OMEGA-3 FATTY ACIDS AND ASTAXANTHIN PRODUCED BY *Chlorella vulgaris* AND *Haematococcus pluvialis* CULTIVATED IN CHICKEN MANURE MEDIUM

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DEDICATION

This thesis is dedicated to my parents, who taught me that the best kind of knowledge to have been that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

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ABSTRACT

Chicken manure contains a high concentration of nutrients such as nitrogen and phosphate, which can be used to replace commercially available medium for growth of microalgae. Cultivating microalgae by using chicken manure as the nutrients can reduce the cost of cultivation and also increase the quality of fish pellet in aquaculture systems. This research was conducted to study the growth of *Chlorella vulgaris* and *Haematococcus pluvialis in* different concentrations of chicken manure on the growth of *C. vulgaris* and *H. pluvialis*. The concentration of omega-3 fatty acids in *C. vulgaris* and astaxanthin in *H. pluvialis* were determined by cultivating *C. vulgaris* and *H. pluvialis* in Bold Basal medium and Rudic medium respectively, and compared with chicken manure medium (CM). 50% of CM concentration was considered the best chicken manure concentration to replace commercial medium in *C. vulgaris* cultivation, although the Bold Basal Medium and 50% of CM has a comparable specific growth rate of 0.178 day^{-1} and 0.512 day^{-1} respectively. However, it will be much cost effective to use a waste product with high nutrients, i.e., CM instead of commercial medium to grow the microalgae. In *H. pluvialis* cultivation, the highest specific growth rate was obtained in 50% of CM and 40% of CM with 0.554 day^{-1} and 0.528 day^{-1}, respectively. For omega-3 fatty acid, the highest concentration was obtained in 14.8% *C. vulgaris* in 10% of CM due to depletion of nitrogen, which causes the increase of fatty acid production in the microalgae. For astaxanthin production, *H. pluvialis* grown in 50% of CM gave highest concentration of astaxanthin (0.082 mg/mL). The increase in astaxanthin was due to low concentration of phosphorus in the growth medium.

ABSTRAK

Najis ayam dikatakan mengandungi kepekatan nutrien yang tinggi seperti nitrogen dan fosfat, yang dapat digunakan untuk menggantikan medium mikroalga yang sudah dikomersial untul tumbesaran mikroalga. Mengkultur mikroalga dengan menggunakan najis ayam sebagai nutrien dapat mengurangi kos mengulkultur mikroalga dan dapat meningkatkan kualiti pelet ikan dalam sistem akuakultur. Penyelidikan ini dilakukan untuk mempelajari pertumbuhan *Chlorella vulgaris* dan *Haematococcus pluvialis* dalam kepekatan najis ayam yang berlainan. Kepekatan asid lemak omega-3 dalam *C. vulgaris* dan astaxanthin dalam *H. pluvialis* ditentukan dengan mengkultur *C. vulgaris* dan *H. pluvialis* dalam medium Bold Basal dan medium Rudic dan dibandingkan dengan medium najis ayam (CM). 50% kepekatan CM dianggap kepekatan najis ayam terbaik untuk menggantikan medium komersial dalam kultur *C. vulgaris* walaupun *Bold Basal Medium* dan 50% CM tidak mempunyai perbezaan yang banyak dalam pertumbuhan mikroalga iaitu 0.178 per hari dan 0.512 per hari masing-masing. Walau bagaimanapun, ia menjimatkan kos dengan menggunakan produk buangan yang mempunyai nutrien yang tinggi, iaitu CM sebagai nutrien untuk mengkultur mikroalga. Sementara dalam kultur *H. pluvialis,* kadar pertumbuhan tinggi diperoleh pada 50% CM dan 40% CM dengan 0.554 day^{-1} dan 0.528 day^{-1}, masing-masing. Kepekatan asid lemak omega-3 tertinggi diperoleh pada *C. vulgaris* 14.8% dalam 10% CM disebabkan oleh kekurangan nitrogen yang menyebabkan peningkatan pengeluaran asid lemak dalam mikroalga. Untuk pengeluaran astaxanthin, 50% CM memberikan kepekatan astaxanthin tertinggi yang dihasilkan (0.082 mg / mL) dalam *H. pluvialis* kerana kepekatan fosforus yang rendah dalam medium.

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LIST OF ABBREVIATIONS

FAO	-	Food and Agriculture Organization of the United Nations
WHO	-	World Health Organization
DHA		Docosahexaenoic
EPA		Eicosapentaenoic
AA		Arachidonic acid
TN		Total nitrogen
TP		Total phosphorus
BBM		Bold basal medium
RM		Rudics medium
GHG		Greenhouse gas
GWP		Global warming potential
Gt		Gigatons
GCMS		Gas chromatograph mass spectrometer
HPLC		High performance liquid chromatograph
FM		Fish meal
FO		Fish oil
dH_2O		Distilled water
OD		Optical density

LIST OF SYMBOLS

A_{FAME}	-	Peak area of fatty acid methyl esters
$\sum A$	-	Total peak area of FAME chromatogram
A_{C17}	-	Peak area of the internal standard (C17:0)
W_{oil}	-	Oil weight
$W_{raw\ material}$	-	Weight of raw material

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Microalgae are aquatic plants that grow by consuming the nutrients in their environment and the energy provided by the sun (Gohman, 2021). Microalgae are live microorganisms made up of carbohydrates, proteins, lipid micronutrients, and other biologically active elements (Dani et al., 2016).

Microalgae has been demonstrated to be a long-term, sustainable source of biomass and oils for fuel, food, feed, and other co-products (Mitchell, 2021). Microalgae have shown potential in fulfilling the population's demand for a more sustainable food supply, particularly protein demand. These potential protein sources provide significant advantages over other currently used raw materials (Caporgno & Mathys, 2018).

Algae can be grown in fresh water or seawater, depending on the species (Morales et al., 2019). There were numerous applications for microalgae, including cosmetics, animal feeds, and supplements. Previous study found that, *Chlorella* and *Spirulina* species were used as health-food supermarkets and the other species like *Tetraselmis, Nannochloropsis and Scenedesmus* were used as animal feed (Koyande et al., 2019). In this research, the microalgae can be used in aquaculture systems since the medium of cultivation was chicken manure.

Microalgae, which was found near the aquatic food chain, are an essential part of the diet of marine animals such as fish. Aquaculture feeding with microalgae produced a primary fresh feed or addition, such as a source of colour. Algae contain almost all of the nutrients required for a fish diet.(Safafar, 2017).

The researchers were investigated microalgae as nutrients substitutes in fish feeds due to nutritional drawbacks and low fillet consistency. Microalgae have been considered alternatives nutrients in aquaculture as it is rich in amino acids, minerals, vitamins, and long-chain n-3 fatty acids (Sarker et al., 2020).

Two types of microalgae were selected for this study, *Chlorella vulgaris* and *Haematococcus pluvialis*. Omega-3 fatty acids is a sources of fatty acids that can be obtained in *C. vulgaris* (Rismani & Shariati, 2017). According to a recent Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) expert consultation, when compared to children of women who do not consume fish, mothers who give birth to children who use fish high in omega-3 fatty acids have reduce risk of poor brain plus neural system development. There was strong evidence that it can reduce the risk of death from coronary heart disease (by 36%) (Globefish, 2021).

Astaxanthin was considered as super anti-oxidant, which can be obtained from highly in *H. pluvialis* (Shah et al., 2016). The importance of astaxanthin as an antioxidant for humans is only now being recognised, and fish tinted with natural astaxanthin could be a good supply of this nutrient for humans. In the United States, only two astaxanthin sources are currently permitted for direct human ingestion as a supplement: krill oil and the algae *H. pluvialis* (Valeri & C., 2007). Each microalga has special nutrients which are likely to improve the fish feed.

To produce high-quality fish, the microalgae must be grown under medium with nutrition-rich conditions. Medium is a chemical mixture that contains nutrients for microalgae to grow. This research used chicken manure as an alternative medium to commercial mediums such as Bold Basal medium (BBM) and Rudic`s medium (RM).

According to (M. A. Yaakob et al., 2021), nitrogen and phosphate were the essential mains components in a medium for algae growth. As a result, this research emphasises the amount of nitrogen and phosphate necessary for microalgae culture and the advantages of nitrogen and phosphate in enhancing the biomass productivity of microalgae. Plant waste and animal waste were two promising alternative mediums.

Chicken manure can be used to substitute the typical culture medium since it is rich in nutrients (e.g., nitrogen and phosphate) (Tan et al., 2017). Chicken manure has long been a traditional organic alternative source of fertiliser that is more cost-effective than chemical fertilisers. It also reduces manure pollution caused by improper disposal, allowing much-needed nutrients to be recovered and re-used (Agwa & Abu, 2014). Chicken manure was a good choice of source because it is readily available, dissolved rapidly and had a well-defined composition (Sipaúba-Tavares et al., 2015).

Table 1.1 shows the total livestock for different states in Malaysia in 2018. Among 10 types of livestock, poultry was recorded the most, nearly 30 times higher compared to ducks, the second most popular livestock. Accordingly, management of poultry production can be challenging (Tan, 2013). Thus, it was the motivation of this study to utilize chicken manure as an alternative culture medium for microalgae rather than being discarded and becoming a source of pollution such as greenhouse effect (Chai et al., 2019).

Table 1.1 Livestock population by states in year 2018 (Jabatan Perkhidmatan Veterinar Malaysia, 2018)

MALAYSIA : BILANGAN TERNAKAN MENGIKUT NEGERI, 2018 ^{E}

Malaysia : Livestock Population By States, 2018 ^{E}

NEGERI *State*	Kerbau *Buffalo*	Lembu *Cattle*	Kambing *Goat*	Bebiri *Sheep*	Babi *Swine*	Ayam *Poultry*	Itik *Duck*	Burung Unta *Ostrich*	Puyuh *Quail*	Rusa *Deer*
Perlis	249	6,718	3,176	1,644		1,784,417	7,838	5	10,203	287
Kedah	5,219	54,297	48,459	10,833	1,588	56,529,903	561,819	11	237,883	1,205
Pulau Pinang	608	11,934	11,008	2,811	320,165	11,342,444	343,397	1	142,930	78
Perak	9,174	45,516	27,167	3,008	545,258	39,897,038	7,463,087		43,197	5,251
Selangor	2,896	25,143	21,515	3,117	262,060	18,064,933	5,236	213	127,110	48
N. Sembilan	1,899	39,667	33,986	16,084	241	21,187,714	32,068	101	384,402	1,108
Melaka	4,408	29,272	38,121	6,902	44,025	27,000,465	84,314		381,794	422
Johor	3,788	105,712	42,463	21,870	228,639	72,686,534	1,057,353	297	1,570,996	1,749
Pahang	11,604	124,530	32,433	18,826	3,893	12,915,104	11,284	1	87,029	2,061
Terengganu	8,935	85,155	29,798	6,892		2,749,331	26,453		78,916	745
Kelantan	5,425	96,109	41,501	33,589	645	3,727,520	62,144		9,349	230
W. Persekutuan		210	108							47
Jumlah S. M'sia *Total For P. M'sia*	**54,205**	**624,263**	**329,735**	**125,576**	**1,406,514**	**267,885,403**	**9,654,993**	**629**	**3,073,809**	**13,231**
Sabah	54,680	75,766	56,346	2,597	101,840	9,814,509	54,861	59	16,762	
Sarawak	5,436	10,452	12,964	1,821	334,074	34,278,682	298,400		340,917	1,121
JUMLAH BESAR *Grand Total*	**114,321**	**710,481**	**399,045**	**129,994**	**1,842,428**	**311,978,594**	**10,008,254**	**688**	**3,431,488**	**14,352**

The study began with the *C. vulgaris* and *H. pluvialis* being grown on a chicken manure medium, followed by analyses of omega-3 fatty acids in *C. vulgaris* and astaxanthin in *H. pluvialis* using both chicken manure and commercial media (Bold Basal Medium and Rudic`s Medium). This study focused on the development and analysis of omega-3 fatty acids and astaxanthin contained in microalgae inside chicken manure medium. Results obtained from this study can be formed into a product such as a fish pellet in an aquaponic system. Figure 1.1 shows the flowchart of this study.

C. vulgaris and *H. pluvialis* were cultivated in commercial (BBM and RM respectively) and chicken manure medium.

→

Omega-3 fatty acid and astaxanthin were analysed using GCMS and spectrophotometer (432 nm) analysis respectively.

→

The specific growth rate was calculated in *C. vulgaris* and *H. pluvialis* to determine the best growth. While omega-3 fatty acids and astaxanthin content were determined by calculating the omega-3 fatty acid content and spectrophotometer value, respectively.

Figure 1.1 The flowchart of the research.

1.2 Problem Statement

The usage of chicken manure as a medium for microalgae is known to be cost-effective. Microalgae cultivation necessitates a considerable medium volume, resulting in high operating costs and adverse environmental effects due to nutrient leakage (Tan et al., 2017). This finding was in agreements with the study by Agwa and Abu (2014) who showed using chicken manure as an alternative medium for the cultivation of microalgae was economical.

Chicken manure can be used to grow microalgae as it provides a source of nutrients to cultivate microalgae. These nutrient sources are an effective technique to boost microalgae growth since the waste includes high concentrations of nutrients such as nitrate and phosphate (Tan et al., 2017) These elements are harmful to the environment if released untreated (Imran et al., 2007). Here, the application of chicken manure to cultivate microalgae, providing a cheap source of nutrients, will be studied.

Secondly, it is due to the nutritional deficiency and poor fillet quality in the aquaculture system. According to Young K. (2009), tilapia contains a high concentration of omega-6 fatty acids (n-6) and a low concentration of omega-3 fatty acids (n-3), this is a potentially unhealthy ratio for humans. Astaxanthin improves the fillet quality by providing colorants of pigmentation to the fish. Besides their role as colorants in food, it also have antioxidant properties, preventing the deterioration caused by oxidation in food products (Elisa et al., 2014).

Hence, this research focused on two primary nutrients, which were omega-3 fatty acids in *C. vulgaris* and astaxanthin in *H. pluvialis*. The omega-3 fatty acids and astaxanthin content in the cultivation of microalgae using chicken manure were investigated and compared with the commercial medium before it can be introduced for fish pellets in the future.

1.3 Objectives of Study

1. To study the effect of different concentration of chicken manure towards the growth of *C. vulgaris* and *H. pluvialis*.
2. To examine and compare the amount of omega-3 fatty acids produced in *C. vulgaris* cultivated in Bold Basal medium and chicken manure medium.
3. To examine and compare the amount of astaxanthin produced in *H. pluvialis* cultivated in Rudic`s medium and chicken manure medium.

1.4 Scope of the Study

The content of nitrogen and phosphorus were determined in commercially available mediums, which were Bold Basal Medium, Rudic`s and chicken manure medium. The growth of microalgae was monitored, and the optical density of microalgae was measured at 750 nm using a spectrophotometer. Omega-3 fatty acids in the *C. vulgaris* was extracted and identified using a gas chromatograph mass spectrometer (GCMS). The content of astaxanthin in the sample was quantified using a spectrophotometer at a wavelength of 432 nm by using the dry mass of *H. pluvialis*.

1.5 Significance of the study

Environmental issues are one of the significant concerns in Malaysia and need to be carefully addressed. Hence, recycling waste can help to reduce pollution. The waste from poultry can be used for nutrients in the cultivation of microalgae. For this study, chicken manure medium was used as a replacement for commercial mediums in the growth of microalgae. Chicken manure contributes to the high growth of *C. vulgaris* and *H. pluvialis* because of the nitrogen and phosphorus elements inside the chicken manure medium itself. Many countries use animal manure to raise plankton, resulting in much more fresh food for fish to eat and increased fish production. (Renalda & Hieromin, 2021). Studies have shown that, by using chicken manure as a culture medium, it can enhance the omega-3 fatty acids and astaxanthin contained in microalgae cells. Thus, it can be used to substitute the nutrients in fish feed.

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