Improving the feed value of seaweed (*Ulva sp*.) for broiler chickens using feed enzymes

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DECLARATION

I, Matshogo Tumisang Ben, duly state that this thesis belongs to me and is my novel work, which I submitted to the North-West University to fulfil the requirements for the degree of Doctor of Philosophy in Agriculture in Animal Science. This work was carried out under the guidance of Prof. Victor Mlambo and Prof. Caven Mguvane Mnisi. Additionally, I affirm that this work has not been previously submitted to any other university or institution for examination. All material and publications that were used as sources of information have been duly acknowledged.

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GENERAL ABSTRACT

The study aimed to enhance the feed value of green seaweed (*Ulva* sp.) meal using exogenous feed enzymes in the diets of Cobb 500 broiler chickens. Chapter One provides the background, problem statement, justification, aims and objectives of the study. Chapter Two gives an overview of broiler chicken production as well as the nutritional profile and importance of seaweed in chicken feeds. It also provides an outline of the use of various exogenous feed enzymes in chicken diets and summarises the gaps and discordance that exist in literature. In Chapter Three, a preliminary feeding trial was conducted using 300, two-week-old male chicks (159.3 ± 11.76 g live-weight) that were bought from Montshego Chicken Farm (25º03'49" S, 26º15'64" E) in Zeerust (North West, South Africa). The objective was to determine the maximum tolerance level of dietary seaweed meal (SWM) in broilers. To this end, this experiment investigated the effect of incremental levels of dietary SWM on growth performance, blood parameters, internal organ sizes, and carcass and meat quality characteristics in Cobb 500 broiler chickens. Five dietary treatments were formulated by including 0, 20, 25, 30, and 35 g of SWM /kg of standard grower and finisher diets. The chicks were randomly allocated to 30 pens (experimental unit), which were replicated six times per dietary treatment. Neither linear nor quadratic trends (P >0.05) were observed for overall feed intake (FI), overall body weight gain, and carcass and meat quality attributes of Cobb 500 broiler chickens as SWM levels increased. However, overall gain-to-feed ratio [G:F, R^2 = 0.192, P = 0.018] and spleen weights [R^2 = 0.182; P = 0.020] linearly declined as dietary SWM levels increased. No linear or quadratic responses (P >0.05) were observed for serum biochemical and haematological parameters except for lymphocytes [R^2 = 0.123, P = 0.042]. For meat shelf life, quadratic trends (P >0.05) were observed for pH on days 4, 5 and 6 of storage. Meat lightness (L*) linearly increased on days 1, 2, 3 and 7 of storage, whereas meat redness (a*) quadratically responded to dietary SWM levels only on day 3 of storage. In

conclusion, the optimum level of dietary SWM inclusion could not be determined based on the growth performance data. However, the linear decline in G:F suggests a need to pre-treat the SWM with exogenous feed enzymes to improve its protein and fibre digestibility. The purpose of Chapter Four was to assess the effect of dietary treatments pre-treated with fibrolytic multi-enzymes on growth performance, haemato-biochemistry, internal organs, and carcass and meat quality characteristics of Cobb 500 broiler chickens. The aim was to use fibrolytic enzymes to break down the fibre components of SWM and subsequently improve its utilisation by broilers. Three hundred and thirty (330), two-week-old male chicks (318.9 ± 16.12 g live-weight) were used in this study and randomly allocated in 30 pens (experimental units), with 11 birds per pen replicated six times. Five dietary treatments were developed as follows: a commercial grower or finisher diet containing 35 g SWM /kg (the maximum inclusion level investigated in Chapter 3) pre-treated with fibrolytic enzymes (Viscozyme® -L) at the rate of 0 (SW35), 2.0 (V20), 5.0 (V50), 7.5 (V75) and 12.0 (V120) g enzymes/kg SWM. No linear or quadratic trends (*P* >0.05) for overall FI, growth performance, blood parameters, and visceral organ weights in response to pre-treatment levels of fibrolytic enzyme were observed. However, linear increases for hot carcass weight [R^2 = 0.176, *P* = 0.033] and cold carcass weight [R^2 = 0.164, *P* = 0.040] were observed, as enzyme levels increased. Seaweed meal pre-treatment with fibrolytic enzyme levels did not influence (*P* >0.05) overall FI, physiological responses, carcass traits, visceral organs and meat quality parameters except for breast meat lightness (L^*_i). The control treatment SW35 promoted the highest L^*_i value (66.33) than diets V75 and V120, which did not differ (*P* > 0.05). The optimum level of the fibrolytic multi-enzyme pre-treatment of SWM could not be determined using growth performance, blood parameters, internal organs, and carcass and meat quality attributes of the Cobb 500 broiler chickens. Seaweed meal pre-treatment with fibrolytic enzymes alone was an ineffective strategy to increase its utilisation in broiler chickens. It was, therefore, hypothesised that poor protein digestibility, and not the

incalcitrant fibre fraction, could be the reason seaweed utilisation is poor in broilers. To test this hypothesis, a combination of the fibrolytic multi-enzymes and a proteolytic monoenzyme was evaluated as a strategy to enhance the feed value of SWM for broiler chickens in Chapter Five. Synergistic effects of fibrolytic multi-enzymes and protease monoenzyme were investigated using growth performance, physiological responses, and meat quality attributes of Cobb 500 broiler chickens. Dietary treatments were formulated as follows: a standard chicken diet with 35 g SWM/kg diet (SW35); SW35 pre-treated with 12 g/kg fibrolytic enzymes (V120); V120 pre-treated with protease monoenzyme at 5 (VP50), 10 (VP100) and 15 (VP150) g protease enzyme/kg SWM. A total of 300, two-week-old chicks (239.3 ± 8.57 g live-weight) were randomly allocated to 30 pens (experimental units) with 10 birds per pen, replicated six times per dietary treatment. At six weeks of age, chickens fed with the SW35 treatment had the highest (P <0.05) feed intake (1144.5 g/bird) when compared to chickens fed with the VP50, V120 and VP150 diets. Neither quadratic nor linear trends (P >0.05) were found in relation to protease pre-treatment levels for growth performance and carcass attributes. Gizzard weight increased linearly (P <0.05), whereas calcium, symmetric dimethylarginine, meat pH_{24}, and hue $angle_{24}$ responded quadratically (P <0.05) to protease pre-treatment levels. Chickens reared on the SW35 treatment had the lowest phosphorus levels (3.37 mmol/L) as compared to the other diets. In conclusion, indicators of physiological and meat quality were not improved by seaweed pre-treated with a combination of fibrolytic and proteolytic enzymes in Cobb 500 broiler chickens. The use of fibrolytic and proteolytic exogenous feed enzymes is an ineffective strategy to improve the feed value of green seaweed meal in broiler chicken diets.

Keywords: Blood parameters; Carcass characteristics; Exogenous enzymes; Growth performance; Meat quality; Seaweed meal

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DEDICATION

I dedicate this thesis to my late younger brother, Khumo Matshogo.

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LIST OF ABBREVIATIONS

AA	:	Amino acids
ADF	:	Acid detergent fibre
ADL	:	Acid detergent lignin
AGPs	:	Antibiotic growth promoters
ANFs	:	Anti-nutritional factors
BWG	:	Body weight gain
CCW	:	Cold carcass weight
CF	:	Crude fibre
FBW	:	Final body weight
FI	:	Feed intake
G:F	:	Gain-to-feed ratio
GLM	:	General linear model
HCW	:	Hot carcass weight
NDF	:	Neutral detergent fibre
NSP	:	Non-starch polysaccharides
OM	:	Organic matter
PCV	:	Packed cell volume
RBC	:	Red blood cells
SAS	:	Statistical analyses system
WBC	:	White blood cells
WHC	:	Water holding capacity

1 CHAPTER ONE – GENERAL INTRODUCTION

1.1 Background

Globally, the poultry industry is predicted to grow by 18%, from producing an average of 118.1 billion kilograms of meat between 2019 and 2021 to an expected 139.0 billion kilograms in 2030 (Henchion *et al.*, 2021), in response to the rising demand of meat. In high-input poultry production systems, feed and associated additives contribute over 70% of total production costs. Therefore, it is important to identify low-cost feed ingredients, which can supply chickens with additional nutrients as well as bioactive compounds that can boost productivity and product quality. One such source of nutraceuticals that can enhance broiler chicken production is green seaweed (*Ulva sp*.).

Seaweeds are primary producers in marine ecosystems that have great ecological and economic significance (Egan *et al*., 2013; Ba-Akdah *et al.*, 2016). The global seaweed animal feed market is worth US$11.34 billion annually and accounted for roughly 2.5% of the global animal feed market in 2016 (Egan *et al*., 2013). South Africa is home to over 800 different seaweed species (Bolton & Stegenga, 2002). Green seaweeds are largely found in areas with abundant light, such as shallow waters. Although most seaweeds are harvested offshore, they can also be produced in separate ponds or as co-products in fish farming (Chopin *et al.*, 2001). Green seaweeds are used in the pharmaceutical industry for the treatment of iodine deficiency and intestinal disorders and as hypoglycemic agents (El Gamal, 2010). They provide several ingredients to the feed or pharmaceutical industries, such as hydrocolloids, pigments, vitamins, chelated micro-minerals and prebiotic substances in the form of complex carbohydrates (Evans & Critchley, 2014).

Seaweeds are rich in amino acids (glycine, alanine, proline, arginine, glutamic and aspartic acids), trace elements, antioxidants, vitamins and other beneficial bioactive compounds (Rey-Crespo *et al*., 2014), thus can play an important role in poultry diets. According to Biesalski *et* *al*. (2009), the use of seaweed as a beneficial source of naturally occurring bioactive compounds such as vitamins and polyphenols has been shown to positively influence animal nutrition and health. Marine plants have a high content of dietary fibre, large amounts of minerals, vitamins, polyphenols and carotenoids (Holdt & Kraan, 2011). Polysaccharide levels in marine plants can range from 4 to 76% of dry weight (Holdt & Kraan, 2011), and they include glucans, cellulose, xylans, galactose, arabinose, xylose and starch (Jmel *et al*., 2018). Protein content varies and the highest concentrations are mainly in green seaweed 5 - 15% when compared to brown and red seaweed (Maa & Lu, 2014). According to de Jesus Raposo *et al.* (2016), marine plants occur naturally and contain a high concentration of dietary fibre, which is found in their physique.

1.2 Problem statement

In 2050, the world human population is expected to reach 9.6 billion, leading to a rapid increase in demand for poultry meat (Steinfeld & Wassenaar, 2007; Henchion *et al*., 2021). However, the rising cost of feeding limits the viability and expansion of poultry enterprises. According to Boland *et al*. (2013), soybean meal and fishmeal are the main sources of dietary protein for simple non-ruminants. However, the economic and ecological sustainability of these protein sources is a growing concern (Godfray *et al*., 2010; Duro, 2020). Consequently, feed resources such as seaweeds, which can supply the required nutrients as well as boost growth performance and quality and safety of poultry products through the action of bioactive compounds could be the solution (Odegard & van der Voet, 2014).

Seaweeds (*Ulva* sp.) offer high yields per unit area and do not require arable land or fresh water (Bolton *et al*., 2009; Mata *et al*., 2016). However, the use of non-conventional feed ingredients, such as seaweeds, can be restricted by the presence of secondary plant compounds and high fibre levels. Despite the potential value of seaweeds as a nutraceutical source in poultry feeds, high fibre content and other anti-nutritional compounds (such as phytic acid, gluten, tannins and oxalates) may reduce their utility. To exploit the bioactive compounds in seaweeds without compromising feed intake and nutrient utilisation of chickens, strategies to decrease the negative effects of fibre and other anti-nutritional factors must be identified and evaluated. High fibre content, poorly digestible carbohydrates, and the high cost of production prevent the inclusion of a substantial amounts of seaweed in monogastric diets (Sharma *et al*., 2018).

To address some of these constraints, seaweed meal can be pre-treated with exogenous enzymes, such as fibrolytic enzymes, to break the fibre components and protease enzymes to enhance protein bioavailability for broilers. The use of feed enzymes to pre-treat seaweeds can increase the economic feasibility of seaweed meal as an ingredient in poultry diets. While many studies have investigated low inclusion levels of seaweed in broiler diets due to high fibre content (Abudabos *et al*., 2013; Choi *et al.*, 2014), pre-treatment with exogenous enzymes to enhance the utility of seaweed meal in broiler diets has arguably not yet been carried out. This approach has the potential to allow the inclusion of larger quantities of seaweed in the diets of chickens, thereby reducing the production costs.

1.3 Justification

Seaweeds are reported to have high concentrations of fibre, vitamins, minerals, protein, antioxidants and fatty acids (Agregán *et al.*, 2017). They also contain bioactive substances such as polyphenols that are known to possess antibacterial, antifungal, antiviral and natural antioxidants properties (Stengel *et al.*, 2011) and thus can be used as nutraceuticals in broiler production (Van Hal *et al.*, 2014). They have also been reported to contain between 26% and 32% of aspartic and glutamic acids, which together constitute a large percentage of the amino acid fraction (Vinoj Kumar & Kaladharan, 2007). The inclusion of seaweeds between 2% and 3% in broiler chicken diets provided the best nutrient availability and apparent metabolisable energy, which could be attributed to the duodenum's high level of amylase (Perali *et al.*, 2020). It improved breast meat quality by increasing feed intake, feed conversion efficiency and average daily growth while decreasing abdominal and subcutaneous fat thickness (Wang *et al.*, 2013). Similarly, El-Deek & Barika (2009) discovered that the inclusion of green seaweeds in broiler finisher diets improved growth and feed consumption and gain-to-feed ratio.

According to Abudabos *et al.* (2013), adding seaweed meal at the rate of 3% in broiler diets (12 – 33 days) proved to not affect feed consumption, body weight gain, feed conversion ratio (FCR) and nutrient absorption. In most studies carried out with chickens, the inclusion level of seaweed meal did not exceed 3%, while in a few studies where higher levels were used, lower feed consumption and slow growth performance were observed (Øverland *et al.*, 2019). In addition to their nutritional value, seaweeds offer numerous technological advantages associated with their high mineral contents and the properties of their fibres (Cofrades *et al.*, 2011). Pre-treatment with exogenous feed enzymes could be a solution to enhance the utilisation of seaweed meal and enable the use of higher inclusion levels (Valente *et al.*, 2021). Indigestible substrates like non-starch polysaccharides (NSP), such as cellulose and xylans, are found in high fibre diets, which do not only impair total feed digestibility but also reduce nutrient utilisation. Thus, the solution to ameliorate the antinutritional effects of high fibre in seaweeds can be the use of exogenous fibrolytic enzymes to pre-treat seaweed. The development of commercially available feed enzymes to target specific substrates and ameliorate anti-nutritive effects has received increased attention in previous years. The use of exogenous substrate-specific enzymes (xylanase, beta-glucanase, amylase, among others) in poultry diets to improve birds' performance has been extensively researched (Bedford, 2000; Acamovic, 2001). However, these improvements relate to greater nutrient digestion and absorption in cereal grains caused by the degradation of cell wall NSP (Bedford & Schulz, 1998). By using exogenous enzymes, the indigestible NSP can be hydrolysed, resulting in improved broiler growth performance.

The use of exogenous enzymes in poultry feeds not only enhances growth performance but also reduces environmental pollution due to decreased nutrient concentration in the excreta. The complex cell wall of seaweed contains polysaccharides intertwined with proteins and other nutrients. Therefore, pre-treatment of dietary seaweed meal with a protease enzyme can also increase protein utilisation and allow its dietary inclusion at higher levels. The use of exogenous fibrolytic enzymes is critical because of their hydrolytic action on heterogeneous algal cell walls to release polysaccharides and proteins (Giovannoni *et al*., 2020). However, to my knowledge, no studies have attempted to improve the feed value of dietary green seaweed meal using fibrolytic multi-enzymes and a protease monoenzyme for broiler chickens. The cost of poultry production is increasing at a fast rate, thus searching for alternative ingredients, such as seaweed with beneficial bioactive compounds, might reduce costs and improve profitability.

1.4 Aim and objectives

The study aimed to enhance the feed value of seaweed (*Ulva* sp.) meal for Cobb 500 broiler chickens using exogenous fibrolytic and proteolytic enzymes. The specific objectives of the study were as follows:

a. The first experiment investigated the growth performance, haematology, serum biochemistry, internal organs and carcass and meat quality parameters of Cobb 500 broilers fed with diets containing incremental levels of seaweed meal.
b. The second experiment evaluated growth performance, blood parameters, internal organs and carcass and meat quality traits in Cobb 500 broilers fed with diets containing fibrolytic enzyme-treated seaweed meal.

c. The third experiment investigated growth performance, haemato-biochemistry, internal organs and carcass and meat quality of Cobb 500 broilers fed with diets containing seaweed meal pre-treated with a combination of fibrolytic enzymes and a protease monoenzyme.

1.5 Hypotheses

a. The first experiment tested the hypothesis that adding different levels of SWM in broiler meals will enhance Cobb 500 broiler chickens' growth performance, physiological responses, and meat quality parameters and allow the determination of an optimum inclusion level.
b. The second experiment tested the hypothesis that fibrolytic enzyme pre-treatment of seaweed meal improves growth performance, blood parameters, carcass and meat quality traits of Cobb 500 broilers.
c. The third experiment tested the hypothesis that pre-treatment of seaweed, with a combination of fibrolytic and proteolytic enzymes, improves growth performance, blood parameters and meat quality traits of Cobb 500 broilers.

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2 CHAPTER TWO - LITERATURE REVIEW

2.1 Introduction

The growing human population as well as the high demand for animal protein have stimulated the search for novel feed sources, including seaweed (Makkar *et al.*, 2016; Buschmann *et al.*, 2017). The predicted global increase in meat, egg, and milk consumption in the coming decades is driven by the rapidly growing human population, which is expected to reach 9.7 billion in 2050 (United Nations, 2017; Duro, 2020), as well as the rising living standards and urbanisation (Boland *et al.*, 2013). Concomitant with this increase is the rise in demand for feed ingredients and, as such, the development of novel sources of biomass for feed production. The extension of marine biomass production by wild harvest and large-scale cultivation of seaweed could significantly contribute to biomass production for feed as well as food. Compared to the year 2000, the need for animal feed is anticipated to rise on a worldwide scale by 70% in 2050 (Alexandratos & Bruinsma, 2012; Boland *et al.*, 2013). According to the United States Department of Agriculture (USDA, 2020), global chicken meat production in 2020 will be higher than in the past years due to high demand because consumers are seeking less expensive animal protein sources amid the economic crisis and Coronavirus Disease 2019 (COVID-19) pandemic. The production of chicken is effective at turning feed into a mass of good quality in a short period (Ezzat Abd El-Hack *et al.*, 2016; Nordhagen & Klemm, 2018). Commercial broiler chickens have been chosen for their rapid growth rates, feed conversion efficiencies and to meet the demands of poultry meat. According to Havenstein *et al.* (2003), improvements in chicken growth rates and carcass yields are due to breeding and selection of quality chicken in a commercial breeding station.

According to Makkar *et al.* (2016), three categories are used to group seaweeds, depending on their colour; the (*Rhodophyceae*) are red; the (*Phaeophyceae*) are brown, and the (*Chlorophyceae*) are green. The world market of seaweed is worth $11.5 billion annually and accounts for roughly 4% of the global animal feed market in 2018 (Chopin & Tacon, 2021). Currently, there is a lot of interest in using seaweeds in poultry feeds and for other industrial applications. Seaweeds are particularly interesting, as they have zero requirements for land-based resources (Ortiz *et al.*, 2006; Øverland *et al.*, 2019). The beneficial bioactive components found in seaweeds can be added to poultry diets as a way of improving performance and health status (Michalak & Chojnacka, 2015).

It is well recognised that green seaweeds are useful functional feed ingredients with the ability to improve feed utilisation efficiency and meat quality parameters. In addition, seaweed contains bioactive compounds, such as minerals, vitamins, carotenoids, polyunsaturated fatty acids, protein and polysaccharides (Wong & Cheung, 2001; Kendel *et al.*, 2015). Seaweed polysaccharides can facilitate many activities, such as growth promotion, appetite stimulation, immune enhancement and anti-pathogenic properties in poultry production (Chen *et al.*, 2003; Gahan *et al.*, 2009; Li *et al.*, 2011). Furthermore, seaweeds are high in phenolic compounds (catechins, phlorotannins, flavonoids, phenolic acids and tannins) that have antioxidant, anti-inflammatory, anti-proliferative and anti-microbial properties (Gullón *et al.*, 2020), which can be used to enhance the development and quality of poultry meat. Seaweeds are also high in sulfur-amino acids like methionine, lysine and threonine, which improve dressing percentage, breast meat yield and carcass characteristics (Wong & Cheung, 2001). In addition, seaweed contains large amounts of complex carbohydrates that have prebiotic effects (Wells *et al.*, 2017). The majority of seaweed have hard cell walls that are indigestible by monogastric animals, thus preventing access to their valuable nutritional compounds (Øverland *et al.*, 2019). In related studies, inclusion of 2.0% seaweed meal improved broiler performance and dressing percentage, while the addition of 4% seaweed in the broiler chicken basal diet led to decreased body weight gain (Abudabos *et al.*, 2013). The common maximum inclusion level of 5% reveal a precaution to avoid the negative impact of fibre and other anti-nutritional compounds on feed utilization.

Supplementing poultry diets with enzymes is ecologically friendly and nutritious (Kamel *et al.*, 2015). Enzymes are used to boost the energy content of feed ingredients and improve the utilisation of protein, lipids, carbohydrates and phosphate from plants, resulting in a lower rate of undigested nutrients being excreted into the environment and, thus, less pollution. Most feed supplement enzymes, particularly proteases, provide this function because lowering nitrogen excretion, a significant pollutant, depends on the digestion of nitrogenous compounds in feeds around the world (Kamel *et al.*, 2015). The application of exogenous enzymes has been largely utilised in animal feeds to improve their nutritive value (Slominski, 2011). Moreover, exogenous enzymes may generate prebiotic oligosaccharides that alter resident micro-biota and promote animal health (Bedford & Cowieson, 2012). Exogenous fibrolytic enzymes have been used to boost the bioavailability of nutrients, giving nutritionists more options when it comes to the ingredients that they can use during formulations (Alemawor *et al.*, 2009). Furthermore, fibrolytic enzymes have shown a significant role in reducing the negative environmental impact of chicken production. Therefore, improvements in the digestion of dietary fibre have the potential to increase the sustainability and efficiency of broiler production. The introduction of fibrolytic enzyme combinations in poultry diets, which largely comprise xylanases and cellulases, could improve the digestion of fibre and digestible energy intake (Beauchemin *et al.*, 2003). The combined effects of a proteolytic monoenzyme and a fibrolytic multi-enzymes have not yet been studied for seaweeds used in broiler diets. The application of green seaweeds might stand as a promising ingredient in chicken diets (El-Hady & El Ghalid, 2018).

2.2 Chicken production and development of feed

The production of poultry meat has been growing at a high rate than that of pork and beef worldwide. According to Davis *et al.* (2021), broiler meat consumption in South Africa was over 1.88 million metric tonnes (t) in 2018 and is expected to rise to around 1.9 million metric t in 2021. Chicken output in South Africa was 1.813 million t (99.96% of total poultry production), including subsistence farming and breeders in the broiler and layer subsectors (SAPA, 2019). According to the South African Revenue Service (SARS) certified numbers, annual broiler imports totalled 511 317 t in 2019, a 5.2%, decline from 2018. Despite this, imports were 5.7% higher in 2019 than the 5-year average (2014 – 2018). The poultry industry has adjusted plans to take into account the higher demand by searching for alternative feedstuffs that can assist in flattening the curve. This has been accomplished through advancements in technology for the production and processing of animals (Hoffmann, 2007). Selective breeding, improved genetics, efficient production methods, a highly specialised diet, and regular veterinary care have produced enormous, fast-growing birds, with high breast muscle yields that may reach market weight as soon as five weeks (Fanatico *et al*., 2007). Combined with greater knowledge of human health and dietary concerns, this has resulted in the growth of specialised feed markets focused on creating free-range or organic products that are free from the use of growth hormones and antibiotic growth promoters (Fanatico *et al*., 2007).

Poultry products are among the most cost-effective foods, as well as being a good source of protein, with no religious limitations. Poultry production has the lowest environmental footprint in terms of water use per kg of meat or eggs produced (Mourad *et al*., 2019). In fact, poultry has the least impact on water use, as well as on land size and environmental stress (De Vries & de Boer, 2010). Chicken meat demand and consumption are expected to reach 165 thousand metric t by 2030, up from 55 thousand metric t in 2000 (Robinson & Pozzi, 2011). It is projected that the demand for animal products could grow by 70% in 2050 (Alexandratos & Bruinsma, 2012), while the demand for animal-based calories could increase from 74 to 114%

(Gouel & Guimbard, 2018). This expansion is due to increased urbanisation, the emergence of the middle class and the proliferation of quick-service eateries.

Access to safe and inexpensive feed in terms of energy, protein, mineral, and vitamin sources is a concern for chicken farmers (Prabakaran, 2003; Ravindran, 2013). Feed expenses can make up as much as 70% of a farmer's overall production costs (Van der Poel *et al*., 2020), leaving farmers with razor-thin profit margins. The most costly ingredient in chicken feed is protein and the supply of highly digestible amino acids is still a limiting factor in achieving peak performance (Alagawany *et al*., 2014). On the other hand, farmers have a problem because of the high cost of protein (Admasu *et al*., 2019). In poultry production, the common feed ingredients for protein are fish, soybean and carcass meals (Selaledi *et al*., 2020; Toomer *et al*., 2020). In several countries, the cost of producing broiler chicken is presently dominated by protein at 60% (Ssepuuya *et al*., 2017), putting a strain on both producers' profitability and the agro-food value chain (Nalunga *et al*., 2021). These poultry production-related aspects have prompted a search for non-conventional feed sources for use as nutraceuticals in chicken diets like seaweeds (Chia *et al*., 2019; Parolini *et al*., 2020).

2.2.1 South African broiler feed production

In South Africa, high feed prices are influenced by the costs of raw materials, an increase in the price of ingredients affects feed prices. Typically, South Africa is a net importer of soybean meal but a net exporter of yellow maize (Murithi *et al*., 2016). Immediate access to feed has been found to benefit broiler chickens. Chickens would benefit from a more balanced nutritional profile, notably protein and highly digestible amino acids. Modern broilers can reach market weights at an early age to achieve the target demand for meat (Kleyn & Chrystal, 2008). As a result, advancements in nutrition will be critical in ensuring this fast development and maintaining long-term broiler output. Thus, the typical nutritional focus of just providing nutrients for maintenance and development has become a thing of the past. Poultry production has been of great assistance in meeting human nutritional needs, by being a constant protein source, strengthening the financial capacity and fitting well into commonly embraced farming schemes (Idowu *et al.*, 2018). Poultry diets must be tailored to offer all essential nutrients in appropriate proportions to ensure maximum growth and production potential of chickens (Daghir, 2009).

2.2.2 Nutrient requirements of broiler chickens

In commercial chicken farming, the cost of feed makes up to 70% of the total production costs (Sugiharto & Ranjitkar, 2019). Because of global increases in conventional feed prices, there has been a shift to using non-conventional feed ingredients to increase profitability (Supriyati *et al.*, 2015). Protein is a crucial component of poultry diets, along with other main nutrients, such as minerals, fat, water, vitamins and carbohydrates. However, protein is the most critical nutrient in chicken diets, hence when formulating broiler diets, crude protein (CP) is given priority (Cheeke, 2005). In the digestive system, proteins are broken down and hydrolysed into amino acids, which are subsequently metabolised to generate proteins that are needed in the synthesis of different physiological tissues (Aviagen, 2009). The method of feeding poultry is based on the stage of production and physiological processes occurring, as the birds progress through the different stages of growth (NRC, 1994). The modified Baker and NRC patterns of changes in amino acid compositions is presented in table 2.1.

Table 2.1. Modified Baker and NRC patterns of changes in amino acid compositions in ideal proteins for 0-56 old chickens (% of lysine in diet)

Amino acid	[1]Baker's modified models (1997) for chicks			NRC (1994) model for chicks
	0 - 21 days[2]	21- 42 days[3]	42 - 56 days[4]	0 - 21 days[5]
Threonine	67	70	70	67
Valine	77	80	80	75
Lysine	100	100	100	100
Methionine	36	37	37	42
Cystine	36	38	38	33
Arginine	105	108	108	104
Tryptophan	16	17	17	17
Isoleucine	67	69	69	67
Leucine	109	109	109	100
Histidine	35	35	35	29
Phe+Tyr	105	105	105	112

[1] These ratios are based on true digestible levels of amino acids in the diet (as-fed basis; 90% dry matter). Adapted from (Baker, 1997). Except for glycine, all amino acids are L-isomers. [2] Patterns of amino acid composition in the ideal protein are the same for male and female chickens. The amounts of digestible lysine in the diet (as-fed basis; 90% dry matter) are 1.12% and 1.02% for male and female chickens, respectively. [3] Patterns of amino acid composition in the ideal protein are the same for male and female chickens. The amounts of digestible lysine in the diet (as-fed basis; 90% dry matter) are 0.89% and 0.84% for male and female chickens, respectively. [4] Patterns of amino acid composition in the ideal protein are the same for male and female chickens. The amounts of digestible lysine in the diet (as-fed basis; 90% dry matter) are 0.76% and 0.73% for male and female chickens, respectively. [5] These ratios are based on total amino acids in a typical corn- and soybean meal-based diet. The amount of digestible lysine in the diet (as-fed basis; 90% dry matter) is 1.2% for 0- to 21-d-old chicks.

2.2.3 Cobb 500 broiler chicken

Broiler chickens (*Gallus gallus domesticus*) have been selected for fast growth rates, high feed utilisation efficiencies and early attainment of market weight. Significant investments in research and the growth of the broiler meat value chain have been drawn by the rising demand for chicken meat, as a healthier and more affordable animal protein than red meats (Mottet & Tempio, 2017). In just five to seven weeks, the commercial breed, Cobb 500, can achieve market weight (1.5 – 2.5 kg). When compared to other commercial chicken breeds across the world, Cobb 500 breed has competitive breast meat yields at various processing ages (Coneglian *et al.*, 2010). The Cobb 500 broiler chickens are bred and raised specifically for meat production. Generally, it can be distinguished by white feathers and yellowish skin and reddish-like combs.

2.3 Overview of seaweeds

Any big benthic algae that is multicellular and macrothallic, as opposed to most algae that are macroscopic in size, is referred to as seaweed (Crawford, 2002). The *Chlorophyceae* class, which includes both microscopic and macroscopic species, has 2200 species of green seaweeds (*Chlorophyta*), which are popularly known as green algae (O'Sullivan *et al.*, 2010; Corino *et al.*, 2019). Chlorophyll pigments utilised in the photosynthetic process, is responsible for the distinctive hue (Gupta & Abu-Ghannam, 2011; Makkar *et al.*, 2016). Their hue is generally determined by the ratio of chlorophylls to other pigments like carotene and xanthophylls. Shallow water and natural ponds are good habitats for green algae because they receive a lot of light. *Ulva* is a widespread green seaweed genus that may also be found in brackish water (mainly in estuaries). Green seaweeds are extremely intriguing to research on a nutritional level, since they are rich in vitamins, proteins and minerals (Jamal *et al.*, 2017). The *Ulva sp.*

has a high concentration of sulphated-polysaccharide, which possesses anticancer, antiviral, anti-inflammatory, anti-hyperlipidemic and immunomodulatory activities (Pereira, 2018). Some *Ulva* species are used as livestock feed (Valente *et al*., 2015), and the quality of the meat and the amylase activity in the chicken duodenal content of diets are improved by adding *Ulva* in powder form (Verland *et al*., 2019). Table 2.2 shows the chemical composition of some *Ulva* species.

Table 2.2. Chemical composition of various *Ulva* species

	Protein (%)	Lipid (%)	Ash (%)	Total dietary fibre (%)	Insoluble fibre (%)	Soluble fibre (%)	Reference
Ulva Chlothrata	21.9-26.9	2.5-35	44.8-49.6	24.8-26.3	8.7-10.7	15.6-16.6	Peña-Rodríguez *et al*. (2011)
Ulva Lactuca	8.46	7.87	19-59	54.9	34.9	20.53	Yaich *et al*. (2011)
Ulva sp.	7-44	0.3-16	11-55	38.0	17.0	21.0	Wong *et al*. (2000)

2.3.1 Seaweed production

In the ocean environment, seaweeds represent a significant renewable resource and have long been connected to human civilisation. The use of seaweeds has been documented as far back as 2500 years (Tseng, 2004). The production of seaweed is increasing at a fast rate. In the year 2000, it was produced at a pace of roughly 10.1 million t wet weight, with a market value of $5.6 billion (FAO, 2002). Large-scale cultivation of the indigenous seaweed has developed fast in South Africa, since its inception in the mid-1990s, and the country is now the world's biggest cultivator of farmed abalone outside of Asia (Troell *et al*., 2006), with poaching of wild abalone populations and high market prices driving its cultivation. Access to relatively inexpensive labour, as well as favourable coastal water quality and infrastructure, aided the abalone industry's fast expansion in South Africa. The biological chemicals found in seaweed are of particular relevance; as compared to terrestrial plants, aquatic plants have functional compounds. Seaweed's bioactive chemicals help prevent illnesses, including arthritis, diabetes, cancer, inflammation and high blood pressure in human beings (Wijesinghe & Jeon, 2012).

2.3.2 Seaweed cultivation, harvest and meal production

Seaweed culture is a burgeoning business in most coastal parts of the world. According to FAO (2012), the production of seaweed increased by 9.5% per year in the 1990s and 7.4% per year in the 2000s, from 3.8 million t in 1990 to 19 million t in 2010. In 2012, the largest producers of seaweed were China, Japan and Indonesia, accounting for over 95% of the total seaweed production (FAO, 2012). Nutrients, water flow, temperature, light, and water salinity are all important factors for each species (Roleda *et al*., 2019). Seaweed pieces are connected to ropes or nets or sunk at the bottom of the pond, unattached or kept in place by a fork-shaped instrument (in sediments) or sand-filled tubes in species that grow vegetatively (in sandy soil) (Makkar *et al*., 2016). When these species are harvested, the plant is either completely removed or only a little piece is kept to be used as seed stock for future production.

Brown seaweeds are only gathered in their large sporophyte stage, which mostly reproduces sexually (McHugh, 2003). For the production of seaweed meal, seaweeds must be processed soon after harvesting, since they have a high moisture and may get mouldy. The wet seaweed is run through hammer mills, with progressively smaller screens, to grind it into tiny pieces. The final moisture level of the meal should be approximately 15%, and it ought to be stored in sealed bags. In the refrigerator, seaweed meals can be kept for up to a year (McHugh, 2003).

Several phytochemicals can be found in seaweeds. Thus, it is recommended that they are dried at a low temperature to ensure that the bioactive compounds are less inactivated.

2.3.3 Seaweed as a nutraceutical

The current trend in poultry nutrition is to use natural ingredients as an alternative to antibiotics, growth hormones, or other related chemicals. Seaweeds are natural feed ingredients, with high nutritional value and, therefore, might stand as a promising ingredient in poultry diets (El-Hady & El Ghalid, 2018). Seaweeds are excellent for use as nutraceuticals in animal feeds, due to a combination of nutritional and pharmaceutical properties (Pomin, 2012; Laudadio *et al*., 2015). *In vitro* and *in vivo* studies have revealed that seaweed has health benefits and essential nutrients (Jiménez-Escrig *et al*., 2011; Saeed *et al*., 2021). Although the available literature makes some seaweed components appealing as functional food ingredients, further animal nutritional studies are needed to evaluate both the nutritional benefits and the efficacy of bioactivities, as well as any potential negative effects (Vilà, 2008). According to Gu *et al*. (1988), the performance and dressing percentage of broilers were improved by adding 2% of green seaweed (*Ulva lactuca*) to their diet. However, high levels of salt can be retained by seaweed, with a spongy thallus like *Codium sp*. and subsequently cause broilers to lose weight because of diarrhoea (Ventura *et al*., 1994).

Studies using a different *Ulva* species, *U. lactuca* (Sea lettuce), which grows along Atlantic coastlines, revealed that broiler diets that included seaweed at a level lower than 3% performed better than the control diet (Morais *et al*., 2020). These findings imply that increased levels of crude protein and amino acids, particularly methionine, found in seaweed, have a significant effect on improving dressing percentage and breast yield. The variation in nutrient composition of seaweed due to seasonal changes should be taken into consideration (Ali & Memon, 2014). With these findings, seaweed meal can be partially incorporated into broiler diets.

2.3.3.1 Nutritional composition of seaweed

Factors such as species, age, location, and environmental conditions are the main determinants of the nutritional composition of seaweed (Fleurence *et al.*, 2012). The protein content of seaweeds can range from 5 - 35% of the dry weight, with high protein seaweeds having a similar protein content to high protein content crops, such as canola meal, which contains about 35% protein on a dry matter basis (Rajauria, 2015). The amino acid profile of seaweed varies, based on the kind of species. Glutamic and aspartic acids in brown seaweeds represent 22 - 44% of the total amino acids content. These two amino acids represent 26 - 32% in green seaweeds of the total amino acid content but are present in much lower concentrations in red seaweeds, ranging from 14 - 19% of the total amino acid content (Fleurence, 1999). In addition, seaweed contains polysaccharides and low levels of lipids. On a dry matter basis, seaweeds contain 1 - 5% lipids, and the total content of polysaccharides is about 76% of the dry weight (Rajauria, 2015). Seaweed mineral content is much greater than terrestrial plants. The macro-minerals and trace elements, such as calcium, sodium, and magnesium, are abundant in seaweeds and play a significant role in the nutrition of animals. Some brown seaweeds contain upwards of 3.25 g calcium/kg dry matter (Fleurence *et al*., 2012).

2.3.3.1.1 Proteins and amino acids

Proteins have a crucial role in establishing the nutritional value of feed. These nitrogenous chemicals are found in large quantities in seaweeds. They also include non-protein nitrogen, which is used to make molecules, such as nitrite nitrogen, free amino acids, chlorophyll, ammonium ions and nucleic acids (Lourenco *et al*., 2002). The search to use seaweed as a source of proteins has persisted for decades due to protein deficiency, particularly in developing countries. In recent years, seaweeds have become a more cost-effective source of protein. Protein content in seaweeds varies depending on the parameters, such as distinct species, ambient circumstances and the protein testing technique used (Fountoulakis & Lahm, 1998; Lourenco *et al*., 2002). The protein value is also influenced by how much nitrogen-to-protein conversion factor is used. The values of the nitrogen-to-protein conversion factor have been given for several taxa of green, brown and red seaweed. The nitrogen-to-protein conversion factor for green algae is 5.13, for brown algae 5.38, and 4.92 for red algae on average (Lourenco *et al*., 2002), seaweed variation can be influenced by the drying methods after harvesting as well as the employed protein conversion factor after analysis. Because of these factors, comparing the protein content of several seaweed taxa is challenging. Nevertheless, a nitrogen-to-protein conversion factor of 6.25 has been used in most studies. Green and red seaweeds are known to have a high protein content, with an average of 10 - 30% dry matter (Burtin, 2003; Ramos *et al*., 2000).

2.3.3.1.2 Dietary fibre

Polysaccharides found in seaweed are a diverse set of substances, with a variety of biological roles. Seaweed polysaccharides have an essential function in nutrition as dietary fibre (DF), which is defined as the skeletal remains of plant cells in the diet that are resistant to breakdown by digestive enzymes (de Borba Gurpilhares *et al*., 2019). Over time, the definition of this word was expanded to include any polysaccharides and lignin that are not broken down by endogenous secretions from the digestive system (Bach Knudsen, 2001). Dietary fibre may be divided into two types: insoluble and soluble fibre. The insoluble components of fibre are celluloses, arabinoxylans, hemicelluloses, and lignin whereas the soluble components of fibre include pectin, xyloglucans, galactomannan, hemicelluloses, gums and waxes (Bach Knudsen, 2001).

2.3.3.2 Seaweed as a source of bioactive compounds

Seaweeds have been utilised as functional feed and medicinal herbs due to their phytogenic properties (Liu, 2012). The potential use of seaweed has increased due to world population growth and climate change (Rao & Mantri, 2006). Seaweeds are becoming more widely used in agriculture and biomedicine due to their high concentration of bioactive compounds, such as carotenoids, peptides, oxylipins, phlorotannins, steroids, essential fatty acids and sulphated polysaccharides (Venkatesan *et al*., 2019). Seaweeds that are found growing under adverse marine conditions will produce different bioactive compounds from those grown either on ponds sub-tidal zones where environmental conditions are not as severe (Yu *et al*., 2014). The composition of laminarin varies seasonally, but laminarin forms about 10 - 32% of the dry matter weight of seaweed (Holdt & Kraan, 2011). Fucoidan constitutes about 10 - 20% of the dry matter of brown seaweed (Holdt & Kraan, 2011). The content of phenols in seaweeds ranges from 1 - 4% and is less abundant in green and red seaweeds than in brown seaweeds (Holdt & Kraan, 2011). Dietary antioxidants in seaweed help in reducing oxidative damage and chronic disease (Nagai & Yukimoto, 2003) and regulate signal transduction at different stages, including the inhibition of oncogenes, activating cancer cell death also known as apoptosis, decreasing inflammation, inhibiting angiogenesis and modulating hormone, such as growth (Russo, 2007). Seaweeds contain main potential antioxidant compounds such as pigments (astaxanthin, carotenoids, and fucoxanthin) and polyphenols (phenolic acid, flavonoid, tannins, etc.), with high antioxidative activities (Siriwardhana *et al*., 2004). The most prevalent secondary metabolites and antioxidants are phenolic compounds found in seaweeds (Kadam *et al*., 2013). Seaweeds contain carotenoids, vitamins C and E, which are considered natural antioxidants and can protect against oxidative stress and associated pathologies, such as inflammation, cancer and coronary heart disease (Tapiero *et al*., 2002). According to Namvar *et al*. (2012), seaweeds have phlorotannins that contain vital bioactive compounds, which are 10 - 100 times more stable and potent antioxidants than any other polyphenols.

2.3.3.2.1 Polysaccharides

Simple sugars linked by glycoside bonds make up the carbohydrate biopolymers known as polysaccharides (Muthukumar *et al*., 2021). They are divided into three types: structural polysaccharides, mucopolysaccharides and storage polysaccharides. Many beneficial biological characteristics and low toxicity, high molecular weight polysaccharides, and the low molecular weight oligosaccharides that are produced during their breakdown are significant from an economic perspective. A lot of attention has been devoted to polysaccharides due to their natural origin, a lack of medication residues and a lack of negative side effects (Tian & Feng, 1994). Polysaccharides are frequently used as stabilizers, thickeners and emulsifiers in the food sector (Tseng, 2001). Several polysaccharides found in seaweeds have been demonstrated to have a range of pharmacological activities, including antioxidant, anticancer and anti-inflammatory effects. Alginates, agarans, carrageenan, fucoidan, laminarin, and ulvans are important polysaccharides found in seaweed (Rengasamy *et al*., 2014).

2.3.3.2.2 Phlorotannins

About 15% of the dry weight of brown algae is made up of phloroglucinol polymers known as phlorotannins and are commonly known as algal polyphenols. *Laminariacea* has been discovered as the marine algae with the highest concentration of phlorotannins. Phlorotannins have molecular weights that vary from 126 kDa to 650 kDa. The isolation and pharmacological characteristics of phlorotannins from brown seaweed, such as *Eisienia bicyclis*, *Ecklonia cava*, *E. stolonifera* and *E. maxima,* have been extensively studied in the past two decades (Kannan *et al*., 2013; Rengasamy *et al*., 2013). Phlorotannins have also been discovered to exhibit a variety of biological or pharmacological characteristics, including antibacterial, anti-inflammatory and antioxidant, antiproliferative, anticancer, antihypersensitive, anticoagulant and radioprotective activities (Moon *et al*., 2008).

2.3.3.2.3 *Protein hydrolysates*

Various protein hydrolysates with strong pharmacological effects have been isolated from seaweeds. Seaweed protein hydrolysates have been shown to exhibit a variety of biological properties, including antiplatelet aggregation (Cian *et al.*, 2012), antibacterial action (Beaulieu *et al.*, 2016), anticoagulant (Indumathi & Mehta, 2016), anti-hypertension (Qu *et al.*, 2010; Pan *et al.*, 2016), antioxidant (Beaulieu *et al.*, 2016) and chelating properties (Cian *et al.*, 2016). The extraction of anti-hypertension inhibitory peptides from seaweed is growing in popularity among researchers worldwide, although protein hydrolysates exhibit a range of biological activities. Table 2.3 provides comprehensive details on the proteins, peptides and various protein hydrolysates from seaweeds.

Table 2.3. Seaweed protein hydrolysates and biological characteristics

Source	Biological properties	Model	Findings	Reference
Palmaria palmate	Antioxidant	*In vitro*	DPPH scavenging, FRAP, and ORAC were seen in hydrolyzed fractions after treatment with either chymotrypsin or trypsin.	Bondu *et al.* (2015)
Undaria pinnatifida	Antihypertensive	*In vitro* & *in vivo*	Shown inhibition *in vitro* against ACE. When given orally to rats with spontaneous hypertension, four tetrapeptides showed antihypertensive efficacy.	Suetsuna & Nakano (2000)
Ulva rigida	Antihypertensive	*In vitro*	ACE was inhibited (IC50 = 0.483 mg/mL) in the hydrolysate produced under ideal proteolysis conditions using pepsin and	Paiva *et al.* (2016)

		bromelain. The greatest ACE inhibition was seen in the fraction below 1 kDa (IC50 = 0.095 mg/mL).

2.3.3.2.4 Alkaloids

Alkaloids are a well-known category of natural macromolecules that have been shown to have significant health and biological activities. Galanthamine is a commercially available anticholinesterase inhibitor that has been used for many years to treat dementia. Despite substantial studies on alkaloids (Aniszewski, 2015), little is known about marine algal-based alkaloids. In 1969, the red seaweed *Phyllophora nervosa* produced the first alkaloid molecule, hordenine (Güven *et al*., 1970).

Several seaweed-related studies have been conducted over the past decade to examine their potential as prospective sources of biocompounds (Khan *et al.*, 2018). Figure 2.1 shows the number of documented bioactive chemicals derived from seaweeds, as well as studies connected to their use in meat products from 2005 to 2020. As can be seen, the research trend on bioactive chemicals from seaweeds has grown in the past 15 years, whereas the literature on their use in animal diets is limiting. Although seaweeds or their extracts are becoming more popular as a source of biologically active compounds, such as antioxidants, pigments, peptides, polysaccharides, and fatty acids, their use to create meat products with improved nutritional and technological qualities as well as functional properties is still low (Gullón *et al*., 2020).

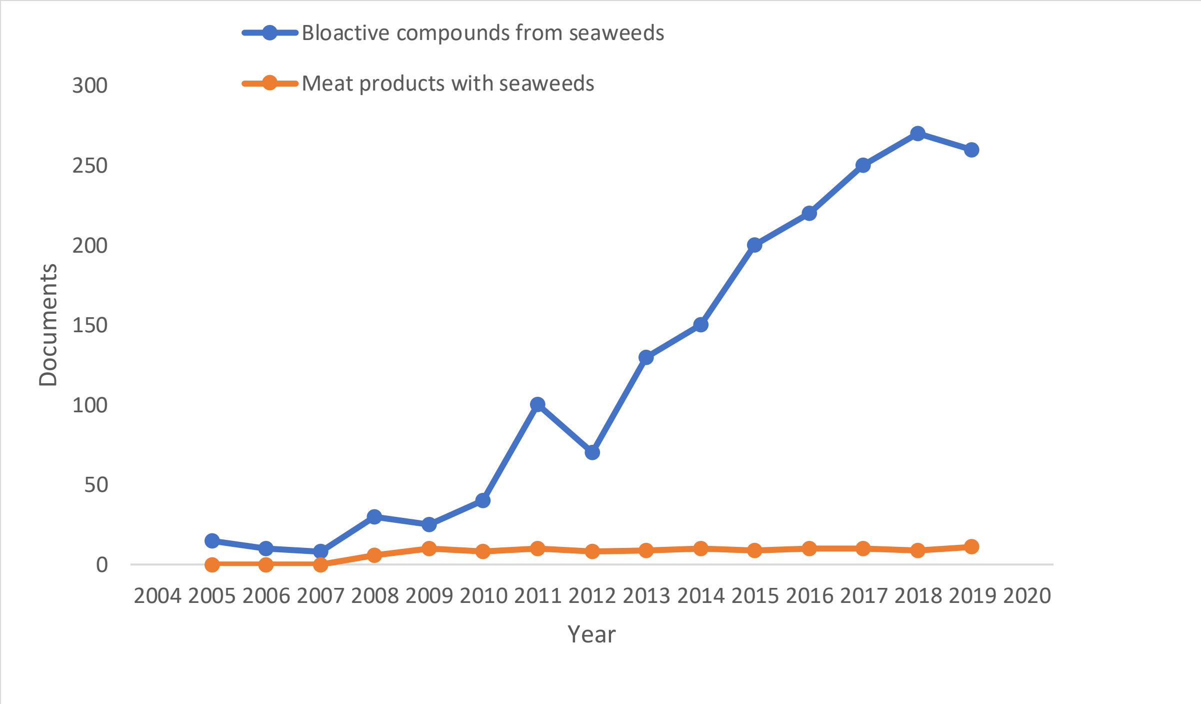

Figure 2.1. Trends in research on bioactive compounds from seaweeds and meat products using seaweed from 2005 to 2020 (Source: Gullón *et al*., 2020).

2.4 Use of seaweeds in the food sector

Countries such as Japan and Vietnam have a long history of using seaweed as animal feed, pest control, fertilizers and medicine (Wang *et al*., 2016). France is reported as the only country in Europe to have specific regulations on how to use seaweed (Mabeau & Fleurence, 1993). Some classes of seaweed, specifically *Sargassum*, *Gracilaria*, *Kappaphycus*, and *Euchema*, are widely used and have great economic benefits in Vietnam (Hong *et al.*, 2007). It appears seaweed is gaining popularity among Western countries, which are now exploring the nutritional benefits of including seaweed in the diet. The phytochemicals present in seaweed have strong biological activities that are of potential benefit to human, animal and plant health. For decades, agricultural industries around the world have utilised seaweed to improve microbial populations in the soil, stimulate root growth, inhibit the growth and proliferation of soil pathogens, treat plant diseases and improve crop yields (Craigie, 2011; Wang *et al*., 2016).

Additionally, using one or more species of seaweed in the diet increases the nutritional content of the feed and may reduce the need for food crops in human diets (Rajauria, 2015).

2.5 The use of seaweed meal in animal diets

The inclusion of seaweed as an animal feed ingredient has gained worldwide acceptance, with Canada and European, and Latin American countries heavily emphasising sustainable animal production (Rajauria, 2015). The high protein, fibre content and well-balanced amino acid profile of seaweed are some of the factors behind the rising interest in using it as a livestock supplement (Rajauria, 2015). In addition, producers have specific interests in feed ingredients that are organically derived, economically viable and globally underutilised, which has put an increased focus on seaweed as a feed source (Chojnacka *et al*., 2012; Rajauria, 2015). Most studies that include seaweeds as a protein source in animal diets have focused on aquatic cattle, especially commercial marine herbivores that consume seaweed naturally (abalone and sea urchins). Seaweeds can give abalone with a complete or partial protein diet (Kemp *et al*., 2015). Incorporating seaweed into the diets of fish (herbivores or carnivores) at inclusion levels higher than 10% reduces growth performance and feed utilisation. Some studies have shown positive outcomes for tilapia (Stadtlander *et al*., 2013).

The use of seaweeds as a source of protein in the diets of pigs and poultry has received relatively little attention. On the other hand, seaweed and its extracts have been studied extensively for their functional benefits on the immune system, gastrointestinal health status and egg and meat quality (Kulshreshtha *et al*., 2014). However, these studies employ modest inclusion rates (5%) that provide a little amount of protein to the diet and, consequently, do not supplement typical protein sources in the feed.

2.6 Potential application of seaweed as a dietary ingredient in poultry

To increase growth rates and production efficiency, the use of various feed additives in intensive animal production is required (Michalak & Chojnacka, 2009). Feeding seaweed to chickens can have a positive impact on the skin and leg colour, as well as the yolk of the eggs (Leng *et al*., 2014). It is very important that the type of feed additive selected for use in animal feeding should be accessible, have a high biological value, be affordable, stable and have a positive effect on the diet (Glencross *et al*., 2007). When these conditions are taken into account, seaweed may become an important feed component in chicken diets. Studies have revealed that seaweeds are constantly submerged in nutrient-rich seawaters, which cause them to absorb large amounts of minerals, making them an important source of animal feed (Dhargalka & Pereira, 2005).

2.6.1 Effect of seaweed on the performance and carcass traits of broilers

The use of seaweed and its extracts to improve animal performance, feed efficiency, and carcass traits has generated variable results (Gardiner *et al*., 2008; Gahan *et al*., 2009; Leonard *et al*., 2010; Michiels *et al*., 2012). The magnitude of response associated with the use of seaweed on animal performance and carcass traits has a strong correlation with the seaweed variety (red, brown, green or yellow), bioactive compounds and the level or rate of inclusion in the diet (Norziah & Ching, 2000). In contrast, *Laminaria hyperborea* and *Laminaria digitata* seaweed extracts had no significant effects on performance when compared to non-supplemented animals (Reilly *et al*., 2008). Previous research found that seaweeds contain bioactive compounds, which affect major carcass characteristics and hot carcass weight (Allen *et al*., 2001; Holdt & Kraan, 2011).

Positive benefits of dietary seaweeds on final body weight, carcass weight, and dressing percentage of broilers have been reported (Pantjawidjaja, 2011). Similarly, Abudabos *et al*.

(2013) showed that diets containing 3% green seaweed produced broilers with better growth rates, higher dressing percentages and lower abdominal fat than control birds. Other studies found that adding seaweed to chicken diets, as a substitute for corn, did not affect feed intake, body weight gain and feed conversion ratio at inclusion rates of 0, 1.0, or 3.0% (Wang *et al.*, 2013). However, the effects of seaweed on broiler production are inconsistent. A lack of significant differences was observed in FI, FCR, growth rate, dressing percentage, muscle weight and gizzard weight at seaweed inclusion levels of 0 and 4% in a broiler starter diet (EL-Deek & Brikaa, 2009). Similarly, the inclusion level of green seaweed at 3% in broiler chickens at the age of 12 – 33 days did not affect FI and FCR (Abudabos *et al.*, 2013). However, adding green seaweed higher than 10% to 21 days old broilers revealed a negative effect by reducing feed intake and growth rate (Ventura *et al.*, 1994).

2.6.2 Effect of dietary seaweed on meat quality

Studies have been conducted on the value of utilising seaweeds or their extracts in meat products to slow down oxidation and microbiological development (Roohinejad *et al.*, 2017). In addition to serving as natural preservatives, using seaweed extracts in meat products offers customers an alternative way to boost the amount of bioactive agents with health advantages in their diets. Factors that affect meat quality are important in poultry production. Meat quality is generally regarded as a very complicated issue that may be tackled from a variety of perspectives. It is essential to consider carcass conformation in addition to good aesthetic, sensory and nutritional attributes when evaluating meat quality (Bogosavljevic-Boskovic *et al.*, 2010). Despite significant improvements in broiler performance and efficiency, a widespread failure to integrate meat quality factors in selection has resulted in the emergence of anomalies in meat products (Souza *et al.*, 2011). Commercial broiler breeders' selection for characteristics like quick development rate and high breast muscle yield has long been thought to have a detrimental influence on the eating quality of broiler meat, as well as the skeletal and cardiovascular health of the live bird (Sandercock *et al*., 2009). According to Sandercock *et al*., (2009) moderate genetic variation for appearance characteristics implies that the variations seen were most likely the product of broiler phenotypic selection. Slower developing birds have been proven to have a harder texture and more intense flavour, making them more popular (Castellini *et al*., 2008). The differences in meat quality between native chickens and conventional broilers may be most clearly seen in the meat quality, including colour, taste and texture (Souza *et al*., 2011). Seaweed contains many bioactive compounds that act as flavour and moisture enhancers (Gullón *et al*., 2020). For example, mannitol, sugar alcohol similar to mannose is found in many species of brown seaweed, especially in *Laminaria* and *Ecklonia* species, and is used in a wide variety of foods, such as candies and chocolate to enhance flavour and taste (Holdt & Kraan, 2011). Because of its absorptive nature, mannitol increases moisture level, shelf life and stability of food products. Increased juiciness and flavor are observed in seaweed-fed pigs due to the presence of mannitol (Holdt & Kraan, 2011). Phycobiliproteins, particularly phycoerythrin, are natural compounds present in red seaweeds, which may influence meat colour (Holdt & Kraan, 2011). For example, phycobiliproteins have diverse applications and are currently used as a natural colourant in the dairy and cosmetic industries (Sekar & Chandramohan, 2008).

According to Abudabos *et al*. (2013), broiler chicken breast yield was enhanced by adding green seaweed up to a concentration of 30 g/kg. This is unsurprising because the composition of seaweeds varies greatly, depending on the type of species, harvesting time, habitat, water temperature, amount of light and nutrient content in water (Makkar *et al*., 2016). The rate of pH change in muscles during slaughter and after slaughter, it has a significant effect on meat quality (Muchenje *et al*., 2009). According to Wang *et al*. (2017), lower pH is frequently correlated with pale meat colour. To delay the lipid oxidation of chicken sausages, Munsu *et* *al*. (2021) investigated the effect of adding red seaweed (*Kappaphycus alvarezii*) powder as an antioxidant to deboned chicken at rates of 2, 4, and 6%. During the storage period (12 days at 4 °C), the presence of seaweed powder decreased the lightness (L^*) and increased the redness (a^*) values when compared to the control sample. The use of seaweed resulted in sausages with superior physicochemical qualities.

2.6.3 Effect of dietary seaweed on gastrointestinal health

Improving and maintaining a healthy gastrointestinal tract (GIT) is fundamental to feed efficiency as well as the overall health of animals, since the GIT is home to a highly diverse and densely populated microflora, which regulate growth and development (Jandhyala *et al*., 2015). Seaweed-derived soluble dietary fibres, such as laminarin, fucoidan, and alginates, have a positive impact on the gut microflora due to their strong antibacterial and antiviral properties that help to maintain a healthy microbial environment (Doherty *et al*., 2010). In addition, dietary fibres, such as laminarin, interact directly with the mucosal layer to increased the production of mucin from goblet cells and to strengthen mucosal stability to maintain intestinal homeostasis (Heim *et al*., 2014). Although the mechanisms by which laminarin stimulates the production of mucin are not yet known, it is hypothesised that laminarin either provides a source of substrate for intestinal microbes or stimulates mucosa cells, which corresponds with increased expression of mucin in the ileum and colon (Smith *et al*., 2011). Furthermore, Smith (2011) discovered that laminarin affected the quantity and quality of mucin produced in the piglets. Mucin is composed of several defensive compounds, which protect against microbial attachment and invasion (Heim *et al*., 2014). Although seaweed extracts demonstrated positive effects on GIT health, deliberate challenge studies are needed to determine the effectiveness of seaweeds on pathogen reduction (Gardiner *et al*., 2008). Furthermore, this would have some implications for feed efficiency, which has a strong correlation with pathogen load (Reilly *et* *al*., 2008). It would also increase the understanding of how pathogens may result in more energy being directed towards disease prevention and not production.

2.6.4 Effect of dietary seaweeds on haematological parameters

The study of the quantity and shape of the cellular components of the blood erythrocytes, leucocytes, and thrombocytes and their application in the diagnosis and monitoring of illness is referred to as haematology (Merck Manual, 2012). Haematological parameters are indicators of an animal's physiological condition (Khan & Zafar, 2005). The negative and positive health effects of seaweed on blood parameters of broiler chickens can be diagnosed by haematological parameters. According to Isaac *et al*. (2013), animals that perform well are more likely to have healthy blood composition. The existence of many metabolites and other elements in the body of animals may be investigated using blood tests, and blood plays an important part in an organism's physiological, nutritional and pathological condition (Aderemi, 2004; Doyle, 2006). In poultry production, blood parameters can give crucial information for the diagnosis and prognosis of illnesses (Olafedehan *et al*., 2010). Feed ingredients can affect the blood profile, as well as the health status of farm animals, the haematological components, such as red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration are important in monitoring feed toxicity (Oyawoye & Ogunkunle, 2004). As part of the immune response, the main functions of the white blood cell and its differentials are to fight infections, defend the body from invasion by foreign organisms through phagocytosis and produce or at least transport and distribute antibodies. Therefore, animals with high white blood cell counts may produce antibodies during phagocytosis and have a greater level of disease resistance, and those with low white blood cell counts are more susceptible to disease infection (Soetan *et al*., 2013). If there is an accident, there will be considerable blood loss, since low platelet concentration makes the coagulation (blood clotting) process take longer. Packed cell volume (PCV), haematocrit or erythrocyte volume fraction are used to describe the percentage of red blood cells in the blood (Purves & Heller, 2003). According to Isaac *et al*. (2013), the PCV is critical for the transportation of oxygen and ingested nutrients. According to Kang *et al*. (2013), adding seaweed (*Chlorella vulgaris*) had no effects on the blood leukocyte counts, such as heterophils, lymphocytes, monocytes, eosinophils, or basophils of broiler chickens. However, there are few leucocyte investigations done on farm animals fed different types of seaweed (Archer *et al*., 2007; Sotoudeh & Jafari, 2015).

2.6.5 Effect of dietary seaweeds on serum biochemical parameters

The effect of seaweed on broiler chickens can be measured by analysing the serum of the chicken. Serum biochemical indicators may be useful in the diagnosis of nutritional problems in broiler chickens (Quintavalla *et al*., 2001). In poultry production, many factors can affect the biochemical values of chicken serum, such as disease and nutrients offered (Kumar *et al*., 2003). According to Abudabos *et al*. (2013), chickens fed with *U. lactuca* at 3% level had lower cholesterol levels. Similarly, Madibana *et al*. (2017) reported that dusky hob fish fed with different graded levels of *Ulva* sp. had decreased cholesterol levels. According to Yu *et al*. (2003), feeding mice 500 mg/kg polysaccharides from *Ulva pertusa*, lowered cholesterol levels. Kulshreshtha *et al*. (2014) reported that serum concentrations of the total protein, glucose and aspartate aminotransferase (AST) of layer hens supplemented with (*Chondrus crispus*) seaweed meal for 28 days were comparable to those of the control group. Furthermore, Weber *et al*. (2013) reported no significant changes in the alanine aminotransferase (ALP) contents of plasma in laying hens fed with brown seaweed, while Choi *et al*. (2018) found no significant differences in the globulin and AST levels when a brown seaweed meal (*Ascophyllum nodosum*) treatment was administered in laying hens. Evenni *et al*. (2016) and

Rizk *et al*. (2017) findings indicated that albumin levels were elevated in layers fed diets containing brown seaweed. Albumin is associated with the functionality of the liver (Evenni *et al*., 2016). Different results were observed in other animal species, for example, a study conducted with rats fed with diets containing brown seaweed resulted in a significant drop in AST (Evenni *et al*., 2016).

2.6.6 Limitation of seaweeds as a dietary ingredient in poultry feeds

Dietary fibre refers to the non-digestible carbohydrate polymers that are edible. Both terrestrial plants and marine seaweeds naturally contain dietary fibre. Naturally, seaweeds have high levels of dietary fibre (Uribe *et al*., 2018). Seaweeds contain between 27% and 70% of their total dietary fibre as soluble fibre (Uribe *et al*., 2018). In animal diets, seaweeds have been used as a source of nutrients and bioactive compounds. However, the presence of non-starch polysaccharides and high levels of fibre have detrimental impacts on nutrient utilisation and performance, such as decreased body weight gains and poor feed conversion efficiencies in poultry. Seaweeds are preferred as a source of cellulose to terrestrial plants, since plant cellulose contains lignin that affects its processing cost, whereas most seaweed species do not contain true lignin, leading to pure cellulose fractions (Trivedi *et al*., 2015). The fibre in chicken diets is mostly used in the hind gut (i.e., caeca and colon). Animals fed high-fibre diets, particularly soluble fibre, have different feacal passage rates, microbiota, metabolites and digestion efficacy (Bach Knudsen, 2001). Due to its detrimental effects on nutrient absorption, dietary fibre has been used sparingly in poultry diets. This is because soluble dietary fibre is known to promote viscosity in the small intestine, inhibiting digestion and absorption of nutrients (Choct *et al*., 1996).

It has long been believed that insoluble fibre serves as a nutritional diluting agent in monogastric diets (Knudsen, 2001). In hens, insoluble fibre degrades slowly or not at all, resulting in a larger amount of digesta in the digestive system. As a result, its impact on the microbial community is negligible (Choct *et al.*, 1996). Due to the low energy content of diets high in insoluble fibre, birds prefer to increase feed consumption to compensate for the lower nutritional concentration in feed (Hill & Dansky, 1954). Feed components high in insoluble fibre induce an increase in the mass of the digesta, which finally leads to rapid digesta transit through the GIT. It has been observed that this impact improves digestibility (Rogel *et al.*, 1987). According to some theories, fibre reduces nutrient digestion by encapsulating nutrients in the plant cell, reducing the action of digestive enzymes. Some fibre sources may promote pancreatic enlargement, thus increasing secretions (Kratzer *et al.*, 1967). Insoluble fibre has been shown to offer certain health benefits. When oat hulls were added to chicken diets, the digestibility of starch from wheat and potatoes improved (Rogel *et al.*, 1987). Even though the nutritional content of the food is lowered, several tests have indicated that if insoluble fibre is provided in chicken diets in low quantities, the performance of the birds is unaffected (Hetland & Svihus, 2001). However, the mechanism behind the effect of modest amounts of insoluble fibre is unknown (Hetland & Svihus, 2001). The fibre in chicken diets is said to impact avian behaviour by decreasing cannibalism by causing birds to spend more time eating rather than pecking each other (Hughes & Duncan, 1972).

Other studies looking at the influence of dietary fibre on bird behaviour found that birds fed oat-based diets do not cannibalise each other (Brunberg *et al.*, 2016). Choct & Hartini (2003) discovered that birds fed a high fibre diet containing 20% NSP during the pre-lay (17-20 weeks) and early lay (21-24 weeks) periods had lower cannibalism mortality than birds fed a commercial diet. As a result, fibre in diets may inhibit pecking in chicks or young birds, resulting in a decrease in pecking-related mortalities. To improve the utilisation of seaweed in poultry, biological pre-treatment approaches, such as the usage of enzymes could be employed.

The use of enzymes to break down polysaccharides, carotenoids, and polyphenols, to mention a few, in a variety of matrices has been studied (Nadar *et al*., 2018).

2.7 Overview of exogenous enzymes

Enzymes are biological catalysts with the ability to speed up chemical reactions. Although animals can produce endogenous enzymes to digest feed, adding exogenous enzymes can increase digestion efficiency (Llamas-Moya *et al*., 2019). Currently, feed enzymes are used to decrease the amount of manure generated and the amount of phosphorus and nitrogen excreted by animals, thereby improving feed efficiency, lowering feed costs and improving environmental health (Barletta, 2010). Therefore, the need to improve the digestion of recalcitrant substrates is the primary rationale for the use of exogenous enzymes (Ravindran, 2013). The use of exogenous enzymes can increase the range of feedstuffs, including non-conventional ingredients that can be used in animal diets, thus allowing flexibility during diet formulations (Bedford & Cowieson, 2012). Additionally, pre-treatment with exogenous enzymes may result in less variation in nutrient content across batches of feed components (Bedford & Cowieson, 2012).

2.7.1 Types of enzymes available for poultry

Enzymes are a type of biological catalyst that is made up of amino acids, vitamins and minerals (Khattak *et al*., 2006). Enzymes are generated in all living organisms, from the most advanced animals and plants to the most primitive unicellular forms of life, since they are required for metabolic processes. Cellulase, ß-glucanase, xylanase, phytase, protease, lipase, and galactosidase are some of the enzymes that have been utilised in chicken feeds to counteract the effects of plant secondary metabolites Antinutritive carbohydrates, such as arabinoxylans and β-glucans, are undesirable because they inhibit the digestion and absorption of all nutrients in the diet, particularly fat and protein. Recently, there has been a lot of interest in using phytase as a feed additive because it enhances phosphate availability in plants and also lowers pollution. Protease to aid protein digestion, lipases to aid lipid digestion, ß-galactosidases to neutralise certain antinutritive components in non-cereal feedstuffs and amylase to aid starch digestion in poultry are among the enzymes being studied in the feed business. However, in most seaweed species, the digestible protein is low in terms of being an appropriate substitute protein source in livestock feed (Øverland *et al*., 2019).

2.7.2 Benefits of exogenous enzymes in poultry diets

The benefits of using exogenous feed enzymes in poultry diets include lowered digesta viscosity, improved digestion and absorption of nutrients, particularly fat and protein and improved apparent metabolisable energy (AME) value of the diet (Odetallah *et al*., 2005; Wang *et al*., 2005). Exogenous enzymes can also result in increased feed intake, weight gain, and gain-to-feed ratio, reduced beak impaction and vent plugging, decreased gastrointestinal tract size and altered microbiota (Odetallah *et al*., 2005; Wang *et al*., 2005). Monogastric animals have a considerably lower capacity to digest water-insoluble NSPs than ruminants (Iji, 1999). These water-insoluble NSPs produce a viscous digesta, which slows the pace of feed transit in the gut (Iji, 1999). Some NSPs in chicken feeds, such as cellulose, xylose, arabinose, and galactonic acid, are difficult for the birds to digest. The antinutritional impact of these NSPs is indicated by stunted development due to reduced nutrient consumption (Annison & Choct, 1991). Exogenous enzymes can be supplemented in the diet to counteract their negative effects (Danicke *et al*., 2000). Enzymes have mostly been used in chicken feeds to aid in the hydrolysis of fibre or NSP fractions. These NSPs are indigestible by poultry endogenous enzymes and may have antinutritive effects. The viscosity of fluids in the digestive system is enhanced as a result of these NSPs' ability to bind significant quantities of water. Increased viscosity creates difficulties in the small intestine because it decreases the substrate-enzyme interaction, lowering nutritional availability, particularly fat (Danicke *et al*., 2000) and resulting in more sticky droppings (Danicke *et al*., 2000).

2.7.3 Application of exogenous enzymes

Enzymes are produced by a selected strain of microorganisms that are grown under strict conditions to target a specific substrate. The rate of an enzyme-catalysed reaction increases with increasing substrate concentration until the enzyme is saturated and no longer reacts, which is an additional key feature of enzymes. As a result, the amount of enzyme must be proportional to the amount of substrate (Acamovic & McCleary, 1996). Enzyme addition to the diet may reduce performance differences and nutritional problems (Maisonnier *et al.*, 2001).

2.7.4 Types of enzymes used in poultry diets

Enzyme classes and their substrates are presented in Table 2.4 for monogastric animals. The different enzymes added to chicken diets are quite selective for certain substrates and their related chemical linkages (Munir & Maqsood, 2013). Most of the enzymes created for animal feeds do not always have activity for these substrates. It is uncommon for commercially manufactured enzymes to have a single activity in animal diets. This can be amply demonstrated when diets pre-treated with carbohydrases were shown to increase the uptake and synthesis of vitamins and fatty acids in chicken tissues and eggs (Maisonnier *et al*., 2001).

Table 2.4. Classes of feed enzymes and their substrates

Enzyme class	Substrates
Pectinases	Pectins
Amylases	Starch
Arabinoxylanases	Arabinoxylans

Cellulases	Cellulose and hemicellulose
Hemicellulases	Hemicellulose
Acid/Alkaline proteases	Proteins
Phytases	Phytic acid esters
Lipases	Lipids
Esterases	Fats and esters
β-Glucanases	β-glucans

2.7.5 Application methods of enzymes in poultry production

Different kinds of enzymes are provided to complement the diets of animals (Asmare, 2014). They can either be supplied as powders and added to the diet before mixing and pelleting or they can be added as granules before the diets are mixed and pelleted. Both of these processes allow the enzymes to interact closely with the feed components, which may help them to react with their substrates more successfully. However, due to their tendency to be thermolabile, many enzymes may have their activity reduced when processed at high temperatures (Acamovic, 2001). An innovation from recent times is the addition of liquid enzymes after pelleting. The drawback of this is that the enzyme is coated onto the surface of the pellets, preventing it from having close contact with all feed particles. When compared to applying the enzyme prior to pellet formation, this may lessen the enzyme pre-ingestion effect. However, adding liquid enzymes to the surface of pellets can frequently have a greater positive impact on both performance and the nutritional content of the diet (Acamovic, 2001).

2.7.6 The use of fibrolytic enzymes in poultry nutrition

Multi-enzyme complexes known as fibrolytic enzymes comprise a variety of carbohydrases, such as arabanase, cellulase, β-glucanase, hemicellulase and xylanase (Kithama *et al*., 2021).

Fibrolytic enzyme is used in a variety of industries to extract valuable products from plant materials and in the processing of grains and vegetables by lowering the viscosity of plant-derived materials, thus enhancing extraction yields. Fibrolytic enzymes were also shown to be effective enzyme for the extraction of polyphenols (Barahona *et al*., 2006).

2.7.7 The use of protease enzymes in poultry nutrition

The actions of proteolytic enzymes may be classified into two groups (Saxena *et al*., 2013). Firstly, restricted proteolysis occurs when a protease cleaves only one or a small number of peptide bonds of a target protein, causing the previously inactive protein to become active. Protease is used primarily to increase protein digestion, energy efficiency and animal productivity. Anti-nutritional substances, such as trypsin inhibitors and lectin, obstruct digestion in monogastric animals (Lalles, 1993; Ghazi *et al*., 2003). Heat during processing can substantially minimise the negative impacts of these anti-nutritional variables, but it also raises processing costs and has the potential to damage other nutrients in soybean meal (Sissons *et al*., 1982; Coon *et al*., 1990; Ghazi *et al*., 2003). Adeola *et al*. (2007) investigated the effects of protease in pekin ducks aged 9 to 10 weeks and reported decreased total dry matter production while increasing nitrogen retention. Protease was added to broiler chicken diets at a rate of 200 mg/kg, resulting in increased apparent digestibility of starch and fat when compared to the control group (Kalmendal & Tauson, 2012). Treating seaweed with protease enzyme can improve the utilisation of the meal in broiler chickens because seaweed can have a high protein content of up to 38% (*Ulva laetevirens* and *Solieria chordalis* seaweed) on a DM basis (Biancarosa *et al*., 2017).

2.8 Summary

Broiler producers strive to provide high-quality meat at a reasonable cost. This goal is difficult to achieve without preparing a nutritionally balanced diet that is cost-effective because feed alone accounts for almost 70% of total production costs. Seaweeds as a rich source of bioactive compounds, when included in poultry diets, can improve their health and performance (Holdt & Kraan, 2011). Nonetheless, the use of seaweeds in poultry feed has generated contradictory and unpredictable effects on broiler performances. In some cases, it has improved performance while in other cases performance has been depressed, especially at higher inclusion levels. There are several reasons for this discordance, including the use of different seaweed species and different dietary inclusion levels, among other factors. Therefore, it is important to enhance the feed value of seaweeds in broiler diets, with the aid of exogenous enzymes to allow for higher dietary inclusion levels. Accordingly, this work aimed to enhance the feed value of seaweed (*Ulva sp*.) for Cobb 500 broiler chickens using exogenous fibrolytic and proteolytic enzymes. Theoretically, the use of exogenous enzymes has several benefits including decreasing undigested components in the diet, improving the availability of amino acid profile, protein digestibility, body weight gain and feed conversion efficiency. Supplementation of feed with exogenous enzymes help to reduce the amount of nutrient excretion, which, if ignored, can result in extra costs to the farmers and feed industries and can be detrimental to the environment (Sheppy, 2010; Ali & Abdelaziz, 2018). Feed accounts for most production costs, thus the use of exogenous enzymes in poultry production can be important in the reduction of feed costs by poorly absorbing other nutrients. While there is potential for exogenous feed enzymes to resolve the problem of poor utilisation of seaweeds in poultry, this strategy has not been extensively evaluated in broiler chickens.

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3 CHAPTER THREE – EFFECT OF DIETARY INCLUSION OF GREEN SEAWEED ON GROWTH PERFORMANCE, BLOOD PARAMETERS AND MEAT QUALITY AND STABILITY TRAITS OF BROILER CHICKENS

This chapter was published in the journal: *Agriculture* (Appendix 3; **Matshogo, T. B**., Mnisi, C. M. & Mlambo, V. **2020**. Dietary green seaweed compromises overall feed conversion efficiency but not blood parameters and meat quality and stability in broiler chickens. Agriculture. 10(11), 547; https://doi.org/10.3390/agriculture10110547)

Abstract

The utilisation of seaweeds as a source of nutrients and beneficial bioactive substances can help to ensure that functional poultry products are produced sustainably. The objective of this experiment was to investigate the effect of dietary green seaweed (*Ulva sp.*) meal on growth performance, physiological and meat quality traits of Cobb 500 broiler chickens. The feeding trial used 300, two-week-old male chicks (159.3 ± 11.76 g live-weight), which were randomly assigned to five experimental diets formulated by diluting a standard broiler diet with seaweed meal (SWM) at 0 (SW0), 20 (SW20), 25 (SW25), 30 (SW30) and 35 g/kg (SW35). There were neither linear nor quadratic trends ($P > 0.05$) for overall feed intake, overall body weight gain, carcass characteristics, and meat quality traits of Cobb 500 broiler chickens. However, overall gain-to-feed ratio [$R^2 = 0.192$, $P = 0.018$] and spleen weights [$R^2 = 0.182$; $P = 0.020$] linearly declined as SWM levels increased. There were no linear or quadratic responses ($P > 0.05$) for all blood parameters, except for lymphocytes, which showed a significant linear and quadratic effect in response to dietary SWM levels [$R^2 = 0.123$, $P = 0.042$]. For meat stability, quadratic trends ($P < 0.05$) in response to SWM were observed for pH on days 4, 5 and 6 of storage. Meat lightness (L*) linearly increased on days 1, 2, 3 and 7 of storage, whereas meat redness (a*) quadratically responded to SWM levels only on day 3 of storage. While there was no optimal inclusion level for dietary SWM based on growth performance, broilers raised on seaweed meal-containing diets showed benefits in several meat shelf-life indices (meat pH, lightness, redness and yellowness). In conclusion, the use of green seaweed meal as a dietary component in broiler diets can reduce the overall feed conversion efficiency without any negative effects on blood parameters and meat quality and stability traits. This may be due to the presence of non-starch polysaccharides, such as hemicellulose and cellulose in seaweeds. However, gain-to-feed ratio responses revealed that high levels of SWM are detrimental to birds. This necessitated the investigation for pre-treatment of seaweed meal using exogenous enzymes to improve its utilisation by broiler chickens.

Keywords: Broilers; Bioactive compounds; Blood parameters; Growth performance; Meat quality; Seaweed meal.

3.1 Introduction

When compared to other commercial broiler breeds produced across the world, the Cobb 500 broiler chicken is a contemporary commercial breed recognised for excellent feed conversion efficiency, quick development rates and competitive breast meat outputs at various processing stages (Coneglian *et al.*, 2020). However, even for this efficient bird, feed expenses account for about 70% of total production costs. According to Mnisi & Mlambo (2018), the utilisation of low-cost, unconventional feed ingredients has attracted research attention worldwide. Functional feed ingredients with growth-stimulating and product-enhancing characteristics, such as seaweeds, can be incorporated into chicken feeds to improve efficiency and sustainability of the South African poultry industry. This is because seaweeds have bioactive compounds with antioxidant and antibacterial properties that can potentially enhance feed utilisation efficiency and meat quality (Nhlane *et al.*, 2020).

In South Africa, nearly 2884 tonnes (wet weight) of seaweed are produced per year (DAFF, 2012) for use as a feed additive in abalone farms (Troell *et al*., 2006). Seaweeds contain polysaccharides, fucoidan and laminarin, which can be used as sources of beneficial bioactive substances due to their diverse biological effects (Sweeney & O'Doherty, 2016). Seaweeds have been used in animal diets as sources of protein, essential amino acids, omega-3 fatty acids, minerals, carotenoids pigments, vitamins, phenolics, phlorotannins and prebiotic substances (Evans & Critchley, 2014; Gullón *et al*., 2020). Antimicrobial, anticoagulant, anti-inflammatory, antioxidant and prebiotic activities have been demonstrated for most of these phytonutrients (Rodrigues, 2015). Other researchers have reported that including seaweeds in animal diets extends the shelf life of meat products and maintains their quality during processing and storage (Kumar *et al*., 2015; Fernández-Segovia *et al*., 2018). Because of the positive bioactivities of seaweed phytonutrients, seaweed-based diets may prevent subclinical and clinical infections, thus improving animal performance (Bocanegra *et al*., 2021). However, the presence of non-starch polysaccharides like cellulose and hemicellulose in seaweeds may limit their use as feed components in chicken production (Gullón *et al*., 2020). When fed at high levels, cellulose and hemicellulose decrease digestion, which may interfere with the absorption of important bioactive substances that are useful for growth performance and meat quality and stability. As a result, maximum tolerance limits for dietary seaweeds must be determined to avoid compromising growth and meat quality characteristics in broilers. Thus, the objective of this study was to test the effect of incremental levels of dietary SWM on growth performance, blood parameters, internal organs, carcass features, and breast meat quality and stability traits in Cobb 500 broiler chickens. The experiment tested the hypothesis that adding different levels of SWM in broiler meals will enhance Cobb 500 broiler chickens' growth performance, physiological responses, and meat quality parameters and allow the determination of an optimum inclusion level.

3.2 Materials and methods

3.2.1 Ethics statement

The procedures used to rear, handle and slaughter the broiler chickens for all the feeding trials were reviewed and approved by the Research Ethics Committee for Animal Production studies of the North West University (approval no. NWU-00356-19-A5; Appendix 1).

3.2.2 Study site and ingredient sources

The study was conducted during the winter season in North-West University Research Farm (26º41′36″ S, 27º05′35″ E) in Mafikeng, South Africa. Temperatures was ranging between 16ºC and 20ºC. The seaweed used in this study was collected from Aquinion Abalone Farm (34º34′58″ S, 19º21′8″ E) in Gansbaai (Western Cape, South Africa). All the other feed ingredients used in the study were purchased from Simplegrow Agric Services (PTY) LTD (Gauteng, South Africa). Seaweed was oven-dried (60 °C) and then milled (2 mm, Retsch grinder, 42781 Haan, Germany) to produce seaweed meal (SWM).

3.2.3 Diet formulation and analyses

The seaweed meal was analysed for its nutritional composition (AOAC, 2005) before diet formulations and was found to contain 856.5 g/kg dry matter (DM), 375.2 g/kg DM ash, 175.9 g/kg DM crude protein (CP), 339.3 g/kg DM crude fat, 322.5 g/kg DM neutral detergent fibre (NDF) and 171.1 g/kg DM acid detergent fibre (ADF). A nutritional software was used to formulate five isonitrogenous and isocaloric diets, in a mash form, according to NRC (1994) guidelines. The experimental diets were formulated (Table 3.1) for grower and finisher phases as follows: 1) SW0 = a standard grower/finisher diet with no seaweed meal, 2) SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, 3) SW25 = a standard grower/finisher diet in with 25 g/kg of seaweed meal, 4) SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and 5) SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal.

Table 3.1. Gross ingredient composition (g/kg, *as fed* basis) of experimental diets in grower and finisher phases

	Grower					Finisher				
	SW0	SW20	SW25	SW30	SW35	SW0	SW20	SW25	SW30	SW35
AxtraPhy10000	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Crude soya oil mixer	7.16	0	0	0	0	15	7.18	3.827	0.76	11.39
DL-Methionine	2.82	2.88	2.88	2.90	2.92	2.08	2.27	2.29	2.32	2.29
Extruded full fat soya	120.0	81.10	61.49	46.59	31.88	120.0	120.0	120.0	120.0	34.57
Finisher premix	0	0	0	0	0	2.0	2.0	2.0	2.0	2.0
Grower premix	2.5	2.5	2.5	2.5	2.5	0	0	0	0	0
Lignobond	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Limestone	11.94	9.66	9.07	8.48	7.89	11.16	8.82	8.26	7. 70	7.05
L-Threonine	0.67	0.84	0.88	0.93	0.98	0.31	0.47	0.52	0.57	0.58
Lysine HCL	2.72	3.01	3.12	3.26	3.40	1.81	1.59	1.69	1.80	1.85
Monocalcium phosphate	7.80	8.05	8.14	8.21	8.28	5.65	5.66	5.70	5.75	5.99
Oil Crude Soya 47%	176.6	192.8	203.0	207.5	211.9	150.6	163.4	160.0	156.8	218.1
Oil Crude Sunflower 36%	30.00	30.00	31.64	36.60	41.51	30.0	30.0	30.0	30.0	30.0
Prime gluten 60	0	0	0	0	0	17.62	0	0	0	0
Salinomycin 12%	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt-fine	2.56	0.56	0.06	0	0	2.55	0.57	0.07	0	0
Seaweed (*Ulva sp.*)	0	20.0	25.0	30.0	35.0	0	20.0	25.0	30.0	35.0
Sodium bicarbonate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Yellow maize 8.0%	630.3	643.7	647.3	648.1	648.8	636.3	633.2	635.7	637.4	646.3
Zinc Bacitracin	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33

SW0 = a standard diet with no seaweed meal, SW20 = a standard diet with 20 g/kg of seaweed meal, SW25 = a standard diet with 25 g/kg of seaweed meal, SW30 = a standard diet with 30 g/kg of seaweed meal, and SW35 = a standard diet with 35 g/kg of seaweed meal.

3.2.4 Diet composition

The calculated values based on diet formulae of experimental diets are presented in Table 3.2. The diets were calculated for dry matter, organic matter, crude protein, amino acids (AP lysine, methionine and threonine), ash, crude fibre, crude fat, metabolisable energy, nitrogen and minerals (calcium, phosphorus, chloride, and sodium).

Table 3.2. Calculated values based on diet formulae (g/kg, unless stated otherwise) of experimental diets in grower and finisher phases

	Grower					Finisher				
	SW0	SW20	SW25	SW30	SW35	SW0	SW20	SW25	SW30	SW35
[1]ME (MJ/kg)	12.92	12.92	12.92	12.92	12.92	13.26	13.26	13.26	13.26	13.26
Dry Matter	884.8	882.5	882.1	881.8	881.6	885.0	883.0	882.3	881.7	882.3
AP Methionine	5.6	5.6	5.6	5.6	5.6	4.98	4.98	4.98	4.98	4.98
AP Lysine	10.65	10.65	10.65	10.65	10.65	9.5	9.5	9.5	9.5	9.5
AP Threonine	6.9	6.9	6.9	6.9	6.9	6.5	6.5	6.5	6.5	6.5
Ash	25.12	30.92	32.39	33.91	35.44	24	30.87	32.32	33.77	35.16
Crude fat	56.16	43.35	40.23	37.87	35.54	64.17	56.7	53.62	50.78	46.87
Crude fibre	35.45	44.0	46.23	49.05	51.87	34.83	44.5	46.8	49.09	49.88
Crude protein	192.2	192.2	192.2	192.2	192.2	189.5	189.5	189.5	189.5	189.5
Chloride	2.4	2.71	2.79	3.15	3.54	2.26	2.49	2.58	2.92	3.29
Sodium	1.8	1.8	1.8	1.97	2.16	1.8	1.8	1.8	1.96	2.16
Total phosphorus	5.48	5.46	5.46	5.46	5.47	4.95	4.97	4.97	4.97	4.94
Avl phosphorus	4.2	4.2	4.2	4.2	4.2	3.8	3.8	3.8	3.8	3.8
Calcium	8.4	8.4	8.4	8.4	8.4	7.6	7.6	7.6	7.6	7.6

SW0 = a standard diet with no seaweed meal, SW20 = a standard diet with 20 g/kg of seaweed meal, SW25 = a standard diet with 25 g/kg of seaweed meal, SW30 = a standard diet with 30 g/kg of seaweed meal, and SW35 = a standard diet with 35 g/kg of seaweed meal.

[1]ME = metabolizable energy.

3.2.5 Experimental design

A total of 300-day-old male Cobb 500 broiler chicks were bought from Montshego Chicken Farm (25º03'49" S, 26º15'64" E) in Zeerust (North West, South Africa). The birds were weighed and allocated randomly and equally to 30 pens, which measured 3.5 m × 1.0 m × 1.85 m (L × B × H), and were designated as experimental units. Sunflower husks were used as bedding and the house was cleaned once every week. For the first three days, the chicks were given vitamins and electrolytes (stress pack) in their drinking water and fed a regular commercial starter meal until they were 10 days old. The birds were acclimated to the experimental meals starting from day 11 to day 13. The five experimental diets were randomly allocated to the 30 experimental units, which were replicated six times per treatment. For the first two weeks, the house temperature was kept at 35°C, using infrared electric bulbs to guarantee a consistent source of heat. Growth performance data were collected from days 14 to 28 and days 29 to 49 for the grower and finisher phases, respectively. The birds had free access to the experimental feeds and clean, fresh water during the feeding trial.

3.2.6 Feed intake and growth performance

All the birds were weighed to determine initial weights and, thereafter, weighed weekly using a weighing scale (ADAM scale, readability 0.5 g to 2 g, South Africa) to determine average weekly body weight gain (ABWG). The difference between the feed offered and refusals collected in the morning before feeding was used to determine daily feed intake. For each pen, the average daily feed intake (ADFI) was determined as follows:

$$ADFI = \frac{Feed\ offered - feed\ refusal}{Number\ of\ birds}$$

Gain-to-feed ratio (G:F) was calculated as average weight gain divided by average feed intake.

3.2.7 *Blood collection and analysis*

At 47 days, two birds from each pen were used for blood collection. Using 23-gauge needles and 5-ml syringes, blood samples were drawn from the brachial vein and promptly transferred to serum and whole blood tubes. An automated IDEXX Laser Cyte Haematology Analyser (IDEXX Laboratories Inc., Gauteng, South Africa) was used to determine haematocrit, white cell count (WCC), platelets, heterophils, lymphocytes and monocytes. An automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories Inc. Gauteng, South Africa) was used to analyse glucose, symmetric dimethylarginine (SDMA), creatinine, albumin, lipase, blood urea nitrogen to creatinine ratio (BUN/CREA), phosphorus, calcium, total protein, gamma-glutamyl transferase (GGT) and amylase.

3.2.8 *Slaughter procedures, carcass traits and internal organ weights*

At 49 days, all the birds were taken to a local abbatoir, and upon arrival, the birds were electrically stunned and slaughtered by cutting the jugular vein with a sharp knife. The plucker machine was used to remove feathers after bleeding, and then the evisceration was done manually. Carcasses were identified in each experimental unit using woollen fibre of various colours attached to the drumstick. The hot carcass weight (HCW) was taken right after slaughter, whereas the cold carcass weight (CCW) was taken 24 hours later. The percentage of HCW on final body weight (FBW) was used to calculate carcass yield. The weights of the breast, drumstick, wing, thigh, gizzard, proventriculus, spleen, liver, duodenum, jejunum and ileum) and large intestines, including the caeca, were measured (ADAM scale, readability 0.001 g to 0.01 g, South Africa).

3.2.9 Meat pH and colour

A portable meat pH meter (HI98163, Hanna Instruments, Romania) equipped with a spear-type electrode was used to measure the pH of the breast 24h post-mortem. The pH meter was calibrated with pH 4, pH 7, and pH 10 standard solutions after every 10 readings. Using a Minolta colour-guide, colour coordinates (L^* = brightness, a^* = redness and b^* = yellowness) were calculated in triplicate on the surface of the breast muscle (BYK-Gardener GmbH, Geretsried, Germany). The colour guide was calibrated and adjusted, as instructed by the manufacturer.

3.2.10 Cooking loss and meat tenderness

Cooking losses were determined following the modified method described by Honikel (1998), wherein pre-weighed breast meat samples (w1) were cooked to a core temperature of 75°C. After allowing the cooked meat samples to cool for 20 minutes, they were then re-weighed to get the cooked weight (w2). Cooking loss was estimated as the difference in weight between raw and cooked meat in proportion to the raw meat weight. The following formula was used to calculate the percentage of cooking loss:

$$Cooking\ loss\ (\%) = \frac{w1 - w2}{w1} \times 100$$

The raw breast meat samples were sheared to determine shear force (N), a measure of meat softness, using a Meullenet-Owens Razor Shear Blade (A/MORS) placed on a Texture Analyser (TA. XT plus, Stable Micro Systems, Surrey, UK).

3.2.11 Water holding capacity, drip loss and shelf-life measurements

The water holding capacity (WHC) of the meat was measured in duplicate samples on the surface of a freshly cut slice of the pectoralis major muscle (PMM) (8-16 grams). The WHC was determined as the amount of water expressed from fresh meat held under pressure (60 kg pressure) using the filter-paper press method developed by Grau and Hamm (1957). The water from the fresh meat was taken up by a pre-weighed filter paper and calculated as a percentage. The meat WHC was then determined using the following formula:

$$\text{WHC } (\%) = 100 - [\frac{\text{initial meat weight} - \text{ meat weight after pressing}}{\text{initial meat weight}} \times 100]$$

Drip loss was determined using a method adapted from Zhang *et al.* (2009). Pieces of muscle from the pectoralis major muscle (PPM) weighing ~ 2 grams (wet weight, w1) were hooked using a wire and suspended in a plastic container so that the samples did not touch the sides of the bottle, which was then sealed. The suspended samples were stored in a cold room at 4°C for 72 hours. The meat samples were then reweighed to obtain weight after drip (w2). The difference in weight of each sample before and after drip was conveyed as percentage drip loss and calculated as follows:

$$\text{Drip loss } (\%) = (\frac{\text{w1} - \text{ w2}}{\text{w1}} \times 100)$$

Where; w1 is the initial weight and w2 is the weight after drip.

3.2.12 Statistical analysis

Data for linear and quadratic effects were evaluated using polynomial contrasts in a response surface regression analysis (PROC RSREG; SAS, 2010). The following quadratic model was used:

$$y = ax^2 + bx + c$$

where y = response variable; a and b are the coefficients of the quadratic equation; c is intercept; x is exogenous enzyme pre-treatment level (%) and $-b/2a$ is the x value for minimum or maximum response.

Repeated measures analysis in the general linear model procedure (GLM PROC) of SAS (2010) was used to analyse weekly feed intake, growth rate, and gain-to-feed ratio and shelf-life data. The following linear statistical model was used:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk}$$

Where, Y_{ijk} = dependant variable, μ = population mean, D_i = effect of diet, W_j= effect of weeks $(D \times W)_{ij}$ = the effect of interaction between diets and weeks and E_{ijk} = random error associated with observation ijk, assumed to be normally and independently distributed.

The GLM procedure of SAS (2010) was also used to analyse overall growth performance, blood parameters, carcass features, and meat quality data using one-way analysis of variance (ANOVA). The following linear statistical model was used:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where, Yij = dependant variable, μ = population mean, Di = effect of meals, and Eij = random error associated with observation assumed to be normally and independently distributed. For all statistical tests, significance was declared at P <0.05. Least squares means (LSMEANS) were compared by using the probability of difference option in the LSMEANS statement of SAS.

3.3 Results

3.3.1 Feed intake and physiological responses

Repeated measures analysis revealed no week (chicken age) × diet interaction effect on FI, BWG and G:F. Table 3.3 shows that there were neither linear nor quadratic trends ($P > 0.05$) for overall feed intake, initial and final body weight as well as overall body weight gain in response to dietary SWM levels. Overall G:F linearly decreased [$y = 0.533\ (\pm 0.014) - 0.002\ (\pm 0.0018)\ x$; $R^2 = 0.192$, $P = 0.018$] with increasing levels of dietary SWM.

Table 3.3. Overall feed intake and growth performance of Cobb 500 broiler chickens offered seaweed meal-containing diets

	[1]Diets						*P* value	
	SW0	SW20	SW25	SW30	SW35	[2]SEM	Linear	Quadratic
Initial BW (g/bird)	157.0	160.8	157.2	159.1	162.3	5.18	0.475	0.863
Overall FI (g/bird)	2311.8	2424.6	2313.8	2397.8	2286.0	74.75	0.846	0.277
Overall BWG (g/bird)	1233.3	1218.2	1144.3	1180.1	1125.1	61.11	0.249	0.710
Overall G:F (g:g)	0.533	0.502	0.493	0.490	0.490	0.014	0.019	0.586
Final BW (g/bird)	1390.3	1379.1	1301.5	1339.2	1287.4	61.29	0.274	0.721

[1]Diets: SW0 = a standard diet with no seaweed meal, SW20 = a standard diet with 20 g/kg of seaweed meal, SW25 = a standard diet with 25 g/kg of seaweed meal, SW30 = a standard diet with 30 g/kg of seaweed meal, and SW35 = a standard diet with 35 g/kg of seaweed meal.
[2]SEM = standard error of the mean.

There were significant linear and quadratic effects for lymphocytes [$y = 0.030\ (\pm 0.014)\ x^2 - 1.329\ (\pm 0.489)\ x + 32.8\ (\pm 3.85)$; $R^2 = 0.123$, $P = 0.042$] in response to dietary SWM levels

(Table 3.4). However, neither linear nor quadratic trends (*P* >0.05) were observed for all the other haematological parameters with dietary SWM levels.

Table 3.4. The effects of seaweed meal-containing diets on haematological parameters of Cobb 500 broiler chickens

	[1]Diets						*P* value	
Blood indices	SW0	SW20	SW25	SW30	SW35	[2]SEM	Linear	Quadratic
Haematocrits (%)	32.02	32.17	31.67	31.42	33	0.884	0.781	0.481
Heterophils (%)	7.43	11.97	12.37	10.45	14.16	3.252	0.181	0.872
[3]WCC ($\times 10^9$/L)	11.03	16.27	16.75	13.66	18.36	2.898	0.124	0.759
Platelets ($\times 10^9$/L)	7.87	10.15	10.05	8.24	11.06	2.04	0.401	0.891
Lymphocytes ($\times 10^9$/L)	3.29	2.19	2.91	2.97	2.46	0.438	0.028	0.042
Monocytes ($\times 10^9$/L)	1.02	1.02	0.81	0.81	0.96	0.332	0.077	0.601

[1]Diets: SW0 = a standard grower/finisher diet with no seaweed meal, SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, SW25 = a standard grower/finisher diet in with 25 g/kg of seaweed meal, SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal.
[2]SEM = standard error of the mean.
[3]WCC = white cell count.

In response to increasing amounts of dietary SWM, there were no linear or quadratic (*P* > 0.05) trends for glucose, SDMA, creatinine, albumin, lipase, BUN/CREA, phosphorus, calcium, total protein, GGT and amylase as shown in Table 3.5.

Table 3.5. The effects of seaweed meal-containing diets on serum biochemical parameters of Cobb 500 broiler chickens

[3]Blood indices	[1]Diets					[2]SEM	*P* value	
	SW0	SW20	SW25	SW30	SW35		Linear	Quadratic
Glucose (mmol/L)	6.34	7.28	4.43	6.438	5.89	0.742	0.839	0.549
SDMA (µg/dL)	3.92	4.92	6.93	4.63	5.52	1.523	0.707	0.409
Creatinine (µmol/L)	24.5	31.49	33.77	24.75	54.83	11.89	0.177	0.064
Albumin (g/L)	32.42	43.3	54.58	37.25	53.8	9.836	0.866	0.954
Lipase (U/L)	3.56	5.46	4.29	3.75	5.59	0.664	0.921	0.053
BUN/CREA	27.63	14.33	23.71	27.33	24.19	5.988	0.987	0.376
Phosphorus (mmol/L)	4.13	2.55	4.37	4.13	3.62	0.485	0.681	0.359
Calcium (mmol/L)	76.82	117.4	82.58	104.5	92.58	11.56	0.132	0.484
Total protein (g/L)	97.5	58.08	94.67	83.08	88.92	10.85	0.499	0.141
GGT (U/L)	320.5	206.6	295.8	330.1	328.8	61.53	0.837	0.64
Amylase (U/L)	303.2	441.3	471	331.5	346.2	0.254	0.492	0.838

[1]Diets: SW0 = a standard grower/finisher diet with no seaweed meal, SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, SW25 = a standard grower/finisher diet in with 25 g/kg of seaweed meal, SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal.

[2]SEM = standard error of the mean.

[3]Blood indices: SDMA = symmetric dimethylarginine; BUN/CREA = blood urea nitrogen /creatinine ratio; GGT = gamma-glutamyl transferase.

3.3.2 Carcass traits and internal organ weights

The effect of graded levels of dietary SWM on carcass trait in Cobb 500 broiler chickens is shown in Table 3.6. There were no significant linear or quadratic trends for the carcass characteristics.

Table 3.6. The effects of seaweed meal-containing diets on carcass characteristics (% HCW, unless stated otherwise) of Cobb 500 broiler chickens

	[1]Diets						*P* value	
	SW0	SW20	SW25	SW30	SW35	[2]SEM	Linear	Quadratic
Breast	21.13	23.19	20.43	21.69	23.92	1.064	0.268	0.531
Drumstick	6.41	6.62	6.13	6.43	6.87	0.27	0.514	0.353
Wing	6.12	5.81	5.61	6.11	6.27	0.252	0.879	0.067
Thigh	7.47	7.04	6.82	7.39	7.56	0.371	0.959	0.135
[3]HCW (g)	967.3	976.9	912.4	929.3	917.9	47.79	0.378	0.748
[4]CCW (g)	952.4	957.7	895.3	913.3	889.7	47.65	0.306	0.675
Carcass yield (%)	69.48	71.22	70.17	69.21	71.18	1.745	0.693	0.869

[1]Diets: SW0 = a standard grower/finisher diet with no seaweed meal, SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, SW25 = a standard grower/finisher diet with 25 g/kg of seaweed meal, SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal.
[2]SEM = standard error of the mean.
[3]HCW = hot carcass weight.
[4]CCW = cold carcass weight.

The effect of incremental levels of dietary SWM on internal organ weights in Cobb 500 broiler chickens is shown in Table 3.7. Except for spleen weights, which linearly declined [y = 0.24

(±0.016)‑0.000 (±0.002) x; R^2 = 0.182; P = 0.020] with SWM levels, there were no significant linear or quadratic trends for the other internal organ weights.

Table 3.7. The effects of seaweed meal-containing diets on internal organ weights (% HCW, unless stated otherwise) of Cobb 500 broiler chickens

	[1]Diets						*P* value	
	SW0	SW20	SW25	SW30	SW35	[2]SEM	Linear	Quadratic
Duodenum	1.97	1.71	2.02	2.13	2.01	0.269	0.736	0.539
Jejunum	3.35	2.39	3.41	3.65	3.39	0.872	0.827	0.504
Ileum	3.75	3.08	2.14	2.97	3.12	0.825	0.419	0.444
Large intestine	0.65	1.17	0.88	0.84	0.49	0.249	0.914	0.052
Caeca	1.04	1.05	1.15	1.16	1.12	0.054	0.141	0.928
Gizzard	2.64	2.64	2.567	2.76	2.61	0.111	0.895	0.947
Proventriculus	0.67	0.65	0.65	0.9	0.76	0.101	0.307	0.469
Spleen	0.24	0.21	0.22	0.21	0.17	0.016	0.02	0.464
Liver	3.57	3.39	3.48	3.58	3.59	0.148	0.894	0.265

[1]Diets: SW0 = a standard grower/finisher diet with no seaweed meal, SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, SW25 = a standard grower/finisher diet with 25 g/kg of seaweed meal, SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal.
[2]SEM = standard error of the mean.

3.3.3 Meat quality and stability

In response to increasing amounts of dietary SWM, there were no linear or quadratic ($P > 0.05$) trends for meat pH, colour, cooking loss, shear force, drip loss and WHC as shown in Table 3.8.

Table 3.8. The effects of seaweed meal-containing diets on meat quality parameters of Cobb 500 broiler chickens

	[1]Diets						*P* value	
	SW0	SW20	SW25	SW30	SW35	[2]SEM	Linear	Quadratic
Cooking loss (%)	25.40	24.45	23.54	25.11	24.67	1.171	0.621	0.469
Shear force (N)	9.33	9.22	9.63	9.03	7.55	1.060	0.388	0.286
Drip loss (%)	7.87	7.45	7.93	7.58	8.55	0.667	0.671	0.342
[3]WHC (%)	12.22	12.33	16.66	12.05	13.10	0.919	0.451	0.278
pH	6.22	6.09	6.36	6.25	6.33	0.091	0.361	0.349
*L** (lightness)	51.90	55.04	51.39	53.13	51.45	1.100	0.906	0.105
*a** (redness)	1.03	0.98	0.92	0.95	1.16	0.153	0.849	0.336
*b** (yellowness)	10.08	9.41	10.11	9.92	10.28	0.505	0.839	0.313

[1]Diets: SW0 = a standard grower/finisher diet with no seaweed meal inclusion, SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, SW25 = a standard grower/finisher diet with 25 g/kg of seaweed meal, SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal.

[2]SEM: Standard error of the mean.

[3]WHC: Water holding capacity.

The data obtained from meat quality parameters measured at room temperature over 7 days revealed no significant effect (*P* >0.05) of dietary treatments (Figures 3.1 – 3.4). The breast meat pH for broiler chickens on SW0, SW20, SW25, SW30, and SW35 diets increased over 7 days. For all treatments, the lightness (*L**) of the breast meat decreased over 7 days. The redness (a*) of the breast meat increased over 7 days among the treatments. The yellowness (b*) of the breast meat decreased for SW0, SW25, SW30 and SW35 while for SW20 increased over 7 days.

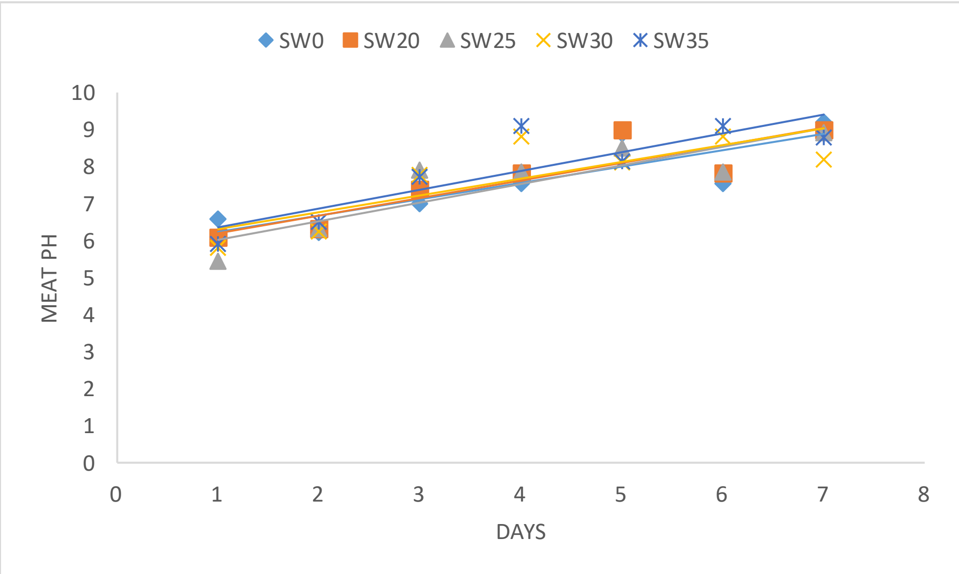

Figure 3.1. Effect of seaweed meal on the stability of breast meat pH upon storage at room temperature for 7 days [SW0 = a standard grower/finisher diet with no seaweed meal, SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, SW25 = a standard grower/finisher diet with 25 g/kg of seaweed meal, SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal].

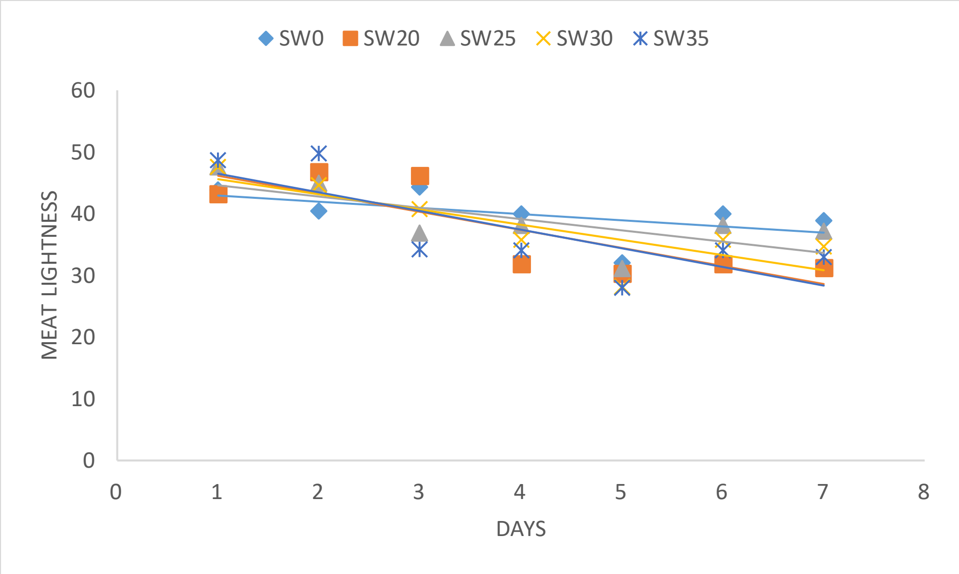

Figure 3.2. Effect of seaweed meal on the stability of breast meat lightness upon storage at room temperature for 7 days [SW0 = a standard grower/finisher diet with no seaweed meal, SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, SW25 = a standard grower/finisher diet with 25 g/kg of seaweed meal, SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal].

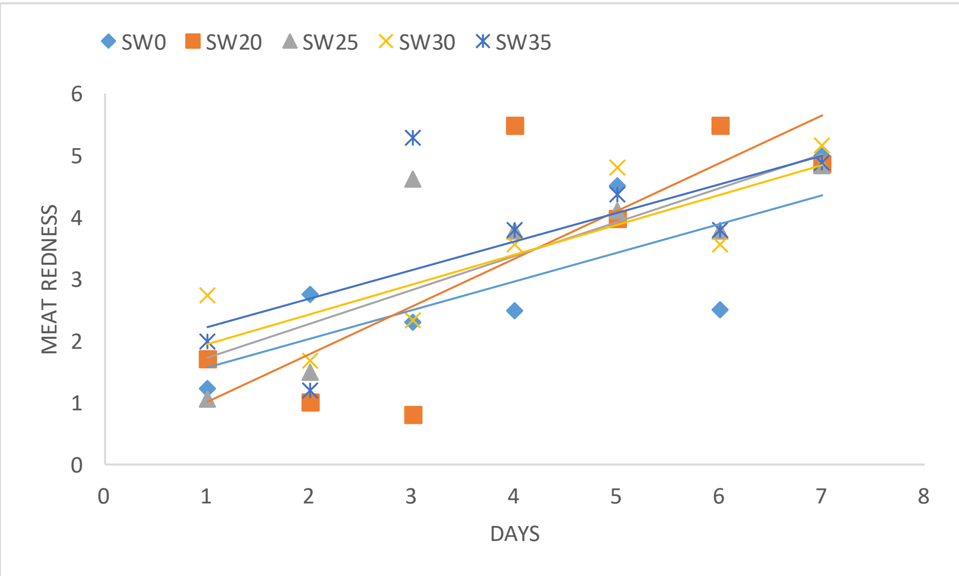

Figure 3.3. Effect of seaweed meal on the stability of breast meat redness upon storage at room temperature for 7 days [SW0 = a standard grower/finisher diet with no seaweed meal, SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, SW25 = a standard

grower/finisher diet with 25 g/kg of seaweed meal, SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal].

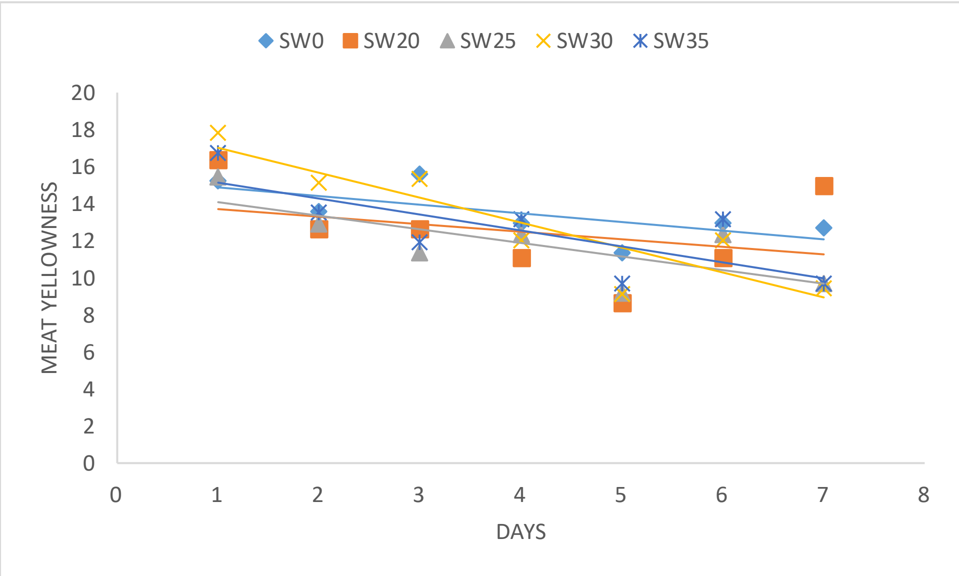

Figure 3.4. Effect of seaweed meal on the stability of breast meat yellowness upon storage at room temperature for 7 days [SW0 = a standard grower/finisher diet with no seaweed meal, SW20 = a standard grower/finisher diet with 20 g/kg of seaweed meal, SW25 = a standard grower/finisher diet with 25 g/kg of seaweed meal, SW30 = a standard grower/finisher diet with 30 g/kg of seaweed meal, and SW35 = a standard grower/finisher diet with 35 g/kg of seaweed meal].

3.4 Discussion

Seaweeds are important functional feed ingredients with nutraceutical properties that may be used to increase feed efficiency and performance in broiler chickens when incorporated into their diets (Yuan & Walsh, 2006). Repeated measures analysis revealed that there was no significant interaction effect between diet and week (bird age) on average weekly feed intake, body weight gain, and gain-to-feed ratio, indicating that experimental diet-induced variation in feed utilisation and growth performance were unaffected by the birds' age. There were no linear or quadratic impacts on overall feed intake or overall body weight gain when different levels of dietary SWM were included in Cobb 500 broiler diets. Abudabos *et al*. (2013) observed that adding *Ulva lactuca* up to 30 g/kg to broiler chicken diets had no influence on cumulative feed intake and body weight gain.

When seaweed meal was added at 30 g/kg to duck diets, El-Deek & Brikaa (2009) found no dietary impacts on growth performance and feed intake. In the current study, the addition of green seaweed meal, on the other hand, resulted in a linear reduction in G:F of the chickens, indicating that the seaweed meal hampered the conversion of feed into body mass. The presence of non-starch polysaccharides, like hemicellulose and cellulose in seaweeds, may explain this impact (Sinha *et al*., 2011). This implies that seaweeds may need to be pre-treated with exogenous fibrolytic enzymes to break down the fibre components and enhance their utilisation by chickens. It is critical to figure out the maximum amount of dietary SWM that may be added to broiler diets without compromising the growth or health status of chickens.

Blood parameters are helpful and effective diagnostic indices for detecting any pathophysiological changes and nutritional condition of animals (Verheyen *et al*., 2007). As a result, haematological and serum biochemical indices were established and utilised as health indicators to monitor the influence of SWM inclusion on the birds' well-being. Except for lymphocytes, no significant linear or quadratic impacts were detected for any of the blood parameters. Nonetheless, all the blood levels found in this investigation were within normal ranges reported for healthy broiler chickens (Animashahun *et al*., 2006; Van Niekerk *et al*., 2020). Similarly, when SWM was added to the diets of laying hens, Kulshreshtha *et al*. (2014) found no dietary impacts on blood serum concentrations. This demonstrates that SWM had no negative impact on the health and nutritional status of the birds.

Carcass yield and carcass cut weights are essential meat industry characteristics since they are used to grade meat products and have a direct impact on market prices. The addition of SWM in the feed of Cobb 500 broiler chickens had no effect on the carcass characteristics that were assessed. The absence of dietary influence on carcass characteristics and dressing percentage corroborate the findings of several scholars. Nikolakakis *et al*. (2005) and Mushtaq *et al*. (2007) found similar effects in broiler chickens. Cold carcass yield is believed to be an excellent predictor of total edible meat during storage, implying that adding SWM did not diminish the amount of edible meat. Except for spleen weights, which exhibited a linear decline in response to SWM levels, no changes in internal organ weights were observed. It is unclear why higher levels of SWM inclusion resulted in decreased spleen weights. The weights of the spleen and the sizes of the other visceral organs were, however, within the normal range for healthy birds (Kumanda *et al*., 2019; Animashahun *et al*., 2006). Moreover, the lack of dietary impacts on the relative weights of visceral organs was consistent with studies by Brenes *et al*. (2008) and Kulshreshtha *et al*. (2014), who found no difference in organ weights of broiler chickens fed with a control diet compared to diets containing natural plant products. These findings show that dietary seaweeds have few anti-nutritional elements that might harm broiler chickens' gastrointestinal tract and auxiliary organs.

Seaweeds have a variety of bioactive chemicals that may be used to create functional poultry products that provide health advantages to customers and act as natural preservatives to extend shelf-life (Sweeney & O'Doherty, 2016). Meat parameters such as pH, cooking loss, shear force, WHC, drip loss, and shelf life are commonly linked with the quality of meat and meat products (Muchenje *et al*., 2009). The outcomes of this study indicated that adding SWM to the diet had no effect on meat quality parameters, which was in line with the findings of Nhlane *et al*. (2020), who found that dietary seaweed had no effect on drip loss, cooking loss or shear force values in indigenous chickens. Meat colour values tested 24 h post-mortem were normal when compared to Barbut (1997) colour categorisation criteria, showing that the inclusion of SWM in the diet had no effect on the end product's freshness or quality. The final pH of the meat offers an accurate indicator of the amount of pH drop 24 hours after slaughter (Muchenje *et al*., 2009). The quantity of glycogen in muscle before slaughter, as well as how quickly the residual glycogen is converted to lactic acid after slaughter, is thought to impact this parameter (Dyubele *et al*., 2010). Thus, the lack of dietary impacts on pH might indicate that dietary SWM had no influence on the birds' glycogen levels. The pH levels discovered in this investigation were in line with the ultimate meat pH values (5.5 - 6.5) reported for broilers by Martins *et al*. (2021).

Seaweeds can be used to inhibit microbial development and delay oxidation processes in meat products (Roohinejad *et al*., 2017). Adding seaweeds to animal diets increases shelf-life during processing and storage (Kumar *et al*., 2015). Repeated measures analysis showed significant diet and time interaction effects on pH, *L** and *a**, demonstrating that dietary impacts on shelf-life indicators were dependent on storage time. The findings revealed that dietary SWM can improve the lightness and redness of meat products at room temperature, but not meat yellowness. Colour indicators play an essential role in customer perception and choice when buying meat products, thus more studies are needed to completely understand the influence of SWM-containing diets on meat stability in broiler chickens (Muchenje *et al*., 2009). After three days of storage at room temperature, the pH readings dramatically increased, possibly indicating that green seaweed supplementation failed to preserve normal meat pH.

3.5 Conclusions

In this study, dietary green seaweed meal compromised the overall gain-to-feed ratio in Cobb 500 broiler chicken diets without causing detrimental impacts on blood parameters, carcass characteristics and meat quality and stability characteristics. Although an optimum of seaweed meal inclusion level could not be established, a negative linear association with G:F shows that excessive levels of seaweed inclusion may reduce feed utilisation. As a result, pre-treatment of seaweed with exogenous fibrolytic enzymes to increase the feed value of seaweeds for broiler chickens should be investigated.

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4 CHAPTER FOUR - EFFECT OF EXOGENOUS FIBROLYTIC ENZYMES PRE-TREATMENT OF DIETARY GREEN SEAWEED ON GROWTH PERFORMANCE, AND PHYSIOLOGICAL AND MEAT QUALITY RESPONSES OF COBB 500 BROILER CHICKENS

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Abstract

In the previous chapter, it was concluded that high levels of dietary green seaweed meal compromised feed utilisation efficiency in Cobb 500 broilers. This was attributed to high levels of fibre, whose negative effects could be ameliorated through pre-treating the seaweed meal with exogenous feed enzymes. Therefore, the effects of an exogenous fibrolytic enzyme pre-treatment of dietary green seaweed (*Ulva sp*.) on growth performance, physiological responses, and meat quality parameters in Cobb 500 broiler chickens were investigated in this study. A total of 330, two-week-old male chicks (318.9 ± 16.12 g live-weight) were evenly and randomly distributed in 30 pens (experimental units) with six replicates. Five isonitrogenous and isoenergetic dietary treatments were assigned randomly to the pens. Commercial grower or finisher diets were formulated to contain 3.5 g/kg seaweed meal pre-treated, with fibrolytic enzyme (Viscozyme® L with enzyme activity of 100 fungal beta-glucanase per gram and a density of 1.2 g/mL) at the rates of 0 (SW35), 2.0 (V20), 5.0 (V50), 7.5 (V75) and 12.0 g/kg (V120). In this study, feed consumption, growth performance, blood parameters, visceral organs, and carcass and meat quality parameters were all measured. Repeated measures analysis revealed no significant week × diet interaction impact on average weekly feed intake (FI), body weight gain (BWG), and gain-to-feed ratio (G:F). In response to incremental levels of fibrolytic enzyme pre-treatment of seaweed meal, regression results revealed no linear or quadratic trends (P >0.05) for overall FI, growth performance, hematological and serum biochemical parameters, as well as visceral organ weights. However, hot [R^2 = 0.176, P = 0.033] and cold [R^2 = 0.164, P = 0.040] carcass weights increased linearly after enzyme pre-treatment of dietary seaweed meal. Breast meat lightness assessed immediately after slaughter (L^*i) [R2 = 0.147, P = 0.022] showed a linear reduction (P >0.05), as enzyme levels increased. Pre-treatment of seaweed meal with fibrolytic enzymes showed no impact (P >0.05) on overall feed consumption, physiological responses, carcass characteristics, visceral organs and meat quality indicators. However, diet SW35 promoted the greatest L^*_i value (66.33) than diets V75 and V120. Pre-treating seaweed with fibrolytic enzyme resulted in similar performance and greater carcass weights than the untreated seaweed-containing diet, showing that the enzymes were ineffective at degrading seaweed fibre components. The responses observed for physiological and meat quality parameters in this study did not allow for the mathematical determination of an optimum enzyme pre-treatment level, suggesting that levels higher than 12 g/kg of fibrolytic treatment may need to be investigated further.

Keywords: Blood parameters; Chicken; Growth performance; Meat quality; Seaweed

4.1 Introduction

The biggest goal of the poultry industry is to provide high-quality meat at a reasonable cost (Nkukwana, 2018). This goal is however difficult to fulfil because feed accounts for about 70% of total production costs (Alqaisi *et al*., 2017). As a result, there has been a shift towards the use of alternative, locally accessible and non-conventional feedstuffs from conventional diets largely composed of maize corn and soybean meal. Feed sources with nutraceutical properties are of particular importance because they can offer nutrients, reduce subclinical illnesses, improve feed utilisation efficiency and improve product quality (Alagawany *et al*., 2021). According to Gullón *et al*. (2020), seaweed is one such possible feed ingredient that has received great attention in both human and animal nutrition. Seaweeds are coastal macroalgae that come in a variety of shapes, sizes, colours and composition (Makkar *et al*., 2016). On the other hand, its nutritional content is influenced by environmental factors and ecological circumstances (Abudabos *et al*., 2013). Seaweeds possess important nutrients, such as lipids, proteins, minerals and vitamins, as well as bioactive compounds, such as polysaccharides, phenolics, amino acids, lipids and phlorotannins (Michalak & Mahrose, 2020). Seaweed bioactive compounds have antibacterial, prebiotic, antioxidant and anti-inflammatory and immunomodulatory effects (Corino *et al*., 2019). Several research studies have investigated its effects on ducks (EL-Deek & Brikaa, 2009), broilers (Abudabos *et al*., 2013; Choi & Oh, 2014), layers (Padhi *et al*., 2003) and indigenous chickens (Nhlane *et al*., 2020). As a functional component in poultry diets, seaweeds have the potential to increase feed utilisation, growth performance and carcass quality. However, high amounts of fibre may limit the utilisation of seaweed in broiler diets (Gullón *et al*., 2020). Indeed, in the previous chapter of this thesis, it was concluded that higher levels of dietary green seaweed meal compromised nutrient utilisation in Cobb 500 chickens. This result could be due to the high fibre content in seaweeds. There are reports that high dietary fibre in poultry diets decreases nutrient digestion and utilisation while slowing the development rate (Mnisi *et al*., 2017). Therefore, the high fibre levels in green seaweed meal can limit the ability of broilers to make use of beneficial bioactive compounds found in seaweed (Abudabos *et al*., 2013) while compromising financial returns by restricting the optimum productivity of the birds.

Pre-treatment with exogenous fibrolytic enzymes is one approach for increasing the feed value of seaweed in broiler chickens. Exogenous fibrolytic enzymes have been utilised to enhance nutrient bioavailability and thus giving nutritionists more options when it comes to the ingredients they use during feed formulation (Alemawor *et al*., 2009). Furthermore, by decreasing waste outflow, fibrolytic enzymes play an essential role in reducing the negative environmental effect of animal production (Beauchemin *et al*., 2003). As a result, increases in fibre digestibility would almost certainly result in an enhancement in the production system's efficiency and sustainability. The use of fibrolytic enzyme products in chicken diets, which generally consist of xylanases and cellulases, might potentially improve fibre content and, as a result, increase digestible energy intake (Beauchemin *et al*., 2003). The scarcity of information on the appropriate dosage of enzyme therapy in broilers is a fundamental barrier to the use of exogenous enzymes to increase seaweed utilisation. This is because most commercial fibrolytic enzyme preparations are unintended for non-traditional feed ingredients like seaweeds. The objective of this study was to evaluate the effect of fibrolytic enzyme pre-treatment of dietary green seaweed meal on growth performance, haemato-biochemical parameters, carcass traits, meat quality attributes and shelf life of Cobb 500 broilers. It was hypothesised that pre-treating seaweeds with exogenous fibrolytic enzymes would improve growth performance, physiological parameters and meat quality characteristics in Cobb 500 broilers.

4.2 Materials and methods

4.2.1 Study area and resources

The study was conducted in summer, temperatures during this time ranged from 11℃ to 31℃. The study area was as described in Chapter 3, Section 3.2.2. The green seaweed was from the same batch as the one described in Chapter 3, Section 3.2.2. Sigma–Aldrich (Modderfontein, South Africa) supplied the fibrolytic enzyme (Viscozyme® L), which is a mixture of a cellulolytic mixture of arabinase, cellulase, β-glucanase, hemicellulase and xylanase enzymes. The enzyme is manufactured from *Aspergillus spp.* and contains an enzyme activity of 100 FBG/g and a density of 1.2 g/ml.

4.2.2 *Enzyme pre-treatment of seaweed*

The seaweed meal was treated with fibrolytic enzymes at the rate of 0, 0.2, 0.5, 0.75 and 1.2% before being incorporated into the commercial broiler diets. Exactly 6800 g of seaweed meal per treatment was sprayed and mixed with 5000 ml of distilled water in which 11.3, 28.3, 42.45, and 68 ml of fibrolytic enzyme (density: 1.2 g/ml) had been dissolved. The untreated seaweed meal (6800 g) were sprayed with 5000 ml of distilled water without the enzyme. The amount of distilled water used to dissolve the enzyme was determined through an iterative process without leaching the seaweed's chemical components. Treated and untreated seaweed meals were kept for 24 hours under room temperature to allow time for the fibrolytic enzymes to react with fibre components in seaweeds, and thereafter, oven-dried at 60°C until constant weight. After oven-drying, the samples were crushed to break up lumps before being incorporated into commercial grower and finisher diets.

4.2.3 *Feed formulation and analyses*

A commercial diet containing untreated seaweed meal from Chapter 3 (SW35) was used as a control diet. The other diets were as follows: V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg, as shown in Table 4.1.

Table 4.1. Ingredient composition (g/kg *as fed*) of diets containing exogenous fibrolytic enzyme-treated seaweed meal

	Grower (14 – 28 d)					Finisher (29 - 42 d)				
Ingredients	SW35	V20	V50	V75	V120	SW35	V20	V50	V75	V120
Viscozyme® L	0	2	5	75	120	0	2	5	75	120
DL-Methionine	2.92	2.92	2.92	2.92	2.92	2.29	2.29	2.29	2.29	2.29
L-Threonine	0.98	0.98	0.98	0.98	0.98	0.58	0.58	0.58	0.58	0.58
Seaweed meal	35	35	35	35	35	35	35	35	35	35
Yellow maize 8.0%	648.8	648.8	648.8	648.8	648.8	646.3	646.3	646.3	646.3	646.3
Full fat soya	31.88	31.88	31.88	31.88	31.88	34.57	34.57	34.57	34.57	34.57
Soya oil cake 47%	211.9	211.9	211.9	211.9	211.9	218.1	218.1	218.1	218.1	218.1
Sunflower oil cake 36%	41.51	41.51	41.51	41.51	41.51	30.0	30.0	30.0	30.0	30.0
Limestone	7.89	7.89	7.89	7.89	7.89	7.05	7.05	7.05	7.05	7.05
[1]MCP	8.28	8.28	8.28	8.28	8.28	5.99	5.99	5.99	5.99	5.99
Sodium bicarbonate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Lysine HCL	3.40	3.40	3.40	3.40	3.40	1.85	1.85	1.85	1.85	1.85
Crude soya oil	0	0	0	0	0	11.4	11.4	11.4	11.4	11.4
Lignobond	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Grower premix	2.5	2.5	2.5	2.5	2.5	0	0	0	0	0
Finisher premix	0	0	0	0	0	2.0	2.0	2.0	2.0	2.0
Axtra phytase	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salinomycin 12%	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Zinc Bacitracin	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33

SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg.
[1]MCP = monocalcium phosphate.

The nutritional composition (g/kg *as fed* basis, unless stated otherwise) of the grower and finisher diet is shown in table 4.2. The diets were calculated for dry matter, metabolisable energy, crude protein, amino acids (AP lysine, methionine and threonine), ash, crude fibre, crude fat, and minerals (calcium, phosphorus, chloride, and sodium).

Table 4.2. Nutritional composition (g/kg *as fed* basis, unless stated otherwise) of the grower and finisher diets

	Grower	Finisher
Dry matter	919.84	921.81
Metabolisable energy (MJ/kg)	12.92	13.26
Crude protein (%)	31.32	33.44
AP Lysine	10.65	9.5
AP Methionine	5.6	4.98
AP Threonine	6.9	6.5
Crude fat	35.54	46.87
Crude fibre	51.87	49.88
Ash	44.99	45.04
Available phosphorus	4.2	3.8
Calcium	8.4	7.6
Chloride	3.54	3.29
Sodium	2.16	2.16
Total phosphorus	5.47	4.94

The proximate composition of treated and untreated seaweed meal (g/kg *as fed* basis, unless stated otherwise) of the grower and finisher diets is shown in table 4.3. For DM determination (AOAC, 2005: method no. 930.15), approximately 1 g of experimental diet were placed in pre-weighed crucibles that were then inserted in an oven set at 105ºC for a duration of 12 h. The DM was calculated as the difference between the initial sample and the moisture weights, while the moisture content was measured as the loss in weight. Samples used to determine DM were incinerated in a muffle furnace set at a temperature of 600°C for 6 h for ash determination. The ANKOM[2000] fibre analyser (ANKOM Technology, Macedon New York) was used to determine the neutral detergent fibre (NDF) and acid detergent fibre (ADF) by refluxing about 0.45 – 0.5 g samples with neutral detergent and acid detergent solutions for 1 h and 1 h 15 min, respectively, according to van Soest *et al.* (1991). A heat-stable α-amylase was used for NDF determination. The lignin content was determined by treating the ADF bags with 72% sulfuric acid to dissolve the cellulose after drying for 24 h (105°C). The standard macro-Kjeldahl method (AOAC, 2005: method no. 984.13) was used to determine the total N content, which was then converted to protein by multiplying percentage N by the factor 6.25.

Table 4.3. Proximate composition of treated and untreated seaweed meal (g/kg *as fed* basis, unless stated otherwise) of the grower and finisher diets

	Grower					Finisher				
	SW35	V20	V50	V75	V120	SW35	V20	V50	V75	V120
DM (g/kg)	917.92	919.62	931.19	922.10	919.54	902.81	920.34	921.5	921.83	925.10
Ash (g/kg)	49.98	48.03	48.17	47.63	46.88	44.77	44.14	45.73	44.52	44.39
NDF (g/kg)	207.14	204.78	203.46	205.46	201.55	204.94	196.63	197.41	205.28	205.30
ADF (g/kg)	185.36	184.05	180.07	183.19	179.28	182.59	174.29	178.37	182.85	182.39

ADL (g/kg)	21.78	20.74	23.40	22.27	22.27	22.35	22.34	19.04	22.44	22.92
Protein (g/kg)	331.70	323.6	317.60	324.6	336.2	364.2	368.2	348.00	353.90	362.7

SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg.

4.2.4 Feeding trial and broiler management

In this study, 330, day-old male Cobb 500 chicks were purchased from Montshego farm in Zeerust (North West, South Africa). The chicks were weighed and randomly allotted into 30 ground floor pens (experimental units), with six replicates per dietary treatment. Each pen (measuring 3.5 m L ×1.0 m B × 1.85 m H) carried 11 birds and the floor was covered with sunflower husk bedding. The birds were given a stress pack for the first three days and were reared on a commercial starter meal until they were 10 days old. From day 11 to day 13, the birds were adapted to the experimental meals. For the growth phase, measurements began on days 14 – 28, and for the finisher phase, measurements began on days 29 – 42. A multi-meter gadget (HTC-1, Xuzhou Sanhe Automatic Control Equipment Co. Ltd, China) was used to monitor and measure the temperature (30 ± 5ºC) and humidity (60%) in the house. Rearing took place under natural lighting, with the curtains opened in the mornings (07h00) and closed in the evenings (18h00). The birds were given unrestricted access to clean, fresh water, as well as the experimental diets. The birds were weighed at two weeks of age (A-E Adam LBK 3, Adam Equipment S.A. PTY, Johannesburg, South Africa) to measure their initial live weight (318.9 ± 16.12 g live weight). In this study feed intake (FI), body weight gain (BWG), and gain-to-feed ratio G:F was calculated as described in Chapter 3, section 3.2.6.

4.2.5 Blood collection and analysis

At 40 days of age, blood samples were collected as described in Chapter 3, section 3.2.7. The automated IDEXX LaserCyte Haematology Analyser and the automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories (Pty) Ltd, Gauteng, South Africa) were used to analyse haematological parameters (reticulocytes, red blood cells, neutophils, mean platelet volume, lymphocytes, monocytes, eosinophils, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, red cell distribution width, platelets, platelet distribution width, procalcitonin and white blood cell count) and serum biochemical parameters (albumin, alkaline phosphase, alanine transaminase, cholesterol, creatinine, gamma-glutamyl, glucose, phosphorus, symmetric dimethylargininase, toatal bilirubin, total protein and urea), respectively.

4.2.6 Slaughter procedure, carcass traits and visceral organs measurements

On day 42, the birds were transported to a local abattoir (Mafikeng, South Africa) for slaughter, after being starved of feed for 13 hours as described in Chapter 3, Section 3.2.8. Hot carcass (HCW) and cold carcass (CCW) weights, carcass yield, carcass cuts and internal organs were measured as described in Chapter 3, Section 3.2.8.

4.2.7 Meat quality measurements

Breast meat pH values were recorded and the meat colour coordinates (*L*, a** and *b*)* of breast meat were determined as described in Chapter 3, Section 3.2.9. Breast meat samples were refrigerated overnight at 4°C and cooking loss and meat tenderness were determined as described in Chapter 3, Section 3.3.10. The water holding capacity (WHC), drip loss, and shelf-life measurements were evaluated as described in Chapter 3, Section 3.2.11.

4.3 Statistical analysis

Response surface regression was used to determine the linear and quadratic coefficients of enzyme-treated seaweed meal using the model described in Chapter 3, Section 3.2.12. The general linear models (GLM) procedure of SAS version 9.3 (SAS, 2010) was used to account for dietary differences as described in Chapter 3, Section 3.2.12. The repeated measures analysis was also employed as described in Chapter 3, Section 3.2.12.

4.4 Results

4.4.1 Growth performance and physiological responses

Repeated measures analysis for average weekly feed intake, average weekly body weight gain, and gain-to-feed ratio of broiler chickens revealed no significant week and diet interaction effect in this study. The overall feed intake, overall body weight gain, and overall gain-to-feed ratio did not reveal any significant linear or quadratic trends, as shown in Table 4.4. In Cobb 500 broiler chickens, dietary treatments had no effect (*P* >0.05) on overall feed intake, overall body weight gain and overall gain-to-feed ratio.

Table 4.4. Overall feed intake (g/bird), body weight gain (g/bird) and gain-to-feed ratio feed conversion efficiency of broiler chickens fed diets containing exogenous fibrolytic enzyme pre-treated seaweed meal

	[1]Diets						*P* value	
	SW35	V20	V50	V75	V120	[2]SEM	Linear	Quadratic
Overall FI	3385.8	3408.1	3421.7	3389.8	3495.9	50.08	0.203	0.899
Overall BWG	1714.2	1746.1	1765.6	1764.6	1802.6	50.30	0.154	0.472

Overall G:F	0.505	0.511	0.516	0.520	0.515	0.015	0.611	0.600

[1]Diet: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg. FI = feed intake, BWG = body weight gain and G:F = gain-to-feed ratio.

[2]SEM: Standard error of the mean.

As the enzyme pre-treatment levels of seaweed meal increased, regression analysis revealed no significant linear or quadratic trend for the haematological parameters (Table 4.5). Pre-treating seaweed meal with fibrolytic enzymes showed no influence (P >0.05) on broiler chicken hematological parameters.

Table 4.5. Hematological parameters of broiler chickens fed diets containing exogenous fibrolytic enzyme pre-treated seaweed meal

	[1]Diets						P value	
[2]Parameters	SW35	V20	V50	V75	V120	[3]SEM	Linear	Quadratic
Reticulocytes (%)	6.98	8.70	3.43	2.84	3.89	3.027	0.375	0.559
WBC ($\times 10^9$/L)	220.6	290.8	254.8	270.1	233.2	34.25	0.733	0.378
Neutrophils (%)	18.66	4.87	6.88	8.36	5.80	6.067	0.227	0.353
MPV (fL)	6.05	3.58	3.93	2.92	2.17	1.348	0.055	0.609
Lymphocytes (%)	57.73	81.34	73.27	70.58	68.59	9.429	0.250	0.312
Monocytes (%)	9.43	12.66	19.22	22.00	17.09	4.694	0.184	0.269

Eosinophils (%)	0.23	0.41	0.50	0.59	0.56	0.153	0.159	0.530
RBC ($\times 10^{12}$/L)	1.61	1.55	1.80	1.49	1.55	0.199	0.674	0.790
Hematocrit (%)	7.23	6.28	8.69	5.18	6.28	1.537	0.405	0.979
Hemoglobin (g/dL)	8.54	8.86	8.62	8.32	8.65	1.45	0.926	0.317
MCV (fL)	42.29	38.76	47.31	27.79	33.37	6.173	0.163	0.988
MCH (pg)	47.69	85.19	51.82	75.99	37.21	22.61	0.557	0.354
RDW (%)	24.89	32.03	31.85	25.04	24.51	4.645	0.583	0.074
Platelets (K/µL)	1469.2	1650.2	2118.2	2065.1	1469.2	296.8	0.367	0.175
PDW (%)	12.74	18.36	16.13	14.76	13.14	4.786	0.742	0.136
Procalcitonin (%)	0.43	0.40	0.47	0.49	0.45	0.083	0.706	0.689

[1]Diet: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg.

[2]Parameters: RBC = red blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; RDW = red cell distribution width; WBC = white blood cell count; MPV = mean platelet volume; PDW = Platelet distribution width

[3]SEM: Standard error of the mean.

With increasing enzyme pre-treatment doses, neither linear nor quadratic trends were detected for any serum biochemical parameters, as shown in Table 4.6. Similarly, fibrolytic enzymes pre-treatment of seaweed meal had no effect ($P > 0.05$) on serum biochemical parameters.

Table 4.6. Serum biochemistry of broiler chickens fed diets containing exogenous fibrolytic enzyme pre-treated seaweed meal

	[1]Diets	*P* value

[2]Parameters	SW35	V20	V50	V75	V120	[3]SEM	Linear	Quadratic
Albumin (g/L)	40.58	41.83	37.75	36.08	47.42	6.328	0.173	0.212
ALKP (U/L)	26.66	38.45	33.54	47.33	29.83	12.08	0.506	0.234
ALT (U/L)	116.5	90.58	94.25	92.00	116.5	22.54	0.452	0.291
Cholesterol (mmol/L)	1.92	1.43	1.23	1.65	1.28	0.244	0.538	0.526
Creatinine (µmol/L)	9.00	8.92	13.08	9.00	7.58	1.748	0.549	0.177
GGT (U/L)	16.0	8.08	7.00	4.67	13.83	6.224	0.478	0.271
Glucose (mmol/L)	7.23	8.30	8.48	8.49	8.23	0.429	0.070	0.221
Phosphorus (mmol/L)	5.09	5.00	4.93	4.94	4.55	0.205	0.079	0.433
SDMA (µg/L)	33.83	32.33	32.58	32.25	25.49	3.767	0.147	0.587
Total bilirubin (µmol/L)	254.7	287.7	216.6	224.1	264.8	57.52	0.528	0.391
Total protein (g/L)	102.6	102.4	97.07	91.75	101.8	11.55	0.704	0.547
Urea (mmol/L)	0.68	0.89	0.89	0.83	0.90	0.194	0.121	0.118

[1]Diet: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg.

[2]Parameters: ALKP = alkaline phosphatase; ALT = alanine transaminase; GGT = gamma-glutamyl transferase; SDMA = symmetric dimethylargininase.
[3]SEM: Standard error of the mean.

4.4.2 Carcass traits, visceral organs and meat quality

Table 4.7 shows that pre-treatment of dietary seaweed meal with enzymes linearly increased HCW [y = 80.68 (±128.3) x + 1562.9 (±32.46); R^2 = 0.176, P = 0.033] and CCW [y = 28.18 (±114.3) x + 60.40 (±143.4); R^2 = 0.164, P = 0.040]. In response to increasing doses of enzyme pre-treatment of dietary seaweed, no linear or quadratic trends were detected for all carcass cuts and yields. No dietary effects (P >0.05) were observed on carcass characteristics of broiler chickens.

Table 4.7. Carcass characteristics (% HCW, unless stated otherwise) of broiler chickens fed diets containing exogenous fibrolytic enzyme pre-treated seaweed meal

	[1]Diets					[2]SEM	*P* value	
	SW35	V20	V50	V75	V120		Linear	Quadratic
Slaughter weight (g)	2034.8	2066.6	2055.4	2070.4	2122.2	55.47	0.082	0.733
Hot carcass (g)	1575.1	1598.1	1644.1	1596.9	1646.4	37.29	0.033	0.954
Cold carcass (g)	1530.1	1520.1	1598.8	1542.3	1594	40.55	0.04	0.807
Carcass yield (%)	77.39	77.43	80.52	77.24	77.62	2.077	0.79	0.723
Drumstick	6.13	6.18	6.29	5.77	6.04	0.182	0.893	0.452
Breast	26.43	26.87	26.98	25.5	27.21	0.726	0.811	0.818
Wing	5.5	5.38	5.6	5.51	5.4	0.152	0.615	0.91
Thigh	6.71	6.53	6.86	6.61	6.59	0.157	0.641	0.757

[1]Diet: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg.

[2]SEM: Standard error of the mean.

Table 4.8 shows that there were no linear or quadratic responses to increasing doses of enzyme pre-treatment of dietary seaweed meal for all visceral organ weights. No dietary effects were observed on the visceral organs of broiler chickens (*P* >0.05).

Table 4.8. Visceral organ weights (% HCW, unless stated otherwise) of broiler chickens fed diets containing exogenous fibrolytic enzyme pre-treated seaweed meal

	[1]Diets						*P* value	
	SW35	V20	V50	V75	V120	[2]SEM	Linear	Quadratic
Liver	2.74	2.69	2.73	2.65	2.66	0.073	0.484	0.862
Gizzard	2.1	2.15	2.13	1.99	2.06	0.099	0.971	0.655
Proventriculus	0.43	0.44	0.43	0.41	0.42	0.013	0.667	0.979
Spleen	0.17	0.17	0.18	0.16	0.17	0.007	0.264	0.257
Duodenum	1.1	1.12	1.24	1.12	1.07	0.064	0.885	0.332
Jejunum	1.84	1.7	1.73	1.89	1.84	0.078	0.363	0.483
Ileum	1.58	1.42	1.42	1.51	1.47	0.075	0.685	0.063
Caeca	0.13	0.12	0.15	0.14	0.12	0.015	0.527	0.879

[1]Diet: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg.

[2]SEM: Standard error of the mean.

Table 4.9 indicates that the lightness of breast meat assessed immediately post-mortem (L^*_i) decreased linearly [y = 64.4 (±0.670) – 0.105 (±0.046) x; R^2 = 0.147, P = 0.022] in response to pre-treatment of seaweed meal with increasing levels of fibrolytic enzyme. On the other hand, the other meat quality parameters did not show any linear or quadratic effects as enzyme levels increased. Pre-treating seaweed meal with fibrolytic enzymes had a significant effect only on L^*_I, where the control treatment SW35 promoted the highest L^*_i value (66.33) than diets V75 and V120, which did not differ ($P > 0.05$).

Table 4.9. Meat quality parameters of broiler chickens fed diets containing exogenous fibrolytic enzyme-treated seaweed meals

[2]Parameters	[1]Diets					[3]SEM	*P* value	
	SW35	V20	V50	V75	V120		Linear	Quadratic
pH_i	6.31	6.35	6.36	6.20	6.30	0.058	0.207	0.120
L^*_i	66.33[b]	63.59[ab]	62.39[ab]	60.91[a]	62.14[a]	1.015	0.029	0.078
a^*_i	1.76	2.14	2.11	2.33	2.35	0.236	0.130	0.606
b^*_i	16.46	15.25	16.75	14.64	15.12	0.635	0.070	0.311
$Chroma_i$	16.57	15.42	16.89	14.84	15.31	0.621	0.078	0.314
Hue $angle_i$	1.46	1.43	1.46	1.41	1.42	0.020	0.095	0.466
pH_{24}	6.17	6.08	6.08	6.08	6.20	0.109	0.606	0.473
L^*_{24}	71.66	72.60	70.16	70.29	70.88	5.014	0.862	0.863
a^*_{24}	3.57	3.62	3.02	3.08	3.75	0.631	0.822	0.415
b^*_{24}	16.63	16.48	15.89	16.05	15.62	1.080	0.552	0.960
$Chroma_{24}$	17.05	16.94	16.19	16.38	16.13	1.126	0.606	0.950

Hue angle$_{24}$	1.37	1.36	1.39	1.39	1.33	0.084	0.408	0.255
Shear force (N)	1.86	1.94	1.81	1.91	1.85	0.092	0.978	0.711
Cooking loss (%)	14.27	14.16	10.54	13.53	16.06	2.15	0.287	0.486
Drip loss (%)	3.57	3.94	4.05	3.93	3.85	0.340	0.913	0.742
[4]WHC (%)	91.26	93.56	90.16	90.96	88.13	1.990	0.163	0.582

[1]Diet: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg.

[2]Parameters: subscript 1 = all parameters were measured 1 hour after slaughter; subscript 2 = all parameters were measured 24 h after slaughter

[3]SEM: Standard error of the mean

[4]WHC = water holding capacity

The data obtained from meat quality parameters measured at room temperature over 5 days revealed no effect (*P* >0.05) of dietary treatments (Figures 4.1 – 4.4). The breast meat pH for broiler chickens on SW35, V20, V50, V75 and V120 diets increased over 5 days. For all treatments the lightness (*L**) of the breast meat decreased over 5 days. The redness (a*) of the breast meat decreased over 5 days among the treatments. The yellowness (b*) of the breast meat increased for SW35, V20, V50, V75 and V120 over 5 days.

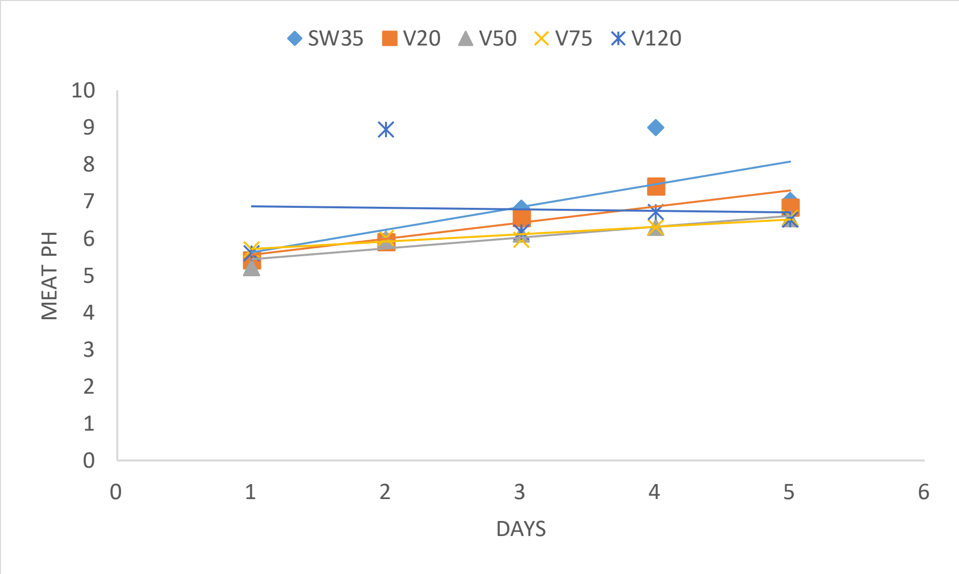

Figure 4.1. Effect of pre-treated seaweed meal on the stability of breast meat pH upon storage at room temperature for 5 days [Diets: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg].

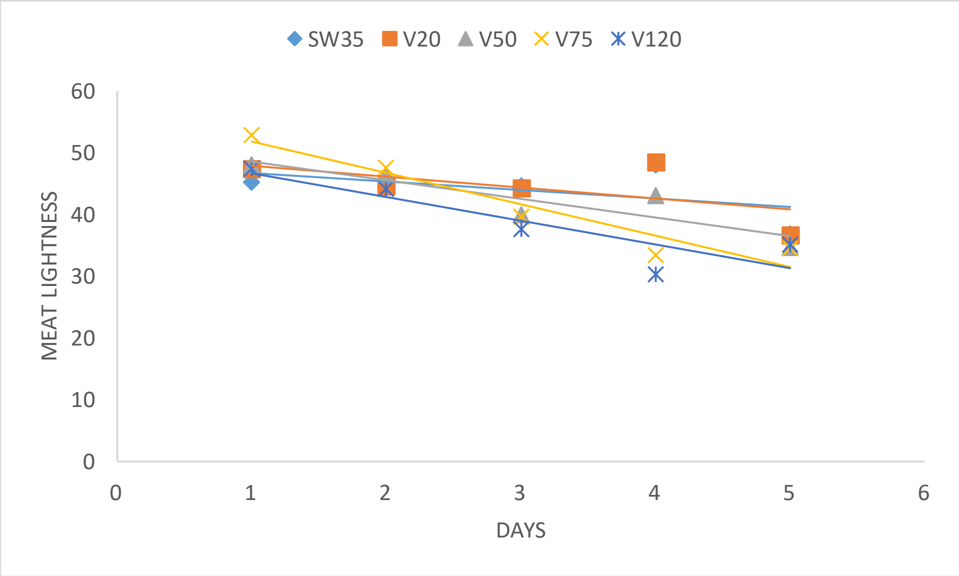

Figure 4.2. Effect of pre-treated seaweed meal on the stability of breast meat lightness upon storage at room temperature for 5 days [Diets: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg].

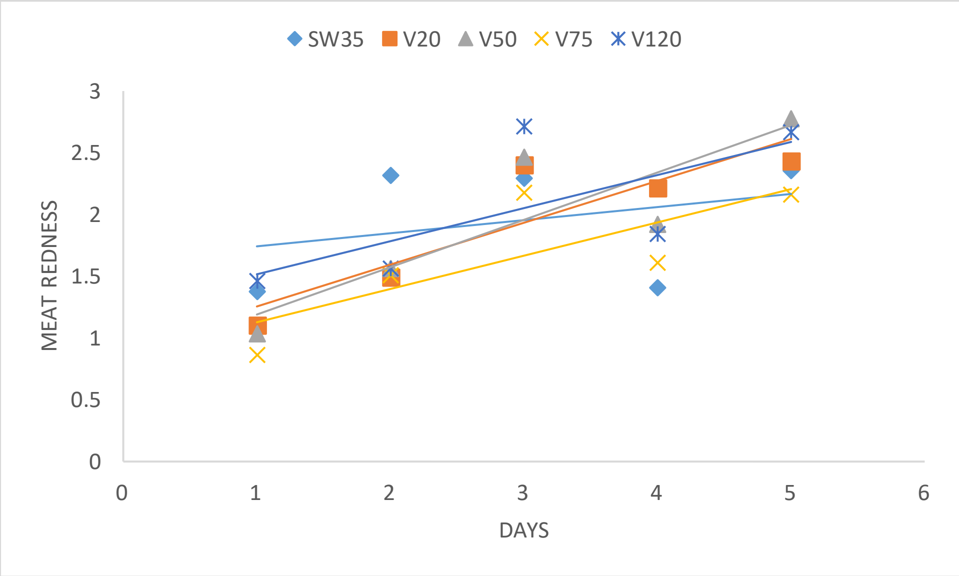

Figure 4.3. Effect of pre-treated seaweed meal on the stability of breast meat redness upon storage at room temperature for 5 days [Diets: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg].

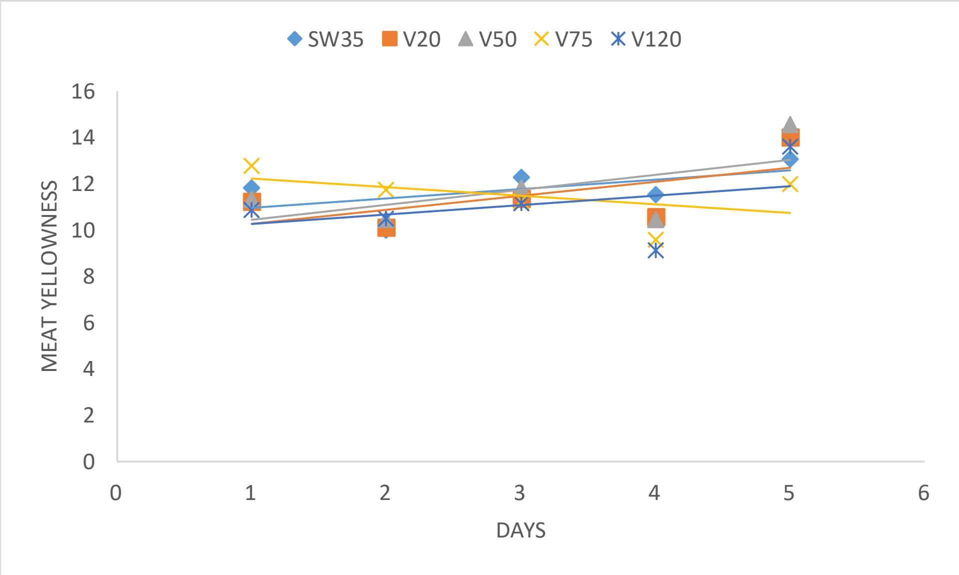

Figure 4.4. Effect of pre-treated seaweed meal on the stability of breast meat yellowness upon storage at room temperature for 5 days [Diets: SW35 = a commercial grower or finisher diet containing untreated seaweed meal; V20 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 2 g/kg; V50 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 5 g/kg; V75 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 7.5 g/kg; V120 = a commercial grower or finisher diet containing seaweed meal pre-treated with the enzyme at the rate of 12 g/kg].

4.5 Discussion

Exogenous fibrolytic enzymes are used to improve the utilisation of extremely fibrous feedstuffs (Beauchemin *et al*., 2004). The use of cell wall disintegrating enzymes in animal feeds is common (Zduńczyk *et al*., 2020). However, a few studies have attempted to investigate the utility of exogenous fibrolytic enzymes to enhance the feed value of seaweeds (*Ulva sp*.) in broiler diets. The results of repeated measures analysis revealed that there was no significant week × diet interaction effect on average weekly feed intake, body weight gain, and gain-to-feed ratio, indicating that the birds' performance was unaffected by their age. Pre-treating seaweed meal with fibrolytic enzyme had no effect on total feed intake or broiler chicken growth performance. Similar findings were reported by Zduczyk *et al*. (2020), who found that pre-treating peas (*Pisum sativum*) with a non-starch polysaccharide-degrading enzyme had no effect on feed consumption or weight gain. In Chapter 3, adding seaweed meal up to 35 g/kg in the feed of Cobb 500 broiler chicks decreased overall G:F. Thus, the similarity between the enzyme-treatment groups and the untreated seaweed-containing diet in terms of overall performance shows that the cellulolytic enzyme mixture is ineffective at degrading ligno-cellulolytic components found in seaweed.

All blood parameters revealed no significant linear or quadratic trends in response to dietary seaweed meal pre-treated with fibrolytic enzymes. These findings were comparable to those of Kumanda *et al*. (2019), who used fibrolytic enzyme to pre-treat red grape (*Vitis vinifera*) pomace at a rate of 1.0 g/kg in Cobb 500 broiler chicken feeds. This means that supplementing the seaweed diet with exogenous fibrolytic enzymes does not compromise the pathophysiological status and well-being of the birds. Furthermore, the hemato-biochemical parameters in this investigation were within the normal range for healthy chickens (Jain, 1986). It is well established that increasing dietary crude protein intake improves blood urea, creatinine and the total bilirubin levels (Bovera *et al*., 2007). However, pre-treatment of seaweed meal with fibrolytic enzymes had no effect on these parameters in this investigation, possibly because the diets had similar protein levels. Furthermore, high fibre levels are known to lower fat, cholesterol, and serum insulin absorption, which minimise cholesterol and lipoprotein synthesis (Rusdi & Arief, 2017). When compared to seaweed-containing diets, the application of fibrolytic enzyme had no influence on serum amylase, glucose or cholesterol levels, suggesting that the use of fibrolytic enzymes had no effect on the energy nutritional profile of the birds. Based on the physiological responses of the broiler chickens, an optimal enzyme treatment level could not be identified, indicating that levels higher than 12 g/kg of fibrolytic treatment should be investigated further.

Exogenous enzyme supplementation has been shown to promote feed metabolism and digestibility, as well as control development and improve carcass features (Sharma *et al*., 2018). The use of exogenous fibrolytic enzymes in diets containing seaweeds enhanced the carcass weights of the birds in this investigation (Gullón *et al*., 2020). Similarly, when red grape pomace was pre-treated with fibrolytic enzymes, Kumanda *et al*. (2019) observed higher carcass weights in broiler chickens. Hajati *et al*. (2009) reported that multi-enzyme supplementation in a corn-soybean meal-wheat-based diet improves carcass yields in broiler chickens. These inconsistencies could be influenced by the application method and microbial strain type of the enzyme. The current findings on dietary effects on breast, wing, thigh, and drumstick weights are consistent with those of Hajati *et al*. (2009), who found no significant differences in carcass characteristics of broilers reared on multi-enzymes pre-treated and untreated diets.

Pre-treatment of seaweed meal with exogenous fibrolytic enzymes had no effect on the size of the visceral organs, which remained comparable to those in the untreated control diet. These findings are similar to those of Hajati *et al*. (2009) and Kumanda *et al*. (2019), who found no significant dietary effects in broiler hens fed with fibrolytic enzyme-treated diets. Feeding high-fibrous diets to simple non-ruminants promote the enlargement of digestive organs (Deniz *et al*., 2007).

Meat colour is one of the most significant qualitative features of meat for customer acceptability (Tang *et al*., 2007). Lokaewmanee & Promdee (2018) classified chicken breast colour as lighter than normal ($L^* > 53$), normal ($48 < L^* > 53$) and darker than normal ($L^* < 48$). In another report, Corzo *et al*. (2005) reported broiler breast muscle with an L^* value of 55 as normal and an L^* value of 60 as pale meat. In this study, all the L^*_i values for all treatment diets exceeded the value of 60, suggesting that the meat was lighter than normal. In comparison to the birds fed with the enzyme pre-treated seaweed-containing diets, those in the control group had lighter breast muscle. According to Harkin *et al*. (2019), seaweeds contain anthocyanins that can influence meat colour, thus, it was expected that broilers would have lighter breast muscle colour. Kumanda *et al*. (2019) found no significant change in L^* value between meat from broilers fed with diets containing fibrolytic enzyme pre-treated red grape pomace and meat from the control diet. The use of fibrolytic enzymes had no effect on pHi, chromai, hue anglei, pH_{24}, L^{*24}, a^{*24} b^{*24}, chroma^{24}, hue angle^{24}, shear force, cooking loss, drip loss, water holding capacity and were comparable to those of broilers fed a control diet, implying that fibrolytic enzymes have no effect on meat quality traits. Similarly, Mnisi *et al*. (2017) in Japanese quail and Nkosi *et al*. (2020) in pigs found that adding enzymes to standard control diets had no effect on meat quality traits. For meat starbility (shelf life), all meat quality parameters measured at room temperature over 5 days period were not significantly affected by the dietary treatment. However, the breast meat pH, increased over 5 days period. Generally the increase in meat pH is associated with improved meat quality and also suggest microbial proliferation.

4.6 Conclusion

Results from the current study showed that growth performance, physiological responses, visceral organ weights, and carcass and meat quality characteristics in broiler chickens fed with exogenous fibrolytic enzyme pre-treated seaweed-containing diets were comparable to those fed with the diet containing untreated seaweed. Furthermore, utilising the growth performance data, an optimal fibrolytic enzyme pre-treatment level could not be established, indicating that levels greater than 12 g/kg of fibrolytic enzymes could be needed to induce non-linear responses. These results may also suggest that factors other than fibre content may have greater effects on diet utilisation in broiler chickens. Such factors require further investigation to optimise seaweeds as functional ingredients in broiler diets.

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5 CHAPTER FIVE - EFFECT OF PRE-TREATING DIETARY SEAWEED MEAL WITH FIBROLYTIC AND PROTEOLYTIC ENZYMES ON GROWTH PERFORMANCE, AND PHYSIOLOGICAL AND MEAT QUALITY PARAMETERS OF BROILER CHICKENS

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ABSTRACT

The high fibre content and low protein digestibility restrict the use of seaweeds as nutraceuticals in poultry feed. The impact of simultaneously pre-treating seaweed (*Ulva sp.*) meal, with a protease mono-enzyme and fibrolytic multi-enzymes on growth performance, physiological parameters and meat quality characteristics in Cobb 500 broiler chicken, was investigated in this study. Five dietary treatments were formulated by mixing a standard chicken diet with fibrolytic enzyme pre-treated (12 g/kg) seaweed meal (35 g/kg) to which 0 (VP0); 5 (VP50), 10 (VP100), and 15 g/kg (VP150) protease mono-enzyme was simultaneously added. A negative control diet in which the standard broiler diet was mixed with untreated seaweed meal at 35 g/kg (SW35) was also formulated. A total of 300, two-week-old chicks (239.3 ± 8.57 g live-weight) were evenly distributed across 30 pens, replicated six times per diet. In response to protease levels, no linear or quadratic changes (P >0.05) in growth performance or carcass characteristics were observed. Six-week-old birds fed diet SW35 had the highest feed intake (1144.5 g/bird) when compared to birds fed with the VP0, VP50, VP100 and VP150 diets. The weight of the gizzard increased linearly [R^2 = 0.201, P = 0.022], whereas symmetric dimethylarginine [R^2 = 0.241, P = 0.013], calcium [R^2 = 0.350, P = 0.005], meat pH_{24} [R^2 = 0.177, P = 0.043] and hue $angle_{24}$ [R^2 = 0.180, P = 0.041] showed quadratic trends (P <0.05) in response to dietary protease pre-treatment levels. Birds fed the control diet (SW35) promoted the lowest (P <0.05) phosphorus levels (3.37 mmol/L) when compared to the other diets. From this study, it was concluded that pre-treatment of green seaweed meal with a combination of exogenous enzymes (proteolytic and fibrolytic enzymes) does not improve growth performance, and physiological and meat quality parameters in Cobb 500 broiler chickens.

Keywords: Broilers; Blood parameters; Growth performance; Meat quality; Seaweed

5.1 Introduction

The poultry industry makes a substantial contribution to global agri-food value chains through the consumption of meat and eggs (Ahaotu *et al.*, 2019). The global output of chicken meat has risen from 99,027 metric tons in 2019 to 100,026 metric tons in 2020 (USDA, 2020). The necessity to meet the nutritional needs of the rising human population, which is expected to reach 9 billion people by 2050, has encouraged this growth (United Nations, 2019). The poultry business has depended largely on high-performing conventional chicken breeds to boost output throughout the years, but the high cost of feed supplies has proven to be a stumbling block in maintaining rising production trends. High feed prices, along with the detrimental impacts of antibiotic growth promoters (AGPs), have sparked global interest in evaluating functional feed ingredients that are generally free, readily available and easy to utilise in chicken production.

Currently, research into the use of different seaweeds as nutraceuticals in animal nutrition has drawn worldwide attention. Seaweeds are significant sources of bioactive metabolites that terrestrial plants do not produce (Gupta & Abu-Ghanna, 2013). Polyphenols, pigments (chlorophyll, fucoxanthin and phycobilins), polysaccharides (starch, glycogen and cellulose), and polyunsaturated fatty acids (i.e., omega-3 fatty acids) are active biocompounds found in seaweeds, which have antioxidative, antibacterial, anti-inflammatory, immunomodulatory, prebiotic, and cholesterol-lowering properties (Holdt & Kraan, 2011; Gullon *et al.*, 2020). Seaweeds have considerably contributed to improving the nutritional condition of societies worldwide due to their rich composition of vital nutrients. They also have wide-ranging applications in diverse industries, such as food, cosmetics and medicine (Michalak & Chojnacka, 2015). Seaweeds have also been used as a rich source of carbohydrates, protein, vital amino acids, minerals and vitamins in animal diets (Evans & Critchley, 2014; Nhlane *et al.*, 2020). According to Evans & Critchley (2014), seaweeds are a source of complex carbohydrates with prebiotic actions that are helpful to the health of hens.

Seaweeds have high fibre levels (33 - 75% of dry weight) due to the presence of non-starch polysaccharides, including cellulose, ulvan, pectin and xylan (Gullon *et al.*, 2020). Thus, its utility in diets of simple non-ruminants could be limited because these animals have a restricted ability to digest high dietary fibre, which can compromise nutrient digestibility and limit development (Jha & Mishra, 2021). Indeed, excessive amounts of dietary fibre hinder birds from gaining access to important nutritional components like protein (Celi *et al.*, 2017). As a result, a negative association between fibre and protein digestibility has been observed (Jha & Mishra, 2021). Furthermore, the presence of condensed tannins in seaweeds (0.91 - 63.36 mg of catechin equivalent (CE)/g dry weight) may interfere with protein consumption and limit digestibility further (Gómez-Guzmán *et al.*, 2018). Thus, the use of both exogenous protease and fibrolytic enzymes to enhance the feed value of seaweeds in broiler chicken diets might be a viable option. This would encourage the inclusion of high quantities of fibrous feed components like seaweed, allowing the birds to benefit from the bioactive compounds. To increase the performance of the birds, exogenous enzymes have been widely used in poultry diets (Zhou *et al.*, 2009; Slominski, 2011). However, because most commercial enzymes are intended for conventional feed components, the synergistic benefits of treating seaweeds with a proteolytic mono-enzyme and fibrolytic enzymes are unknown. The objective of this study was to determine the effect of simultaneously pre-treating green seaweed (*Ulva sp*.) meal with a combination of protease mono-enzyme and fibrolytic multi-enzyme on growth performance, blood parameters, internal organs, and carcass and meat quality traits in Cobb 500 broilers. It was hypothesised that pre-treating dietary green seaweed meal with exogenous enzymes would enhance growth traits, physiological parameters, and meat quality characteristics of the birds.

5.2 Materials and methods

5.2.1 Study site and ingredient sources

The study was carried out between October and November (temperatures during this time ranged from 11°C to 31°C) in the North-West University farm (North West, South Africa). The calculated value based on diet formulae of the experimental diet of the green seaweed was as described in Chapter 3, Section 3.2.2. The fibrolytic enzyme mixture containing cellulase, hemicellulase, xylanase, glucanase, and arabinase enzymes was bought from Sigma-Aldrich (Modderfontein, South Africa). The fibrolytic enzyme is miscible with water and has a density of 1.2 g/ml and an enzyme activity of 100 FBG/g. The protease mono-enzyme was procured from DSM (PTY) LTD (Pretoria, South Africa), while the other feed components were bought from Simplegrow Agric Services (Pretoria, South Africa). The protease mono-enzyme has 600,000 µ/gram enzyme activity.

5.2.2 Feed formulation

The experimental diets were formulated (Table 5.1) as follows: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes only; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme. The SW35 and VP0 treatments were pre-determined in Chapters 3 and 4, respectively, with SW35 being the diet with the highest seaweed inclusion level in Chapter 3 while VP0 was the diet with the highest level of fibrolytic enzyme treatment in Chapter 4.

Table 5.1. Ingredient composition (g/kg, as fed basis) of the experimental diets

	Grower					Finisher				
Ingredients	SW35	VP0	VP50	VP100	VP150	SW35	VP0	VP50	VP100	VP150
Viscozyme® L	0	12.0	12.0	12.0	12.0	0	12.0	12	12.0	12.0
Protease	0	0	5.0	10.0	15.0	0	0	5.0	10.0	15.0
Seaweed	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Yellow Maize 8.0%	648.8	648.8	648.8	648.8	648.8	646.3	646.3	646.3	646.3	646.3
Extruded FF Soya	31.88	31.88	31.88	31.88	31.88	34.57	34.57	34.57	34.57	34.57
Soya O/C 47%	211.9	211.9	211.9	211.9	211.9	218.1	218.1	218.1	218.1	218.1
Sunflower O/C 36%	41.51	41.51	41.51	41.51	41.51	30.00	30.00	30.00	30.00	30.00
Limestone	7.89	7.89	7.89	7.89	7.89	7.05	7.05	7.05	7.05	7.05
Monocalcium Phos	8.28	8.28	8.28	8.28	8.28	5.99	5.99	5.99	5.99	5.99
Sodium Bicarbonate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-Methionine	2.92	2.92	2.92	2.92	2.92	2.29	2.29	2.29	2.29	2.29
L-Threonine	0.98	0.98	0.98	0.98	0.98	0.58	0.58	0.58	0.58	0.58
Lysine HCL	3.40	3.40	3.40	3.40	3.40	1.85	1.85	1.85	1.85	1.85
Crude Soya Oil Mixer	-	-	-	-	-	11.4	11.4	11.4	11.4	11.4
Lignobond	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Premix	2.5	2.5	2.5	2.5	2.5	2.0	2.0	2.0	2.0	2.0
AxtraPhytase Broiler	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salinomycin 12%	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Zinc Bacitracin	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33

SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme.

The nutritional composition (g/kg *as fed* basis, unless stated otherwise) of the grower and finisher diet is shown in table 5.2. The diets were calculated for dry matter, metabolisable energy, crude protein, amino acids (AP lysine, methionine and threonine), ash, crude fibre, crude fat, and minerals (calcium, phosphorus, chloride, and sodium).

Table 5.2. Nutritional composition (g/kg *as fed* basis, unless stated otherwise) of the grower and finisher diets containing seaweed powder

Parameters	**Grower**	**Finisher**
Dry matter	903.41	905.88
Metabolisable energy (MJ/kg)	12.92	13.26
Crude protein (%)	36.09	35.01
AP Lysine	10.65	9.50
AP Methionine	5.60	4.98
AP Threonine	6.90	6.50
Crude fat	35.54	46.87
Crude fibre	51.87	49.88
Ash	43.39	44.99
Available phosphorus	4.20	3.80
Calcium	8.40	7.60
Chloride	3.54	3.29
Sodium	2.16	2.16
Total phosphorus	5.47	4.94

The proximate composition of grower and finisher diets containing pre-treated and untreated seaweed meal (g/kg *as fed* basis, unless stated otherwise) of the grower and finisher is shown in table 5.3. The proximate composition was analysed as described in chapter 4, section 4.2.3

Table 5.3. Proximate composition of grower and finisher diets containing pre-treated and untreated seaweed meal

	Grower					Finisher				
Parameters	SW35	VP0	VP50	VP100	VP150	SW35	VP0	VP50	VP100	VP150
DM (g/kg)	920.92	923.43	923.87	908.83	925.23	926.17	923.51	920.11	923.76	926.01
Ash (g/kg)	48.03	49.03	48.19	47.62	46.69	44.68	44.11	45.68	44.91	44.61
NDF (g/kg)	206.11	204.78	202.46	204.46	201.62	203.94	196.63	197.45	205.11	205.28
ADF (g/kg)	186.33	183.05	180.11	183.18	179.28	181.58	174.21	178.37	182.61	182.22
ADL (g/kg)	21.69	20.67	22.40	22.87	22.42	22.48	22.34	19.81	22.44	22.11
CP (g/kg)	320.1	333.6	327.4	324.4	337.7	362.9	368.2	346.8	351.8	361.7

SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme. DM = dry matter, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin, CP = crude protein.

5.2.3 Feeding and broiler management

Three hundred, one-day-old male Cobb 500 chicks were purchased from Monshego farm in Zeerust (North West, South Africa). The raising management of chicks was performed as described in Chapter 3, Section 3.26. The initial body weights (239.3 ± 8.57 g live-weight) of the birds were measured at 2 weeks of age and thereafter weighed weekly as described in Chapter 3, Section 3.2.6.

5.2.4 Blood sample collection and analysis

At 40 days of age, blood samples were collected as described in Chapter 3, Section 3.2.7. The automated IDEXX LaserCyte Haematology Analyser and the automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories (Pty) Ltd, Gauteng, South Africa) were used to analyse haematological parameters (reticulocytes, red blood cells, neutophils, mean platelet volume, lymphocytes, monocytes, eosinophils, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, red cell distribution width, platelets, platelet distribution width, procalcitonin and white blood cell count) and serum biochemical parameters (albumin, alkaline phosphase, alanine transaminase, cholesterol, creatinine, gamma-glutamyl, glucose, phosphorus, symmetric dimethylargininase, toatal bilirubin, total protein and urea), respectively.

5.2.5 Slaughter procedures

On day 42, the birds were transported to a local abattoir (Mafikeng, South Africa) for slaughter, after being starved of feed for 13 hours, as described in Chapter 3, Section 3.2.8.

5.2.6 Internal organs, carcass traits and meat quality measurements

Hot carcass weight (HCW), cold carcass weight (CCW), dressing percentage, carcass parts, internal organs and meat quality parameters were determined as described in Chapter 3, Sections 3.2.8 – 3.2.11.

5.3 Statistical analysis

Response surface regression was used to determine the linear and quadratic coefficients of enzyme-treated seaweed meal using the model described in Chapter 3, Section 3.2.12. The general linear models (GLM) procedures of SAS version 9.3 (SAS, 2010) were used to account for dietary differences, as described in Chapter 3, Section 3.2.12. The repeated measures analysis was also employed, as described in Chapter 3, Section 3.2.12.

5.4 Results

5.4.1 Growth performance and blood parameters

There was no significant week × diet interaction effects on average weekly BWG (P = 0.946) and average weekly G:F (P = 0.124), as revealed by repeated measures analysis. However, a significant interaction effect was observed on average weekly feed intake (P = 0.018). Table 5.4 demonstrates that throughout the feeding trial period, there were no significant linear or quadratic impacts on average weekly feed intake as protease pre-treatment levels increased. However, significant dietary effects were observed in week 6 of age, where birds fed with diet (SW35) had the highest (P < 0.05) feed intake (1144.5 g/bird) than those fed with diets (VP0), (VP50) and (VP150), which did not differ.

Table 5.4. Average weekly feed intake (g/bird) of Cobb 500 chickens fed diets containing seaweed meal pre-treated with fibrolytic and protease enzymes

	[1]Diets					[2]SEM	*P* value	
	SW35	VP0	VP50	VP100	VP150		Linear	Quadratic
Week 3	513.4	499.0	475.3	480.8	494.2	14.58	0.892	0.207
Week 4	852.9	809.5	754.3	770.2	774.2	28.83	0.528	0.355
Week 5	1062.0	985.7	989.1	983.4	1015.6	27.86	0.514	0.616
Week 6	1144.5^{b}	1006.6^{a}	989.9^{a}	1031.6^{ab}	988.1^{a}	29.60	0.916	0.645

^{a,b} In a row, means with similar superscripts do not differ (P > 0.05).
[1]Diets: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet

containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme.
[2]SEM = standard error of the mean.

Overall body weight gain (BWG), overall G:F, and final body weight (FBW) did not show any linear or quadratic trends (*P* >0.05) in response to proteolytic enzyme treatment levels (Table 5.5). No significant dietary influences were found on overall BWG, overall G:F and FBW of Cobb 500 broiler chickens.

Table 5.5. Overall body weight gain, overall gain-to-feed ratio, and final body weight (g/bird) of Cobb 500 chickens fed diets containing seaweed meal pre-treated with fibrolytic and protease enzymes

	[1]Diets						*P* value	
	SW35	VP0	VP50	VP100	VP150	[4]SEM	Linear	Quadratic
[2]Overall BWG (g/bird)	1708.0	1658.6	1646.1	1645.3	1653.6	40.00	0.926	0.782
[3]Overall G:F (g/bird)	0.478	0.503	0.513	0.505	0.505	0.007	0.951	0.501
Final body weight (g/bird)	1951.3	1896.4	1884.4	1885.1	1893.6	38.82	0.966	0.792

[1]Diets: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme.
[2]Overall BWG = body weight gain per bird from 2 to 6 weeks of age; [3]Overall G;F = gain-to-feed ratio per bird from 2 to 6 weeks of age.
[3]SEM = standard error of the mean.

There were no linear or quadratic responses for haematological parameters, as shown in Table 5.6. No significant dietary influences were found on haematological parameters of Cobb 500 broiler chickens.

Table 5.6. Haematological parameters of Cobb 500 chickens fed diets containing seaweed meal pre-treated with fibrolytic and protease enzymes

	[1]Diets						*P* value	
[2]Parameters	SW35	VP0	VP50	VP100	VP150	[3]SEM	Linear	Quadratic
Hematocrits (%)	34.16	34.41	33.58	36.33	35.91	1.057	0.551	0.061
White blood cells (×109/L)	13.08	12.61	11.66	13.36	12	1.501	0.492	0.681
Heterophils (×109/L)	11.36	11.25	11.1	11.31	9.753	1.321	0.715	0.605
Platelets (×109/L)	35.75	41.98	41.36	40.09	32.33	4.46	0.632	0.23
Monocytes (×109/L)	0.62	0.463	0.486	0.66	0.388	0.119	0.716	0.692
Lymphocytes (×109/L)	1.185	1.611	2.136	1.271	1.555	0.362	0.178	0.137
Eosinophils (×109/L)	0.068	0.008	0.008	0.015	0.008	0.029	0.885	0.747

[a,b] In a row, means with similar superscripts do not differ (*P* >0.05).
[1]Diets: SW55 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme.
[2]Parameters:
[3]SEM = standard error of the mean.

Except for symmetric dimethylarginine and calcium, there were no linear or quadratic responses for serum biochemistry, as shown in Table 5.7. Some blood parameters were affected by dietary treatments, such as (SDMA) [$y = 10.71 (\pm 1.759) + 1.23 (\pm 0.565) x - 0.098 (\pm 0.036)$

x^2; $R^2 = 0.241$, $P = 0.013$] and calcium [$y = 1.03 (\pm 0.139) + 0.148 (\pm 0.045) x - 0.01 (\pm 0.003) x^2$; $R^2 = 0.350$, $P = 0.005$], which quadratically responded to protease pre-treatment levels. Dietary treatments only influenced ($P < 0.05$) SDMA, calcium and phosphorus levels of the broiler chickens. Birds fed with diet VP50 (16.0 µg/dL) had higher ($P < 0.05$) SMDA levels than those fed with diet VP150 (7.50 µg/dL), but were both significantly similar to the other treatment groups. The untreated seaweed control diet (SW35) promoted the lowest phosphorus levels (3.37 mmol/L) compared to all the other dietary treatments, which did not differ ($P > 0.05$). Diet VP0 promoted the lowest ($P < 0.05$) calcium content (1.03 mmol/L) compared to diets VP50 (1.51 mmol/L) and VP100 (1.57 mmol/L).

Table 5.7. Serum biochemistry parameters of Cobb 500 chickens fed diets containing seaweed meal pre-treated with fibrolytic and protease enzymes

	[1]Diets						*P* value	
[2]Parameters	SW35	VP0	VP50	VP100	VP150	[3]SEM	Linear	Quadratic
Glucose (mmol/L)	2.338	2.346	2.416	3.258	2.375	0.246	0.48	0.113
SDMA (µg/dL)	10.08[ab]	10.16[ab]	16.0[b]	11.50[ab]	7.50[a]	1.494	0.136	0.012
Creatinine (µmol/L)	12.75	12.5	15.33	18.62	15.25	2.354	0.326	0.239
Urea (mmol/L)	16.02	17.34	13.26	19.26	16.5	1.526	0.66	0.711
BUN/CREA	145.4	167.6	121	157.4	158.8	33.46	0.941	0.428
Phosphorus (mmol/L)	3.37[a]	4.72[b]	4.86[b]	4.71[b]	4.60[b]	0.202	0.514	0.486
Calcium (mmol/L)	1.18[ab]	1.03[a]	1.51[b]	1.57[b]	1.09[ab]	0.134	0.727	0.003
Total protein (g/L)	99	103.4	96.66	105.9	92.75	11.44	0.613	0.749
Albumin (g/L)	31.91	37.75	39.91	48.08	34.16	4.946	0.915	0.147
Globulin (g/L)	60.33	45.08	29.41	29.58	49.08	9.738	0.76	0.059
ALB/GLOB	0.658	0.35	5.541	0.733	0.475	2.022	0.697	0.291

ALT (U/L)	164.2	282.5	203.2	223.7	217.3	54.01	0.519	0.548
ALKP (U/L)	33.58	34.58	40.66	36	46.75	13.75	0.654	0.883
Total bilirubin (µmol/L)	105.5	161	185.2	237.6	165.8	28.89	0.649	0.152

[a,b] In a row, means with similar superscripts do not differ (P >0.05).
[1]Diets: SW55 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme.
[2]Parameters: SDMA = symmetric dimethylarginine; BUN/CREA = blood urea nitrogen/creatinine ratio; ALB/GLOB = Albumin/Globulin, ALT = Alanine transaminase, and ALKP = Alkaline phosphate.
[3]SEM = standard error of the mean.

5.4.2 Carcass characteristics, internal organs and meat quality

The results from this study show that there were no significant linear or quadratic trends for carcass characteristics, as shown in Table 5.8. Dietary treatments had no influence (P >0.05) on carcass traits.

Table 5.8. Carcass characteristics (% WCW, unless stated otherwise) of Cobb 500 chickens fed diets containing seaweed meal pre-treated with fibrolytic and protease enzymes

	[1]Diets						*P* value	
[2]Parameters	SW35	VP0	VP50	VP100	VP150	[3]SEM	Linear	Quadratic
Carcass yield (%)	74.11	73.5	74.18	72.78	73.15	0.71	0.486	0.844
WCW (g)	1445.8	1394.3	1397.5	1372.7	1385.1	32.13	0.726	0.891
CCW (g)	1405.5	1357.8	1355.2	1340.1	1346.8	30.78	0.737	0.884
Wing	5.38	5.6	5.27	5.73	5.7	0.152	0.297	0.364
Breast	23.83	26.9	26.91	27.4	25.73	1.315	0.464	0.365

Drumstick	5.95	6.2	6.05	6.25	6.23	0.115	0.518	0.535
Thigh	7.4	7.67	7.37	7.73	7.42	0.186	0.722	0.968

[a,b,c] In a row, means with similar superscripts do not differ ($P > 0.05$).
[1]Diets: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme.
[2]Parameters: WCW = warm carcass weight; CCW = cold carcass weight.
[3]SEM = standard error of the mean.

The results from this study show that gizzard weights, were increasing linearly as protease pre-treatment levels increased. There were no significant linear or quadratic trends for internal organs, as shown in Table 5.9. The gizzard weights [$y = 0.055 (\pm 0.022) x + 1.88 (\pm 0.069)$; $R^2 = 0.201$, $P = 0.022$], as protease pre-treatment levels increased. Likewise, dietary treatments had no influence ($P > 0.05$) on internal organ weights, except for gizzard weights, where birds on treatments VP50, VP100 and VP150 had the highest ($P < 0.05$) gizzard weights, followed by birds fed with treatment SW35, and the lowest was from birds on treatment VP0.

Table 5.9. Internal organs (% WCW, unless stated otherwise) of Cobb 500 chickens fed diets containing seaweed meal pre-treated with fibrolytic and protease enzymes

	[1]Diets						*P* value	
Parameters	SW35	VP0	VP50	VP100	VP150	[2]SEM	Linear	Quadratic
Gizzard	1.99^{b}	1.87^{a}	2.10^{c}	2.15^{c}	2.12^{c}	0.088	0.022	0.079
Proventriculus	0.46	0.45	0.47	0.46	0.471	0.021	0.552	0.79
Spleen	0.1	0.1	0.11	0.11	0.1	0.006	0.69	0.108

Duodenum	0.65	0.68	0.7	0.72	0.71	0.035	0.545	0.765
Ileum	1.31	1.36	1.29	1.29	1.33	0.055	0.707	0.301
Jejunum	1.35	1.39	1.41	1.4	1.43	0.056	0.693	0.961
Caecum	0.98	1.04	0.96	1	1.03	0.072	0.97	0.415
Liver	1.93	1.81	1.97	1.96	1.91	0.088	0.481	0.246

[a,b,c] In a row, means with similar superscripts do not differ (P >0.05).
[1]Diets: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme.
[2]SEM = standard error of the mean.

Table 5.10 shows that there were significant quadratic trends for meat pH at 24 hours after slaughter [y = 5.79 (±0.019) + 0.011 (±0.006) x - 0.001 (±0.0004) x^2; R^2 = 0.177, P = 0.043] and 24 hour-hue angle [y = 1.38 (±0.022) + 0.016 (±0.007) x - 0.001 (±0.0005) x^2; R^2 = 0.180, P = 0.041] in response to protease treatment levels. There were no dietary effects observed on meat quality parameters of Cobb 500 broiler chicken.

Table 5.10. Breast meat quality parameters of Cobb 500 chickens fed diets containing seaweed meal pre-treated with fibrolytic and protease enzymes

	[1]Diets						P value	
[2]Parameters	SW35	VP0	VP50	VP100	VP150	[3]SEM	Linear	Quadratic
WBC (%)	86.08	85.01	88.41	88.48	87.69	1.307	0.181	0.125
Drip loss (%)	1.14	1.41	1.82	1.41	1.48	0.378	0.864	0.522
Cooking loss (%)	8.78	7.00	7.43	9.14	8.74	1.306	0.192	0.718
pH_1	6.23	6.01	6.09	6.04	5.92	0.130	0.689	0.587

L^*_{1}	44.30	40.06	40.71	36.62	30.74	5.594	0.205	0.558
a^*_{1}	3.27	3.24	4.18	3.67	4.17	0.460	0.293	0.640
b^*_{1}	14.40	12.36	15.57	14.59	13.96	1.081	0.390	0.062
Chroma$_{1}$	14.78	12.79	16.19	15.07	14.60	1.055	0.332	0.061
Hue angle$_{1}$	1.35	1.31	1.30	1.32	1.28	0.036	0.617	0.585
pH$_{24}$	5.79	5.80	5.83	5.83	5.77	0.017	0.469	0.043
L^*_{24}	56.86	57.62	57.64	58.14	54.75	1.863	0.395	0.422
a^*_{24}	2.56	3.74	2.62	2.53	2.79	0.421	0.173	0.154
b^*_{24}	18.02	18.56	20.80	18.39	16.88	17.83	0.276	0.223
Chroma$_{24}$	18.20	18.99	20.97	18.58	17.13	20.97	0.248	0.265
Hue angle$_{24}$	1.43	1.37	1.45	1.43	1.40	0.022	0.447	0.041
Shear force (N)	1.90	1.92	1.86	1.93	1.90	0.040	0.869	0.766

[1]Diets: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme.
[2]Parameters: subscript 1 = all parameters were measured 1 hour after slaughter; subscript 2 = all parameters were measured 24 h after slaughter; WBC = water-binding capacity.
[3]SEM = standard error of the mean.

The data obtained from shelf-life parameters measured at room temperature over 5 days revealed no significant effect (*P* >0.05) of dietary treatments (Figures 5.1 – 5.4). The breast meat pH for broiler chickens on VP0, VP50, VP100 and VP150 diets increased, while breast meat from broiler chickens on SW35 diets decreased at day 4. For all treatments, the lightness (*L**) of the breast meat decreased over 5 days. The redness (a*) of the breast meat increased over 5 days, except for VP0, which decreased on day 3. The yellowness (b*) of the breast meat decreased for SW35, VP0, VP50 and VP100, while it increased for VP150 on day 4.

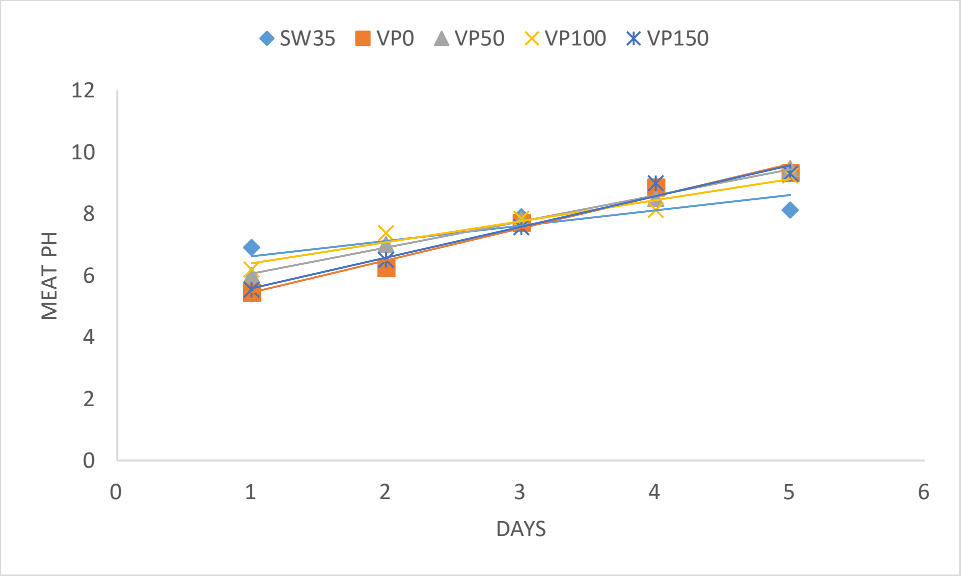

Figure 5.1. Effect of fibrolytic and protease enzyme-treated seaweed meal on the stability of breast meat pH upon storage at room temperature for 5 days [Diets: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme].

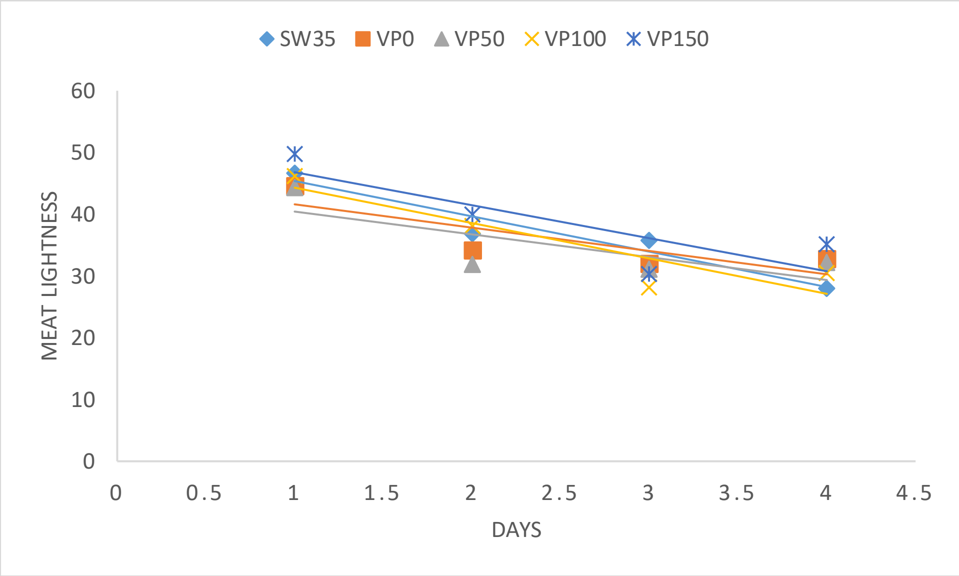

Figure 5.2. Effect of fibrolytic and protease enzyme-treated seaweed meal on the stability of breast meat lightness upon storage at room temperature for 5 days [Diets: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme].

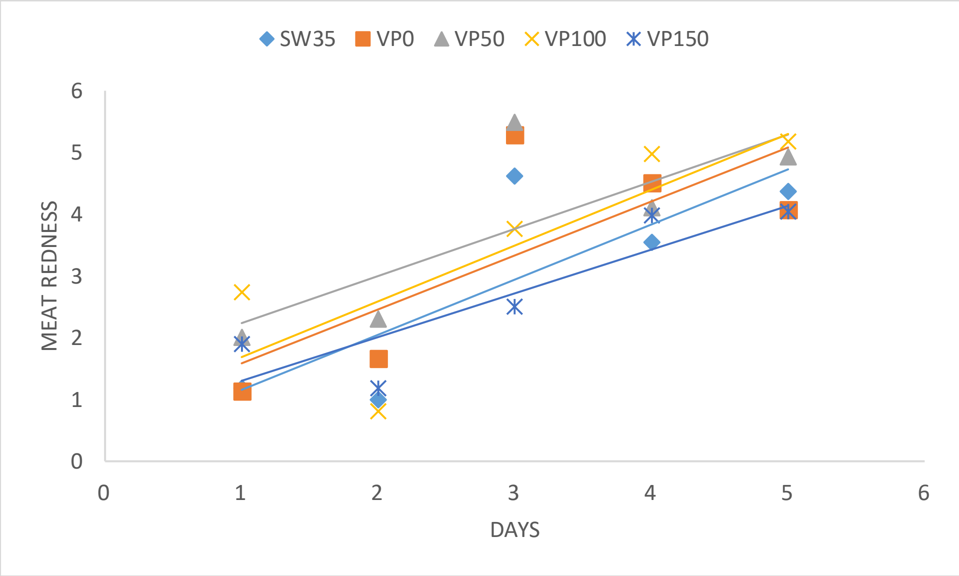

Figure 5.3. Effect of fibrolytic and protease enzyme-treated seaweed meal on the stability of breast meat redness upon storage at room temperature for 5 days [Diets: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme].

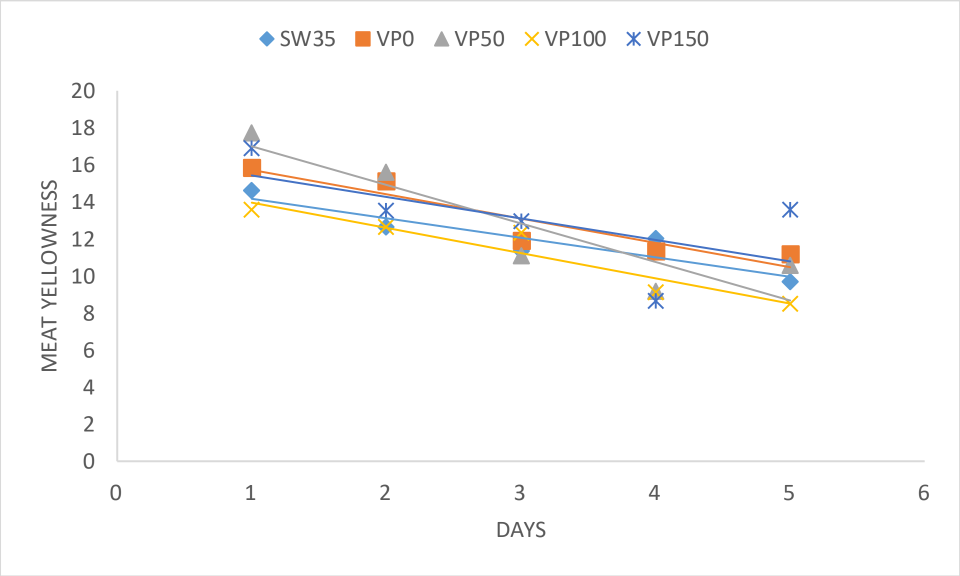

Figure 5.4. Effect of fibrolytic and protease enzyme-treated seaweed meal on the stability of breast meat yellowness upon storage at room temperature for 5 days [Diets: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed meal; VP0 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes; VP50 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed meal pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme].

5.5 Discussion

The effects of dietary seaweed meal pre-treated with a combination of exogenous proteolytic and fibrolytic enzymes on broiler chicken performance, blood parameters, and meat quality traits were examined in this chapter. The repeated measures analysis revealed a significant diet and week interaction impact only on feed intake, indicating that the birds' capacity to use the diet was dependent on their age. The results indicated that pre-treating green seaweed meal with exogenous enzymes had no effect on the birds' live performance. In Chapter 3, it was discovered that adding 35 g/kg of untreated green seaweed meal to Cobb 500 broiler diets had a negative effect on the birds' performance and feed utilisation efficiency. The presence of indigestible non-starch polysaccharides, such as cellulose, hemicellulose, xylans, and ulvans, in the seaweed (Kraan, 2012) seems to have been the reason for this adverse effect. Furthermore, large amounts of condensed tannins in green seaweeds may decrease protein digestibility by creating tannin-protein complexes that are indigestible by endogenous digestive enzymes. Indeed, adding 30 g/kg of untreated green seaweed (*Ulva lactuca*) to broiler meals had no effect on the birds' body weight increase or feed conversion ratio (Abudabos *et al.*, 2013). Pre-treating the seaweed meal with both the protease mono-enzyme and the cellulolytic multi-enzyme was, therefore, envisaged to increase the utilisation of the meals, resulting in improved growth performance. The results showed that pre-treatment of seaweed with a combination of exogenous enzymes (protease and fibrolytic enzymes) did not improve the physiological and meat quality parameters in Cobb 500 broiler chickens. Similarly, Mnisi *et al*. (2017) and Mnisi & Mlambo (2018), found no improvement in growth characteristics of Japanese quail-fed canola-containing diets treated with fibrolytic enzymes and a protease mono-enzyme, respectively. Furthermore, Sayyazadeh *et al*. (2006) reported no significant effects on body weight and feed efficiency of chickens fed cereal-based diets supplemented with exogenous enzymes. Nonetheless, when exogenous enzymes were used in poultry diets, numerous studies found positive results (Zhou *et al.*, 2009).

The use of a mixture of amylase, xylanase, and protease has been demonstrated to improve nutritional digestibility and animal performance (Singh *et al.*, 2019). The varied types of feed substrates utilised in these research studies, as well as varying application techniques, enzyme activity, and treatment doses, might explain the discrepancies (Kim & Rajapakse, 2005). The lack of changes in feed intake of six-week-old broilers between the control treatment group (SW35) and the treatment group (VP100) further indicates the enzymes' ineffectiveness in improving the seaweeds' value.

In agricultural animals, hematological profiles have been used to predict nutritional responses (Madubuike *et al*., 2006). The findings of this study show that enzyme pre-treatment of seaweed had no effect on the birds' hematological parameters, with all reported values falling within the normal range for healthy chickens (Animashahun *et al*., 2006). The lack of side effects suggests that the enzymes provided enough nourishment for broiler chickens. Furthermore, serum biochemical measurements give valuable information on an animal's health status by identifying different metabolic changes that occur after eating non-traditional feed components (Cupisti *et al*., 2017). The results of this investigation revealed that seaweed meal treated with exogenous enzymes had no negative effects on the blood parameters of the birds. The quadratic responses and variability in broiler performance with enzyme supplementation in terms of calcium, phosphorus, and SDMA might be attributable to the related advantage in improving protein/amino acid digestibility (Cowieson & Bedford, 2009). Similar to the current findings, a few studies (Shalash *et al*., 2009; Slominski, 2011) found that adding an enzyme combination (glucanase, amylase, and/or protease) to broiler diets had no effect on blood parameters. The reaction to a multienzyme complex is likely to be influenced by several parameters, including the chemical composition of the feed, enzyme dosage, genetic strain and the age of the birds (Cowieson *et al*., 2006). As reported by Rizk *et al*. (2017), supplementation of hen diets with green, brown, and red seaweed (0.1% and 0.2%) lowered the concentration of serum total cholesterol and improved serum total protein, albumin, globulin, liver and kidney functions than the control group.

Poultry birds are mostly chosen for their fast development, larger breast weight and enhanced muscular mass. The effect of exogenous enzyme supplementation on broiler chicken carcass traits has received little attention. Hussein *et al*. (2017) found no impact of exogenous enzyme supplementation on broiler chicken carcass weight, abdominal fat or breast meat weight. The effects of exogenous enzyme treatments on meat quality and organ weight in broilers have been variable in previous studies (Cho *et al*., 2012; Ndazigaruye *et al*., 2019). Symeon *et al*. (2009) reported the benefits of enzyme administration, whereas others found no improvement when a protease was administered (Zakaria *et al*., 2010).

The addition of green seaweed at a rate of 2, 3 or 4% of dry algal powder from *Enteromorpha prolifera* improved the quality of the breast meat (Wang *et al*., (2013). Michalak & Mahrose (2020) showed no significant changes in carcass weight and yield of Arbor Acres broilers fed varying amounts of *Ulva rigida* (air dried and ground), inclusion at different dietary levels of 2, 4 and 6% (Michalak & Mahrose 2020). Dietary treatments had a significant effect on gizzard weights in the digestive system. When seaweed meal was pre-treated with fibrolytic and protease enzymes, the findings revealed a substantially increased influence on the gizzard weight of broiler chickens. However, incorporating seaweed meal at a high level may raise fibre levels and be linked to substantial structural and morphological changes in the gut, as an adaptive mechanism to accommodate fibre and phytochemicals. Adding exogenous enzymes to feed can be useful by breaking down the feed and making nutrients available for animals.

The seaweed pre-treated with fibrolytic and protease enzymes had no effect on the carcass traits of the birds in the current investigation. However, the findings might indicate that the greater quantities of fibre in seaweed harmed the digestive tract and reduced nutritional absorption, resulting in comparable carcass weights. According to Muchenje *et al.* (2009), the most important signal when customers buy meat products is meat colour, which is a crucial parameter that influences consumer acceptability of meat. Seaweed pre-treated with fibrolytic and protease enzymes had no effect on the meat colour of the birds in the current investigation. The findings revealed that seaweed pre-treated with fibrolytic and protease enzymes can improve the pH and redness of meat products at room temperature, but not meat yellowness and lightness. The pH readings dramatically increased, possibly indicating that seaweed pre-treated with fibrolytic and protease enzymes failed to preserve normal meat pH.

5.6 Conclusion

It was concluded that the use of a combination of a protease mono-enzyme and fibrolytic multi-enzymes to pre-treat dietary green seaweed meal did not enhance feed intake, physiological, or meat quality measures in Cobb 500 broiler chickens. Furthermore, utilising the growth performance data, an optimal protease treatment level could not be established, indicating that levels greater than 15 g/kg of protease mono-enzyme are needed to induce non-linear responses.

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6 CHAPTER SIX - GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

6.1 General discussion

The growing human population and high demand for animal protein have stimulated the search for novel feed sources, including seaweed (Makkar *et al.*, 2016; Buschmann *et al.*, 2017). According to Biesalski *et al.* (2009), the use of seaweed as a source of naturally occurring beneficial bioactive compounds, such as vitamins and polyphenols, has been shown to positively influence animal nutrition and health. According to Holdt & Kraan, (2011), seaweeds contain bioactive compounds, such as terpenoids, polyunsaturated fatty acids, carrageenan, sulphated polysaccharides and fucoidan. These bioactive compounds are known to boost immune responses in food animals, and thus can be incorporated into animal feeds (Khalid *et al*., 2018). Accordingly, seaweeds can meet the growing demand for food and land resources, since they are quick growing and produce high biomass than other conventional biomass feedstocks like maize or soybean.

Until now, debatably, seaweeds have not been nutritionally evaluated in Cobb 500 broilers using growth performance, blood parameters and meat quality as indicators. Therefore, the first experiment of this study investigated the growth performance, haematology, serum biochemistry, internal organs and carcass and meat quality parameters of Cobb 500 broilers fed with diets containing incremental levels of green seaweed meal. This experiment explored the hypothesis that growth performance, and physiological and meat quality parameters in Cobb 500 broilers would respond to incremental levels of dietary seaweed meal in a non-linear fashion and thus allowing the determination of an optimum dietary inclusion level for untreated seaweed. Five isonitrogenous and isoenergetic diets were formulated by diluting a commercial broiler diet for the grower and finisher phases, with graded levels of seaweed meal at a rate of 0 (SW0), 20 (SW20), 25 (SW25), 30 (SW30) and 35 g/kg (SW35).

The results obtained from this study revealed that dietary treatments affected overall gain-to-feed ratio (G:F), spleen weights, blood lymphocytes, and breast meat pH, lightness and redness. However, the hypothesis for the first experiment was rejected because no non-linear responses were observed, and thus, the optimum dietary inclusion level could not be determined for the untreated seaweed meal. In addition, a linear decrease in the G:F, as the levels of dietary seaweeds increased, indicating that higher levels of seaweeds were detrimental to nutrient utilisation for growth.

Armed with this information, it was speculated that this effect could be due to high levels of fibre that are known to have antinutritional effects in simple non-ruminants like broiler chickens. The desire to improve the digestibility of seaweed meal-containing diets in broiler chickens led to the formulation of an experiment where these negative fibre effects would be ameliorated using exogenous fibrolytic enzymes. Therefore, the second experiment tested the hypothesis that fibrolytic enzyme pre-treatment of seaweed meal improves growth performance, blood parameters, internal organs, and carcass and meat quality traits of Cobb 500 broilers. Five different isonitrogenous and isoenergetic dietary treatments were formulated and assigned randomly to the birds. A commercial grower or finisher diet comprising 35 g/kg untreated seaweed meal (SW35) and a commercial grower or finisher diet, including seaweed meal treated with fibrolytic enzyme at the rates of 2.0 (V20), 5.0 (V50), 7.5 (V75) and 12.0 g/kg (V120) were used. The results obtained from this experiment showed that hot carcass weight (HCW), cold carcass weight (CCW), and breast meat redness were affected by dietary treatments. Exogenous enzyme supplementation has been shown to promote food metabolism and digestibility, as well as control development and improve carcass features (Sugiharto *et al.*, 2019). In this experiment, the hypothesis was also rejected because the responses for growth performance, and physiological and meat quality parameters could not allow the determination of an optimum enzyme treatment level. Seaweed pre-treatment, with fibrolytic enzyme alone, was an ineffective strategy to increase its utilisation in broiler chicken. It was, therefore, hypothesised that the poor protein digestibility and not the recalcitrant fibre fraction, maybe the reason seaweed utilisation performed poorly in broiler chicken. This suggests that seaweed may need to be pre-treated with a combination of the fibrolytic multi-enzymes and proteolytic monoenzyme in the third experiment to valorise the feed value of seaweed meal in broiler chicken.

The third experiment tested the hypothesis that pre-treatment of seaweed meal with a combination of fibrolytic and proteolytic enzymes improves growth performance, blood parameters, internal organs, carcass characteristics, and meat quality traits of Cobb 500 broilers. The experimental diets were formulated as follows: SW35 = standard grower or finisher diet containing 35 g/kg untreated seaweed; VP0 = standard grower or finisher diet containing 35 g/kg seaweed pre-treated with 12 g/kg fibrolytic enzymes only; VP50 = standard grower or finisher diet containing 35 g/kg seaweed pre-treated with 12 g/kg fibrolytic enzymes and 5 g/kg protease mono-enzyme; VP100 = standard grower or finisher diet containing 35 g/kg seaweed pre-treated with 12 g/kg fibrolytic enzymes and 10 g/kg protease mono-enzyme; and VP150 = standard grower or finisher diet containing 35 g/kg seaweed pre-treated with 12 g/kg fibrolytic enzymes and 15 g/kg protease mono-enzyme.

The results from this experimental trial showed that gizzard weight, symmetric dimethylarginine, calcium, meat pH_{24} and hue $angle_{24}$ were affected by dietary treatment. The hypothesis in this study was rejected because results showed that pre-treatment of seaweed meal, with a combination of exogenous enzymes (protease and fibrolytic enzymes), did not improve the performance, physiology, and meat quality parameters in Cobb 500 broiler chickens. However, the findings suggest that higher levels of seaweed (35 g/kg) compromised digestive tract function and thus reduced nutritional absorption, resulting in lower carcass weights. This suggests that seaweed should be used with caution in broiler chicken diets. The combination of fibrolytic and protease enzymes did not improve the feed value of seaweed in broilers, poor protein digestibility and fibre fraction could be the reason seaweed utilisation is poor in broilers. The high levels of salt in seaweed can be retained and cause broilers to lose weight because of diarrhoea. This suggests that further investigation is needed for comparing unwashed and washed of seaweed in order to reduce seaweed salt.

6.2 General conclusion

The cost of poultry production is increasing at a fast rate, thus searching for alternative ingredients, such as seaweed with beneficial bioactive compounds, might reduce costs and improve profitability. Although an optimum dietary seaweed inclusion level could not be established in the first study, a negative linear association with G:F shows that excessive levels of seaweed meal inclusion may reduce feed utilisation in broilers. It was found that pre-treatment with exogenous fibrolytic enzymes had no effect on seaweed meal utilisation, but more research is needed to determine the optimal inclusion level of fibrolytic enzymes. Similarly, a combination of protease mono-enzyme and fibrolytic multi-enzymes to pre-treat dietary green seaweed meal did not enhance feed intake, physiological or meat quality measures in Cobb 500 broiler chickens. In addition, utilising the growth performance data, an optimal fibrolytic and proteolytic treatment level could not be established, indicating that levels greater than 12 g/kg of fibrolytic multi-enzyme and 15 g/kg of protease mono-enzyme could be needed to induce non-linear responses.

6.3 Recommendation and future studies

Future research is required to establish other effective, and possibly less expensive, methods to mitigate the antinutritional effects of tannins and fibre in seaweed meals. Feeding of different seaweed species and inclusion levels is required in future studies. Future research should also consider the use of tannins-binding agents to ameliorate the negative effects of tannins. Another important investigation is to perform a fatty acid profile, sensory evaluation, and oxidative stability on breast meat of broiler chickens fed a diet containing seaweed. Future research is required to test the effect of seaweds on nutrient digestibility, gut histomorphology and metagenomics of broiler chickens. Further studies should carry out cost-benefit analyses when seaweed is used as a nutraceutical for broilers.

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7 LIST OF APPENDICES

7.1 Appendix: Ethics Approval

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Research Ethics Regulatory Committee
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Email: nkosinathi.machine@nwu.ac.za

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC)** on 04/07/2019, the NWU Animal Production Sciences Research Ethics Committee hereby **approves** your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Improving the feed value of seaweed (*Ulva armoricana*) for broiler chickens using feed enzymes
Study Leader/Supervisor (Principal Investigator)/Researcher: Prof V Mlambo & Dr C Mnisi
Student: Matshogo T

Ethics number:

N	W	U	-	0	0	3	5	6	-	1	9	-	A	5
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: 2019/08/01 **Risk Category:** 2
Expiry date: 2020/07/31

Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the NWU-AnimProdREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-AnimProdREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-AnimProdREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and NWU-AnimProdREC reserves the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*

1

7.2 Appendix turtin report

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ORIGINALITY REPORT

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7.3 Appendix: Published from Chapter Three

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Article

Dietary Green Seaweed Compromises Overall Feed Conversion Efficiency but not Blood Parameters and Meat Quality and Stability in Broiler Chickens

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Abstract: Using seaweeds as sources of nutrients and beneficial bioactive compounds can promote sustainable production of functional poultry products. This study investigated the physiological and meat quality responses of Cobb 500 broiler chickens to graded levels of green seaweed (*Ulva* sp.) meal (SWM). Three hundred, two-week-old male chicks (159.3 ± 11.76 g live-weight) were randomly assigned to five diets formulated by diluting a standard broiler diet with SWM at 0 (SW0), 20 (SW20), 25 (SW25), 30 (SW30) and 35 g/kg (SW35). There were neither linear nor quadratic trends ($p > 0.05$) for overall feed intake, overall growth performance and carcass and meat quality traits. Overall feed conversion efficiency ($R^2 = 0.192$, $p = 0.018$) and spleen weights ($R^2 = 0.182$; $p = 0.020$) linearly declined as SWM levels increased. Linear and quadratic responses ($p > 0.05$) were observed for lymphocytes. There were linear effects for meat pH except on day 7 of storage. Meat lightness (L^*) linearly increased whereas meat redness (a^*) quadratically responded to SWM levels (day 3 of storage). While an optimum inclusion level could not be established for seaweed based on growth performance, improvements in some meat shelf life indicators were observed in the broilers reared on seaweed-containing diets.

Keywords: chicken; growth performance; hematology; meat quality; seaweed; serum biochemistry

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1. Introduction

The Cobb 500 broiler is a modern commercial chicken hybrid known for high feed efficiency, fast growth rates and competitive breast meat yields at various processing stages when compared to other commercial hybrids currently produced around the world [1]. Under intensive poultry production systems, feed costs contribute close to 70% of the total production cost, which is due to increases in global feed prices. Consequently, the use of inexpensive unconventional feed ingredients has received worldwide interest [2]. Additional challenges include the ban of in-feed antibiotic growth promoters in several dozen countries, which results in the uncontrolled proliferation of pathogenic bacteria to the detriment of growth performance and product quality [3]. The presence of antibiotic residues in poultry products is undesirable for consumers who continue to demand high quality and safe foods with functional properties [4]. This has led to the search for alternative feed additives with growth-boosting and product-enhancing properties such as seaweeds. Indeed, these marine macroalgae have been reported to exhibit growth-stimulating, antioxidant, antimicrobial and meat quality-boosting properties in an indigenous chicken strain [5].

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7.4 Appendix: Published from Chapter Four

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Effect of pre-treating dietary green seaweed with fibrolytic enzymes on growth performance, blood indices, and meat quality parameters of Cobb 500 broiler chickens

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HIGHLIGHTS

- Seaweeds are functional feed ingredients with putative growth-stimulating and meat quality-enhancing effects.
- Effectiveness of exogenous fibrolytic enzyme pre-treatment in enhancing seaweed feed value was investigated.
- Fibrolytic enzymes did not improve feed intake, physiological parameters, and meat quality in broiler chickens.

ARTICLE INFO

Keywords:
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Meat quality
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ABSTRACT

Seaweeds are functional feed sources that contain biocompounds with growth-stimulating, antimicrobial and meat-enhancing properties. However, the presence of non-starch polysaccharides such as cellulose, hemicellulose, pectin and xylan may limit its utilization in broiler diets. Thus, this study evaluated the effects of pre-treating dietary green seaweed (*Ulva sp.*) with a fibrolytic enzyme mixture on physiological and meat quality parameters of Cobb 500 broiler chickens. Three hundred and thirty, two-week old male chicks (318.9 ± 16.12 g live weight) were evenly and randomly placed in 30 replicate pens (experimental units) to which five iso-nitrogenous and isoenergetic diets were randomly allotted. Experimental diets were commercial grower and finisher diets containing seaweed pre-treated with a fibrolytic enzyme mixture of cellulase, hemi-cellulase, arabanase, β-glucanase and xylanase, at the rate of 0 (EZ0), 2.0 (EZ20), 5.0 (EZ50), 7.5 (EZ75) and 12.0 g/kg (EZ120). Growth performance, blood indices, visceral organs, and carcass and meat quality parameters were determined. No week × diet interaction effect ($P > 0.05$) on average weekly feed intake (FI), body weight gain (BWG), and feed conversion efficiency (FCE) was observed. Neither linear nor quadratic trends ($P > 0.05$) were observed for overall FI, growth performance, haematological and serum biochemical parameters as well as visceral organ weights in response to incremental levels of fibrolytic enzyme pre-treatment of seaweed. However, there was a linear increase in hot [$R^2 = 0.176$, $P = 0.033$] and cold [$R^2 = 0.164$, $P = 0.040$] carcass weights in response to increased enzyme pre-treatment rates. Breast meat lightness measured immediately after slaughter (L^*_1) linearly decreased [$R^2 = 0.147$, $P = 0.022$] as enzyme levels increased. Diets with enzyme-treated seaweed promoted higher ($P < 0.05$) carcass weights compared to the untreated seaweed-containing control diet. It was concluded that pre-treatment of seaweed meal with an exogenous fibrolytic enzyme mixture did not improve growth performance, blood parameters, and meat quality traits in broiler chickens. An optimum fibrolytic enzyme treatment level for seaweed could not be determined, suggesting a need to investigate levels beyond the maximum treatment level of 12 g/kg used in this study.

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7.5 Appendix: Published from Chapter Five

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Article

Effect of Pre-Treating Dietary Green Seaweed with Proteolytic and Fibrolytic Enzymes on Physiological and Meat Quality Parameters of Broiler Chickens

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Abstract: The use of seaweeds as nutraceuticals in chicken diets is limited by high fibre levels and low protein digestibility. Therefore, we tested the effect of pre-treating dietary seaweed (*Ulva* sp.) with a combination of protease and fibrolytic enzymes on physiological and meat quality parameters of Cobb 500 broilers. Five dietary treatments were formulated by including untreated (T1); fibrolytic (12 g/kg) enzyme-treated (T2); fibrolytic (12 g/kg) and protease (5 g/kg) enzyme-treated (T3); fibrolytic (12 g/kg) and protease (10 g/kg) enzyme-treated (T4); fibrolytic (12 g/kg) and protease (15 g/kg) enzyme-treated (T5) seaweed (35 g/kg) in a standard broiler diet. Three hundred, two-week-old chicks (239.3 ± 8.57 g live weight) were evenly distributed to 30 replicate pens to which the diets were then randomly allocated. Birds fed diet T1 had the highest feed intake (1144.5 g/bird). Neither linear nor quadratic trends were recorded for growth performance and carcass traits in response to protease pre-treatment levels. Gizzard weight linearly increased, while symmetric dimethylarginine, calcium, meat pH_{24}, and hue $angle_{24}$ quadratically responded to protease levels. Diet T1 promoted the lowest serum phosphorus levels (3.37 mmol/L). In conclusion, pre-treatment of seaweed with a combination of protease and fibrolytic enzymes did not improve diet utilization, physiological parameters, and meat quality in broilers.

Keywords: blood parameters; chickens; growth performance; carcass characteristics; meat quality

1. Introduction

The poultry industry significantly contributes to agri-food value chains around the world [1]. Chicken meat production has been increasing worldwide [2], driven by a growing human population, which is projected to breach the 9 billion mark by 2050 [3]. Over the years, poultry producers have relied heavily on high-performing conventional chicken strains to meet the increasing demand for animal protein. However, these chicken strains have high nutrient requirements resulting in high feeding costs and lower profit margins. In addition, intensive production systems predispose these chickens to a variety of stressors leading to a high incidence of diseases. Consequently, antibiotic growth promoters (AGP) have traditionally been used to control infections and enhance feed utilization efficiency [4]. However, the emergence of antimicrobial resistance as well as the threats posed by antibiotic residues to consumer health has seen AGPs being banned in some countries [4,5]. However, due to a lack of effective alternatives, the use of AGPs has continued in low-income countries that are burdened by high incidences of infectious disease outbreaks. A possible solution is the use of readily available plant-based feed additives with nutraceutical properties as AGP alternatives in poultry production.

7.6 Appendix: Language editing certificate

7.5 LANGUAGE EDITING CERTIFICATE

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26 August 2022

TITLE: Improving the feed value of seaweed (Ulva sp.) for broiler chickens using feed enzymes

This serves to confirm that I edited substantively the above document. The document was returned to the author with various tracked changes intended to correct errors and to clarify meaning. It was the author's responsibility to attend to these changes.

Yours faithfully

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