UNIVERSITI PUTRA MALAYSIA

EFFECTS OF BROWN AND GREEN SEAWEEDS ON NUTRIENT DIGESTIBILITY, GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND HEALTH PERFORMANCE IN BROILER CHICKENS

AZIZI MOHAMMAD NAEEM

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By

AZIZI MOHAMMAD NAEEM

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

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DEDICATION

This work is dedicated to the gentle soul of my beloved father and to my mother, who is the origin of my success. To my dear wife for her encouragement, prayers, and patience. To my dear brothers and sisters for their cordial support and prayers.

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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Chairman: Prof. Loh Teck Chwen, PhD
Faculty: Agriculture

It has widely reported that seaweed has bioactive molecules that have been examined for health-promoting effects on livestock. Whereas there is still a lack of research to describe the effects of seaweed on broiler apparent ileal digestibility of nutrients, production and health performances. Two experiments were conducted to study the effects of brown seaweed and green seaweed on broiler chickens' nutrient digestibility, growth performance, carcass characteristics, small intestine histomorphology, blood plasma profiles, antioxidant and immune statuses.

In the first experiment of this study, the nutritional properties of brown and green seaweed were analysed. A digestibility trial was conducted to investigate the effect of seaweeds on crude protein, crude fibre, ether extract, dry matter, organic matter, amino acids and minerals apparent ileal digestibility. A total of 36 broiler chickens (Cobb 500) were randomly distributed into two dietary treatments containing 90.30% of each seaweed type. The data analysis for both experiments was conducted using the General Linear Model of the Statistical Analysis System by one-way ANOVA. Duncan's Multiple Range Test was used to compare the significant difference between the treatments at $p < 0.05$. The results showed that brown seaweed and green seaweed had 59.8 and 55.88% crude protein, 1.28 and 0.30% ether extract, 5.78 and 5.19% crude fibre, 29.19 and 34.68% carbohydrates, and 9.7 and 9.14% ash contents, respectively. Brown seaweed and green seaweed contained a high amount of micromineral K (2.96 and 2.20%) and trace elements Fe (14.67 and 11.73 mg. 100 g^{-1}). The gross energy value of brown seaweed and green seaweed was 6171.53 and 6150.26 kcal/kg, respectively. The digestibility results showed that the apparent ileal digestibility of brown seaweed and green seaweed was 88.82 and 86.8% for ether extract, 82.03 and 80.6% for organic mater, 60.69 and 57.80% for crude protein, 48.56 and 44.02% for crude fibre, and 17.97 and 19.40% for ash contents, respectively. The apparent ileal digestibility of various amino acids was 40.96 to 77.54%. The results of Ca, Na, K, Mg, Zn, Cu, Fe and Mn digestibility showed that among the selected minerals, Ca had the highest level (96.91, 97.61%) apparent ileal digestibility. Furthermore, both seaweed types exhibited a remarkable antioxidant activity.

In the second experiment, a feeding trial was conducted to determine the effects of brown and green seaweed on growth performance, carcass characteristics and health performance in broiler chickens. A total of 504 one-day-old male broiler chicks (Cobb 500) were randomly distributed into twelve dietary treatments, containing: Basal diet (T1), basal diet + vitamin E (100 mg/kg feed) (T2), and basal diet + 0.25, 0.50, 0.75, 1 and 1.25% brown seaweed and green seaweed inclusions (T3-T12). The result demonstrated that different brown seaweed and green seaweed inclusion levels increased ($p < 0.05$) the growth performance of broiler chickens during the starter period. Different brown and green seaweed levels increased ($p < 0.05$) the crude protein digestibility during the starter period. The birds fed 0.25 and 0.50% brown seaweed, and 0.25, 0.75 and 1% green seaweed had increased ($p < 0.05$) breast meat crude protein content. In week three, birds fed 0.50, 0.75, and 1.25% brown seaweed had significantly higher jejunum villi height. Regarding the plasm lipid profile, the total cholesterol and high-density lipoprotein levels were higher ($p < 0.05$) for birds fed 0.75 and 1% brown seaweed compared to the negative and positive control groups. Different levels of green seaweed (0.75, 1 and 1.25%) significantly improved plasma immunoglobulin A and immunoglobulin G concentrations. Birds fed 0.50% brown seaweed, and 0.25% and 0.50% of green seaweed increased ($p < 0.05$) the caecal *Lactobacillus* population. Meanwhile, the 0.75% inclusion level of brown seaweed and green seaweed decreased ($p < 0.05$) the *Escherichia coli* population compared to the negative control group. Brown and green seaweed also increased propionic acid and total volatile fatty acids in the caecal digesta. Various brown and green seaweed inclusion levels significantly upregulated the interleukin-6, interleukin-10 and interferon gamma gene mRNA expression. The result showed that the mRNA expression of the growth hormone receptor gene was higher ($p < 0.05$) for birds fed 0.50% and 0.75% of green seaweed. Furthermore, birds feed 0.50% brown seaweed and 0.25%, 0.50% and 0.75% of green seaweed significantly upregulated the hepatic insulin-like growth factor-1 mRNA expression. The hepatic superoxide dismutase-1 mRNA expression was higher for birds fed 0.50% and 0.75% of brown seaweed.

In conclusion, the crude protein content was the major component of dried brown and green seaweed. Regarding the digestibility of 90.30% seaweed-based diets, the results showed that broiler chickens utilised brown and green seaweed with higher apparent ileal digestibility of organic matter, ether extract and minerals, moderate crude protein and amino acids, and low apparent ileal digestibility of crude fibre and ash contents. The results suggested that adding green and brown seaweed up to 1.25% in broiler chicken diet can improve bird growth performance and nutrient digestibility during the starter period. Brown and green seaweed also improved bird's meat crude protein content. Different brown seaweed and green seaweed levels showed immunostimulating effects due to enhancing immunoglobulins concentrations, regulating the balance of intestinal microbiota, and increasing inflammatory mediators without adverse effects on meat quality, carcass characteristics, and plasma lipid profile. Discoveries from the current research are helpful for further studies investigating the mechanisms and components underlying the growth and digestibility enhancing effects during the starter period of birds.

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

KESAN RUMPAI LAUT TERHADAP KEBOLEHCERNAAN NUTRIEN, PRESTASI TUMBESARAN, MORFOLOGI USUS KECIL, PROFIL DARAH PLASMA DAN STATUS DAYA TAHAN TERHADAP AYAM PEDAGING

Oleh

AZIZI MOHAMMAD NAEEM

November 2021

Pengerusi: Prof. Loh Teck Chwen, PhD
Fakulti: Pertanian

Rumpai laut telah dilaporkan mengandungi pelbagai bahan aktif yang mempunyai kesan penambahbaikan terhadap kesihatan haiwan ternakan. Namun begitu, kajian mengenai kesan rumpai laut terhadap kebolehcernaan nutrisi di usus, pengeluaran dan kesihatan ayam pedaging adalah masih tidak mencukupi. Dua ujikaji telah dijalankan untuk mengkaji kesan rumpai laut perang dan rumpai laut hijau terhadap kebolehcernaan nutrisi, prestasi pertumbuhan, ciri-ciri karkas, histomorfologi usus kecil, profil plasma darah dan status imun ayam pedaging.

Ujikaji pertama adalah untuk menganalisis ciri-ciri khasiat rumpai laut perang dan hijau. Satu kajian kebolehcernaan telah dijalankan untuk menyiasat kesan rumpai laut terhadap penghadaman ketara ileum, protin kasar, ekstrak eter, jisim kering dan jisim organic, asid amino dan mineral. Sebanyak 36 ekor ayam pedaging (baka Cobb 500) telah diagihkan secara rawak kepda dua diet rawatan yang mengandungi 90.30% rumpai laut. Kedua-dua eksperimen menggunakan General Linear Model dan ANOVA sehala daripada Sistem Analisis Statistik. Duncan Multiple Range Test digunakan untuk menentukan perbezaan bererti antara rawatan pada $p < 0.05$. Hasil kajian menunjukkan rumpai laut perang dan rumpai laut hijau mengandungi masing-masing 59.8% dan 55.88% protein kasar, 1.28% dan 0.30% ekstrak eter, 5.78% dan 5.19% serat kasar, 29.19% dan 34.68% karbohidrat, dan 9.70% dan 9.14% isi abu. Rumpai laut perang dan rumpai laut hijau juga mengandungi jumlah mikromineral K (2.96 dan 2.20%) yang tinggi dan unsur surih Fe (14.67 dan 11.73 mg/100 g-1). Nilai tenaga kasar rumpai laut perang dan rumpai laut hijau masing-masing adalah sebanyak 6171.53 and 6150.26 kcal/kg. Hasil dapatan menunjukkan penghadaman ketara ileum rumpai laut perang dan hijau masing-masing adalah sebanyak 88.82% dan 86.80% untuk ekstrak eter, 82.03% dan 80.60% untuk jisim organik, 60.69% dan 57.80% untuk protein kasar, 48.56% dan 44.02% untuk serat kasar serta 17.97% dan 19.4% untuk isi abu. Untuk penghadaman ketara ileum bagi pelbagai asid amino adalah sebanyak 40.96% hingga 77.54%. Keputuan penghadaman ketara ileal untuk Ca, Na, K, Mg, Zn, Cu, Fe dan Mn menampilkan Ca mencatatkan nilai tertinggi

iaitu antara 96.91% hingga 97.61%. Selain itu, kedua-dua jenis rumpai laut mengandungi aktiviti antioksidan yang memuaskan.

Dalam ujikaji kedua, satu kajian pemakanan telah dilakukan untuk menentukan kesan pemberian rumpai laut perang dan hijau terhadap prestasi tumbesaran, ciri-ciri karkas dan tahap kesihatan dalam ayam pedaging. Sebanyak 504 anak ayam berumur sehari (Cobb 500) diagihkan secara rawak kepada 12 rawatan diet, mengandungi diet asas (T1), diet asas + vitamin E (100mg/kg baja) (T2), dan diet asas + 0.25, 0.50, 0.75, 1 dan 1.25% rumpai perang atau hijau (T3-T12). Dapatan kajian mencatatkan perbezaan tahap penambahan rumpai laut perang dan hijau meningkatkan ($p < 0.05$) prestasi tumbesaran ayam pedaging pada peringkat permulaan. Perbezaan tahap penambahan rumpai laut perang dan hijau menambahkan ($p < 0.05$) penghadaman protein kasar pada pringkat permulaan. Ayam yang diberi 0.25% dan 0.50% rumpai laut peranf dan 0.25%, 0.75% dan 1% rumpai laut hijau telah meningkatkan ($p < 0.05$) kandungan protein kasar di daging dada. Pada minggu ketiga, ayam yang diberi baja mengandungi 0.50%, 0.75% dan 1.25% rumpai laut perang mempunyai ketinggian bererti pada villi jejunum. Merujuk kepada profil lipid plasma, jumlah kolesterol dan tahap lipoprotein ketumpatan tinggi adalah lebih tinggi ($p < 0.05$) untuk ayam diberi baja merangkumi 0.75% dan 1.00% rumpai laut perang berbanding dengan kawalan kumpulan postitif dan negatif. Perbezaan tahap rumpai laut hijau (0.75%, 1.00% dan 1.25%) mempunyai peningkatan bererti terhadap kepekatan plasma immunoglobulin A dan immunoglobulin G. Ayam diberi makan 0.50% rumpai laut perang, serta 0.25% dan 0.50% rumpai laut hijau meningkatkan ($p < 0.05$) populasi Lactobacillus di sekum. Sementara itu, penambahan 0.75% rumpai laut perang dan hijau mengurangkan ($p < 0.05$) populasi Escherichia coli berbanding dengan kawalna kumpulan negatif. Rumpai laut perang dan hijau juga menambahkan asid propionic dan jumlah asid lemak meruap pada sekum digesta. Penambahan pelabgai tahap rumpai laut perang dan hijau membawa penigkatan bereti pada ungkapan mRNA gen inleukin-6, interleukin-10 dan interferon gamma. Hasil kajian menunjukkan ungkapan mRNA untuk gen reseptor hormon pertumbuhan adalah lebih tinggi ($p < 0.05$) pada ayam pedaging diberi makan 0.50% dan 0.75% rumpai laut hijau. Tambahan lagi, ayam diberi makan 0.50% rumpai laut perang dan 0.25%, 0.50% dan 0.75% rumpai laut hijau juga telah meningkatkan ($p < 0.05$) ungkapan gen hepatic insulin seperti faktor pertumbuhan-1. Ungkapan mRNA gen hepatik superoxide dismutase-1 juga adalah lebih tinggi dalam kalangan ayam yang diberi makan 0.50% dan 0.75% rumpai laut perang.

Kesimpulannya, kandungan protein kasar merupakan komponen utama untuk rumpai laut perang dan hijau yang kering. Berkenaan ujian penghadaman yang menggunakan 90.30% rumpai laut, keputusan mencatatkan ayam pedaging memilih rumpai laut yang mengandungi penghadaman ketara ileal yang tinggi untuk jisim organic, ekstrak eter dan mineral, sederhana untuk protein kasar dan asid amino serta rendah serat kasar dan isi abu. Hasil kajian juga mencadangkan penambahan rumpai laut perang dan hijau sehingga 1.25% ke dalam diet ayam pedaging dapat meningkatkan pretasi tumbesaran dan penghadaman nutrient pada peringkat permulaan. Rumpai laut perang dan hijau juga meghasilkan daging yang mengandungi protein kasar yang lebih tinggi. Tahap penambahan rumpai laut mempengaruhi kesan daya tahan yang disebabkan oleh peningkatan kepekatan immunoglobulin, mengawal microbiota usus dan menambah pengantara keradangan tanpa kesan buruk terhadap kualiti daging, ciri-ciri karkas dan profil lipid plasma. Penemuan daripada kajian semasa membantu kajian akan datang untuk menyiasat mekanisma dan komponen yang berfungsi dalam tumbesaran dan penghadaman khususnya pada peringkat permulaan ayam.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date: 9 March 2022

Declaration by graduate student

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%	Per cent
µL	Microlitre
µM	Micromolar
a*	Redness
ADG	Average daily gain
AID	Apparent ileal digestibility
APN	Aminopeptidase N
b*	Yellowness
BS	Brown seaweed
CAT	Catalase
CD	Crypt depth
CFU	Colony-forming units
CP	Crude protein
CWG	Cumulative weight gain
DM	Dry matter
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-picryl-hydrazyl
EDTA	Ethylenediaminetetraacetic acid
EE	Ether extract
FBW	Final body weight
FCR	Feed conversion ratio
FI	Feed intake
g	Gram
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GHR	Growth hormone receptor

GIT	Gastrointestinal tract
GLM	General linear model
GPX	Glutathione peroxidase
GS	Green seaweed
h	Hour
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HDL	High-density lipoprotein
IFN	Interferon
Ig	Immunoglobulin
IGF-1	Insulin like growth factor 1
IL	Interleukin
kg	kilogram
l*	Lightness
LAB	Lactic acid bacteria
LDL	Low density lipoprotein
M	Molar
mg	Milligram
min	Minute
mL	Millilitre
mM	Millimolar
NC	Negative control
ng	Nanogram
nm	Nanometre
OM	Organic matter
PC	Positive control
pb	Base pair

PepT1	Oligopeptide transporter
ppm	Parts per million
RNA	Ribonucleic acid
rpm	Revolution per minute
SAS	Statistical analysis system
sec	Second
SGLT5	Glucose transporter
SOD	Superoxide dismutase
TiO_2	Titanium dioxide
TNF-α	Tumour necrosis factor-alpha
VL	Villi height
VLDL	Very low-density lipoprotein
w/w	Weight/weight
WHC	Water holding capacity
α	Alpha
β	Beta
γ	Gamma

CHAPTER 1

INTRODUCTION

The poultry industry is an essential global industry that has shown rapid growth over the last decades. This industry has relative advantages of simple management, higher productivity, and fast investment than other livestock industries (Butterworth et al., 2021). Currently, poultry products are high around the world and tend to be increased. Poultry meat is a vital animal protein source in human diets and is permitted and respected globally in many food cultures and traditions. Its nutrient composition makes it an essential part of nutritious diets. Furthermore, poultry meat is moderately cheap and readily available.

The aquatic environment is a source of numerous valuable food and feed components such as macroalgae (seaweed) (Bonos et al., 2017). Malaysia is one of the seaweed producer countries. Seaweed cultivation has been implemented in Sabah Malaysia since 1978 and has become an increasingly important natural resource for Malaysia (Ahemad, 2010). Seaweed is mainly aquatic, non-flowering photosynthetic macroalgae that occur in streaming sections of oceans, seas, and rivers (Rao et al., 2018). Seaweed is divided into three different groups; brown seaweed, green seaweed, and red seaweed, which have been scientifically distinguished based on the colour (Hayes, 2019). Seaweed is rich in macro and micronutrients and different biological bioactive components (Garcia-Vaquero & Hayes, 2016; Corino et al., 2019). Recently, the interest in marine algae as a source of nutrients and bioactive components has been increased (Cherry et al., 2019). Seaweed is rich in bioactive compounds such as; carotenoids, phenolic, alkaloids, sterols, essential fatty acids, vitamins, polyphenols, dietary fibres, polysaccharides, and proteins (Aziz et al., 2019).

Seaweed has been used directly for human or food industries, chemical and pharmaceutical industries to produce chemicals and medicine. In Asian countries such as China, Japan, and Korea, around 66.5% BS, 5% GS, and 33% RS have been consumed by humans (Muhamed, 2011). Whereas seaweed has a long history of use as livestock feed (Makkar et al., 2016).

Seaweed or its compounds have been using in animal feeding to increase their performances (Michalak & Mahrose, 2020). Seaweed could be used as feed additives or as a prebiotic substitute in animal nutrition (Cabrita et al., 2016; J. Fleurence et al., 2018). Issues such as safe usage, supply sustainability, efficient drying and processing methods, and innovative extraction technologies make seaweed an effective agricultural product for livestock feed (Garcia-Vaquero & Hayes, 2016). Seaweed has been used as a supplemental or primary feed for livestock due to its low calorie and fat content, among other high-quality bio components (Kim, 2012).

Antibiotic residues in poultry products are a significant concern among medical health professionals (Shareef et al., 2009). Hence, natural feed supplements can lead to producing antibiotic-free chicken meat that will be helpful for foodborne disease (Kleter & Marvin, 2009; Kareem et al., 2016). It has revealed that bioactive molecules containing seaweed function as a healthy prebiotic that may improve the growth of animals (Holdt & Kraan, 2011; Corino et al., 2019).

Furthermore, numerous free radical species can damage organic molecules, including carbohydrates, lipids, and proteins. It also damages DNA in different mechanisms such as disrupting DNA duplication, interfering with DNA maintenance, breaking open the molecule, or altering the structure by reacting with the DNA bases (Gupta, 2015). Natural antioxidants have been high in interest in recent years. Seaweed contains dietary antioxidants that can be used as medicinal and preventative agents (Rao et al., 2018; Hayes, 2019).

Conclusively, It has widely reported that seaweed has numerous bioactive molecules that have been examined for several health-promoting effects such as; prebiotic, antimicrobial, antioxidant, anti-inflammatory, immunomodulatory and lipids lowering effects on livestock (El-Deek et al., 2011; Corino et al., 2019; Kidgell et al., 2019; Øverland et al., 2019). In contrast, there is still a lack of research to describe the effects of seaweed on broiler apparent nutrient digestibility, meat quality, intestinal morphology, blood plasma profiles, antioxidant status and immune status.

1.1 Research aim

To analyse the effects of brown and green seaweed on the nutrient digestibility and performances of broiler chickens.

1.1.1 Specific objectives

1. To analyse the chemical compositions of brown and green seaweed and its effect on the nutrient digestibility in broiler chickens.
2. To determine the effects of different levels of brown and green seaweed on growth performance and carcass characteristics, apparent nutrient digestibility, meat quality and meat composition, intestinal morphology, blood plasma profiles, antioxidant status, and immune status in broiler chickens.

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