Effect of Treated Quail Dung on the Growth Performance of Freshwater Microalgae

By

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DECLARATION

I hereby declare that the work embodied in this report is the original research and has not been submitted for a higher degree to any universities or institutions.

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Date :

I certify that the Report of this final year project entitled **"Effect of Treated Quail Dung on the Growth Performance of Freshwater Microalgae"** by Hiew Jion Long, matric number F14A0085 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science), Faculty of Agro Based Industry, Universiti Malaysia Kelantan.

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Effect of Treated Quail Dung on the Growth Performance of Freshwater Microalgae

ABSTRACT

For decades, freshwater microalgae have been used in various fields for human food, animal feed, and fertilizer. In aquaculture industry, freshwater microalgae use as live feed for all growth stages of bivalve molluscs likes oysters or scallops, larval stages of abalone or some fish species and for zooplankton in aquaculture food chains. The purpose of the study is to compare the effect of treated quail dung (TQD) and the commercial fertilizer (NPK) on the growth performance of freshwater microalgae. The filtered pond water sample was added into six conical flasks which contained different concentrations of the fertilizer. The TQD was obtained from Universiti Malaysia Kelantan (UMK). The concentrations of TQD were 3 g, 6 g, and 9 g while same concentrations of NPK fertilizer were used which acted as control treatment. The data were collected every morning for 7 days using the spectrophotometer at 650 nm. The statistical analysis of the optical density (OD) and the specific growth rate per day (SGR d^{-1}) of the freshwater microalgae were done by using one-way ANOVA and post hoc Turkey test at $p < 0.05$. Based on the result obtained, treatment with TQD fertilizer had significantly higher peak OD at 1.27 ± 0.018 and SGR d^{-1} at 0.70 ± 0.016 of freshwater microalgae compared to NPK fertilizer. As a conclusion, TQD can be used as an alternative fertilizer in freshwater microalage cultivation in order to reduce the cost of cultivation.

Keywords: Treated quail dung (TQD), commercial fertilizer (NPK), specific growth rate per day (SGR d^{-1}), freshwater microalgae and concentration.

Kesan Tahi Puyuh yang telah Dirawat kepada Prestasi Pertumbuhan Mikroalgae Air Tawar

ABSTRAK

Selama beberapa dekad, mikroalga air tawar telah digunakan dalam pelbagai jenis bidang untuk makanan manusia, makanan haiwan dan baja. Dalam industry akuakultur, mikroalga air tawar digunakan sebagai makanan langsung kepada semua peringkat pertumbuhan moluska kerang seperti tiram atau kekapis, peringkat larva abalone atau beberapa spesies ikan dan untuk zooplankton dalam rangkaian makanan akuakultur. Tujuan kajian ini adalah untuk menbandingkan kesan tahi puyuh yang telah dirawat (TQD) dan baja komersial (NPK) kepada prestasi pertumbuhan mikroalga air tawar. Sampel air tasik yang ditapis telah ditambah ke dalam enam konical flask yang mengandungi kepekatan baja yang berlainan. TQD diperolehi dari Universiti Malaysia Kelantan (UMK). Kepekatan TQD ialah 3 g, 6 g dan 9 g manakala kepekatan yang sama juga digunakan oleh baja NPK yang bertindak sebagai rawatan terkawal. Data telah dikumpul pada setiap pagi sepanjang 7 hari dengan menggunakan spektrofotometer pada 650 nm. Analisis statistic kepadatan optik (OD) dan spesifik kadar pertumbuhan sehari (SGR d^{-1}) mikroalga air tawar dilakukan dengan menggunakan satu cara ANOVA dan ujian poc hoc Turkey pada $p < 0.05$. Berdasarkan keputusan yang diperolehi, rawatan dengan baja TQD mempunyai puncak tertinggi OD pada 1.27 ± 0.018 dan SGR d^{-1} mikroalga air tawar pada 0.70 ± 0.016 yang lebih tinggi dan ketara apabila berbanding dengan baja NPK. Sebagai kesimpulan, TQD boleh digunakan sebagai baja alternatif dalam kultivasi mikroalga air tawar bagi mengurangkan kos kultivasi.

Kata Kunci :Tahi puyuh yang telah dirawat (TQD), baja komersial (NPK), spesifik kadar pertumbuhan sehari (SGR d^{-1}), mikroalga air tawar, dan kepekatan.

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LIST OF ABBREVIATIONS

Ca	Calcium
CO_2	Carbon Dioxide
d	Days
dH_2O	Distilled Water
FYM	Farmyard Manure
g	Gram
gL^{-1}	Gram per Liter
h	Hours
H_2O	Water
i.e.	That is
K	Potassium
lux	Luminous Flux per Unit Area
mL	Milliliter
mL/L	Milliliter per Liter
N	Nitrogen
NH_4^+	Ammonium
NH_3-N	Unionised Ammonia
nm	Nanometer
NPK	Commercial Fertilizer
OD	Optical Density
P	Phosphorus
pH	Potential of Hydrogen
PUFA	Polyunsaturated Fatty Acid
SGR d^{-1}	Specific Growth Rate per Day
TQD	Treated Quail Dung

UMK	Universiti Malaysia Kelantan
µm	Micrometer

LIST OF SYMBOLS

~	Approximately
ºC	Degree Celcius
<	Less Than
>	More Than
%	Percentage
±	Plus-minus
-	to

CHAPTER 1

INTRODUCTION

1.1 Research Background

Microalgae are the autotrophic (self-feeding) components of the plankton community in which they contain chlorophyll and require solar energy, carbon dioxide (CO_2) and water (H_2O) for photosynthesis (Harun et al., 2010). Hence, microalgae comprise the basic of food chain and act as the primary producer in aquatic food web due to the photosynthesizing properties. Microalgae are classified as prokaryotic or eukaryotic microscopic organisms that can grow faster and float in the euphotic zone (surface layer) of the seas, freshwater and other body of water where sunlight penetrates the water (Mata et al., 2009). Li et al. (2008) stated that Cyanobacteria (*Cyanophyceae*) are examples of prokaryotic microorganisms while *Chlorophyta* (known as green algae) and *Bacillariophyta* (known as diatoms) are example of eukaryotic microalgae. Green (2015) mentioned microalgae is a constantly available food source for man, land-based farm animals and cultured aquatic species likes molluscs and early larval or juveniles stages of different species of fish and crustaceans. Thus, microalgae are known as valuable resource for aquaculture and reduce the production cost of fish farmers because they can be cultured easily and economically (Das et al., 2012).

Quail dung refers to the combination of accumulated manures, feathers, spilled feed and bedding materials (Tawadchai et al., 2012). Iwamoto et al. (2008) reported that due to rapid growth, high laying, fecundity and environmental resistance as compared to chicken, the *Coturnix japonica or* normally known as Japanese quail

is an excellent birds for commercial domestication. Amanullah et al. (2010) stated that utilization of poultry litter has been a common practice in India since long time as manure in agriculture or in aquaculture. Poultry manure is rich organic manure since solid and liquid excreta are excreted together which contains about 3 - 5 % nitrogen (N), 1.5 - 3.5 % phosphorous (P) and 1.5 - 3.0 % potassium (K) and acceptable amount of micro-nutrients (Amanullah et al., 2010).

The aim of this study is to determine the growth performance of freshwater microalgae using treated quail dung (TQD) and commercial fertilizer (NPK).

1.2 Problem Statements

In aquaculture industry, larval rearing is one of the most challenge tasks but it also could be the most money-making investment. Thus the farmers concern about the provision of a reliable, nutritionally complete and low costing feed source for the fish larvae (Das et al., 2012). Mandal et al. (2009) reported formulated larval feed in the market is different compared to the freshwater microalgae in terms of cost, size, acceptance, and nutrient level.

Freshwater microalgae also can acts as an alternative feed source for fish meal used in aquaculture feed. This is because freshwater microalgae can be culture using low cost fertilizer such as livestock waste or animal waste and hence lower production cost of fish farmer.

1.3 Hypothesis

There is difference on the growth performance of freshwater microalgae using treated quail dung (TQD) and commercial fertilizer (NPK).

1.4 Objective of Study

To compare the effect of treated quail dung (TQD) and commercial fertilizer (NPK) on the freshwater microalgae growth performance.

1.5 Scopes of Study

The scope of study was to observe and compare the growth performance of freshwater microalgae using treated quail dung (TQD) and commercial fertilizer (NPK) at 3 g, 6 g and 9 g. The freshwater microalgae were cultured in 500 mL conical flask in the laboratory. The fresh quail dung was treated for odor removal and antimicrobial treatment. The optical density (OD) of freshwater microalgae was determined using spectrophotometer (Genesys 20, Thermo Scientific, UK).

1.6 Significances of Study

Most of the microalgae are cultured in marine or saltwater which is known as open system cultivation. In open system, the requirements for microalgae cultivation and water parameters such as temperature are difficult to control because it depends on the weather. This study can be regarded as an improve cultivation of freshwater microalgae because they were cultured in a closed system with organic fertilizer, which is treated quail dung (TQD). The requirements for freshwater microalgae cultivation and water parameters in closed system can be monitored easily.

This study is also an eco-friendly research because the TQD were provided to freshwater microalgae cultivation. The quail dung has less negative effect to environment compared to the chemical fertilizer.

This study helps fish farmers to reduce their production cost by using low cost fertilizer like TQD in freshwater microalgae cultivation. Farmers do not need to purchase expensive live feed from the market.

1.7 Limitation of Study

Due to the lack of proper facility for freshwater microalgae cultivation in campus, there was a need to set-up a temporary space for algae culture.

CHAPTER 2

LITERATURE REVIEW

2.1 Algae and Microalgae

The term algae has no formal taxonomic standing because algae is normally define as oxygen-producing aquatic protists or photosynthetic which the organism use light energy to produce organic compound that serve as cellular building blocks and energy reserves (Linda, 2009). With the definition above, algae could be considered a plant division as they produce the same storage compounds as well as use similar defense systems against predators and parasites. However the difference between algae and plants are algae do not have roots, stems, leaves, nor well-defined vascular tissues, even though many seaweeds are plant-like in appearance (Laura and Paolo, 2014). Laura and Paolo (2014) stated that some of algae showed specialization and differentiation of their vegetative cells which their reproductive structures made up of cells that are all potentially fertile and lack sterile cells covering or protecting them.

Algal can be divided into two body types which are macroalgae and microalgae. Macroalgae have coenocytic, parenchymatous, or pseudoparenchymatous bodies that can be seen with the unaided eye (macroscopic). In contrast to macroalgae, microalgae species occurs as solitary cells (unicells) that need a microscope to observe them (Linda, 2009). Aquatic algae can be found from freshwater spring to salt lakes with tolerance of pH, temperature, turbidity, oxygen and carbon dioxide (CO_2) concentration (Laura and Paolo, 2014).

2.2 Microalgae Utilization in Aquaculture Industry

Microalgae have been utilized in aquaculture as live feeds for all growth stages of bivalve molluscs, for the larval or early juvenile stages of abalone, crustaceans, and some fish species, and for zooplankton (Catarina et al., 2015). Almost hundred microalgae species have been tested as animal feed but probably < 20 have gained widespread use in aquaculture over the last 4 decades (Laura and Paolo, 2014). Microalgae not only help to enrich zooplankton for feeding fish and other larvae. In addition to providing essential amino acids and energy, they also provide other nutrients such as vitamins, essential polyunsaturated fatty acids (PUFA), pigments and sterols, which are transferred through the food chain (Pronob et al., 2012). Hence microalgae is known as “living capsules of nutrition” in order to achieve high survival rate of aquatic animals. Pronob et al. (2012) mentioned that supplying live food continuously to the cultured stock along with supplemented artificial feed can produce a disease free healthy stock because supplemented artificial feed cannot meet all the elements required for the growth of fish.

Pronob et al. (2012) stated besides chlorophyll, microalgae also contain various carotenoid pigments which determine its colours such as Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). Brown and red algae are marine algae while green algae are culture in freshwater and free floating type (Patil et al., 2008). Catarina et al. (2015) mentioned that microalgae have been used in many new applications like wastewater treatment, nutrient recycling, bioconservation of solar energy and so on. In recent years, mass culture of unicellular algae such as *Chaetoceros* and *Skeletonema* (diatoms) and *Isochrysis, Tetraselmis and Chlorella* (small phytoplankters) is famous for feeding larvae of fishes, prawns, shrimps and molluscs in aqua hatcheries (Pronob et al. 2012). Helm et al. (2004) stated besides nutritional attributes of microalgae in aquaculture

hatcheries, microalgae also meets the feeding size for early stages of aquatic animals as their size ranging from 5 - 25 µm and this increase survival and growth rates.

Laura and Paolo (2014) stated that the nutritional value of microalgae is affected by its size and shape, digestibility, and biochemical composition (such as nutrients, enzymes and toxins if present). Microalgae grown to late-logarithmic growth phase typically contain 30 - 40 % protein, 10 - 20 % lipid and 5 - 15 % carbohydrate (Laura and Paolo, 2014). When cultured through to stationary phase, the proximate composition of microalgae can change significantly (Harrison et al., 1990). PUFAs derived from microalgae, i.e. docosahexaenoic acid, eicosapentaenoic acid and arachidonic acid are known to be essential for various larvae (Sargeant et al., 1997). The content of vitamins can vary between microalgae.

2.3 Parameters for Freshwater Microalgae Cultivation

The purpose of microalgae cultivation is to propagate the microalgae cell in the shortest time (LeRoy, 2010). In order to obtain pure culture of freshwater microalgae, a room specially for algal culture is needed to avoid contamination by cultures of other organisms; temperature in the algal room should maintained between 20 - 24 ºC by air conditioning; algal culture should provided with light by fluorescent tubes and aeration continuously; and the transferring of algae from one flask to another should carry out in a laminar flow or simple glass cabinet for sterile purpose (Chisti, 2007; Johnson, 2009).

2.3.1 Light Source

Like all other plants, microalgae are photosynthesizing in which they convert inorganic carbon into organic matter. Light source is an important energy source that accelerates photosynthesis reaction. In this reaction, light intensity, spectral characteristics and photoperiod needed to be strictly controlled (Johnson, 2009; Lavens and Sorgeloos, 1996). LeRoy (2010) described these requirements vary in the culture depth and the cell concentration of microalgae culture. In other words, higher culture depth and cell densities required higher light intensity to pass through the culture.

2.3.2 Temperature Control

Temperature is another limiting factor for both open and closed microalgae cultivation systems (Chisti 2007). The effects of temperature in many species of laboratory cultured microalgae were well documented but the effects of temperature in outdoor cultivation systems were limited. When exceeding the optimum temperature by 2 - 4 °C may caused total culture loss (Mata et al., 2009).

2.3.3 Aeration and Mixing

Lavens and Sorgeloos (1996) and LeRoy (2010) described aeration and mixing in microalgae cultivation ensured:

I. All cell of population exposed evenly to the light and nutrients.
II. Reduced self-shading or photoinhibition occurred in which photosynthesis rate decreasing caused by excess light.

III. Boosted gas exchange rate of the culture medium with the air because the air is a carbon source (from CO_2) needed by photosynthesis process. CO_2 also help in pH stabilization.

2.3.4 pH and Salinity

The range of pH for microalgae species is stated in Table 2.1. Carbon dioxide (CO_2) plays a dual role in microalgae culture in which act as the carbon source and maintain pH level at optimum.

Every species of microalgae has its own optimum salinity range. High evaporation rate during hot weather can increase the salinity range. The effects of salinity changes were osmotic stress; ion (salt) stress; and changes of the cellular ionic ratios caused by permeability selective ion membrane (Moheimani, 2005). Dilution or adding salt could control the salinity level of microalgae culture (Mata et al., 2009).

Table 2.1 : General conditions for culturing microalgae (Andersen, 2005).

CONDITIONS	RANGE	OPTIMAL
Temperature (^oC)	16 - 27	18 - 24
Salinity (gL^{-1})	12 - 40	20 - 24
Light intensity (lux)	1000 -10000 (depends on volume and density)	2500 - 5000
Photoperiod (light:darks, h)		16:8 (minimum) 24:0 (maximum)
pH	7 - 9	8.2 - 8.7

2.4 Inorganic Nutrients Required for Freshwater Microalgae Cultivation

The nitrogen (N) sources for most algae are nitrate, nitrite or ammonium (NH_4^+). The pH of the culture may fall firmly especially in high cell concentration culture during hot weather when NH_4^+ is used, thus causes decrease in growth rate and mortality of cell of population (Laura and Paola, 2014). Inorganic phosphorus ($H_2PO_4^-$ and HPO_4^{2-}) are the phosphorus (P) source for microalgae intake. Laura and Paolo (2014) claimed those algae which can dominate organic phosphate compounds hydrolyse these extracellularlly by the action of phosphoesterase or phosphatase enzymes. The end product of these actions is inorganic phosphorus. Potassium (K) is a cofactor for a variety of enzymes and is probably required by all algae.

2.5 Application of Poultry Manure in Agriculture

Manure is organic by-products that containing organic matter which can be used as organic fertilizer in agricultural or aquaculture (Green, 2015). Jeff et al. (2014) stated animal manures consist of feces which in the forms of farmyard manure (FYM) or farm slurry. Slurry means manure in liquid form and is produced by more intensive livestock rearing systems where concrete or slats are used while FYM also contains plant material (often straw), which has been used as bedding for animals with the function to absorb the feces and urine. In fact, untreated manure is simply animal feces while properly treated or processed manure is a valuable and marketable organic residual (Liandong and Erkki, 2014). The amount and consistency of manures varies with animal type, climate, feed ration, animal age and health, and other factors.

Poultry manure refers to the excreta (composed of mainly feces and urine) of birds such as chicken or quail that have faster fermentation rate (Reza, 2016). Besides birds' dropping, poultry manure that mixed with used bedding likes sawdust or rice hulls, spilled feed, feathers and other substances is known as poultry litter (Reza, 2016).

2.6 Poultry Waste Utilization in Freshwater Microalgae

There has been increased interest in using poultry litter as fertilizer since commercial fertilizer prices have skyrocketed (Funderburg, 2009). Fertilization is an important part in the yield of increasing production or providing nutrients to the microalgae cultivation (Michael et al., 2013). Fertilization in microalgae cultivation can be carried out using chemical fertilizer continuously with increasing dose or organic fertilizer. Michael et al. (2013) mentioned continuous chemical fertilizer application is inadvisable due to environmental friendly, human health and adverse effects of agrichemical on the environment. The use of organic fertilizer likes poultry waste (dung from chicken or quail) is enhanced in the aquaculture or agriculture industry (Funderburg, 2009).

In aquaculture industry, the nutrients in fresh or after processing poultry waste can stimulate the production of natural food organism likes phytoplankton and detritus (David and Kriengkrai, 2012). Chisti (2007) stated in tropical country like Malaysia, the average water temperature > 25 ºC are ideal for culturing fish using poultry wastes as fertilizer to ensure the growth of both fish and their natural feeds. The carps and tilapias obtain the nutrients released from the poultry wastes due to they can utilize plankton, benthic and detrital food organism effectively (David and Kriengkrai, 2012). However, the use of poultry manure is classified as hazardous

organic matter which gives a risk to the water environment (Mlejnkova and Sovova, 2012).

2.7 Nutrient Contents in Poultry Manure

Poultry manure enriched with essential plant nutrients like N, P, K and many trace elements like zinc, copper, iron and others (Amanullah et al., 2010). It is important to process poultry manure immediately to avoid rapid decomposition and save its nutrients properties because Funderburg (2009) stated the nutritional value of fresh poultry manure deteriorates very quickly. For example, nearly 40 % of nitrogen is lost within 30 days (d) if poultry manure left exposed (Amanullah et al., 2010). Liandong and Erkki (2014) stated that nitrogen content in manure varies with the type of animal and feed ration, amount of litter, bedding or soil included, and amount of urine concentrated with the manure. Moisture content is also a major consideration. For example: The moisture content of fresh manure is around 70 - 85 %. The moisture content of air-dried manure is around 9 - 15 % (Edward and David, 2010).

Freshwater Microalgae which is *Daphnia magna* contained high nutritional value and acted as the natural feed for fish larvae (Herawati et al., 2015). The growth and quality of *D. magna* nutrient value depend on its culture medium. Herawati et al. (2017) stated the use of organic fertilizers in culture media including the wastes or feces of chicken, goat and quail mixed with the rejected bread and tofu waste fermented with the probiotic bacteria had not so far been conducted as the use of organic fertilizer could impact the growth performance and content of *D. magna*. The highest nutrients - particularly for the content of N, P and calcium (Ca) in organic fertilizer were the food sources of *D. magna*.

In the nutrient analysis, the chicken waste contained N (4.75 %), P (3.57 %) and Ca (4.80 %); the quail waste contained 4.06 % of N, 2.96 % of P and 2.57 % of Ca; the goat feces contained N, P and Ca with 2.36 %, 2.96 % and 3.41 % respectively (Herawati et al., 2017). The researcher in this paper found that the organic material in the animal manures mixed with expired bread and waste tofu gave a result of increase for its nutrient quality with 1.2 % of N, 1 % of P and K.

CHAPTER 3

METHODOLOGY

3.1 Sampling Site and Collection of Treated Quail Dung (TQD) Sample

The pond water sample was collected from a tilapia fish pond at Agro Techno Park of Universiti Malaysia Kelantan (UMK), Malaysia. Temperature and pH of the sample were measured using digital thermometer (310C Pocket Digital Thermometer, Test Products International, S. Korea) and pH tester (CHECKER pH Tester, Hanna Instruments, Romania). The sample of fresh quail dung was obtained from Amigous Quail Enterprise at Agro Techno Park, UMK, Malaysia and a quail farm located in Ayer Lanas, Kelantan, Malaysia. The impurities such as sawdust and feed waste attached to the quail dung were roughly cleaned by hand.

3.2 Preparation of Microalgae Culture

All apparatuses and materials used such as 500 mL conical flask, 100 mL and 50 mL measuring cylinder, 1000 mL Schott bottle and distilled water (dH_2O) were autoclaved to avoid any contamination. The concentrations of treated quail dung (TQD) and commercial fertilizer (NPK) were measured into 3 g, 6 g and 9 g. The N:P:K ratios of TQD and NPK fertilizers was approximately 1 %. Then, the TQD fertilizer was placed into three different tea bags and labeled according to their weight. Each tea bag was wrapped with another 3 tea bags layer by layer to minimize the residue of TQD fertilizer that may diffused out into the culture during the experiment which may affect the data collection. 360 mL of autoclaved dH_2O was

poured into each conical flask. A total of 6 conical flasks were prepared and labeled as stated in Table 3.1.

Table 3.1 : Label and description of treatments.

Treatments	Description
1 (T1)	360 mL of dH_2O and 3 g of NPK fertilizer
2 (T2)	360 mL of dH_2O and 6 g of NPK fertilizer
3 (T3)	360 mL of dH_2O and 9 g of NPK fertilizer
4 (T4)	360 mL of dH_2O and 3 g of TQD fertilizer
5 (T5)	360 mL of dH_2O and 6 g of TQD fertilizer
6 (T6)	360 mL of dH_2O and 9 g of TQD fertilizer

All conical flasks containing different types of fertilizer in each treatment were autoclaved again to be sterilized and let to cool until room temperature in the laminar flow (AHC-301, ESCO Laminar Flow Cabinet, US).

1500 mL of pond water sample was obtained immediately from a tilapia fish pond at Agro Techno Park, UMK before mixing. The temperature of pond water sample and the mixture in all treatments were check immediately using digital thermometer (310C Pocket Digital Thermometer, Test Products International, S. Korea). The temperature difference was maintained at < 5 °C to avoid heat shock towards microalgae. The pond water sample was filtered with 100 µm mesh size of plankton net to get rid of unwanted substances. 10 % of the total volume i.e. 40 mL of filtered pond water sample was poured into all treatments and swirled gently to form homogenize mixture. Then the mouths of all conical flasks were closed tightly with cotton wool to prevent contamination during the experiment. All the processes mentioned above were carried out in laminar flow.

All treatments were placed on a rack and provided with artificial light (fluorescent bulb) as well as aeration for 24 h for 7 d. The mouth of the conical flasks were stuffed with some cotton wool to avoid evaporation of treatments. The water parameters of all treatments such as pH and temperature were monitored and recorded once a day using digital thermometer and pH tester (CHECKER pH Tester, Hanna Instruments, Romania). Similarly, the reading of optical density (OD) was also recorded on a daily basis using spectrophotometer (Genesys 20, Thermo Scientific, UK). All treatments were carried out in triplicates.

The experiments were carried out in 3 cycles with each cycle had a period of 7 d. The aeration tube was changed and the apparatuses were autoclaved for every new cycle to minimize the occurrence of contamination. The autoclaved dH_2O was added if evaporation of treatments occurred or the total volume decreased. The pHs of all treatments were maintained in the range from 6 - 8. In acidic condition (pH < 6), lime powder was added to neutralize the mixture. In contrast to alkaline condition (pH > 8), hydrochloric acid was added to neutralize the mixture.

3.3 Analysis Methods

3.3.1 Microalgae Growth Performance

The data were collected and recorded once a day for every cycle. Li et al. (2011) stated the microalgal density was determined by using by measuring the optical density of algal culture at 650 nm (OD_{650}) using spectrophotometer (Genesys 20, Thermo Scientific, UK). The relationship between microalgal density per mL (D mL^{-1}) and OD_{650} is as shown in Equation (1) (Li et al., 2010):

$$D = 9.52 \times 10^6 OD_{650} + 70957, R = 0:997 \quad (1)$$

Liang et al. (2010) mentioned the specific growth rate per day (SGR d^{-1}) was calculated by fitting the OD of microalgal culture to an exponential function as shown in Equation (2):

$$SGR = (\ln OD_t - \ln OD_0)/t \quad (2)$$

where OD_0 is the optical density at the initial day, OD_t is the optical density measured on day t. Each recorded OD_t was corrected by taking away that of the corresponding blank sample.

3.3.2 Data Analysis

Data collected in the current study were analyzed using One-Way Analysis of Variance (ANOVA) and post hoc Turkey test at $p < 0.05$ using Statistical Package for the Social Science (SPSS) version 23. The curve graph was generated using Microsoft Excel 2007 (Kamal ,2012; Ilknur ,2010).

CHAPTER 4

RESULTS

4.1 Correlation between pH, Temperature and Optical Density (OD) in Freshwater Microalgae Cultivation

The effect of all treatments on the growth of the freshwater microalgae was studied. Freshwater microalgae cultivation were treated with different types of fertilizers at the same volume, temperature, light, in the same environment in the laboratory. The parameters such as temperature and pH were recorded once a day for every cultivation cycle. Correlation between pH, temperature and OD in freshwater microalgae cultivation was shown in Table 4.1.

Table 4.1 : Correlation between pH, temperature and optical density (OD) in freshwater microalgae cultivation.

	Temperature	pH	OD
Temperature	1		
pH	-0.085	1	
OD	-0.065	0.728**	1

Note : ** Correlation is significant at the 0.01 level.

Based on Table 4.1, there a negative relationship between temperature and pH and OD in microalgae cultivation as r = -0.0865 and r = -0.065 respectively. However, there was strong positve relationship between the pH and OD (r = 0.728) at $p < 0.01$. This showed that the OD of freshwater microlagae culture was affected by pH.

4.2 Concentration Yield of Freshwater Microalgae Culture

Figure 4.1 showed the peak optical density (OD) for all treatments in freshwater microalage cultivation.

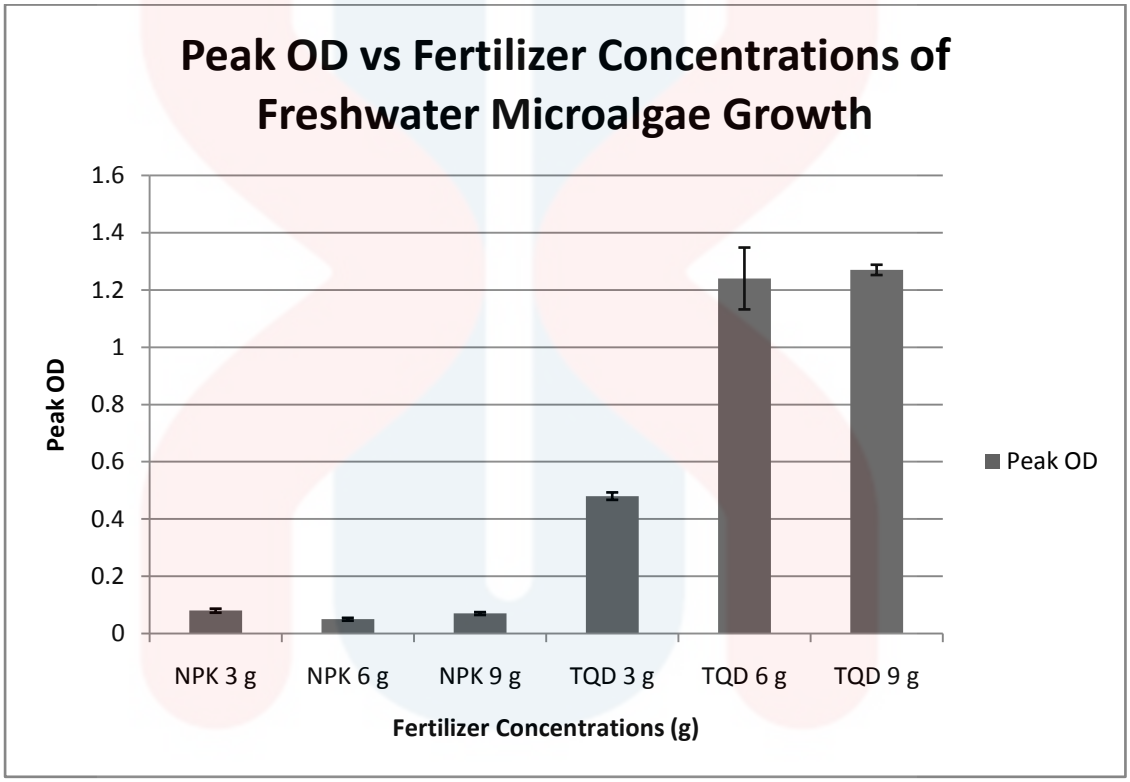

Figure 4.1 : Peak OD against fertilizer concentrations in freshwater microalage cultivation.

TQD 9 g had the highest peak OD (1.27 ± 0.018) followed by TQD 6 g (1.24 ± 0.108). Meanwhile, TQD 3 g had moderate peak OD (0.48 ± 0.013). The lowest peak OD was recorded by all the microalgae culture treated with commercial fertiliser (NPK) with peak OD 0.08 ± 0.007 for NPK 3 g; 0.05 ± 0.005 for NPK 6 g and 0.07 ± 0.005 for NPK 9 g. However TQD 9 g was not significantly different from TQD 6 g. Both TQD 6 g and 9 g were significantly different from TQD 3 g and all the microalgae treated with NPK fertilizer. There were no significant difference between all NPK fertilizer treatments.

4.3 Growth Phase of Freshwater Microalgae Culture

The optical density (OD) of freshwater microalgae growth that were treated with different fertilizers were indicated in Figure 4.2.

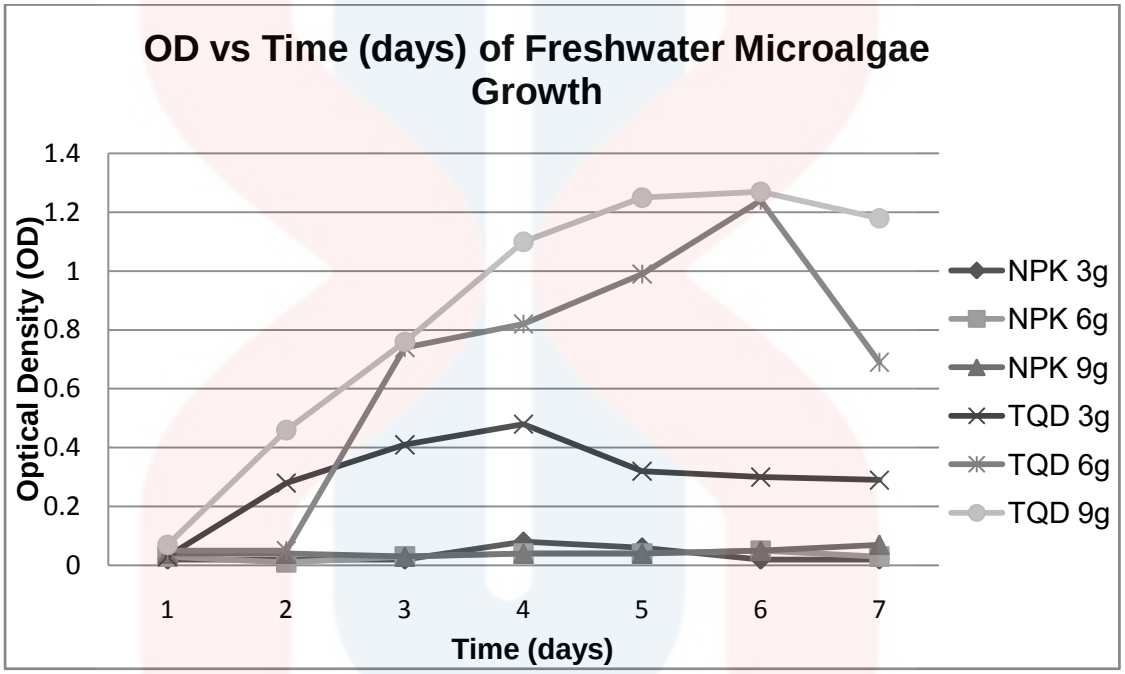

Figure 4.2 : OD against time (days) of freshwater microalgae growth for NPK and TQD treatments.

Among the all concentrations of TQD fertilizer in Figure 4.2, the highest OD reading of TQD 3 g was in Day 4 (0.48 ± 0.013). TQD 6 g and 9 g showed highest OD reading in the same day which was Day 6 with values 1.24 ± 0.108 and 1.27 ± 0.018 respectively. TQD 3 g and 9 g showed the lowest OD reading in Day 1 with values 0.03 ± 0.001 and 0.07 ± 0.006 respectively. Meanwhile, TQD 6 g showed the lowest OD reading in both Day 1 and Day 2 (0.05 ± 0.012). From Figure 4.1, NPK 3 g had highest OD reading in Day 4 (0.08 ± 0.007) and in Day 2 and Day 3 showed lowest OD reading with value 0.02 ± 0.002. The highest OD reading of NPK 6 g was recorded in Day 6 (0.05 ± 0.005) and the lowest OD reading was recorded in Day 2

(0.01 ± 0.002). NPK 9 g showed the highest OD reading in the last day with value 0.07 ± 0.005 and lowest OD reading in Day 3 (0.03 ± 0.008).

Based on Figure 4.2, the death phase of freshwater microalgae cultivation had not be identified in the current study. In the treatment NPK 3 g, the lag phase started from day 1 to day 3; the exponential phase started from day 3 to day 4; the declining phase started from day 4 to day 6 and the stationary phase started from day 6 to day 7. The lag phase started from day 1 to day 3; the exponiential phase started from day 4 to day 6 and declining phase started from day 6 to day 7 in the treatment NPK 6 g. In the treatment NPK 9 g, the freshwater microalgae culture had lag phase started from day 1 to day 3 and started to propagate exponentially from day 4 to day 7.

The freshwater microalage cultivation in treatment TQD 3 g experienced similar growth pattern with NPK 3 g which were lag phase started from day 1 to day 2; freshwater microalage cells started to propagate exponentially day 3 to day 4; declining phase started from day 5 to day 6 and stationary phase started from day 6 to day 7. The treatments TQD 6 g and TQD 9 g had the same growth pattern in freshwater microalage culture. The freshwater microalgae cells had lag phase on the frist two days; then the cells propagated exponentially from day 3 to day 6 and lastly the cells started to decline from day 6 and day 7.

4.4 Specific Growth Rate per Day (SGR d^{-1}) of Freshwater Microalgae Culture

Figure 4.3 showed the specific growth rate per day (SGR d^{-1}) against fertilizer concentrations in freshwater microalgae cultivation.

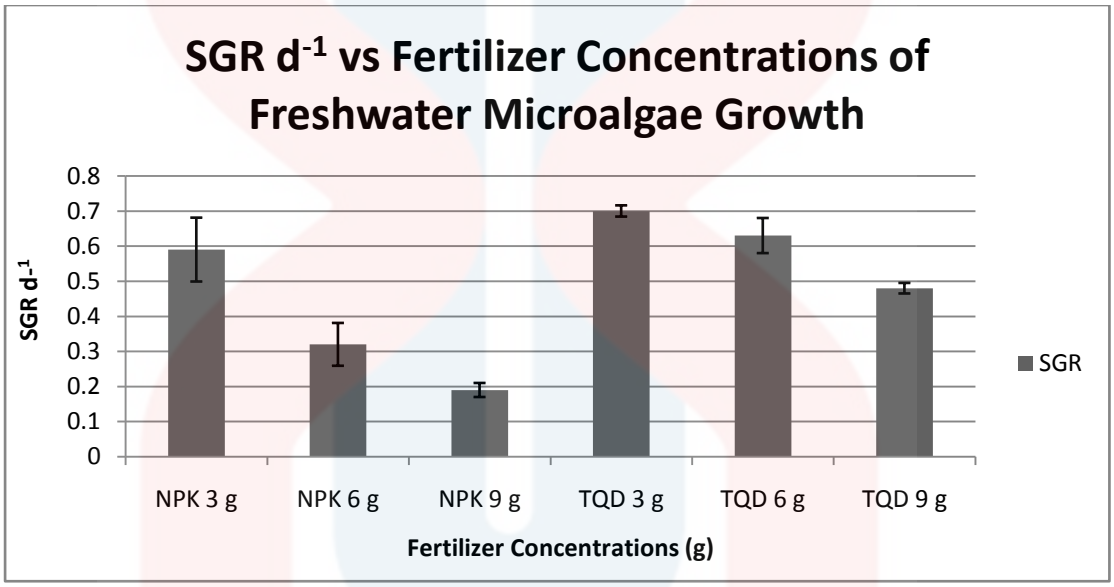

Figure 4.3: SGR d^{-1} against fertilizer concentrations of freshwater microalgae for NPK and TQD treatments.

TQD 3 g had the highest SGR d^{-1} (0.70 ± 0.016) followed by TQD 6 g (0.63 ± 0.050) and then NPK 3 g (0.59 ± 0.091). Meanwhile, TQD 9 g had moderate SGR d^{-1} (0.48 ± 0.015) followed by NPK 6 g (0.32 ± 0.061). The lowest SGR d^{-1} was recorded by NPK 9 g (0.19 ± 0.020). However, TQD 3 g was significantly different from NPK 3 g. TQD 6 g was not significantly different from TQD 3 g and NPK 3 g. Both TQD 6 g and NPK 3 g were significantly different from TQD 9 g. TQD 9 g was significantly different from NPK 6 g. NPK 6 g was significantly different from NPK 9 g.

CHAPTER 5

DISCUSSION

Based on Figure 4.1 and Figure 4.2, the peak optical density (OD) and the specific growth rate per day (SGR d^{-1}) of freshwater microalgae treated with TQD were higher compared to the NPK treatments. This result is supported by a study carried out by Ilknur (2010) in which the best growth of the microalgae was observed in organic agriculture fertilizer treatment at concentration 0.01 mL/L. Furthermore, Herawati et al. (2017) reported that the other organic materials in the animal manures increased available nutrients that helped in the growth of freshwater microalgae compared to the pure combination of commercial fertilizer. Thus TQD fertilizer had a better effect on the growth performance of freshwater microalgae compared to NPK fertilizer. This could be proven when the pale-brown colour of TQD treatments in all concentration turns to dark greenish-brown which showed the growth of microalgae and also the higher peak OD and SGR d^{-1} of freshwater microalgae.

In the current study, the wavelength of optical density (OD) used was 650 nm because the OD readings of freshwater microalgae sample were consistent when compared to trials at 600 nm, 680 nm and 700 nm. The OD at wavelength 650 nm was also supported in a study carried out by Li et al. (2011). Blauch (2009) stated that a spectrophotometer is an instrument that measures the amount of photons (the intensity of light) absorbed after it passes through sample solution. Spectrophotometry is a measurement of how much a chemical substance absorbs or transmits over a certain range of wavelength. Gore (2000) mentioned that the absorption or the transmission of a sample solution can be determined by the colour in visible spectrophotometry. For example, a solution sample that absorbs light

over all visible ranges (i.e., transmits none of visible wavelengths) appears black in theory. If all visible wavelengths are transmitted, the solution sample appears white. If a solution sample absorbs red light (~ 650 nm), it appears green.

According to Edward and David (2010), the microalgae growth curve was characterized by 5 phases, i.e. lag phase, exponential phase, declining phase, stationary phase and death phase. In the current study, the growth phases of freshwater microalgae were determined by the values of OD. NPK 3 g and TQD 3 g had the same growth pattern which consists of lag phase, exponential phase, declining phase and stationary phase. NPK 6 g and TQD 6 g also had same growth pattern which were lag phase, exponential phase and declining phase. However, the lag phase and exponential phase were observed in both NPK 9 g and TQD 9 g but declining phase only exhibited by microalgae culture in TQD 9 g. The declination of microalgae cell density may be due to the nutritional level in TQD, light, carbon dioxide (CO_2), inconsistency of pH or other parameters that acts as limiting factors in microalgal growth (Lavens and Sorgeloos, 1996). In stationary phase, the nutrient available in the culture and the SGR were balanced which results in a relatively constant cell density (Coutteau, 2015).

Based on Figure 4.2, the SGR d^{-1} of freshwater microalgae in both treatments decreased when the fertilizer concentrations were increased. The highest SGR d^{-1} in NPK treatment was recorded by NPK 3 g (0.59 ± 0.091) followed by NPK 6 g (0.32 ± 0.061) and the lowest SGR d^{-1} was NPK 9 g (0.19 ± 0.020). Meanwhile, the highest SGR d^{-1} in TQD treatment was recorded by TQD 3 g (0.70 ± 0.016) followed by TQD 6 g (0.63 ± 0.050) and the lowest SGR d^{-1} was TQD 9 g (0.48 ± 0.015). Masautso and Confred (2014) mentioned that the use of quail manure which was rich in proteins underwent decomposition and the end product was ammonia which contained unionized ammonia. Ammonia exists in unionised ammonia (NH_3-N), and

ionised ammonia (NH_4+-N), the sum of these two is called total ammonia nitrogen (Molleda et al., 2007). Pillay and Kutty, 2005 stated that the relative concentration of ammonia is primarily a function of water pH, salinity and temperature. The higher toxicity levels of NH_3-N and CO_2 in water depends on the water's pH controls acid-base equilibrium; as an example, at 20 ^oC and a pH of 7, the mole fraction of NH_3-N is 0.004, but at a pH of 10, the NH_3-N increase to 0.8 at the same temperature (Timmons et al., 2002).

Masqood (2012) stated that the best pH for green algal growth ranged between 7 - 7.9 which is in neutral condition. In this experiment, the range of pH recorded was 6 - 8.9 where microalgae growth had occurred. This stated that microalgae can grow between slightly acidic and slightly alkaline condition. This result is supported by Lavens and Sorgeloos (1996) as shown in Table 2.1. Laura and Paolo (2014) also stated that some freshwater microalgae species are able to tolerate acid-base environment.

Some problems were faced during the study. The first problem was monitoring and maintaining the pH of the culture. Based on Table 4.1, it showed that the OD and pH of culture were significantly correlated. The pH of TQD treatments almost reached 9 which exceeded the optimum pH range of microalgae culture. The culture was assumed to have become alkaline due to the increase in ammonia content in TQD treatments than in NPK treatments which lead to ammonia toxicity. The ammonia toxicity can affect the growth rate of any aquatic animals and plants. According to Timmons et al. (2002), the normal ammonia level should be maintained at < 0.05 mgL^{-1}. When the pH increased to alkaline, hydrochloric acid was added to neutralize the pH. In this experiment, different volumes of hydrochloric acid were added into different concentrations of TQD treatments. Distilled water (dH_2O) was

also added with the purpose of neutralizing the culture besides maintained the volume of culture loss caused by evaporation.

Another problem was contamination of culture. The factors of contamination were the environment of the freshwater microalgae culture was not sterile, the experiment was not cultured in a closed room, and the spore or fungi attached to the tea bags before experiments were carried out. To minimize the chances of contamination, all conical flasks used and the medium (360 mL of dH_2O with the fertilizer) were autoclaved before the pond water was added. The pond water was filtered with 100 µm mesh size of plankton net in order to prevent other microorganisms from contaminating the culture before being placed in the respective conical flasks. The aeration tubes were also wiped with 70 % ethanol for hygiene purposes.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

In the conclusion, the use of treated quail dung (TQD) is the best alternative fertilizer that can affect the growth of the freshwater microalgae. TQD contains more nutrients than commercial fertilizer that required by the freshwater microalgae. TQD fertilizer is recommended for the farmer especially for those who wants to reduce the cost of using the commercial fertilizer in the culture of the freshwater microalgae. The quail dung can also be easily obtained as there are many quail farms in the state of Kelantan. TQD is also an eco-friendly fertilizer compared to commercial fertilizer.

It is suggest that future microalgae cultivation must be carried out in a closed room with strict biosecurity measures. This is to ensure the cultivation is free from pathogen, diseases or any contamination. In the current study, the microalgae cultivation was carried out in laboratory but no biosecurity procedures were applied which led to the high chances of contamination of the cultures.

In the current study, TQD and NPK fertilizer were supplied to the general freshwater microalgae population without identifying their species. This is due to the fact that freshwater sample was taken from a tilapia fish pond that contains various microalgae species. Therefore, it is recommended that future researchers identify specific freshwater microalgae species and carry out the research to find out the growth performance of that particular species treated with TQD and NPK fertilizer.

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APPENDICES A

Table A.1 : Pearson correlation between temperature (ºC), pH and optical density (OD) of freshwater microalgae cultivation.

Correlations

		°C	pH	AU
°C	Pearson Correlation	1	-.085	-.065
	Sig. (2-tailed)		.540	.639
	N	54	54	54
pH	Pearson Correlation	-.085	1	.728**
	Sig. (2-tailed)	.540		.000
	N	54	54	54
AU	Pearson Correlation	-.065	.728**	1
	Sig. (2-tailed)	.639	.000	
	N	54	54	54

**. Correlation is significant at the 0.01 level (2-tailed).

Table A.2 : Descriptives data of peak OD of freshwater microalgae for NPK and TQD treatments.

Descriptives

PEAKOD

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NPK3G	9	.0751	.00688	.00229	.0698	.0804	.06	.08
NPK6G	9	.0458	.00487	.00162	.0420	.0495	.04	.06
NPK9G	8	.0689	.00544	.00192	.0643	.0734	.06	.08
TQD3G	9	.4811	.01279	.00426	.4713	.4909	.46	.50
TQD6G	9	1.2414	.10752	.03584	1.1588	1.3241	1.09	1.32
TQD9G	9	1.2686	.01771	.00590	1.2549	1.2822	1.24	1.29
Total	53	.5388	.54159	.07439	.3896	.6881	.04	1.32

Table A.3 : One-way ANOVA of peak OD of freshwater microalgae for NPK TQD treatments.

ANOVA

PEAKOD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.156	5	3.031	1467.463	.000
Within Groups	.097	47	.002		
Total	15.253	52			

Table A.4 : Homogeneous subsets of Post Hoc tests of peak OD of freshwater microalgae for NPK and TQD treatments.

PEAKOD

Tukey HSD[a,b]

		Subset for alpha = 0.05		
FERTCONC	N	1	2	3
NPK6G	9	.0458		
NPK9G	8	.0689		
NPK3G	9	.0751		
TQD3G	9		.4811	
TQD6G	9			1.2414
TQD9G	9			1.2686
Sig.		.753	1.000	.809

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.816.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table A.5 : Peak OD of freshwater microalgae for NPK and TQD treatments.

Treatment	Peak OD
NPK 3 g	0.08 ± 0.007^{c}
NPK 6 g	0.05 ± 0.005^{c}
NPK 9 g	0.07 ± 0.005^{c}
TQD 3 g	0.48 ± 0.013^{b}
TQG 6 g	1.24 ± 0.108^{a}

TQD 9 g	1.27 ± 0.018[a]

Note : a = Highest peak OD
b = Moderate peak OD
c = Lowest peak OD

Table A.6 : Descriptives data of OD reading of freshwater microalgae for NPK and TQD treatments.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
DAY1	NPK3G	9	.01556	.002698	.000899	.01348	.01763	.013	.019
	NPK6G	9	.02978	.003383	.001128	.02718	.03238	.025	.036
	NPK9G	8	.04175	.003284	.001161	.03900	.04450	.036	.045
	TQD3G	9	.02944	.001424	.000475	.02835	.03054	.028	.032
	TQD6G	9	.05389	.012232	.004077	.04449	.06329	.031	.074
	TQD9G	9	.07222	.006180	.002060	.06747	.07697	.066	.081
	Total	53	.04042	.019690	.002705	.03499	.04584	.013	.081
DAY2	NPK3G	9	.01778	.001563	.000521	.01658	.01898	.016	.021
	NPK6G	9	.00933	.002179	.000726	.00766	.01101	.007	.013
	NPK9G	8	.03600	.004243	.001500	.03245	.03955	.030	.042
	TQD3G	9	.28167	.028094	.009365	.26007	.30326	.244	.315
	TQD6G	9	.05389	.012232	.004077	.04449	.06329	.031	.074
	TQD9G	9	.45633	.036739	.012246	.42809	.48457	.420	.508
	Total	53	.14451	.172092	.023639	.09708	.19194	.007	.508
DAY3	NPK3G	9	.02333	.002449	.000816	.02145	.02522	.019	.026
	NPK6G	9	.03356	.003779	.001260	.03065	.03646	.025	.038
	NPK9G	8	.03238	.007818	.002764	.02584	.03891	.024	.042
	TQD3G	9	.41122	.011595	.003865	.40231	.42013	.399	.428
	TQD6G	9	.73522	.032163	.010721	.71050	.75994	.700	.784
	TQD9G	9	.76478	.015458	.005153	.75290	.77666	.747	.793
	Total	53	.33909	.327929	.045044	.24871	.42948	.019	.793
DAY4	NPK3G	9	.07511	.006882	.002294	.06982	.08040	.060	.080
	NPK6G	9	.04222	.004353	.001451	.03888	.04557	.034	.049
	NPK9G	8	.04363	.005902	.002087	.03869	.04856	.038	.052
	TQD3G	9	.48111	.012791	.004264	.47128	.49094	.463	.496
	TQD6G	9	.81622	.091393	.030464	.74597	.88647	.691	.901
	TQD9G	9	1.10122	.012882	.004294	1.09132	1.11112	1.077	1.119

	Total	53	.43381	.419970	.057687	.31805	.54957	.034	1.119
DAY5	NPK3G	9	.06411	.011773	.003924	.05506	.07316	.051	.080
	NPK6G	9	.03789	.002934	.000978	.03563	.04014	.034	.043
	NPK9G	8	.03900	.001604	.000567	.03766	.04034	.037	.042
	TQD3G	9	.31978	.011498	.003833	.31094	.32862	.304	.337
	TQD6G	9	.98511	.196436	.065479	.83412	1.13611	.710	1.283
	TQD9G	9	1.24911	.017120	.005707	1.23595	1.26227	1.223	1.269
	Total	53	.45691	.500149	.068701	.31905	.59476	.034	1.283
DAY6	NPK3G	9	.02256	.006521	.002174	.01754	.02757	.012	.033
	NPK6G	9	.04578	.004868	.001623	.04204	.04952	.040	.057
	NPK9G	8	.05488	.003871	.001368	.05164	.05811	.050	.060
	TQD3G	9	.30144	.024439	.008146	.28266	.32023	.266	.336
	TQD6G	9	1.24144	.107521	.035840	1.15880	1.32409	1.085	1.322
	TQD9G	9	1.26856	.017714	.005905	1.25494	1.28217	1.237	1.290
	Total	53	.49730	.558365	.076697	.34340	.65121	.012	1.322
DAY7	NPK3G	9	.02311	.002892	.000964	.02089	.02533	.020	.028
	NPK6G	9	.03333	.009042	.003014	.02638	.04028	.019	.046
	NPK9G	8	.06888	.005436	.001922	.06433	.07342	.063	.079
	TQD3G	9	.28867	.037733	.012578	.25966	.31767	.248	.341
	TQD6G	9	.69422	.072692	.024231	.63835	.75010	.619	.799
	TQD9G	9	1.17878	.125167	.041722	1.08257	1.27499	1.037	1.350
	Total	53	.38706	.436016	.059891	.26688	.50724	.019	1.350

Table A.7 : Optical density (OD) of freshwater microalgae for NPK and TQD treatments.

Treatment	Optical Density (OD)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
NPK 3 g	0.02 ± 0.003	0.02 ± 0.002	0.02 ± 0.002	0.08 ± 0.007	0.06 ± 0.012	0.02 ± 0.007	0.02 ± 0.003
NPK 6 g	0.03 ± 0.003	0.01 ± 0.002	0.03 ± 0.004	0.04 ± 0.004	0.04 ± 0.003	0.05 ± 0.005	0.03 ± 0.009
NPK 9 g	0.04 ± 0.003	0.04 ± 0.004	0.03 ± 0.008	0.04 ± 0.006	0.04 ± 0.002	0.05 ± 0.004	0.07 ± 0.005

TQD 3 g	0.03 ± 0.001	0.28 ± 0.028	0.41 ± 0.012	0.48 ± 0.013	0.32 ± 0.011	0.30 ± 0.024	0.29 ± 0.038
TQD 6 g	0.05 ± 0.012	0.05 ± 0.012	0.74 ± 0.032	0.82 ± 0.091	0.99 ± 0.196	1.24 ± 0.108	0.69 ± 0.073
TQD 9 g	0.07 ± 0.006	0.46 ± 0.037	0.76 ± 0.015	1.10 ± 0.013	1.25 ± 0.017	1.27 ± 0.018	1.18 ± 0.125

Table A.8 : Descriptives data of SGR (d^{-1}) of freshwater microalgae for NPK and TQD treatments.

Descriptives

SGR

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
NPK3G	9	.5851	.09111	.03037	.5150	.6551	.42	.70
NPK6G	9	.3219	.06078	.02026	.2752	.3686	.23	.42
NPK9G	8	.1889	.01988	.00703	.1723	.2056	.15	.21
TQD3G	9	.6986	.01611	.00537	.6862	.7110	.68	.72
TQD6G	9	.6317	.05026	.01675	.5931	.6704	.54	.71
TQD9G	9	.4782	.01475	.00492	.4668	.4895	.45	.49
Total	53	.4896	.18382	.02525	.4390	.5403	.15	.72

Table A.9 : One-way ANOVA of SGR (d^{-1}) of freshwater microalgae for NPK TQD treatments.

ANOVA

SGR

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.634	5	.327	125.151	.000
Within Groups	.123	47	.003		
Total	1.757	52			

Table A.10 : Homogeneous subsets of Post Hoc tests of SGR (d^{-1}) of freshwater microalgae for NPK and TQD treatments.

SGR

Tukey HSD[a,b]

FERTCONC	N	Subset for alpha = 0.05				
		1	2	3	4	5
NPK9G	8	.1889				
NPK6G	9		.3219			
TQD9G	9			.4782		
NPK3G	9				.5851	
TQD6G	9				.6317	.6317
TQD3G	9					.6986
Sig.		1.000	1.000	1.000	.405	.085

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 8.816.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table A.11 : Specific Growth rate per day (SGR d^{-1}) of freshwater microalgae for NPK and TQD treatments.

Treatment	SGR d^{-1}
NPK 3 g	0.59 ± 0.091^{b}
NPK 6 g	0.32 ± 0.061^{d}
NPK 9 g	0.19 ± 0.020^{e}
TQD 3 g	0.70 ± 0.016^{a}
TQD 6 g	$0.63 \pm 0.050^{a, b}$
TQD 9 g	0.48 ± 0.015^{c}

Note : a = Highest SGR d^{-1}
b = Second highest SGR d^{-1}
c = Moderate SGR d^{-1}
d = Low SGR d^{-1}
e = Lowest SGR d^{-1}

APPENDICES B

Figure B.1 : Place for freshwater microalgae cultivation.

Figure B.2 : Odor removal and antimicrobial treatment of quail dung.

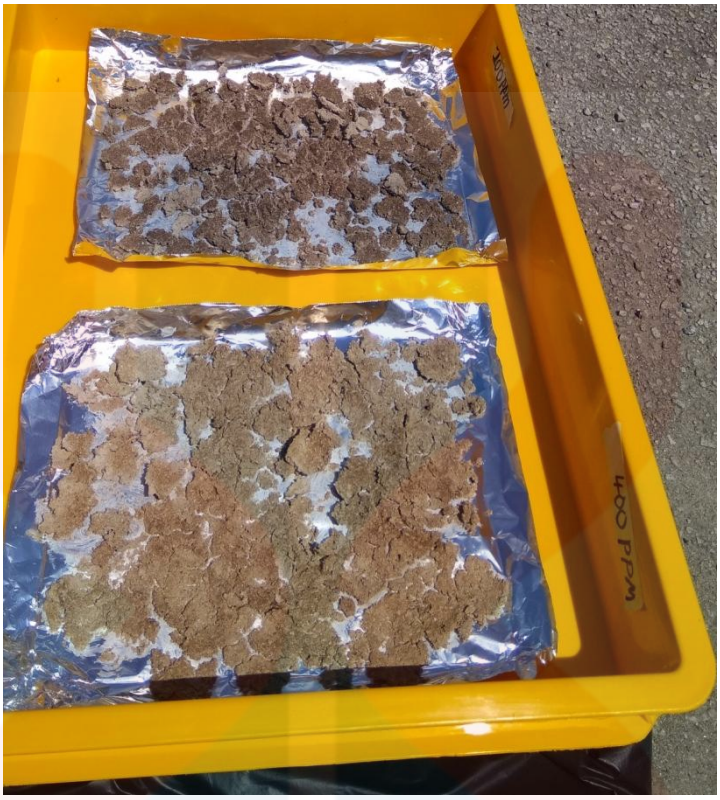

Figure B.3 : Drying of TQD under sunlight for 24 - 48 h.

Figure B.4 : Immersed tea bag contained fertilizer into conical flask.

Figure B.5 : Collect pond water from Agro Techno Park.

Figure B.6 : Mixing pond water and the dH_2O homogenised in connical flask.

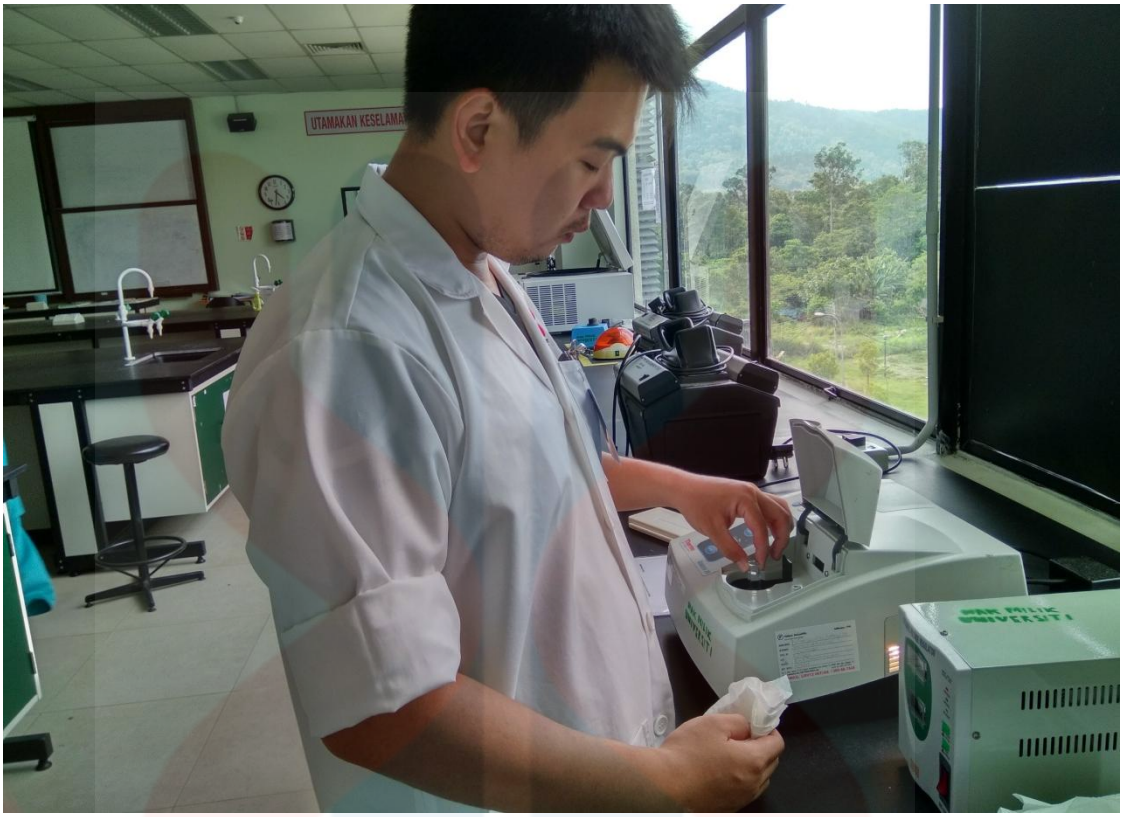

Figure B.7 : Checking OD reading using spectrophotometer.

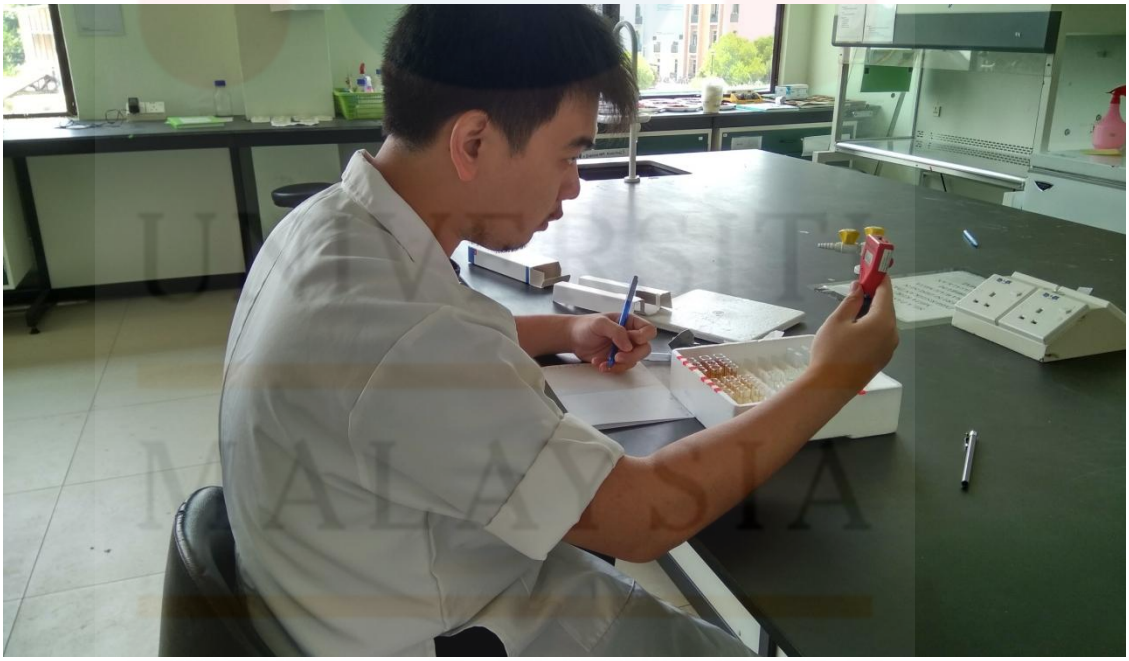

Figure B.8 : Checking pH of the microalage culture.

Figure B.9 : Checking temperature of the culture.

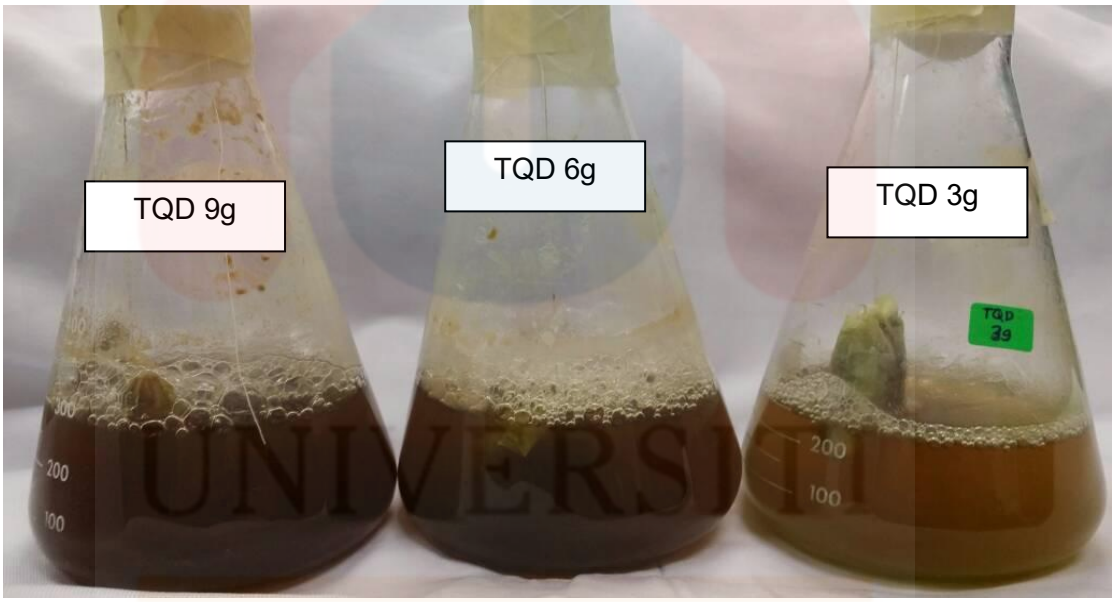

Figure B.10 : The sample of freshwater microalgae cultivation for TQD treatments after 7 days.

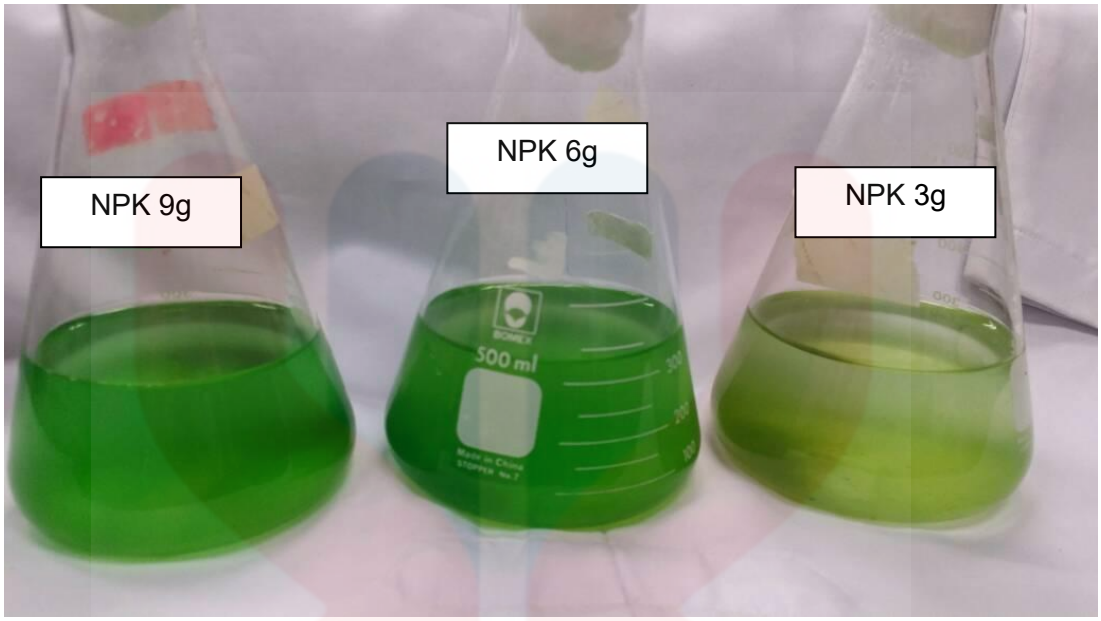

Figure B.11 : The sample of freshwater microalgae cultivation for NPK treatments after 7 days.