



**UNIVERSITI PUTRA MALAYSIA**

**EVALUATION OF DIFFERENT STRAINS OF *Spirulina* ON SELECTED CULTURE MEDIA AS POTENTIAL FEED SUPPLEMENT FOR BROILER**

**HASFAR SYAFIQAH BINTI ABDUL GHOFAR**

**IPTSM 2019 4**



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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science**

**January 2019**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**January 2019**

**Chairman: Associate Professor Anjas Asmara Samsudin, PhD**  
**Faculty: Institute of Tropical Agriculture and Food Security**

Poultry is one of the important livestock industries and has become the staple meat in Malaysia. Due to high demand, the production for the broiler had increased and has met the level of self-sufficiency with the aid in technological progress in animal husbandry, particularly nutritional aspect. A variety of synthetic feed additives has been used to maximize the production. However, due to public concern on the antibiotic residues in the meat, the feed business tends to use natural ingredients as supplement. *Spirulina* (*Athrospira* sp.) is a planktonic photosynthesis filamentous cyanobacterium consists of highly nutritious, a potential feed resource for many agriculturally important animal species. In this work, five difference strains of *Spirulina* sp.; *Spirulina platensis* TBSH1-5, *Spirulina platensis* TBSHX-1, *Spirulina platensis* M1, *Spirulina maxima*, *Spirulina platensis* were grown on three different culture media, namely; Zarrouk media, OFERR media and Revised media (6). The growth rate and dry weight after 30 days of cultivation was determined. No significant difference was shown to be affected by the either strains nor medias on the growth and dry weight but with more outstanding results in the OFERR media and Revised medium (6). Large scale cost production of *Spirulina* is expensive; thus, it is more cost effective to use a cheaper medium. Animal wastewater has demonstrated to be one of the best nitrogen sources to produce a low-cost medium. Therefore, *Spirulina* was cultivated in four different sources of wastewater, namely; goat, poultry, seawater and tap water at dilution of 25%, 50%, 75% and 100% for 30 days. Cultivation of *Spirulina* sp. in difference wastewater was found to significantly ( $P < 0.05$ ) affected by different type of wastewater with more growth of *Spirulina* was notified in goat wastewater and tap water at 25% dilution. The similar result was also observed on dry weight, where seawater was found to be significantly higher compared to another wastewaters, 0.27 g (25%), 0.29 g (50%), 0.18 g (75%) and 0.08 g (100%) respectively, followed by tap water and goat wastewater medium. The *in-vitro* fermentation analysis was carried out by incubating the commercial diet supplemented with *Spirulina platensis* and *Spirulina platensis* TBSH-5 with cecal digesta of broiler for 72 hours at  $39 \pm 5$  °C. The gas production and end products of the fermentation were examined. Commercial starter and finisher diet supplemented with or without *Spirulina platensis* TBSH1-5 and *Spirulina platensis* has demonstrated that interaction between treatment and *Spirulina* strains has no significant. While, the *in-vitro* dry matter degradability of both starter and finisher diet were improved ( $P < 0.05$ ), with more outstanding results observed in the

*Spirulina* sp. supplementation at 0.25 g and 0.75 g. Thus, it is suggested that *Spirulina* has a potential to be used as a supplement for poultry when it is cultivated in dilution of media with tap water and a better digestibility when supplemented at 0.75 g (starter) and 0.25 g (finisher).



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master sains

**PENILAIAN STRAIN *Spirulina* YANG BERBEZA TERHADAP MEDIA KULTUR TERPILIH SEBAGAI POTENSI MAKANAN TAMBAHAN BAGI AYAM PEDAGING**

Oleh

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Ayam itik merupakan salah satu industri ternakan terpenting dan telah menjadi daging ruji di Malaysia. Disebabkan permintaan yang tinggi, pengeluaran untuk ayam pedaging telah bertambah dan telah mencapai tahap sara diri dengan bantuan kemajuan teknologi dalam bidang penternakan, terutamanya aspek nutrisi. Pelbagai jenis bahan makanan tambahan sintetik telah digunakan untuk meningkatkan pengeluaran. Walaubagaimanapun, disebabkan oleh kebimbangan umum terhadap sisa antibiotik dalam daging, peniaga makanan ternakan cenderung untuk menggunakan bahan asli sebagai makanan tambahan. *Spirulina* (*Athrospira* sp.) ialah sianobakteria berfilamen fotosintesis plankton yang mengandungi nutrisi yang amat berkhasiat, satu sumber makanan berpotensi bagi kebanyakan spesies haiwan yang penting di dalam pertanian. Dalam kajian ini, lima jenis *Spirulina* sp.; *Spirulina platensis* TBSH1-5, *Spirulina platensis* TBSHX-1, *Spirulina platensis* M1, *Spirulina maxima*, *Spirulina platensis* telah dibiak didalam tiga kultur media yang berbeza, iaitu; *Zarrouk media*, *OFERR media* dan *Revised media* (6). Kadar pertumbuhan dan berat kering selepas 30 hari pembiakan telah dikenal pasti. Tiada perbezaan signifikan telah ditunjukkan yang memberi kesan kepada pertumbuhan dan berat kering oleh jenis mahupun media tetapi hasil yang lebih menonjol di dalam *OFERR media* dan *Revised media* (6). Kos pengeluaran *Spirulina* bagi skala besar amat mahal; maka, ia akan lebih menguntungkan apabila media kos rendah digunakan. Air sisa buangan telah ditunjukkan sebagai salah satu sumber nitrogen yang terbaik bagi penghasilan medium kos rendah. Lantaran itu, *Spirulina* telah dibiak dalam empat jenis sumber air sisa buangan, iaitu; kambing, ayam, air laut dan air paip pada cecair 25%, 50%, 75% dan 100% selama 30 hari. Pembiakan *Spirulina* sp. dalam air buangan yang berbeza didapati terkesan dengan signifikan ( $P < 0.05$ ) oleh jenis air buangan berbeza dengan hasil yang lebih tinggi bagi pertumbuhan *Spirulina* dilaporkan didalam air buangan kambing dan air paip di 25% cecair. Hasil kajian yang serupa juga dilihat pada berat kering, dimana air laut didapati signifikan lebih tinggi berbanding dengan sisa buangan yang lain, 0.27 g (25%), 0.29 g (50%), 0.18 g (75%) dan 0.08 g (100%), diikuti oleh sisa buangan air paip dan kambing. Analisis *in-vitro* telah dijalankan

dengan mengikubasi komersial diet dengan tambahan *Spirulina platensis* dan *Spirulina platensis* TBSH-5 dengan digesta sekum ayam pedaging selama 72 jam di  $39\pm 5$  °C. Pengeluaran gas dan produk akhir fermentasi telah diperiksa. Komersial diet pemula dan penamat dengan tambahan atau tanpa *Spirulina platensis* TBSH-5 dan *Spirulina platensis* telah menunjukkan bahawa interaksi antara rawatan dan jenis *Spirulina* tidak mempunyai signifikan. Manakala, penguraian bahan kering *in-vitro* bagi kedua-dua diet pemula dan penamat telah diperbaiki ( $P < 0.05$ ), dengan hasil lebih tinggi diperhatikan dalam *Spirulina* sp. penambahan pada 0.25 g dan 0.75 g. Maka, ia disarankan bahawa *Spirulina* mempunyai satu potensi digunakan sebagai penambah dalam makanan ayam itik apabila ia diusahakan dalam pencairan media dengan air paip dan kebolehcernaan lebih baik apabila diperlengkapkan pada 0.75 g (pemula) dan 0.25 g (penamat).



## ACKNOWLEDGEMENTS

Here I would like to express my deepest gratitude to my supervisor Assoc. Prof. Dr. Anjas Asmara @ Abd. Hadi bin Samsudin, for his selfless time despite his tight schedule, he still able to give his full commitment throughout my studies. Thank you for all your guidance, knowledge, encouragement and supports throughout my studies from the start of my project until completing my thesis. I would like to extend my gratitude to both of my co-supervisor Dr. Mohammad Faseleh Jahromi whose currently in Iran and Dr. Natrah Fatin Mohd Ikhsan from Department of Aquaculture for all the shared knowledge as well as idea for my project.

To my beloved parent and sisters, Abdul Ghofar bin Abdul Mubin, Hasemah binti Omar, Hasfar Arynurliyana binti Abdul Ghofar and Hasfar Syazwani binti Abdul Ghofar, thank you so much for all your supports, care and patience throughout my studies. Mama your words will always remain in my hearts and will always be the reasons for me to stay strong, "*Mama tak ada harta untuk diberi tetapi pelajaran ialah harta mama*". It will always remind me the importance of knowledge.

Lastly, I would like to thank my friend, Arthy Surendran whose is like an older sister to me, for always staying besides me through thick or thin, giving me supports and guidance when I am lost. My sincerest thanks to all the lecturer and staffs of Institute of Tropical Agriculture and Food Security (ITAFOs) and Departments of Animal Science for the assistance and providing facilities needed in completing my research.



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## LIST OF ABBREVIATION

AA	Amino acid
AABA	$\alpha$ -amino-N-butyric acid
Ca	Calcium
CH <sub>4</sub>	Methane
CO <sub>2</sub>	Carbon dioxide
DM	Dry matter
FAME	Fatty acid methyl ester
g	Gram
HCl	Hydrochloric acid
HPLC	High pressure liquid chromatography
H <sub>2</sub>	Hydrogen
hr	Hour
IVDMD	<i>In-vitro</i> dry matter digestibility
IVGPT	<i>In-vitro</i> gas production technique
IVOMD	<i>In-vitro</i> organic matter digestibility
KOH	Potassium hydroxide
kg	Kilogram
L	Litre
mL	Millilitre
min	Minute
Mm	Millimolar
mg	Milligram
M	Molarity
Mg	Magnesium
m	Metre
mm	Millimetre
NaOH	Sodium hydroxide
NH <sub>4</sub> Cl	Ammonia chloride
NSP	Non-starch polysaccharides
O <sub>2</sub>	Oxygen
ppm	Part per million
ppt	Part per thousand
rpm	Round per minute
sp.	Species
VFA	Volatile fatty acids
vol	Volume
Wt	Weight
$\mu$ l	Microlitre
$\mu$ m	Micrometre

## CHAPTER 1

### INTRODUCTION

Broiler meat is a primary protein source for most of Malaysian populations. The overall production of broiler has expanded consistently with the growth in local demand and exported to some countries. However, to reach the expected demand, a variety of synthetic feed additives such as drugs and antibiotics that are responsible to maximize the production, control disease and product quality of the poultry has been used in poultry feed (Jamil et al., 2015). The use of antibiotics in poultry industry act as antimicrobial compounds to control infections, improve meat and eggs production (Kaoud, 2012) as well as growth promoter. However, there is a widespread disagreement caused by antibiotic used as it can presence in the most meat and can cause bacterial resistance in the poultry (Jamil et al., 2015; Kaoud, 2012).

Nowadays, most of the poultry industry player tend to use natural ingredients as an alternative to the synthetic colors, antibiotics as well as other chemicals (Mariey et al., 2014) to overcome the public concerns regarding the developments of these antibiotics-resistant bacteria in humans (Jamil et al., 2015). One of the natural ingredients that are gaining popularity due to its highly beneficial nutrients is an algae known as *Spirulina*. *Spirulina* are multicellular and filamentous blue-green algae that grows in water containing high alkaline condition, easily harvested and processed and consist of high macro- and micronutrient content. The blue green algae, *Spirulina*, contain high quality natural feed additives that can be used in animal and poultry nutrition due to high content of protein, vitamins, essential amino acids, minerals, essential fatty acids and high carotenoid content (Kharde et al., 2012).

Feeding chicken supplemented with *Spirulina* has been found to enhance the defence systems by increasing microbial killing, antigen processing and greater T-cell activity (Mariey et al., 2014) as well as enhancing the yolk, flesh and color (Mariey et al., 2014; Ross & Dominy, 1990). Moreover, based on Kaoud, (2012) and Kharde et al., (2012), they stated that the feed conversion ratio is significantly lower by the dietary supplements with *Spirulina*.

Zarrouk media was known as the standard medium for the cultivation of *Spirulina* (Habib et al., 2008). However, there are other mediums which had been modified and able to produce a biomass compatible with the Zarrouk medium. The nutritional content of *Spirulina* may also vary depending on the medium composition. However, the cost for the biomass production of *Spirulina* can vary for various nutrition composition and nutrient environments (Habib et al., 2008). Therefore, it may not be profitable to be used as poultry supplements due to highly cost for broiler production. Therefore, another acceptable way in the production of microalgae is by using a low-cost medium to reduce the cost production of *Spirulina*. Several research has evaluated the potential of using animal waste as a low-cost nitrogen sources (Cheunbarn & Peerapornpisal, 2010; Ungsethaphand et al., 2009; Yilmaz & Sezgin, 2014).

Animal wastewater consist of highly essential nutrients that can alternatively use not only as a source of fertilizer, but also proven to be beneficial for microalgae growth. It was one of the alternative ways to use organic nutrient sources as nutrient for the *Spirulina* to growth. Therefore, this study was conducted to study the effect of different culture media and the effect of different source of wastewater as low-cost culture media on the biomass production of *Spirulina* and the *in-vitro* ceecal fermentation profiles on commercial diet supplemented with *Spirulina*.

## 1.2 Research hypothesis

The cultivation of *Spirulina* in different culture media and vary dilution of wastewater will influence the nutritional composition, increase dry weight and growth performance of *Spirulina* and supplementation of *Spirulina* in the commercial diet will also influence the caecal fermentation characteristic of commercial diet.

## 1.3 Objectives

- i. To determine the efficacy of five strains of *Spirulina* sp.; *Spirulina platensis* TBSH1-5, *Spirulina platensis* TBSHX-1, *Spirulina platensis* MI, *Spirulina maxima*, *Spirulina platensis* in Zarrouk media, OFERR media and Revised media (6).
- ii. To determine the effect of varying dilution level of wastewater in the cultivation of *Spirulina* sp.
- iii. To determine the feed digestibility of broiler diet supplemented with *Spirulina* by using the *in-vitro* gas production technique.

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## LIST OF PUBLICATIONS

Ghofar, H. S. A., Jahromi, M. J., Ikhsan, F. N. M., Samsudin, A. A. (2017). Biomass production of *Spirulina sp.* strains on different media as supplement for broiler. Proceeding of the 38<sup>th</sup> Malaysian Society of Animal Production (MSAP) Annual Conference, Senai, Johor, Malaysia, August 27-30, 2017.

Ghofar, H. S. A., Jahromi, M. J., Ikhsan, F. N. M., Samsudin, A. A. (2018). Effects of different level of wastewater dilution on *Spirulina sp.* cultivation. Proceeding of the 18<sup>th</sup> Asian-Australasian Animal Production Congress (AAP) Annual Conference, Kuching, Sarawak, Malaysia, August 1-5, 2018.





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