soybean meal (PKD) was partly replaced by of *M. oleifera* leaf meal protein (PBL) in the diet, following: 100% soybean meal + 0% *M. oleifera* leaf meal (Treatment1); 70% soybean meal + 30% *M. oleifera* leaf meal (Treatment2); 60%

soybean meal + 40% *M.oleifera* leaf meal (Treatment4), 50% soybean meal + 50% *M. oleifera* leaf meal (Treatment5). In totally, *M. oleifera* leaf meal consisted with 0%; 8,0%; 10,7% and 13,5% in the chicken diet.

Formula and nutritional value of concentration feed met the nutrient requirements for coloured broilers.

* Observation parameters

Egg laying rate, yield, egg productivity; Feed conversion rate/10 eggs, 10 beeding eggs, feed conversion ratio and feed cost for 1 class newly hatched chicks.

Egg quality was observed following parameters of egg weight, morphological index, egg yolk weigh, egg albumen weight, shell weight; ratio of yolk and albumen, and egg shell; albumen index, yolk index.

Chemical compositions of egg were analysed for dry matter, crude protein, crude lipids of yolk and albumen; yolk depth.

The hatching abilities was observed, including: ratio of embryo eggs /incubated eggs, hatching rate/ embryos eggs, ratio of type I chicks/incubated eggs and embryos.

* Observation methods

The chickens feed intake and the hen-day eggs production in each group were monitored and recorded. 6 eggs/treatment were weekly collected to investigate egg quality and averaged; a total consisted of 16 investigation in 16 weeks of experiment. Eggs that collected at 3rd, 6th, 9th, 12th and 15th weeks of experiment were used for analysing the chemical composition (n = 5).

From 38 week – 42 week of laying, 300 eggs/treatment were incubated once a week. The incubation trays of each treatment were marked to calculate the number of embryo eggs, hatching ratio, and type 1 newly hatched chicks of each treatment.

Dry matter, protein, lipids of yolk, and egg albumen were analysed according to TCVN.

Yolk carotenoids was analysed by using high-pressure liquid chromatography (HPLC). Yolk colour was measure by couloured comparision with Roche colorimetric fan.

The analysis was carried out at the Institute of Life Sciences – Thai Nguyen University of Agriculture and Forestry.

2.4. Data processing methods

Analysis of Variance (ANOVA) and DUNCAN Comparison of experiments 1, 2, 3 data was performed by the Software IRRISTAT 5.0 according to the guideline of Do Thi Oanh and Hoang Van Phu (2012).

Statistical analytical model of the analysis was as follows:

$$y_{ij} = + Ti + Rj + eij$$

In which: y_{ij} : study criteria

µ : : Population average

Ti : Effect of the experimental formula (i = 1 → n) (n depends on the number of treatments of each experiment)

Rj : Effect of repetition (i = 1 → 5)

eij : Effect of randomness

Analysis of variance (ANOVA) and Tukey *Test for* Pairwise *Mean c*omparisons of experiments 4, 5, 6 data were carried out in Minitab software version 18.1 following the guidline of Truong Huu Dung et al. (2018).

The model of the statistical analysis s as as follows:

Yij = + Tij+ eij

Where: Yij: is the dependent variable

μ: Population average

Tij : Effect of treatment i = 1 → n (n depends on the number of treatments of each experiment)

eij : Effect of random factors

Chapter 3

RESULTS AND DISCUSSION

3.1. Meteorology and chemical composition of soil of the experimental area

The average temperature in the year of the study area was 23,9^{0}C; the temperatures from April - September was optimum for plant growth; and the previous year October to next year March had adverse effects on the growth and yield of forage plants. The average humidity of the area was 81,3%; The average rainfall of the monitoring two years was 1857,9 mm/year.

The experimental soil pH is 6.51, while its fertility is medium.

3.2. Determination of optimum cultivation density for *M. oleifera*

3.2.1. Effect of different cultivation densities on M. oleifera yield

Table 3.3. ***Effect of different cultivation densities on M. oleifera yield*** **(***ton/hectare***,** n=5**)**

Indicator	**NT1** (125[(1)])	**NT2** (100[(1)])	**NT3** (83,5[(1)])	**NT4** (71,5[(1)])	***SEM***	***P***
* Average total biomass yield/year						
Year 1	121,842	121,127	120,447	119,900	*5,960*	*0,959*
Year 2	87,138	89,585	92,519	95,160	*4,627*	*0,071*
$\overline{X}$	104,490	105,356	106,483	107,080	*5,288*	*0,815*
*Average fresh leaf/year						
Year 1	47,128	46,852	46,589	46,377	*2,305*	*0,959*
Year 2	33,705	34,652	35,786	36,808	*1,789*	*0,071*
$\overline{X}$	40,417	40,752	41,188	41,593	*2,046*	*0,815*
*Average dry-matter production/ year						
Year 1	10,269	10,209	10,152	10,106	*0,502*	*0,959*
Year 2	7,344	7,551	7,798	8,020	*0,390*	*0,071*

$\overline{X}$	8,807	8,880	8,975	9,063	*0,446*	*0,815*
*Average protein production/ year						
Year 1	3,516	3,496	3,476	3,460	*0,172*	*0,959*
Year 2	2,515	2,585	2,670	2,746	*0,134*	*0,071*
$\overline{X}$	3,015	3,040	3,073	3,103	*0,153*	*0,815*

Note: : $\overline{X}$ is the average yield of both years $\overline{X} = (\overline{X}1 + \overline{X}2)$. Crude protein yield = dry matter yield x crude protein ratio in dry matter of the ration. The ratio of crude protein/ dry matter is 34,24%; (1) a thousand plants/hctare.

Low density cultivation tended to reduce average dry matter and crude protein yield/ hectare/ year in the first year and increase in the second year and average value of two years, but there was no significantly defference between all treatment (*P>0,05*).

The cost for producing 1 kg of leaf meal was calculated. The cost of seedlings pots accounted to large ratio of total caltivation cost, lead to enhancing the payment for 1 kg of leaf meal in low density caltivation comparing to high density caltivation. The cost for producing 1 kg of leaf meal in the treatments NT2, NT3 and NT4 was 88,64%, 80,39% and 75,34% of NT1, respectively. Therefore, planting *M. oleifera* to produce fodder with a density of about 71,5 – 83,5 thousand plants/ha seemed to be the most effective.

3.3. Determining the optimum nitrogen fertilization level for *M. oleifera*

3.3.1. Effect of nitrogen fertilization level on M. oleifera yield

Table 3.6. ***Effect of nitrogen fertilization level on M. oleifera yield***

(ton/hectare/year)

Index	**NT1** 0N	**NT2** 20N	**NT3** 40N	**NT4** 60N	**NT5** 80N	***SEM***	***P***
*Average Total biomass production/year							
Năm 1	90,205[f]	100,445[d]	110,915[c]	120,445[b]	126,095[a]	5,470	0,000
Năm 2	67,510[d]	76,312[cd]	84,860[bc]	92,519[ab]	97,100[a]	5,068	0,000
$\overline{X}$	78,858[d]	88,378[cd]	97,888[bc]	106,483[ab]	111,598[a]	5,363	0,000
* Average fresh leaf production/ year							
Năm 1	34,890[f]	38,850[d]	42,900[c]	46,590[b]	48,775[a]	2,116	0,000
Năm 2	26,113[d]	29,517[cd]	32,824[bc]	35,786[ab]	37,558[a]	1,960	0,000
$\overline{X}$	30,502[d]	34,185[cd]	37,863[bc]	41,188[ab]	43,166[a]	2,027	0,000
* Average dry-matter production/ year							
Năm 1	8,075[d]	8,805[c]	9,540[b]	10,152[a]	10,395[a]	0,468	0,000
Năm 2	6,043[c]	6,692[bc]	7,300[ab]	7,798[a]	8,004[a]	0,434	0,000
$\overline{X}$	7,058[c]	7,750[bc]	8,421[ab]	8,975[a]	9,199[a]	0,449	0,000
* Average protein production/ year							
Năm 1	2,590[f]	2,885[d]	3,196[c]	3,475[b]	3,658[a]	0,158	0,000
Năm 2	1,938[d]	2,193[cd]	2,446[bc]	2,670[ab]	2,816[a]	0,146	0,000
$\overline{X}$	2,264[d]	2,540[cd]	2,821[bc]	3,073[ab]	3,237[a]	0,151	0,000

Notes: In the horizontal row, numbers In the horizontal row, numbers carrying different letters have statistically significant differences with P < 0,001. Average yield of 2 years = (average yield of year 1 + average yield of year2)): 2

Increasing the level of nitrogen fertilization from 0 – 80 kg N/hectare/time resulted in the increase of total biomass production, fresh leaves, dry matter, crude protein content of *M. oleifera*. Based on the dry matter yield, fertilizating 60N and 80N had highest dry matter yields. However, based on the efficiency of ration nitrogen fertilizer per dry matter production, the fertilizating 20, 40 and 60 kg nitrogen /hectare/ time showed the higher effective production in comparision to 80N treatment, but there was no significantly difference between those treatments. In addition, based on the production cost for each kg leaf meal, fertilizing 60 kgN show the lowest cost. In conclusion, fertilizating at 60 kg nitrogen/hecta was most optimum for *M. oleifera* cultivation.

3.3.2. Effect of nitrogen fertilization levels on the M. oleifera leaf quality

Increasing the level of nitrogen fertilization caused to the decrease of crude fiber and the increase of crude protein. These results may increase feed intake and feed digestibility of livestock. The total amino acids content was different among treatments, which was higher in 40N treatment in comparision with control and 80N treatments ($P<0,001$). The essential amino acid index (EAAI) was better for the 40N treatment in comparision with 0N and 80N treatments ($P<0.001$). Thus, optimum nitrogen fertilization improved protein quality of *M. oleifera* fodder.

3.4. Determination of the optimum cutting interval for *M. oleifera*

3.4.1. Effect of cutting interval on the M. oleifera yield

Table 3.12. ***M. oleifera*** **yield** ***at diferent intervals cutting***

(*ton/hectare/year*, n = 5)

Indicator	**NT1** 30days	**NT2** 40 days	**NT3** 50 days	**NT4** 60 days	**NT5** 70 days	***SEM***	***P***
Average Total biomass production/year							
Year 1	91,476^{f}	105,366^{d}	120,445^{c}	136,188^{b}	152,580^{a}	*5,624*	*0,000*
Year 2	74,834^{c}	89,570^{b}	92,519^{b}	106,742^{a}	108,430^{a}	*5,106*	*0,000*
$\overline{X}$	83,156^{c}	97,469^{b}	106,483^{b}	121,465^{a}	130,505^{a}	*5,337*	*0,000*
Average new leaf production/year							
Year 1	39,144^{b}	46,278^{c}	46,590^{c}	39,876^{b}	37,720^{a}	*2,025*	*0,000*
Year 2	32,021^{c}	39,339^{a}	35,786^{b}	31,254^{c}	26,804^{d}	*1,867*	*0,000*
$\overline{X}$	35,582^{b}	42,808^{a}	41,188^{a}	35,565^{b}	32,261^{b}	*1,934*	*0,000*
Average dry-matter production/ year							
Year 1	7,700^{c}	9,570^{ab}	10,150^{b}	8,900^{a}	8,932^{d}	*0,434*	*0,000*
Year 2	6,299^{b}	8,135^{a}	7,798^{a}	6,976^{b}	6,347^{b}	*0,395*	*0,000*
$\overline{X}$	6,999^{c}	8,853^{a}	8,975^{a}	7,938^{b}	7,639^{bc}	*0,412*	*0,000*
Average protein production/ year							

Year 1	2,650^{f}	3,360^{b}	3,475^{a}	2,982^{c}	2,832^{d}	*0,149*	*0,000*
Year 2	2,168^{c}	2,856^{a}	2,670^{ab}	2,477^{b}	2,013^{c}	*0,136*	*0,000*
$\overline{X}$	2,409^{c}	3,108^{a}	3,073^{ab}	2,819^{b}	2,422^{c}	*0,141*	*0,000*

Note: In the horizontal rows, the numbers carrying different letters are statistically different with (P<0,001).

Increasing the interval cutting period from 30–70 days/cutting led to the increase of dry matter and crude protein yield. Crude protein yeild was highest for 40 and 50 days cutting interval treatments (*P<0,05*). Thus, *M. oleifera* fodder for animal feed purpose should be interval harvested at 40 or 50 days.

3.4.2. Effect of cutting interval on M. oleifera leaf quality

The dry matter in the leaves increased (from 19,67 – 23,68%) with the increase of interval cutting days. However, there was no significantly different with every 10 days (30 – 40; 40 – 50; 50 – 60) interval cutting days. The dry matter in fresh leaves was not significantly different between all treatments, except for 70 days interval cutting. The crude protein was highest in the period of 30 – 40 days (from 34,42 – 35,11%), then gradually decliced and was lowest at the 70 - days interval cutting (only 31,70%). The fiber content increased from 7,02 to 10,35% when increased the cutting interval days (*P<0,001*).

3.5. Determination of digestibility and metabolic energy of *M. oleifera* leaf meal

The ileal protein, lipid, fiber and free nitrogen extract digestibility of *M. oleifera* leaf meal in broiler chickens were 67,97%, 78,15%, 25,48%, and 72,84%, respectively.

M. oleifera leaves and leaf meal (90,68% dry matter) metabolic energy adjusted for nitrogen accumulation in the body of each kg dry matter were 2480 kcal and 2249 kcal, respectively.

3.6. Study on potential of replacing soybean meal with *Moringa oleifera* leaf meal in the diet of Luong Phuong broilers

3.6.1. Body weight and average daily gain of experimental chickens

Table 3.20. Chicken body weight and average daily gain, (n=9)

Index	NT1 0%	NT2 20%	NT3 30%	NT4 40%	NT5 50%	*SEM*	*P*
Body weight (g/chicken)							
15 days old	196 ^{a}	196 ^{a}	196 ^{a}	196 ^{a}	196 ^{a}	*1,160*	*1,000*
42 days old	1020 ^{b}	1073 ^{a}	1027 ^{b}	999 ^{c}	973 ^{d}	*10,689*	*0,000*
70 days old	1928 ^{c}	2059 ^{a}	2008 ^{b}	1913 ^{c}	1861 ^{d}	*19,132*	*0,000*
Average daily gain (g/ chicken/ day)							
15 - 42 days old	29,43^{b}	31,32^{a}	29,68^{b}	28,68^{c}	27,75^{d}	*0,346*	*0,000*
43 - 70 days old	32,43^{b}	35,21^{a}	35,04^{a}	32,64^{b}	31,71^{c}	*0,311*	*0,000*
15 - 70 days old	30,93^{c}	33,27^{a}	32,36^{b}	30,66^{c}	29,73^{d}	*0,323*	*0,000*

Note: In the horizontal rows, the numbers carrying different letters are statistically different at P<0,05

In the 15 – 42 days old period, the replacing 20% and 30% soybean meal protein with *M. oleifera* leaf meal protein showed the highest body and average daily gain in broiler.

In the 43 – 70 days old period, the replacing 20% to 40% soybean meal protein with *M. oleifera* leaf meal protein had not disadvantages effect on broiler performance. Thus, the ratio of *M. oleifera* leaf meal protein in the broiler diets should depend on the age of chicken.

3.6.2. Feed intake and metabolic efficiency

Table 3.21. Feed intake, feed utilization efficiency of experimental chickens, (n=9)

Index	NT1 0%	NT2 20%	NT3 30%	NT4 40%	NT5 50%	*SEM*	*P*
Feed intake (g/chicken/day)							
15 - 42 days old	65,91^{ab}	66,82^{a}	65,82^{b}	65,07^{bc}	64,36^{c}	*0,676*	*0,000*
43 - 70 days old	113,26^{bc}	115,04^{a}	114,32^{ab}	113,46^{bc}	112,43^{c}	*1,171*	*0,000*
15 - 70 days old	89,59^{bc}	90,93^{a}	90,07^{ab}	89,27^{bc}	88,39^{c}	*0,924*	*0,000*
Feed conversion ratio, FCR (kg)							
15 - 42 days old	2,24^{c}	2,13^{f}	2,22^{d}	2,27^{b}	2,32^{a}	*0,012*	*0,000*
43 - 70 days old	3,49^{b}	3,27^{c}	3,26^{c}	3,48^{b}	3,55^{a}	*0,018*	*0,000*
15 - 70 days old	2,90^{b}	2,73^{d}	2,78^{c}	2,91^{b}	2,97^{a}	*0,014*	*0,000*

Note: In the horizontal rows, the numbers carrying different letters are statistically different with (P < 0,001)

In the 15 – 42 days old period, replacing 20 – 30% soybean meal protein (PKD) with *M. oleifera leaf meal* protein *(*PBL) % in broiler diets had possitive effect on the feed conversion ratio (FCR) of experimental chickens. However, replacing 40 – 50% soybean meal protein (PKD) with *M. oleifera leaf meal* protein showed negative effect on its FCR.

In the 43 – 70 days old period, the FCR of treatment 1 (NT1) was higher than these of treatment 2 (NT2) and 3 (NT3), but its was similar with the treatment 4 (NT4) FCR. Futher, FCR of treatment 5 (NT5) was significantly highest (*P<0,001*). Thus, replacing 40% soybean meal protein with *M. oleifera leaf meal* protein in the 43 – 70 days old broiler diet did not affect feed converted ratio. In summary, the 15 – 70 days old broiler could be fed diet containing 40% *M. oleifera* leaf meal protein.

3.6.3. Meat productivity and quality

3.6.3.1. Meat productivity

Table 3.22. Some slaughter parameters of experimental chickens at 70 days old, (n = 5)

Index	**Unit**	**NT1 0%**	**NT2 20%**	**NT3 30%**	**NT4 40%**	**NT5 50%**	***SEM***	***P***
Carcass weight	g	1486 [c]	1590 [a]	1554 [b]	1473 [c]	1429 [d]	*13,866*	*0,000*
Carcass weight/live weight	%	76,80 [a]	77,00 [a]	77,10 [a]	76,70 [a]	76,40 [a]	*0,371*	*0,059*
Breast+whole leg/carcass	%	38,84 [ab]	39,48 [a]	39,46 [a]	38,94 [ab]	38,62 [b]	*0,421*	*0,009*
Liver/Carcass	%	2,46	2,43	2,45	2,47	2,48	*0,029*	*0,057*
Abdominal fat/Carcass	%	2,63 [a]	1,72 [b]	1,55 [c]	1,36 [d]	1,31 [e]	*0,016*	*0,000*

Note: In the horizontal rows, the numbers carrying different letters are statistically different

Relacing 20% and 30% soybean meal protein with *M. oleifera* leaf meal protein significantly affected on the ratio of breast and whole leg, while replacing 40% and 50% soybean meal protein with *M. oleifera* leaf meal protein did not show significant effect of these ratio, in comparision with treatment 1 (NT1). The ratio of abdominal fat significantly decreased with the increase of *M. oleifera* leaf meal protein in the diets (*P<0,01*).

3.6.3.2. Meat quality

Table 3.23. chemical composition of experimental chicken meat, (n = 5**)**

Index	**Unit**	**NT1** 0%	**NT2** 20%	**NT3** 30%	**NT4** 40%	**NT5** 50%	***SEM***	***P***
a, Breast muscle								
Dry matter	%	25,41	25,30	25,21	25,14	25,05	*1,881*	*0,990*
Protein	%	22,98	23,02	23,04	23,10	23,10	*1,840*	*0,884*
Lipid	%	$1,44^{a}$	$1,28^{b}$	$1,16^{c}$	$1,03^{d}$	$0,95^{e}$	*0,274*	*0,000*
b, Whole leg muscle								
Dry matter	%	23,35	23,24	23,20	23,16	23,02	*1,804*	*0,823*
Protein	%	19,82	19,84	19,92	19,98	20,01	*1,718*	*0,820*
Lipit	%	$2,31^{a}$	$2,15^{b}$	$2,03^{c}$	$1,91^{d}$	$1,75^{e}$	*0,045*	*0,000*
c,Liver Carotenoid	mg %	$0,51^{e}$	$1,15^{b}$	$1,22^{b}$	$1,64^{a}$	$1,78^{a}$	*0,124*	*0,000*
d,Jaundice level	point	$1,60^{c}$	$3,17^{b}$	$3,67^{ab}$	$4,00^{a}$	$4,33^{a}$	*0,658*	*0,000*

Note: In the horizontal rows, the numbers carrying different letters are statistically different

Muscle dry matter and lipid tended to decline, while muscle protein tended to increase with the increase of *M. oleifera* leaf meal protein in the broiler diets.

In conclusion, the broiler could be fed the diet containing 40% *M. oleifera* leaf meal protein replace to soybean meal protein without effects on growth performance and meat quality. However, replacing 20

– 30% soybean meal protein with *M. oleifera* leaf meal protein in the broiler diets showed highest growth performance and meat quality.

3.7. Study on replacing soybean meal with *Moringa oleifera* leaf meal in the breeder Luong Phuong diets

3.7.1. Survival rate and laying rate of experimental breeder.

Table 3.26. Laying rate of experimental breeder (n=3**)**

Experimental week	**NT1** (0%)	**NT2** (30%)	**NT3** (40%)	**NT4** (50%)	***SEM***	***P***
1	71,43^{a}	71,75^{a}	70,63^{a}	70,48^{a}	*0,659*	*0,125*
8	69,05^{ab}	70,32^{a}	69,21^{ab}	67,94^{b}	*0,630*	*0,012*
16	53,49^{c}	62,54^{a}	60,00^{b}	53,81^{c}	*0,700*	*0,000*
1 – 16	66,08^{c}	69,48^{a}	67,90^{b}	65,83^{c}	*0,210*	*0,000*

During 16-weeks of the experiment, no mortality rate of chickens was found, the survival rate of all 4 treatments was 100%.

Replacing soybean meal protein with *M. oleifera* leaf meal protein up to 50%, the laying rate of chickens did not affect the laying rate of breeder, in comparision with treatment 1. However, replacing 30 – 40% soybean meal protein with *M. oleifera* leaf meal protein increased the laying rate of breeder in comparision with treatment 1. This results proved that *M. oleifera* leaf meal protein could replace to soybean meal protein in the breeder diets. On the other hand, *M. oleifera* leaf meal may provide number of pigment, such as carotenoids, that increase the laying rate in poultry (Tu Quang Hien et al., 2013; Hien et al., 2016).

3.7.2. **Egg production and yield of experimental breeder layer**

Table 3.27. Egg production and yield of experimental breeder, (n=3)

Indicator	**Unit**	**NT1** 0%	**NT2** 30%	**NT3** 40%	**NT4** 50%	***SEM***	***P***
Total egg production	Egg/group	6661^{c}	7004^{a}	6844^{b}	6636^{c}	*7,071*	*0,000*
Hen-day egg yield	Egg/layer/	74,01^{c}	77,82^{a}	76,04^{b}	73,73^{c}	*0,236*	*0,000*

Comparison		100	105,1	102,7	99,6	-	-
Breeding egg production	Egg/group	6426 [c]	6775 [a]	6649 [b]	6457 [c]	*8,775*	*0,000*
Breeding egg ratio	%	96,47 [b]	96,73[ab]	97,15 [ab]	97,30[a]	*0,268*	*0,018*
Breeding egg yield	Egg/layer	71,40 [c]	75,28 [a]	73,88 [b]	71,74 [c]	*0,292*	*0,000*
comparison	%	100	105,4	103,5	100,5	-	-

Note: In the horizontal rows, the numbers carrying different letters are statistically different

Replacing 30 and 40% soybean meal protein with *M. oleifera* leaf meal protein showed higher hen-day egg production, total egg production, and breeding egg production in comparision to treatment 1. Replacing 50% soybean meal protein with *M. oleifera* leaf meal protein did not affect on performance of breeder. In summary, 50% soybean meal protein in Luong phuong breeder diet could be replaced with *M. oleifera* leaf meal protein without effect on laying egg performance. However, replacing 30 – 40% soybean meal protein with *M. oleifera* leaf meal protein seem to be most optimum.

3.7.3. Results of surveying seveeral egg parametters

Table 3.28. Survey of some egg parametters (n=16**)**

Index	**Unit**	**NT1** 0%	**NT2** 30%	**NT3** 40%	**NT4** 50%	***SEM***	***P***
Egg weight	gram	55,454[a]	55,537 [a]	55,595 [a]	55,744 [a]	*0,838*	*0,796*
morphological index	%	1,29 [a]	1,29 [a]	1,30 [a]	1,30 [a]	*0,130*	*0,992*
Egg albumen weight	gram	31,314 [a]	31,321 [a]	31,338 [a]	31,411 [a]	*0,744*	*0,982*
Egg yolk weight	gram	17,684 [a]	17,742 [a]	17,762 [a]	17,814 [a]	*0,687*	*0,961*
Egg shell weight	gram	6,456 [a]	6,474 [a]	6,495 [a]	6,519 [a]	*0,352*	*0,962*
Egg albumen ratio	%	56,47 [a]	56,40 [a]	56,37 [a]	56,35 [a]	*1,073*	*0,990*

Egg yolk ratio	%	31,89 [a]	31,95 [a]	31,95 [a]	31,96 [a]	*1,221*	*0,998*
Egg shell ratio	%	11,64 [a]	11,66 [a]	11,68 [a]	11,69 [a]	*0,561*	*0,995*
Egg albumen index	%	0,078 [b]	0,085 [a]	0,086 [a]	0,087 [a]	*0,003*	*0,000*
Egg yolk index	%	0,463 [c]	0,471[bc]	0,485[ab]	0,492 [a]	*0,017*	*0,000*

Note: In the horizontal rows, the numbers carrying different letters are statistically different

Replacing soybean meal protein with *M. oleifera* leaf meal protein increased albumen and yolk index in comparision to treatment 1. Replacing *M. oleifera* leaf meal protein at all ratio was higher albumen index than treatment 1. However, yolk index of treatment 3 and 4 were significantly higher than other treatments (*P< 0,001*).

3.7.4. Results of analysis of some chemical parameters of eggs

Table 3.29. Some chemical parameters of eggs (n=5)

Indicator	**Unit**	**NT1** 0%	**NT2** 30%	**NT3** 40%	**NT4** 50%	***SEM***	***P***
a. Egg yolk							
Dry matter	%	50,92 [a]	51,18 [a]	51,06 [a]	50,89 [a]	*0,186*	*0,088*
Protein	%	15,74 [b]	16,12 [a]	16,18 [a]	16,22 [a]	*0,125*	*0,000*
Lipid	%	33,84 [a]	33,73 [ab]	33,54 [ab]	33,36 [b]	*0,219*	*0,016*
Carotenoids	mg%	2,19 [d]	4,62 [c]	6,73 [b]	7,55 [a]	*0,201*	*0,000*
b, Egg albumen							
Dry matter	%	13,39 [a]	13,26 [a]	13,18 [a]	13,14 [a]	*0,201*	*0,251*
Protein	%	11,82 [a]	11,68 [a]	11,63 [a]	11,59 [a]	*0,214*	*0,380*
Lipid	%	0,210 [a]	0,206 [a]	0,195 [a]	0,192 [a]	*0,048*	*0,070*
C, Egg yolk colour	point	8,55[d]	13,41 [c]	14,17 [b]	14,62 [a]	*0,246*	*0,000*

Note:In the horizontal rows, the numbers carrying different letters are statistically different

Replacing soybean meal protein with *M. oleifera* leaf meal protein in the breeder chicken diets significantly increase yolk protein (*P<0,001*) while it tended to increase yolk protein (*P=0,088*). Egg yolk colour was significantly improve with the increase of *M. oleifera* leaf meal protein content (*P<0,001*). These results showed that replacing soybean meal protein with *M. oleifera* leaf meal protein had advantages effect on egg quality.

3.7.5. Effect of substitution of soybean meal with M. oleifera leaf meal on egg quality

Table 3.30. Results of monitoring several parameters of incubated eggs, (n=3)

Index	**Unit**	**NT1** 0%	**NT2** 30%	**NT3** 40%	**NT4** 50%	***SEM***	***P***
Number of incubated eggs	egg	1500	1500	1500	1500	-	-
Number of embryonated eggs	egg	1377 [a]	1409 [a]	1412 [a]	1415 [a]	*7,106*	*0,184*
Percentage of embryonated eggs	%	91,80 [a]	93,93 [a]	94,13 [a]	94,33 [a]	*1,421*	*0,184*
Number of hatching eggs	egg	1296 [b]	1351 [a]	1360 [a]	1365 [a]	*4,573*	*0,001*
Percentage of hatching embryo eggs	%	94,12 [b]	95,88 [a]	96,32 [a]	96,47 [a]	*0,658*	*0,008*
Number of class 1 newly hatched chicks	Chick	1277 [b]	1332 [a]	1343 [a]	1349 [a]	*4,528*	*0,001*
Percentage of class 1 newly hatched chicks	%	85,13 [b]	88,80 [a]	89,53 [a]	89,93 [a]	*0,906*	*0,001*
comparison	%	100	104,31	105,17	105,64	-	-

Note:In the horizontal rows, the numbers carrying different letters are statistically different

Replacing soybean meal protein with *M. oleifera* leaf meal protein in the breeder chicken diet increased the ratio of embryo egg and type I newly hatched chicks haching. The increase of dry matter, protein and carotenoid in the breeding egg may enhance the ratio of hatching eggs.

3.7.6. Effect of replacing soybean meal with M. oleifera leaf meal on feed efficiency for egg production

Table 3.31. Feed conversion ratio for egg and hatched chicks production, (n=3)

Index	**Unit**	**NT1** 0%	**NT2** 30%	**NT3** 40%	**NT4** 50%	***SEM***	***P***
Egg production/group	egg	6661 ^{c}	7004 ^{a}	6844 ^{b}	6636 ^{c}	*7,071*	*0,000*
Breeeding Egg production/ group	egg	6426 ^{c}	6775 ^{a}	6649 ^{b}	6457 ^{c}	*8,930*	*0,000*
Number of class I newly hatched chicks/ group/	chick	5470 ^{d}	6016 ^{b}	5953 ^{a}	5807 ^{c}	*7,001*	*0,000*
Feed conversion rate/10 eggs	Kg	2,401 ^{a}	2,284^{c}	2,323^{b}	2,410 ^{a}	*0,007*	*0,000*
Conversion rate/breeding eggs	Kg	2,489 ^{a}	2,361^{c}	2,405^{b}	2,477 ^{a}	*0,011*	*0,000*
Conversion rate/type 1 newly hatched chicks	Kg	0,292 ^{a}	0,266 ^{c}	0,269^{c}	0,275 ^{b}	*0,002*	*0,000*
Feed cost/ type 1 newly hatched chicks	VND	2.646^{a}	2.395 ^{c}	2.430^{c}	2.483 ^{b}	*17,251*	*0,000*

Note: In the horizontal rows, the numbers carrying different letters are statistically different

Replacing 30% and 40% soybean meal protein with *M. oleifera* leaf meal protein in the Luong Phuong breeder diets increased the ratio of breeding egg quality, feed convertion rate and feed cost for type I newly hatched chicks in comparision to treatment 1. Even replacing 50% soybean meal protein with *M. oleifera* leaf meal protein did not affect breeding egg performance. However, the replacement at 30 – 40% seem to have highest economic efficience in Luong phuong breeder production.

CONCLUSION AND RECOMMENDATION

1. Conclusion

1. Cultivation density of *M. Oleifera* at 71.500 plants/hectare showed highest dry matter and crude protein yield (9,063 and 3,103 tons/hectare/year, respectively) and lowest production cost for each kg leaf meal (7950 VND/kg).

2. Applied fertilization of 60 kg nitrogen/hectare was had highest dry matter and crude protein yield (8,975 and 3,073 tons/ha/year respectively), and lowest leaf meal cost production (8.482 VND/kg). The efficient production was of 2,45 kg leaf protein/kg N fertilization.

3. 40 days cutting interval showed the highest the dry matter and crude protein yield (8,853 and 3,108 tons/hectare/year).

4. The ileal proteins, lipids, fibers and nitrogen free extract digestibility of *M. oleifera* leaf meal in broilers were 67,97%, 78,15%, 25,48% and 72,84% respectively. The metabolizable energy corrected with nitrogen accumulation in the chicken body of each kg of dry matter and *M. oleifera* leaf meal (with 90,68% dry matter) was 2480 kcal and 2249 kcal respectively.

5. Replacing 20% soybean meal protein with *M. oleifera* leaf meal protein in the Luong Phuong broiler diet had highest 70 days old body weight (2.059g), daily weight gain (33,27 g/day); feed conversion ratio (2,73kg feed/kg weight gain).

6. Replacing 30% soybean meal protein with *M. oleifera* leaf meal protein in the Luong Phuong breeder diet had highest egg production (77,82 eggs/hen in 112 days), the ratio of class I newly hatched chicks (88,80%), feed converssion ratio (0,266 kg feed/newly hatched chick) and feed cost (2.395 VND/ newly hatched chick).

2. Recommendation

Apply the results of this study into poultry production.

Further studies on pig, fish, etc... should be considered to have comprehensive view of *Moringa oleifera* potential for livestock production.