Towards the optimization of brown seaweed (*Ecklonia maxima*) for indigenous chickens using oyster mushrooms

G. Mhlongo

ORCID.org/ **0000-0002-9806-914X**

Thesis accepted in fulfilment of the requirements for the degree *Doctor of Philosophy in Animal Science* at the North-West University

Promoter: Prof. C.M. Mnisi

Graduation ceremony: May 2024

DECLARATION

I, Godfrey Mhlongo, declare that this thesis titled "**Towards the optimization of brown seaweed (*Ecklonia maxima*) for indigenous chickens using oyster mushrooms**" is my original work. I confirm that proper citation of all sources has been duly acknowledged in the form of comprehensive chapter-specific reference lists. Additionally, I affirm that this thesis has not been previously presented to any university other than the North-West University.

Candidate: G. Mhlongo (26735670)

Signed: …………………………………. Date: …………………………………….

Promoter: Prof. C.M. Mnisi

Signed ………………………………….. Date: ……………………………………

GENERAL ABSTRACT

Ecklonia maxima is a type of brown seaweed, commonly known as sea bamboo or Osbeck, which is found in aquatic ecosystems. Sea bamboo meal (SBM) could be used in chicken diets as a natural source of nutrients and phytochemicals such as polyphenols and terpenoids. However, its high fibre and secondary metabolites such as lectins, trypsin and alpha-amylase inhibitors, alginate, and phlorotannins content restricts the amount that can be incorporated into Boschveld cockerels' diets. Thus, this thesis aimed to enhance the feed value of SBM in the diets of Boschveld cockerels using oyster mushroom (*Pleurotus ostreatus*) spawn (OMS). A total of 225, four-week-old Boschveld cockerels (316.4 ± 23.01 g live-weight) were randomly assigned to 25 pens (experimental units), with 9 birds per pen replicated five times per dietary treatment (SBM: 0, 20, 40, 60 and 80) in a completely randomised design (CRD). The objective was to determine the effect of including varying levels of dietary SBM in a diet of Boschveld cockerels on growth performance, apparent retention (AR) of feed components, physiological (blood and viscera morphometry) responses, carcass characteristics, and meat quality . Five dietary treatments were formulated as follows: a standard grower diet containing 0 (SBM0), 20 (SBM20), 40 (SBM40), 60 (SBM60), and 80 g/kg (SBM80). The cockerel's initial body weights were measured at 5 weeks of age, and then subsequently weighed weekly to calculate average weekly body weight gain (BWG). Feed intake was measured daily and used to determine average weekly feed intake (FI). The gain-to-feed ratio (G:F) was calculated using the weekly BWG and FI data measured during the 11-week feeding trial. At 15 weeks and 6 days of age, blood collected from wing vein of two randomly selected birds per pen was used to determine the haematology and clinical biochemistry. At 11 weeks of age, two cockerels per pen were randomly selected for measurements of AR, while the remaining birds were slaughtered, visceral morphometry and meat quality were determined. The results showed neither linear nor quadratic effects (P >0.05) for overall BWG, G:F, AR of dry matter, organic

matter, crude protein, neutral detergent fibre and acid detergent fibre, haematology and clinical biochemistry and meat quality in Boschveld cockerels as SBM levels increased. However, linear increases were recorded for overall FI [R^2 = 0.397; P = 0.021], relative ceca weight [R^2 = 0.417; P = 0.013], duodenum length [R^2 = 0.537; P = 0.04] and small intestine length [R^2 = 0.305; P = 0.04]. Birds reared on the SBM0 diet had higher blood calcium levels (2.675 mmol/L) than those reared on diet SBM60 (1.834 mmol/L). In conclusion, dietary inclusion of SBM in Boschveld cockerels' diets increased overall FI, masses and lengths of proventriculus, caeca, duodenum, ileum and small intestines but did not have an effect on growth metrices, blood profiles, AR of proximate components, carcass characteristics, meat quality and stability attributes. From the previous experiments, the optimum inclusion level of dietary SBM could not be generated, thus, the inclusion level of 150 g SBM /kg diet was evaluated further in the next experiment. To counteract the anticipated negative effects of fibre, the SBM was inoculated with different levels of OMS. This experiment evaluated the proximate composition of SBM spent mushroon substrate and its potential impact when included in a diet of Boschveld cockerels using growth performance, physiological responses, and meat quality. A total of 324, four-week-old Boschveld cockerels (310.8 ± 13.98 g live-weight) were used. The cockerels were randomly assigned to 36 pens (experimental units), with 9 birds per pen to give six replicates per dietary treatment in a CRD. Six experimental diets were formulated as follows: a standard grower diet (CON); and CON containing 150 g/kg of SBM after inoculating with 0 (SMS0), 20 (SMS20), 30 (SMS30), 40 (SMS40) and 50% OMS/kg SBM (SMS50). Body weight gain increased linearly (P <0.05) in weeks 8, 11, 14 and 15 in response to SMS inclusion in the diet. Furthermore, the inclusion of SMS in the diet induced linear increases for final body weight [R^2 = 0.197; P = 0.017] and breast weight [R^2 = 0.197; P = 0.020]. However, a linear decline was noted for relative caecal weight [R^2 = 0.176; P = 0.035]. Quadratic responses (P <0.05) were also recorded for white cell counts, platelets, lymphocytes, heterophils,

monocytes, relative spleen weight, and large intestine lengths. Birds reared on SMS40 diet had a longer (*P* <0.05) caeca (21.12 cm) than those reared on CON and the other SMS treatment groups. For breast meat stability, there were significant dietary effects on meat pH on day 3, where all the SMS treatments promoted the least (*P* <0.05) pH values than CON. In conclusion, growth performance, slaughter and breast weights were improved by feeding Boschveld cockerels with a diet containing SBM-spent oyster mushroom substrate. Furthermore, the inclusion of SBM spent oyster mushroom substrates altered haematological parameters and relative caeca weights without affecting the cockerel's overall health, serum biocheemistry and meat quality. The results of this study showed that the use of oyster mushroom spawn is an effective strategy to improve the feed value of sea bamboo meal for use in the diets of Boschveld cockerels.

Keywords: Chickens, Fibre, *Pleurotus ostreatus*, Seaweed, Spent mushroom substrate

ACKNOWLEDGEMENTS

First and foremost, I want to express my gratitude to the Almighty God for giving me life and the strength to work and complete this project.

A special thanks to my supervisor, Prof. C.M. Mnisi, for his constant guidance and support throughout this research project which turned out to be a success against all odds. Prof. Mnisi, thank you for sharing your writing and research expertise. I sincerely appreciate your assistance throughout the entire process.

Special appreciation to my colleagues and friends: Messrs M. Marareni, L.C. Mahlobo, A. Gila, J. Moloto and A.N. Matabane and Dr T.B. Matshogo for their help and support throughout my doctoral study. I am immensely grateful to the National Research Foundation (NRF grant number: 130600), the North-West University Postgraduate and the Faculty of Natural and Agricultural Science bursaries for the financial assistance towards this research.

A special thanks to my family for their unwavering love, support and encouragement.

Lastly, I would like to express my heartfelt gratitude to Spesfed (PTY) Ltd, RawKelp and Yara International for the assistance with the feed formula, the sea bamboo, and the dicalcium phosphate used in this study, respectively.

DEDICATION

I dedicate this thesis to my family, particularly my mother, Nompumelelo Ngomane, who has always supported me in my academic pursuits and encouraged me to follow my dreams. To my partner, Kedibone Sethole, who has been by my side throughout the challenges and joys of this project, and to my son, Celumusa Mhlongo, who fills my life with love and happiness. You are the most important people in my life, and I am grateful for your presence and guidance. Thank you for everything.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AA	: Amino acids
ADF	: Acid detergent fibre
AgriLASA	: Agri-Laboratory Association of Southern Africa
AOAC	: Association of Official Analytical Chemists
BWG	: Body weight gain
CCW	: Cold carcass weight
CP	: Crude protein
DM	: Dry matter
FCR	: Feed conversion ratio
FI	: Feed intake
G:F	: Gain-to-feed ratio
GLM	: General linear model
HCW	: Hot carcass weight
LSMEANS	: Least square means
NDF	: Neutral detergent fibre
NRC	: National Research Council
WCW	: Warm carcass weight
WHC	: Water holding capacity
SAS	: Statistical analysis system
SBM	: Sea bamboo meal
SMS	: Spent mushroom substrate

PEER-REVIEW ARTICLES PUBLISHED FROM THIS THESIS

1. **Mhlongo, G.** & Mnisi, C.M., 2023. Effect of seaweed (*Ecklonia maxima*) on apparent nutrient digestibility, growth performance, and physiological and meat quality parameters in Boschveld cockerels. *Poultry Science*, 102(2), 102361. **(Published).** https://doi.org/10.1016/j.psj.2022.102361
2. **Mhlongo, G.** & Mnisi, C.M., 2023. Dietary incorporation of brown seaweed spent oyster mushroom substrate alters growth performance, physiological responses and meat quality parameters in Boschveld roosters. *Scientific reports*. (**Under review**).

1 CHAPTER ONE – GENERAL INTRODUCTION

1.1 Background

The poultry industry is a crucial source of high-quality animal protein for human consumption, contributing significantly to national food security and zero-hunger goals (Bureau for Food Agricultural Policy & National Agricultural Marketing Council, 2016). Many African communities are keeping a small flock of indigenous chickens in their backyards as a source of meat, eggs, and income. Indigenous chickens have valuable traits, including resistance to various poultry diseases, thermo-tolerance, good meat flavour, high egg quality, and elevated fertility rates (Atela *et al*., 2019). However, modern-day poultry production has primarily focused on conventional broiler and layer birds, neglecting indigenous chickens (Nhlane *et al*., 2021). This is due to their inherent slow growth rates, low feed utilisation efficiency, delayed sexual maturity, and late attainment of market weight compared to their conventional counterparts (Atela *et al*., 2019). Additionally, the cost of rearing indigenous chickens intensively has become a significant hurdle in utilising them as a commercial source of animal protein (Nhlane *et al*., 2021).

Matshogo *et al*. (2020) reported that feed costs, which constitute over 70% of total production costs, pose a significant challenge, which is driven by global increases in feed prices within high-input poultry production systems. Nkukwana (2018) highlighted that the reliance on highly demanded feed ingredients like maize and soybean, as well as antibiotic growth promoters (AGPs) during feed formulations has negative impacts on the economic sustainability of poultry production. This has necessitated the urgency to explore alternative feed ingredients, such as seaweeds, specifically sea bamboo (*Ecklonia maxima*) meal (SBM), to contribute to sustainable intensification of indigenous chicken production.

Sea bamboo, a type of brown seaweed belonging to the *Lessoniaceae* family and commonly known as kelp, is the largest among seaweeds, dominating coastal inshore areas of the south-west coast regions of the Southern Hemisphere, including South Africa, Australia, and New Zealand (Anderson *et al*., 2006; Rothman *et al*., 2015). Sharma *et al*. (2014) noted that sea bamboo is naturally rich in the macro-elements, calcium, magnesium, potassium, sodium, phosphorus and sulphur as well as other trace elements, iron, manganese, copper and zinc. Generally, brown seaweeds are abundant sources of minerals, soluble fibres and bioactive phytochemicals (O'Sullivan *et al*., 2010). Sea bamboo contains more phytochemicals, including phlorotannins, alginates, fucoidans, terpenoids, mannitol and laminarin, with the potential to produce value-added compounds like organic fertilizers (Bordoloi & Goosen, 2020). These bioactive phytochemicals can be exploited to reduce over-reliance on antibiotic growth promoters, and subsequently help reduce the development of antibiotic resistance and environmental pollution. Seaweeds have been utilised in poultry at dietary inclusion rates of 10 - 50 g/kg to enhance bird immune status, reduce microbial load in the digestive tract, and improve the quality of poultry meat and eggs (Abudabos *et al*., 2013; Wang *et al*., 2013).

1.2 Problem Statement

Rural communities in tropical regions often rely on small free-range chicken flocks for essential dietary amino acids, fatty acids, and micronutrients to meet nutritional requirements and to alleviate poverty (Riise *et al*., 2004; Manyeula *et al*., 2019). However, Manyelo *et al*. (2020) noted that these birds face exclusion from high-input production systems due to their perceived genetic inferiorities typified by slow growth rates and poor productivity compared to conventional breeds. Nonetheless, Padhi (2016) stated that indigenous breeds are preferred based on their tropical adaptability, succulent taste and disease resistance. For sustainable large-scale production of indigenous chickens, Nhlane *et al*. (2021) highlighted that there is currently a global interest in the use of non-conventional and readily available feed ingredients with health-promoting and growth-stimulating attributes. Sea bamboo, being one such ingredient, holds the potential for incorporation into indigenous chicken diets to provide nutritional and pharmaceutical benefits. However, a literature gap exists regarding the use of SBM as a source of bioactive compounds to enhance growth performance, blood parameters, and carcass and meat quality characteristics of indigenous chickens.

Further, high fibre levels, primarily non-starch polysaccharides (NSPs), in SBM could limit its use in chicken diets (Gullón *et al*., 2020). For instance, *Ascophyllum nodosum* contains 88 g/kg DM crude fibre, 152 g/kg DM neutral detergent fibre (NDF), 298 g/kg DM acid detergent fibre (ADF) and 48 g/kg DM acid detergent lignin (ADL) (Bikker *et al*., 2020). *Ecklonia maxima* contains 392 g NDF /kg DM, 232 g ADF /kg DM, and 93 g ADL /kg DM (Ahmed *et al*., 2022), while *Saccharina latissima* contains 323 g NDF /kg DM, 241 g ADF /kg DM, and 6 g ADL /kg DM (Muizelaar *et al*., 2023). Ventura *et al*. (1994) observed a reduced feed intake and growth rate in three-week-old broiler chickens with the inclusion of *Ulva rigida* seaweed above 100 g/kg. The reduced feed intake and growth rate could be attributed to the presence of NSPs such as agar, alginic acid, soluble sulphated galactan, cellulose, laminarin and xylans (Gullón *et al*., 2020). Moreover, the presence of secondary metabolites such alginate, lectins, phlorotannins, trypsin and alpha-amylase inhibitors may have a negative effect on poultry performance (Oliveira *et al*., 2009). Indeed, Kim *et al*. (2021) reported that NSPs are the least digestible components of poultry feeds due to the absence of endogenous NSP-hydrolases in birds. Therefore, to fully exploit the bioactive compounds of SBM at higher inclusion levels without compromising the bird’s performance, it is imperative to first determine its maximum inclusion level in indigenous chicken diets followed by its pre-treatment with oyster mushroom spawn (OMS) to mitigate the antinutritional effects of fibre.

1.3 Justification

Seaweeds have historically served as livestock feed during feed scarcity in coastal regions (Makkar *et al*., 2016). Recent interest has surfaced in utilising seaweeds as rich sources of proteins, lipids, vitamins, minerals, low-molecular-weight nitrogenous compounds, volatile compounds (hydrocarbons, ketones and aldehydes), polysaccharides, pigments (chlorophylls, carotenoids and xanthophylls) and bioactive substances with various organic applications (Makkar *et al*., 2016). Seaweed polysaccharides have been utilised as prebiotic promoters in broiler and layer chicken diets (Ramnani *et al*., 2012). Additionally, seaweeds are a rich source of polyphenols, acknowledged for their antioxidant and antimicrobial properties, making them potential nutraceutical additives in poultry production (Stengel *et al*., 2011; Van Hal *et al*., 2014). Positive outcomes, including improved feed intake, feed conversion efficiency, body weight gain and breast meat quality, have been reported when incorporating *Enteromorpha prolifera* green seaweed in broiler diets (Burgin, 2016). Notably, Yan *et al*. (2011) observed inhibition of *Salmonella enterica* colonization resulting in reduced body weight loss and mortality in broiler chickens fed a diet containing 2 g/kg of sodium alginate oligosaccharides, which is abrown seaweed carbohydrate extracts.

However, challenges arose with the inclusion of green seaweeds (*Ulva* sp.) up to 35 g/kg in broiler chicken diets, as it interferes with the utilisation of beneficial bioactive phytochemicals, leading to reduced feed conversion efficiency (Matshogo *et al*., 2020). Moreover, Marareni *et al*. (2023) noted lower weight gain and a linear decrease in gain-to-feed ratio in Jumbo quail fed with diets containing 60 g/kg of green seaweeds (*Ulva* sp.), which could be attributed to the presence of long-chain β-glucans, cellulose, arabinoxylans and pectins in seaweeds (Gullón *et al*., 2020). These findings underscore the need to optimize sea bamboo's feed value by using OMS as a strategy to reduce fibre and allow for higher inclusion levels.

Oyster mushroom mycelia are one of the most efficient lignin-degrading fungal among all white-rot fungi because of their ability to produce lignin peroxidase (LiP), manganese peroxidase (MnP) and laccases, which are crucial extracellular lignin-modifying enzymes

(Adebayo *et al*., 2012). According to Dashtban *et al*. (2010), the process of lignin biodegradation involves LiP oxidizing non-phenolic lignin substructures by abstracting one electron and generating cation radicals, which are subsequently decomposed chemically. Further, MnP oxidizes Mn^{+2} to Mn^{+3}, which then oxidizes phenol rings to phenoxy radicals that lead to the decomposition of phenolic and non-phenolic aromatic compounds (Dashtban *et al*., 2010). Additionally, white-rot fungi possess antimicrobial, immune-modulating and antitumor activities that are known to have various pharmacological attributes such as anticancer, antioxidants and anti-inflammatory effects that may enhance poultry meat quality (Elhusseiny *et al*., 2021). Existing literature demonstrates that pre-treating feed substrates with mushroom spawn enhances the nutritive and feed value of the spent substrates (Niu *et al*., 2018; Olagunju *et al*., 2023). For example, Machado *et al*. (2007) reported an improvement in weight gain and feed conversion efficiency of broilers when 10 ppm of avilamycin was substituted with 20 and 21 g/kg of spent *Agaricus blazei* mushroom substrate in broiler chick diets. It is, therefore, imperative to assess the utility of OMS in enhancing the feed value of SBM in Boschveld cockerel diets. However, to the best of our knowledge, no previous studies have undertaken such an endeavour, particularly in Boschveld cockerels.

1.4 Aim and objectives

The study was designed to evaluate the potential of oyster mushroom spawn in enhancing the nutritive and feeding values of sea bamboo (*Ecklonia maxima*) meal in a diet of Boschveld cockerels. The following specific objectives guided the experiments:

a) Determine the effect of graded levels of sea bamboo meal on growth performance, apparent retention (AR) of feed proximate components, haemo-biochemical parameters, internal organs, carcass characteristics and breast meat quality traits of Boschveld cockerels.

b) Determine the effect of graded levels of oyster mushroom spawn on proximate composition, amino acid profile, and mineral contents of the sea bamboo spent substrates.

c) Evaluate the effect of including sea bamboo spent oyster mushroom substrates in a diet on growth performance, physiological responses and carcass and meat quality attributes of Boschveld cockerels.

1.5 Overall hypothesis

a. H_A: The inclusion of graded levels of sea bamboo meal in a diet of Boschveld cockerels would improve growth performance, AR of feed proximate components, physiological responses, and meat quality parameters of the birds.

b. H_A: Pre-treatment of sea bamboo meal with different levels of oyster mushroom spawn would enhance the proximate components, amino acid profile, and mineral contents of the spent substrates.

c. H_A: The incorporation of sea bamboo meal spent oyster mushroom substrate in a diet of Boschveld cockerels would improve growth performance, blood parameters and carcass and meat quality attributes of the birds.

1.6 References

Abudabos, A.M., Okab, A.B., Aljumaah, R.S., Samara, E.M., Abdoun, K.A. & Al-Haidary, A.A., 2013. Nutritional value of green seaweed (*Ulva lactuca*) for broiler chickens. Ital. J. Anim. Sci. 1, 177–181.

Adebayo, E.A., Oloke, J.K., Achana, Y. & Bora T.C., 2012. Improvement of laccase production in *Pleurotus pulmonarius*-LAU 09 by mutation. J. Microbiol. Res. 2(1), 11–17.

Ahmed, E., Batbekh, B., Fukuma, N., Hanada, M. & Nishida, T., 2022. Evaluation of different brown seaweeds as feed and feed additives regarding rumen fermentation and methane mitigation. Fermentation. 8(10), 504.

Anderson1, R.J., Rothman, M.D., Share, A. & Drummond, H., 2006. Harvesting of the kelp *Ecklonia maxima* in South Africa affects its three obligate, red algal epiphytes. J. Appl. Phycol. 18, 343–349.

Atela, J., Mlambo, V. & Mnisi, C.M., 2019. A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chicken. Anim. Nutr. 5, 179–184.

Bikker, P., Stokvis, L., van Krimpen, M.M., van Wikselaar, P.G. & Cone, J.W., 2020. Evaluation of seaweeds from marine waters in North-Western Europe for application in animal nutrition. Anim. Feed Sci. Technol. 263, 114460.

Bordoloi, A. & Goosen, N.J., 2020. A greener alternative using subcritical water extraction to valorize the brown macroalgae *Ecklonia maxima* for bioactive compounds. J. Appl. Phycol. 32, 2307–2319.

Bureau for Food and Agricultural Policy & National Agricultural Marketing Council, 2016. Evaluating the competitiveness of the South African broiler value chain. Pretoria, South African. pp. 1–76.

Burgin, R., 2016. Benefits of seaweed in poultry diets. Poultry World. Doetinchem, Nederland, https://www.poultryworld.net/Nutrition/Articles/2016/2/Benefits-of-seaweed-in-poultry-diets–2761921W. Accessed 05 May 2021.

Dashtban, M., Schraft, H., Syed, T.A. & Qin, W., 2010. Fungal biodegradation and enzymatic modification of lignin. Int. J. Biochem. Mol. Biol. 1(1), 36.

Elhusseiny, S.M., El-Mahdy, T.S., Awad, M.F., Elleboudy, N.S., Farag, M.M., Yassein, M.A. & Aboshanab, K.M., 2021. Proteome analysis and *in vitro* antiviral, anticancer and antioxidant capacities of the aqueous extracts of Lentinula edodes and Pleurotus ostreatus edible mushrooms. Molecules, 26(15), 4623.

Gullón, B., Gagaoua, M., Barba, F.J., Gullón, P., Zhang, W. & Lorenzo, J.M., 2020. Seaweeds as promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products. Trends Food Sci. Technol. 100, 1–18.

Kim, E., Morgan, N.K., Moss, A.F., Li, L., Ader, P. & Choct, M., 2022. The flow of non-starch polysaccharides along the gastrointestinal tract of broiler chickens fed either a wheat- or maize-based diet. Anim Nutr. 9, 138–142.

Machado, A.M.B., Dais, E.S., Dos Santos, E. & De Freitas, R.T.F., 2007. Spent mushroom substrate of *Agaricus blazei* in broiler chick's diet. R. Bras. Zootec. 36(4), 1113–1118.

Makkar, H, P, S., Tran, G., Heuze, V., Giger-Reverdin, S., Lessire, M., Lebas, F. & Ankers, P., 2016. Seaweeds for livestock diets: A review. Anim. Feed Sci. Technol. 212, 1–17.

Manyelo, T.G., Selaledi, L., Hassan, Z.M. & Mabelebele, M., 2020. Local Chicken Breeds of Africa: Their description, uses and conservation methods. Animals. 10, 2257.

Manyeula, F., Mlambo, V., Marume, U. & Sebola, N.A., 2019. Nutrient digestibility, haemo-biochemical parameters and growth performance of an indigenous chicken strain fed canola meal–containing diets. Trop. Anim. Health Prod. 2019, 51, 2343–2350.

Marareni, M., Mhlongo, G. & Mnisi, C.M., 2023. The effect of incorporating dietary green seaweed (*Ulva* sp.) on growth performance, blood parameters, and carcass and meat quality characteristics of Jumbo quail. Heliyon. 9(9), e19603.

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.

Muizelaar, W., van Duinkerken, G., Khan, Z. & Dijkstra, J., 2023. Evaluation of 3 northwest European seaweed species on enteric methane production and lactational performance of Holstein-Friesian dairy cows. J. Dairy Sci. 106(7), 4622–4633.

Nhlane, L.T., Mnisi, C.M., Mlambo, V. & Madibana, M.J., 2021. Effect of seaweed-containing diets on visceral organ sizes, carcass characteristics, and meat quality and stability of Boschveld indigenous hens. Poult. Sci. 100(2), 949–956.

Niu, D., Zuo, S., Jiang, D., Tian, P., Zheng, M. & Xu, C., 2018. Treatment using white rot fungi changed the chemical composition of wheat straw and enhanced digestion by rumen microbiota *in vitro*. Anim. Feed Sci. Technol. 237, 46–54.

Nkukwana, T.T., 2018. Global poultry production: Current impact and future outlook on the South African poultry industry. S. Afr. J. Anim. Sci. 48 (5), 1–16.

O'Sullivan, L., Murphy, B., McLoughlin, P., Duggan, P., Lawlor, P.G., Hughes, H. & Gardiner, G.E., 2010. Prebiotics from marine macroalgae for human and animal health applications. Marine Drugs. 8, 2038–2064.

Olagunju, L.K., Isikhuemhen, O.S., Dele, P.A., Anike, F.N., Essick, B.G., Holt, N., Udombang, N.S., Ike, K.A., Shaw, Y., Brice, R.M. & Orimaye, O.E., 2023. *Pleurotus ostreatus* can significantly improve the nutritive value of lignocellulosic crop residues. Agriculture, 13(6), 1161.

Oliveira, D.M.N., Freitas, A.L.P., Carvalho, A.F.U., Sampaio, T.M.T., Farias, D.F., Teixeira, D.I.A., Gouveia, S.T. & Pereira, J.G., 2009. Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. Food Chem. 115(1), 254–259.

Padhi, M.K., 2016. Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance. Scientifica. 1–9.

Ramnani, P., Chitarraria, R., Tuohy, K., Grant, J., Hotchkiss, S., Philp, K., Campbell, R., Gill, C. & Rowland, I., 2012. *In vitro* fermentation and prebiotic potential of novel low molecular weight polysaccharides derived from agar and alginate seaweeds. Anaerobe. 18, 1–6.

Riise, J.C., Permin, A. & Kryger, O.N., 2004. Strategies for developing family poultry production at village level. Experiences from West Africa and Asia. World Poult. Sci. J. 61, 15–22.

Rothman, M.D., Mattio, L., Wernberg, T., Anderson, R.J., Uwai, S., Mohring, M.B. & Bolton, J.J., 2015. A molecular investigation of the genus *Ecklonia* (*Phaeophycae, Laminariales*) with special focus on the southern hemisphere. J. Phycol. 51, 236–246.

Sharma, S.H.S., Fleming. C., Selby, C., Rao, J.R. & Martin, T., 2014. Plant biostimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. J. Appl. Phycol. 26, 465–490.

Stengel, D.B., Connan, S. & Popper, Z.A., 2011. Algal chemo diversity and bioactivity: sources of natural variability and implications for commercial application. Biotechnol. Adv. 29 483–501.

Van Hal, J.W., Huijgen, W.J.J. & Lopez-Contrera, A.M., 2014. Opportunities and challenges for seaweed in the bio-based economy. Trends Biotechnol. 32(5), 231–233.

Ventura, M.R., Castanon, J.I.R. & McNab, J.M., 1994. Nutritional value of seaweed (*Ulva rigida*) for poultry. Anim. Feed Sci. Technol. 49(1), 87–92.

Wang, S.B., Jia, Y.H., Wang, L.H., Zhu, F.H. & Lin, Y.T., 2013. *Enteromorpha prolifera* supplemental level: effects on laying performance, egg quality, immune function and microflora in faeces of laying hens. Chin. J. Anim. Nutr. 25, 1346–1352.

Yan, G.L., Guo, Y.M., Yuan, J.M., Liu, D. & Zhang, B.K., 2011. Sodium alginate oligosaccharides from brown algae inhibit *Salmonella enteritidis* colonization in broiler chickens. Poult. Sci. 90, 1441–1448.

2 CHAPTER TWO – LITERATURE REVIEW

2.1 Introduction

The evolution of the South African poultry industry over the last fifty years has seen a transformative shift from small-scale backyard farming to a highly integrated and efficient high-input system (Bolton, 2015). Presently, South Africa stands as the leading commercial poultry producer on the African continent, surpassing nations such as Egypt, Morocco, Nigeria, and Algeria (Nkukwana, 2018). Notably, in 2019, poultry production contributed significantly, constituting 20% of the total agricultural gross value and 41% of the gross value of animal products (SAPA, 2019). The South African Poultry Association (SAPA, 2016) underscores the pivotal role of poultry products as the most consumed and affordable animal protein, highlighting its profound impact on the nation's dietary landscape. Beyond its nutritional significance, the poultry industry serves as a vital source of direct and indirect employment, sustaining over 113 547 individuals across various peripheral businesses and the downstream value chain, including the feed industry (SAPA, 2019).

The poultry industry stands as a dominant force in the animal agricultural sector, contributing approximately 65% of locally produced animal protein consumption (excluding milk) in the country (SAPA, 2021). As noted by Rodic *et al*. (2011), this industry not only ensures the provision of affordable, high-quality protein for consumers but also creates valuable investment opportunities for both subsistence and commercial producers. While the large-scale commercial sector constitutes the primary source of chicken production in South Africa, challenges persist on a global scale, with countries grappling to maintain competitiveness due to narrow profit margins and soaring feed costs, which often exceed 70% of total production costs (Wickramasuriya *et al*., 2015).

Nkukwana (2018) stated that despite increasing demand for poultry meat, South Africa's poultry supply falls short, leading to the reliance on net imports of broiler meat from Brazil, the United States and the European Union. These imports are often priced lower than the cost of production per bird in South Africa with substandard quality. To increase local production, there is a dire need to identify and evaluate locally available feedstuffs with functional properties such as sea bamboo (*Ecklonia maxima*). This has the potential to enhance both growth performance metrices and product quality. However, Kraan (2012) reported that the use of seaweed in chicken diets faces limitations due to high fibre content, potentially hindering nutrient digestion and absorption in chickens. Moreover, Gullón *et al*. (2020) stated that high levels of non-starch polysaccharides (xylan, laminarin, fucoidan, hemicellulose and cellulose) in seaweeds may reduce energy and protein utilisation efficiency in chickens, which would consequently affect muscular development and fat deposition.

2.2 Indigenous chickens

Indigenous chickens (*Gallus domesticus*) are predominately grown under traditional family-based-environmental conditions, encompassing of free-range, small-scale and organic production systems. These chickens are sometimes referred to as backyard, traditional, scavenging, and village family chickens (Mahendra, 2016). Mtileni *et al*. (2009) noted that these chickens are very popular amongst resource-limited rural communities of South Africa and the African continent due to their minimal input requirements. McAinsh *et al*. (2004) emphasized the significant roles of these chickens in subsistence farming systems through the provision of meat and eggs for home consumption in African countries including South Africa. Delany (2003) reported that these birds play a key role in improving the socio-economic status of many rural communities.

According to Tarwireyi *et al*. (2013), indigenous chickens serve as a valuable income source in rural areas. However, the lack of knowledge in terms of disease control interventions, poor housing and feeding, and the lack of conservation strategies are the main challenges faced by local chicken production systems (Mtileni *et al*., 2009). Additionally, Alders *et al*. (2018) stated that poor genetic potential, slow-growth rates, and low hatchability of indigenous chickens compounded by poor management and lack of supplemental feeding are the major reasons for suboptimal productivity. Despite these challenges, Manyelo *et al*. (2020) emphasized the resilience of indigenous chickens as reliable income sources and affordable protein sources for rural communities in developing countries.

2.2.1 Nutritional factors influencing poultry production

Poultry production requires a balanced array of nutrients in their feed for maximum growth and health. The specific nutrients required by birds differ based on factors such as age, species and the purpose of production, whether the birds are kept for eggs or meat (Ravindran, 2013). Dietary protein, essential amino acids, minerals and energy content are key determinants of poultry productivity, feed efficiency and body composition (Whitehead, 2002). It is crucial to provide an adequate protein supply in poultry diets, especially at a young age, as it stimulates development of the gastrointestinal tract (GIT) digestive and immune function and enhances immunity and growth (Beski *et al*., 2015). Kingori *et al*. (2003) reported that indigenous chickens require 160 g/kg per day of protein in their diets to improve feed intake, body weight gain and feed efficiency. The amount of protein necessary to supply desired amino acids depends on the amino acid composition of the feed ingredients as well as the availability and cost of synthetic amino acids (Whitehead, 2002). However, meeting the bird's amino acid and dietary protein requirements poses economic challenges because providing a diet with a balanced amino acid composition is costly and significantly contributes to feed costs (Skinner *et al*., 1992).

In modern poultry production, fat and oil are commonly used to improve the energy content of the diets (Poorghasemi *et al*., 2013). Dietary fat, as noted by Baião & Lara (2005) slows the rate of digesta flow through the GIT, enabling better nutrient utilization and absorption. Despite being a reliable source of metabolizable energy (Baião & Lara, 2005), fat plays a crucial role in cardiovascular health, tissue growth, and development and maintenance of immune and enteric nervous systems (Alagawany *et al*., 2021). Various studies have reported that improved utilization of unsaturated fats results in greater metabolizable energy than saturated fats (Celebi & Utlu, 2004). However, realising these benefits depends on the digestion and absorption of fats, which are regulated by the source, type of fat and the age of the birds. In addition to providing energy, the inclusion of fat in a diet also enhances the absorption of fat-soluble vitamins, reduces pulverulence and improves the palatability (Poorghasemi *et al*., 2013), thereby influencing feed intake. Table 2.1 shows some of the nutrient requirements of different poultry breeds. Reported studies on the effects of feeding seaweed on growth performance, physiological (blood and viscera) responses, and meat quality parameters in various poultry breeds are presented in Table 2.2.

Table 2.1. Nutrient requirements of indigenous chickens versus conventional poultry chickens (% DM, unless shown otherwise)

Nutrients	Indigenous(Boschveld and Aseel) chickens	Broilers	Layer hens	Breeders
Protein	19.22[a]	21.47 – 24.6[cd]	15.59 – 17[ef]	15.5 – 17.0[h]
ME (MJ/kg)	12.55 – 12.9[ab]	12.55 – 13.21[cd]	11.30 – 11.92[ef]	11.54 – 12.01[h]
Lysine	1.05 – 1.35[b]	1.15 – 1.28[d]	0.82 – 1.49[ef]	0.71 – 0.83[h]
Methionine	0.48 – 0.52[g]	0.47 – 0.51[d]	0.42[e]	0.31 – 0.36[h]
Threonine	0.75 – 0.95[b]	0.77 – 0.86[d]	0.62 – 0.65[e]	0.47[I]
Arginine	1.07 – 1.42[b]	1 – 1.25[I]	1.05 – 1.10[e]	0.70[I]
Tryptophan	0.16 – 0.22[b]	0.16 – 0.20[I]	0.21[e]	0.16 – 0.21[h]
Valine	0.77 – 1.02[b]	1 – 1.10[d]	0.88[I]	0.70[I]
Calcium	0.84[a]	0.72 – 0.96[cd]	0.43 – 4.17[ef]	2.9 – 3.2[h]
Sodium	0.18[a]	0.14 – 0.20[cd]	0.2 – 0.27[e]	0.16 – 0.18[h]
Phosphorus	0.55[a]	0.55 – 0.60[cd]	0.35 – 0.65[ef]	0.40 – 0.47[h]
Magnesium (mg)	370 – 570[I]	170 – 180[c]	25[I]	500[I]
Iron (mg)	28 – 56[I]	80[I]	56[I]	30 – 80[h]
Iodine (mg)	0.33[I]	0.35[I]	0.044[I]	0.8 – 1.1[h]

Sources: [a]Nhlane *et al*. (2020); [b]Hussain *et al*. (2018); [c]Akinyemi & Adewole (2022); [d]Biesek *et al*. (2020); [e]Al-Harthi & El-Deek (2012); [f]Kulshreshtha *et al*. (2017); [g]Li *et al*. (2020); [h]Leeson & Summers (2010); [I]NRC (1994).

ME = Metabolizable energy.

Table 2.2. Results of feeding different levels of seaweeds on chicken productive performance and health

Breed type	Diet composition	Crude protein levels (g/kg DM)	Metabolizable energy levels (Kcal/kg)	Findings	References
Aseel chickens	Addition of 0, 50 g/kg of *Sargassum wightii* and 50 g/kg of *Gracilaria corticata*	191 and 193	3847 and 3945	Feeding a diet containing 50 g/kg of *G. cortica* improved the body weight gain but did not affect the blood profile of the birds.	Vijayalingam *et al*. (2023)
Ross 308 chickens	Starter and finisher diets supplemented with 0, 0.5, 0.1, 1.5 and 2.5 g/kg of *Halymenia palmate*	229.9 and 205.1	12.56 and 12.98 (MJ/kg)	*Halymenia palmate* supplementation improved the meat quality attributes, such as reducing the water holding capacity, drip loss and cooking loss, while enhancing relative organ weights.	Balasubramanian *et al.* (2021).
Boschveld indigenous chickens	Diets supplemented with 0, 20, 25, 30 and 35 g/kg of *Ulva* spp.	192.2	12.9 (MJ/kg)	Feeding diets containing green seaweed enhanced the feed intake and weight gain.	Nhlane *et al*. (2020)
Male Ross broilers	Substituting corn with 0, 10 and 30 g/kg of *Ulva lactuca*	211 and 215	3127 and 3113	Feeding diets containging 10 and 30 g/kg reduced the abdominal fats, serum total lipid, and cholesterol and uric acid concentrations.	Abudabos *et al*. (2013)
Broiler chcikens	Addition of 0, 20, 40 and 60 g/kg of *Sargassum dentifebium*	205	3100	The inclusion of up 60 g/kg resulted in poor growth performance but improved the polyunsaturated fatty acid and n-3 fatty acids.	El-Deek *et al*. (2011)

2.2.2 Breed of choice: Boschveld chicken

The Boschveld indigenous chicken is a South African breed that was developed by Mike Bosch at the Mantsole Ranch near Radium in the Limpopo province in 1998 (Plate 2.1). Boschveld chickens resulted from crossbreeding three indigenous African breeds: the Venda, Ovambo and Matabele chickens (Manyelo *et al*., 2020). Reports indicate that the Boschveld breed inherited 50% of its traits from the Venda breed, 25% from the Ovambo breed, and 25% from the Matabele breed (McCullough, 2017). Generally, they exhibit plumage colours ranging from red to light brown with white patches on their feathers, providing the Boschveld chicken with a distinct appearance that blends with the African landscape. According to Nhlane (2020), these colours aid in camouflaging the chickens from predators.

This breed has retained the hardiness of Ovambo chicken, size of Matabele chicken, and egg production capacity of Venda chicken. One notable advantage of indigenous breeds, including the Boschveld chicken, is their ability to free-range on homemade feeds, thus reducing the farmer's feed input (Okoro *et al*., 2017). The cockerels or roosters weigh approximately 1.6 kg at 12 weeks and 2.6 kg at 20 weeks, whereas the hens weigh 1.5 kg and 1.7 kg at 12 and 20 weeks of age, respectively. Depending on nutritional levels, cockerels are usually ready for slaughter at 12 weeks of age. According to FAOSTAT (2010), such breeds are preferred over exotic breeds by rural, urban and peri-urban consumers due to their taste, hardiness, pigmentation, leanness and absence of antimicrobial growth promoters. Boschveld chickens reach sexual maturity at 21 weeks of age, with the hens producing four eggs per week, each weighing an average of 53.4 g (Ferreira, 2017).

Plate 2.1. Boschveld chicken (Source: AgriOrbit, 2022).

2.3 Seaweed overview

Seaweeds are a group of photosynthetic, non-flowering plant-like organisms called macroalgae that grow in the sea (Plate 2.2). Classified by their dominant pigmentation, seaweeds are categorized into red (*Rhodophyta*), brown (*Phaeophyta*) and green (*Chlorophyta*) algae (Gomez-Zavaglia *et al*., 2019). Brown seaweeds can range from a few millimetres or centimetres in size, with the largest kelp growing up to 35 - 60 m. They are followed by red seaweeds, which are generally smaller than brown seaweeds, ranging from a few centimetres to a metre; and lastly, the green seaweed, which are also smaller and can grow up to a metre (McHugh, 2003). Seaweed has been utilized for centuries around the world as a source of food for coastal communities in countries like China, Japan and North Korea. Apart from their many wide-ranging uses in the food, cosmetic, industrial and medical industries, seaweeds significantly contribute towards the nutritional status of communities due to their rich composition of macrominerals (such as sodium, calcium, magnesium, potassium and phosphorus), microminerals (iodine, iron, zinc, copper, fluoride and manganese) and vitamins A and B_{12} (FAO, 2018). Jiménez-Escrig *et al*. (2012) reported that macro-algae are a rich source of minerals, bioactive phytochemicals and soluble fermentable fibres, with high levels found in brown seaweeds. Furthermore, Misurcova (2012) reported that seaweed exhibit a highly variable chemical composition with significant differences in fibre, proteins, lipids and minerals, depending on the species, time of harvesting, growth site, water temperature, light intensity and water nutrient concentration.

One unique feature of fresh seaweeds is that they contain high-water content (70 – 90%), meaning that they need to be consumed quickly (approximately in 3 days), while thicker brown seaweeds can last up to 7 days if they are stored in a refrigerator or dried. Brown algae such as *Ecklonia maxima*, have lower protein and higher mineral contents but contain several bioactive compounds that can be beneficial in poultry diets (Forbes, 2009; Misurcova, 2012). *Eckonia maxima* is rich in valuable biomolecules such as polysaccharides (alginate, fucoidan and laminarin), both macro- and micro-minerals (Ca, K, Mg, Zn, Cu, Co, I, B), vitamins (B_{12}, K, C, E, A, D), lipids, polyunsaturated fatty acids (PUFA), pigments like carotenoids (carotene and xanthophyll), chlorophylls, phycobilins and various antioxidant polyphenolic compounds, contributing to the potential development of a blue bio-economy in South Africa (Zhang *et al*., 2021). For instance, in 2015, the global market size of alginate production was estimated at US$ 345 million (Qin, 2018). This is because these molecules have prebiotic, antioxidant, antimicrobial (antibacterial, antifungal and antivirus), and anti-inflammatory immunomodulatory effects (Michalak & Chojnacka, 2015).

Plate 2.2. Sea bamboo (Source: Afrikelp, 2020).

2.3.1 Seaweed production, harvesting and processing

Seaweeds stand out as the millennium's most promising marine resource, offering a plethora of social, economic and ecological benefits (Sultana *et al*., 2022). Their utilisation dates back as far as 2500 years (Tseng, 2001). Seaweeds are prominent commercial marine biota known for their diverse applications as biochemical raw materials (agar, agarose, alginate, and carrageenan), colours, enzymes, food, animal feed, textiles, medications, cosmetics, and fertilizers. Seaweed biotechnology includes study areas such as cell fusion, gene manipulation, callus induction, tissue culture, protoplast production and regeneration (Kim, 2019; Sultana *et al*., 2022). The Food and Agriculture Organization (FAO) estimates that global seaweed output (wild and aquaculture) tripled from 118,000 tons to 358,200 tons from 2000 to 2019 (FAO, 2021). Artificial farming accounted for 97% of global aquaculture output in 2019, with Asia accounting for 97.38% of the seaweed output from the five major continents. The global seaweed market produced around US$ 11.8 billion in that year, with a projected value of US$ 22.13 billion by 2024, driven by the anticipated annual growth rate of 8.9% (Mac Monagail *et al*., 2017; Cotas *et al*., 2020).

Seaweed harvesting typically involves selective cutting from monospecific stands of seaweed (e.g., kelps and rockweeds) or collecting storm-cast fronds (which would result in several mixed species, as well as contaminated jetsam and flotsam) near the shorelines (Mac Monagail *et al*., 2017). In addition, Mac Monagail *et al*. (2017) stated that harvesting seaweeds using nets, bulldozers, horses, or tractors, is very unpredictable and unreliable, although seaweeds contribute significantly to local income along shorelines worldwide. Harvesting with boats, rakes, or diving, as suggested by Rebours *et al*. (2014), is considered more economically viable, as it generates higher income than hand harvesting from the shore at low tide. Due to their high moisture content and susceptibility to mould growth, seaweeds must be processed into a meal soon after harvesting. Hammer mills, equipped with progressively smaller screens are used to crush wet seaweed into small pieces. The resulting meal should be stored in sealed bags with a moisture content of approximately 15%, allowing for refrigeration for up to a year (McHugh, 2003).

2.3.2 The generation of seaweed heaps and their effect on the environment

In recent years, research has revealed massive pelagic seaweed blooms (especially free-floating *Sargassum* seaweed) across aquatic bodies and beach landings around the world, posing new environmental concerns (Langin, 2018). These massive seaweed depositions on beaches have led to various environmental, human health, and ecological issues, negatively impacting local economies (Rodríguez-Martínez *et al*., 2019). Seaweed blooms are known to impede nesting turtles and neonatal hatchlings seeking ocean access, and reducing light penetration in the water column, affecting benthic populations (McGlathery, 2001). Additionally, seaweed blooms cause significant socioeconomic impacts on aquaculture and tourism industries, such as clogging fishing gear and limiting fishing grounds, resulting in reduced income and revenue increasing maintenance costs (Solarin *et al*., 2014; Fidai *et al*., 2020).

Accumulation and compaction of seaweed heaps on beaches result in anaerobic decomposition, releasing volatile and non-volatile chemicals, including ammonia and hydrogen sulfide gas, posing a health risk for both locals and tourists, although the effects have not been extensively described (Resiere *et al*., 2020). Additionally, Rasiere *et al*. (2020) reported that *Sargassum* decomposition produces ammonia and hydrogen sulfide gas, which can induce skin, respiratory and neurological problems in both locals and tourists. The toxic exposure usually occurs after decomposition, about 48 hours after it washes along the shore, with excessive exposure (50 - 400 parts per million) causing agitation, breathing difficulties, high blood pressure, nausea, confusion, vomiting and even unconsciousness (Rasiere *et al*., 2023). However, more information is needed on the effects of seaweed heaps on the environment and human health.

2.3.3 Types of seaweed species

2.3.3.1 Brown seaweed

Brown seaweeds are primarily found in shallow waters or on rocky shorelines, with stems that exhibit remarkable flexibility, allowing them to withstand the continuous battering of the waves (Ghosh *et al*., 2012). These seaweeds have been reported to be the most investigated and utilized in animal feeds compared to the other algae species due to their larger size, which allows ease of harvesting (Makkar *et al*., 2016). One such seaweed is *Ecklonia maxima,* commonly known as Osbeck or sea bamboo, which is amongst the dominant kelp species on the west coast of South Africa, with an estimated total biomass of approximately 530 000 tons of fresh weight (Anderson *et al*., 2007). Over the past decade, around 6,000 tons per annum of *E. maxima* have been harvested for various purposes, including alginate production, animal feed, fertilizers, and agricultural bio-stimulants (Anderson *et al*., 2003). However, in 2014, more than 12,000 metric tons of fresh *E. maxima* were harvested, primarily utilised as feed for abalone farms and as a plant growth stimulant in local and international markets (Zhang *et al*., 2021). Besides *E. maxima*, other commonly cultivated genera include *Ascophyllum*, *Macrocystis*, *Laminaria*, *Nereocystis*, *Sargassum* and *Saccharina* kelp (Murty & Banerjee, 2012). These brown seaweed species are widely distributed and harvested in Asia, Europe, Canada, South America, Southern Africa, Australia and New Zealand for the production of alginate, food, animal feeds and pharmaceutical additives (Cruz-Suarez *et al*., 2008; Evans & Critchley, 2014).

2.3.3.2 Green seaweed

Green seaweeds are commonly found in light-rich environments, such as shallow seas and rocky shores where they are attached to rocks and stones (Baweja *et al*., 2016). They can also be found in contaminated estuaries and brackish water. According to Sherwood (2016), the *Chlorophyta* and *Streptophyta* divisions are generally green in colour because of the presence of chlorophyll *a* and *b* within their double membrane-bound chloroplast and their main storage product for photosynthesis is true starch which is stored in chloroplasts. McHugh (2003) noted that the overall colouration of green seaweeds is determined by the ratio of chlorophylls to other pigments like beta-carotene and xanthophylls. The *Ulva*, *Codium*, *Chaetomorpha*, *Cladophora* and *Enteromorpha* are the most common genera. The *Ulva* genera, commonly known as sea lettuce due to its deep green and thin fronds, is one of the most harvested species (Makkar *et al*., 2016). Among the *Ulva* species, *Ulva lactuca* is the most researched, although it's worth noting that various *Ulva* species, including *U. armoricana*, *U. fasciata*, *U. pertusa*, *U. prolifera*, *U. rigida*, and *U. rotundata*, appear to be similar (Yabe *et al*., 2008; Rybak, 2018).

2.3.3.3 Red seaweed

Red seaweeds (Rhodophyta) constitute a diverse group of unicellular to multicellular photoautotrophic aquatic plants known for their vibrant pink colour, which is generated by the biloprotein pigments R-phycoerythrin and R-phycocyanin (Pereira, 2021). The primary genera of red algae include *Pyropia*, *Porphyra*, *Chondrus*, and *Palmaria*, which are predominantly found at low tide marks to 100 m deep, where sunlight penetration is limited (Makkar *et al*., 2016). Pereira *et al*. (2012) reported that red seaweeds have a vast variety of morphologies, basic anatomy, and life cycles of which 98% are marine species, 2% are freshwater species, and a few uncommon terrestrial or sub-aerial species. In the phylogenetic context, red algae are considered genuine plants as they share a common ancestor with green algae and higher plants (Adl *et al*., 2005). The phylum Rhodophyta, on the other hand, can be differentiated from other algae by various characteristics, such as the absence of centrioles, the flagellate phase, parenchyma, and the presence of chlorophylls, phycobilins, and pit connections, which are holes in the septum between two cells (Pereira, 2012).

2.4 Seaweed as a source of biologically active compounds in poultry

Seaweed species are commonly utilised in human diets, medicine, animal feeds, and agricultural soil development as fertilizers in an unprocessed form (Jamal *et al*., 2017). The distinct nutritional composition of several seaweed species can play significant roles in poultry nutrition. Seaweeds are a source of various polysaccharides (alginate, laminarin, fucoidan, agar, cellulose, carrageenan, pectin inulin, porphyran, ulvan and xylan), essential amino acids, proteins, minerals, lipids, vitamins (B_{12}, K, A, C, D and E), PUFA, pigments (carotenoids, phycobilins, chlorophylls), and many antioxidant compounds including mainly polyphenols (Michalak & Mahrose, 2020). The bioactive compounds in seaweeds possess antimicrobial (antibacterial, antifungal and antivirus), anti-inflammatory, immunomodulatory, prebiotic and antioxidant effects (Michalak & Chojnacka 2015; Corino *et al*., 2019). At low inclusion levels, these compounds can be essential in regulating the intestinal environment, enhancing immunity, suppressing oxidative stress and inflammation, and promoting growth performance in poultry production (Mlambo *et al*., 2022). Additionally, they can serve as an alternative to antibiotic growth promoters (AGP) used in poultry production (Kulshreshtha *et al*., 2020).

However, to the best of our knowledge, there are no studies on the use of active biological compounds extracted from *E. maxima* in poultry production.

2.4.1 Alginates

Alginates are anionic hydrophilic hetero-polysaccharides found in various brown seaweed (*Phaeophyceae*) species (Hecht & Srebnik, 2016). Alginates are composed of homogeneous (MM or GG) and heterogeneous (MG or GM) blocks of (1,4) linked β-D-mannuronic and α - L-guluronic acids in pyranosic conformation (Khajouei *et al*., 2018), which results in a wide range of structural, molecular weight, and physicochemical properties. Alginate composition, availability, and monomeric ratio, as noted by Abka-Khajouei *et al*. (2022), not only vary based on the age and species of the algae but also on the natural alginate source, plant location, season, and geographical region. Commercially, *E. maxima*, *Durvillea antarctica*, *Laminaria hyperborea*, *Macrocystis pyrifera*, *Laminaria digitata*, *Ascophyllum nodosum*, *Sargassum* spp, *Lessonia trabeculata* and *laminaria Saccharina* are some of the commonly cultivated species to produce alginates (McHugh, 2003; Fernando *et al*., 2020).

Alginates have numerous applications in the food and medical industries such as stabilizers, and emulsifiers, including thickeners and pharmaceutical additives. Additionally, they have received more attention in the pharmaceutical industry due to their low molecular weights and stronger biological activity, often associated with health benefits. Alginates possess antimicrobial, antioxidant, anti-lipid, antihypertensive, immunomodulatory, anti-tumour, anti-diabetic, antibacterial, anticoagulant, anti-hypertensive, probiotic, light-protecting and anti-hypoglycemic properties, in addition to their ability to suppress obesity and enhance cell proliferation (Liu *et al*., 2019; Xing *et al*., 2020). These attributes show that alginates have the potential to be utilized as immune boosters and growth promoters in birds.

2.4.2 Carotenoids

Carotenoids are pigments that comprise approximately 8 - 14% of the biomass in brown, green and red seaweeds, which play a crucial role in light harvesting during photosynthesis (Priyadarshani & Biswajit, 2012; Mulders, 2014). Carotenoids are categorised into two groups: xanthophylls and carotenes. Xanthophylls, including β-cryptoxanthin, lutein, astaxanthin, fucoxanthin, peridinin, and zeaxanthin, feature oxygen atoms in carboxylic, hydroxy, aldehyde, carbonyl, epoxide, and furanoxide groups (Maoka, 2020). Meanwhile, carotenes, such as α-carotene, γ-carotene, *β*-carotene, and lycopene, are essential precursors of vitamin A, potentially aiding in the prevention of cataracts and night blindness (Hobbs & Bernstein, 2014).

Moreover, carotenes may contribute to poultry production by assisting glycoprotein formation, cell differentiation, mucus secretion from epithelial tissues, reproduction, and overall bone and body development (Kumar *et al*., 2021). Carotenoids, with their distinctive linear C40 chain that contains up to 11 conjugated bonds (allenic bonds), are considered one of the most important antioxidant compounds found in seaweeds (Miyashita *et al*., 2011; Mikami & Hosokawa, 2013). These allenic bonds may participate in antioxidant activities by transferring surplus energy of singlet oxygen (O) in the long central allenic chain (Miyashita *et al*., 2011). Furthermore, several researchers have reported that carotenoids provide significant functional benefits to seaweed-based diets through their immune-boosting qualities and antioxidants, which may lower inflammation, cardiovascular disease, obesity, neurological disorders, cancer and age-related muscle diseases (Miyashita *et al*., 2020; Mlambo *et al*., 2022).

2.4.3 Fucoidan

Fucoidan is a sulphated polysaccharide commonly extracted from the cell walls of brown seaweeds (*Phaeophyceae*) such as *Undaria pinnatifida, Cladosiphon okamuranus, Fucus vesiculosus, Laminaria japonica* and *E. maxima* (Rothman *et al*., 2015; VijaySankar *et al*., 2023). Like other fucans, fucoidan contains fucose and sulphate groups; but, unlike other fucans, fucoidan contains up to 10% of additional monosaccharides (mannose, glucose, galactose, xylose and rhamnose), uronic acids, or branches of one or more monosaccharides (Li *et al*., 2008). Structurally, it is a polymer composed of 1,2-linked L-fucose-4-sulphaate units and, in rare cases, 1,3- or 1,4-linked fucan sulphate and side chains of galactose, uronic acid, and xylose as residues (Kumar *et al*., 2021). Each algal group's overall composition is distinct; fucoidans in brown seaweeds, galactans in red seaweeds, arabinogalactans and rhamnoglucuronans in green seaweeds (van den Hoek *et al*., 1996).

However, more or minor variations occur depending on the family, order, genus and species, season, geographical location and stage of reproduction (Ponce & Stortz, 2020). From a commercial point of view, fucoidan plays a pivotal role in food manufacturing and cosmeceuticals due to its extraction from low-cost resources, enabling the creation of functional food and innovative medication (Ahmed *et al*., 2014). Notably, fucoidan from *E. maxima* displays potent and robust biological bioactivities such as anticancer, antidiabetes and antioxidant properties like other brown seaweeds (Daub *et al*., 2020) that can be exploited and utilized as potential growth promoters and immune boosters in poultry.

2.4.4 Phlorotannins

Phlorotannins are a group of polyphenolic compounds generated as secondary metabolites to alleviate oxidative stress by brown seaweed and biosynthesized through the acetate malonate pathway (Leyton *et al*., 2016). They exist in the cells of algae either in their free form or as complexes with various elements of the cell walls, such as alginic acid (Montero *et al*., 2016). Phlorotannins primarily constitute of phloroglucinol (1,3,5-trihydroxybenzene) units with varying degrees of polymerization and a group of heterogeneous polymeric compounds (Li *et al*., 2017). Brown seaweeds such as *E. maxima, Ascophyllum nodosum*, *Gongolaria usneoides, Fucus spiralis*, *Gongolaria nudicaulis*, *Pelvetia canaliculata*, *Ericaria selaginoides*, *Fucus* *vesiculosus* and *Saccharina longicruris* produce these various phlorotannin compounds, namely phlorofucofuroeckol, dieckol, 7-phloroeckol, fucotriphloroethol, fucodiphloroethol and fucophloroethol (Ferreres *et al*., 2012). Brown seaweeds can adapt to harsh environments by producing phlorotannins, which prevent the oxidative damage caused by the formation of free radicals and other powerful oxidizing agents (Dang *et al*., 2018). Phlorotannins can display antioxidant activity due to their molecular structure, which has various phenol rings (Kumar *et al*., 2022). Kumar *et al*. (2022) stated that free radicals that cause oxidative stress, are scavenged by phenol rings, which act as electron traps. As a result, phlorotannins possess a wide range of biological properties with antioxidant, anti-tumour, anti-hypertensive, antiviral, antibacterial, anti-obesity, hypoglycemic, anti-diabetic, anti-inflammatory, anti-allergic and immunomodulating activities (Cotas *et al*., 2020).

2.4.5 Laminarin

Laminarin, also known as laminarans, is an active component that is extracted from the dry thallus of brown seaweeds such *Eisenia bicyclis*, *Ecklonia kurome* and *Laminaria japonica* (Déléris *et al*., 2016). It is made up of (1,3)-β-D-glucan linkage and is primarily composed of (1,3)-β-D-glucopyranose residues with some 6-*O*-branching in the main chain and some β-(1,6)-intrachain linkages. They have a molecular mass of approximately 5 kilodaltons (Rioux *et al*., 2007; Kadam *et al*., 2014). Depending mostly on the level of branching, laminarin exists in both water-soluble and insoluble forms (Miao *et al*., 1995). Laminarin extraction typically entails grinding, precipitation in a mild acid media, ultra-filtration and dialysis (Kadam *et al*., 2015). Karuppusamy *et al*. (2022) revealed that due to their biodegradable, non-toxic and biocompatible properties, laminarins are being increasingly investigated for the creation of nutraceuticals and functional foods, as well as functional material in biomedical applications. In addition, laminarin has been reported to possess therapeutic properties such as anticancer, antioxidant, anti-coagulant, anti-obesity, immunomodulatory, anti-tumour, anti-inflammatory, anti-diabetic, neuroprotective and wound healing potential (Karuppusamy *et al*., 2022). Nonetheless, no studies have explored the biologically active compounds that can be isolated from *E. maxima* and used in poultry production. The different kinds of bioactive compounds available in brown seaweed are presented in Table 2.3 below.

Table 2.3. Availability of natural bioactive compounds in brown seaweeds

Brown seaweed types	Bioactive compounds	References
Ecklonia maxima	Phenolic acid, flavonoids, phlorotannins, alginates and fucoxanthin	Olasehinde *et al*. (2019)
Ecklonia cava	Phlorotannins, fucoidan	Shin *et al*. (2021)
Ascopyllum nodusum	Laminarin, Phlorotannins, fucoidans, mannitol, alginic acid	Holdt & Kraan (2011); Moreira *et al*. (2017); Shukla *et al*. (2019)
Saccharina japonica	Carotenoids, fucoidan, laminarin, fucoxanthin, alginate	Islam et al. (2013); Kumar *et al*. (2021)
Fucus spp.	Fucoidan, fucoxanthin, polyphenols	Catarino *et al*. (2018); Obluchinskaya *et al*. (2022)
Laminaria spp.	Alginate, laminarin, mannitol	Kumar *et al*. (2021)
Undaria pinnatifida	Phlorotannins, peptides, polyphenols, phytosterols	Boulom *et al*. (2014); Wan-Loy & Siew-Moi (2016)
Sargassum spp.	Carotenoids, phlorotannins, phytosterols	Catarino *et al*. (2023)

2.5 Uses of seaweeds

The primary purpose of seaweed harvesting is for their direct use as human foods, food supplements, edible hydrocolloids, cosmetics, animal feeds, biodegradable packaging, biofertilizers, and nutraceutical products (McHugh, 2003; FAO, 2018). The majority of seaweed species are regarded as edible since they do not contain any intrinsic toxins. Approximately 700 species, comprising roughly 345 red, 195 brown and 125 green seaweeds, have been recommended as human food (Pereira, 2016). The use of seaweed in culinary applications is currently the leading market for algae harvesting globally. This is primarily owing to the high consumption that exists in Asian countries, where they are most consumed (Holdt & Kraan, 2011).

According to Gomez-Zavaglia *et al*. (2019), algae products can be eaten in a variety of ways, including sushi, soups, bread, tea, salads, mustard, pasta and a variety of other foods. Holdt & Kraan (2011) reported that the main seaweeds used as human food are Nori (*Porphyra* spp.), aonori (*Monostroma* spp.), kombu (*Laminaria japonica*), wakame (*Undaria pinnatifida*), Irish moss (*Chondrus crispus*), Hiziki (*Hizikia fusiforme*), mozuku (*Cladosiphon okamuranus*), sea grapes (*Caulerpa lentillifera*), dulse (*Palmaria palmata*), winged kelp (*Alaria esculenta*) and carola (*Callophyllis variegate*). These seaweeds are a low-calorie food that is high in vitamins, minerals, proteins, polyunsaturated fatty acids, polysaccharides, and dietary fibres as well as bioactive metabolites (Ganesan *et al*., 2019).

Seaweeds also have pharmaceutical properties that are used to treat or prevent goitre, a condition caused by iodine deficiency (Rosenfeld, 2000). Moreover, seaweeds have anti-inflammatory, anti-obesity, anti-diabetic, anti-hypertensive and anti-viral properties (Rajauria *et al*., 2016). Teas *et al*. (2013) found that regular consumption of *Undaria* seaweed can effectively reduce the risk of breast cancer in women. Furthermore, oral administration of seaweed extracts (*Macrocystis pyrifera*, *Fucus vesiculosus* and *Laminaria japonica*) with manganese, zinc and vitamin B_6 potentially reduced osteoarthritis symptoms in different ethnic groups (Myers *et al*., 2016). In crop production, seaweeds have been reported to contain beneficial extracts with major and minor nutrients (amino acids and vitamins), auxin, cytokinins, and abscisic acid-like growth-promoting substances that have been shown to stimulate plant growth by developing tolerance to environmental stress, enhancing antioxidant properties, and increasing nutrient uptake from soil (Rathore *et al*., 2009).

Recently, there has been a surge of interest in employing seaweeds as potential adsorption agents for wastewater treatment. Arumugam *et al*. (2018) reported that the presence of sulphated polysaccharides in the seaweed's cell wall, specifically its fibril matrix and intercellular gaps, enables it to bind contaminants including trace metals. The polysaccharide hydroxyl, sulphate, and carboxyl groups are powerful ion exchangers and thus the key sites of metal cation complexation (Vasconcelos & Leal, 2001). Ashkenazi *et al*. (2019) reported that the *Ulva* (green algae) and *Gracilaria* (red algae) are well-established marine species with high nutrient uptake and growth rates when compared to other seaweed species, making them best suited for bioremediation.

2.6 Seaweeds as a feed source for animals

Seaweeds have been for a long time used as a valuable feed source for livestock and aquaculture (Rajauria, 2015). In the 1900s, seaweeds were either dried and preserved in barns or stored as silage for use as winter feed for cattle and sheep (Evans & Critchley, 2014). According to Rajauria (2015), seaweed's nutritional importance in the diets of poultry and livestock has been demonstrated in numerous research. For instance, Carrillo *et al*. (2009) found that 100 g/kg inclusion of either *Sargassum sinicola*, *M. pyrifera* or *Enteromorpha* sp. seaweeds in a diet of laying Leghorn hens enhanced n-3 fatty acids, albumen height, and yolk colour. In another study, Carrillo *et al*. (2012) reported that including 30 or 60 g/kg of brown seaweed (*Sargassum dentifebium*) in a diet of laying hens reduced triglycerides and cholesterol levels in egg yolk. However, improvements in zeaxanthin, total carotene and lutein contents of the eggs were noted.

In aquaculture, seaweeds not only appeal to fish taste but also stimulate weight gain by enhancing triglyceride and protein deposition in their muscles (Fleurence *et al*., 2012). Wassef *et al*. (2001) revealed that the addition of 50 g/kg *P*. *capillacea* and *U*. *lactuca* seaweeds into gilthead seabream and seabass fish meals enhanced the survival rates, growth rate and nutrient composition. When compared to control-fed rohu fish (*Labeo rohita*), a higher protein efficiency ratio (PER), food absorption efficiency, nutrient digestibility and a lower FCR were noted when rohu fish were fed diets that partially replaced fishmeal with *Spyridia insignis*, *Sargassum wightii* and *Ulva fasciata* seaweeds (Bindu & Sobha, 2004). The existence of substantial levels of the dimethyl sulfonyl propionate molecule in green seaweed, particularly in the Ulvales group, could explain this improvement because it acts as an attractant for fish, which enhances feed intake (Van Alstyne *et al*., 2001). Valente *et al*. (2006) also reported that feeding juvenile seabass (*Dicentrarchus labrax*) a diet containing *Gracilaria cornea*, *U. rigida*, and *Gracilaria bursa-pastoris* seaweeds had no effect on PER. Le Blé & Morice (2013) stated that the blending of seaweed species could improve the palatability of a diet and growth performance of aquatic species compared to a diet consisting of one type of seaweed species. The chemical composition of the types of seaweed species is presented in Table 2.4 below.

Table 2.4. Chemical composition (g/kg DM, unless stated otherwise) of the three kinds of seaweeds

Parameters	Brown seaweed	Red seaweed	Green seaweed
Dry matter (g/kg)	874 – 918[ab]	883 – 949[b]	842 – 880[b]
Crude protein	30 – 150[g]	100 – 470[g]	87 – 327[d]
Neutral detergent fibre	91 – 220[abc]	190 – 392[b]	208 – 385[be]
Acid detergent fibre	126 – 331[bc]	40 – 53[b]	135 – 143[be]
Acid detergent lignin	7 – 180[b]	5 – 14[b]	69 – 70[bc]
Crude fat	10 – 45[i]	7 – 30[i]	6.0 – 42[i]
Ash	139 – 351[ach]	107 – 217[h]	127 – 483[ch]
Calcium	4 – 20[a]	0.99 – 3.30[j]	1.89 – 6.47[j]
Phosphorous	1 – 3[ac]	2.72 – 3.16[k]	2.7[c]
Potassium	24 – 67.5[ac]	41.73 – 86.33[k]	15.1 – 29[c]
Sodium	25 – 36[cj]	10.02 – 39.66[cj]	11.0 – 29.3[cj]

Sources: [a]Erickson *et al*. (2012); [b]Bikker *et al*. (2020); [c]Makkar *et al*. (2016); [d]Fleurence (2004); [e]Denis *et al*. (2010); [f]Mwalugha *et al*. (2015); [g]Abdul Khalil *et al*. (2017); [h]Tayyab *et al*. (2016); [i]Leandro *et al*. (2020); [j]El-Said & El-Sikaily (2013); [k]Benjama & Masniyom (2012).

2.6.1 The potential use of seaweeds on poultry performance and meat quality

The utility of brown seaweeds as a source of bioactive chemicals can play a beneficial role in the poultry industry (El-naga & Megahed, 2018). Evans & Critchley (2014) revealed that seaweed such as *spirulina* has the potential to be utilised in cattle and poultry feeds. However, the utility of seaweed can be limited by the high fibre content, which necessitates the need for prior treatment in simple non-ruminant diets. Consequently, Abudabos *et al*. (2013) reported that the addition of seaweeds (*Ulva lactuca*) at 10 and 30 g/kg in broiler diets increased body weight gain (BWG) of broilers. Wang *et al*. (2013) noted that the use of *Enteromorpha prolifera* seaweed up to 30 g/kg in Highland brown laying hens diets as a substitute ingredient to corn had no effect on feed intake, BWG and FCR.

EL-Deek *et al*. (2011) recorded poor performance when broilers were fed diets containing up to 60 g/kg of seaweed in their diets compared to those fed the control diet. Erum *et al*. (2017)

observed marginal linear increases in ADG (1.54, 1.93, 1.96, and 1.97 g/bird, respectively) and improvements in feed utilization as measured by FCR (2.17, 0.54, 0.69, and 0.72, respectively) when *Sargassum muticum* seaweed (0, 50, 100, and 150 g/kg) was used as a functional feed ingredient in broiler chickens. Likewise, Kumar (2018) discovered that supplementing a broiler diet with 40 g/kg *Sargassum wightii* powder resulted in a higher final body weight than the birds fed a control diet. The beneficial effects of *S. wightii* powder on weight gain and feed utilization efficiency can be ascribed to its high nutrient content and palatability, as well as its capability to promote digestion and absorption of nutrients in the gut. This could be attributed to the presence of essential amino acids, vitamins, sterols, minerals, long-chain fatty acids and fucoidan in *S. wightii* powder that may positively affect broilers (Kumar, 2018).

Abudabos *et al*. (2013) reported improved growth performance, carcass characteristics and serum constituents when up to 30 g/kg of dietary green seaweed was added in broiler chicken diets. Moreover, Mamata *et al*. (2018) recorded a positive impact on body weight and net returns per bird when 50 – 100 g/kg of *Azolla pinnata* inclusion levels were used in broiler chicken diets. In a study by Athis Kumar (2018), meat from broilers fed with 10 or 20 g/kg *Sargassum wightii* inclusion in the basal diet received the highest score when consumers evaluated the meat parameters such as colour, tenderness, flavour, juiciness and taste. In addition, the inclusion of this brown seaweed up to 40 g/kg caused the enhancement of carcass traits such as the weight of the breast, legs, thigh and dressing percentage.

Wang *et al*. (2013) reported that the inclusion of *Enteromorpha prolifera* as dry algal powder at 20, 30 or 40 g/kg improved breast meat quality and reduced the fat content, the thickness of subcutaneous fat and abdominal fat. Moreover, El-Deek & Brikaa (2009) reported that ducks fed a mash diet containing 150 g/kg of *Polysiphonia* spp. increased the weight of breast muscles, while the 50 g/kg and 100 g/kg inclusion levels increased the relative weight of breast meat. Currently, more information is reported on broiler chickens, with limited information on the feed value of dietary seaweeds for indigenous chickens.

2.6.2 Effect of seaweed containing diets on carcass characteristics

Seaweeds contain high concentrations of bioactive chemicals, particularly the sulphur containing amino acids threonine, methionine and lysine, which have positive benefits on dressing percentage, breast meat yield and carcass characteristics (Wong & Cheung, 2001). Indeed, Abudabos *et al*. (2013) revealed that broilers fed diets containing 30 g/kg of *Ulva lactuca* produced higher dressing percentages and lower abdominal fat than control-fed birds. Similarly, Kumar (2018) observed enhanced carcass characteristics (dressing breast, thigh and leg weights) with increasing *Sargassum wightii* levels (0, 10, 20, 30, and 40 g/kg) in broiler diets. Qadri *et al*. (2018) reported improvements in carcass traits (dressing and eviscerated percentage) and organ weight (liver, gizzard and heart) of broilers fed a diet containing incremental levels of *Kappaphycus alvarezii* (2.5, 5, 7.5, 10, 12.5 and 15 g/kg).

Moreover, Martínez *et al*. (2019) revealed that adding 30 g/kg of red seaweed powder (*Chondrus crispus*) to broiler chicken diets enhanced the breast and carcass yield while lowering the abdominal fat yield. These improvements could be attributed to the presence of phenolic compounds (flavonoids, catechins, phenolic acids, tannins and phlorotannins) with anti-inflammatory antioxidant, antimicrobial and antiproliferative activities (Gullón *et al*., 2020). However, Cañedo-Castro *et al*. (2019) discovered no effect on carcass yield and carcass weights of Arbor Acres broilers fed incremental levels of ground and air-dried *Ulva rigida* (0, 20, 40, and 60 g/kg). Regardless, more research is focused on feeding seaweeds to broiler chickens, with minimal information reported on their utility as ingredients in indigenous chicken feeds.

2.6.3 Effect of seaweed containing diets on haematological and serum indices

Blood parameters play critical roles in disease prognosis, diagnosis and treatment by assaying various factors impacting haematological and serum biochemical indices (Yokus *et al*., 2006). Viana *et al*. (2022) reported that the biochemical makeup of blood plasma represents the metabolic status of the tissues, allowing for the evaluation of changes in organ function and the animal's adaptability to abiotic stressors. In a study by Matshogo *et al*. (2020), no dietary effects were observed on blood indices except for lymphocytes, which quadratically increased to incremental levels (20, 25, 30 and 35 g/kg) of *Ulva* sp. meal in broiler chickens.

Bai *et al*. (2019) observed increased lymphocyte numbers when a combination of 30 g/kg *Laminaria japonica* powder and 0.3 g/kg cecropin was supplemented into broiler diets. When two dietary levels (30 and 60 g/kg) of *Sargassum dentifebium* were processed using different methods (boiling, autoclaving and sun-drying) and added to layer rations (23 – 40 weeks of age), lower plasma total cholesterol level and low-density lipoprotein (LDL) were reported compared to the control group. The decrease in plasma total cholesterol and LDL was due to the proximate composition of *S. dentifebium*, which is rich in sterols, fibre and other bioactive chemicals with antioxidant properties (Al-Harthi & El-Deek, 2012).

Additionally, Frasiska *et al*. (2016) observed that adding 125 g/kg of *Gracilaria* sp. and multi-enzyme additive in a diet reduced LDL, cholesterol and triglycerides levels while increasing the concentration of high-density lipoprotein (HDL) in the blood of 22-week-old ducks. These effects can be attributed to the fibre content of *Gracilaria* sp., which acted as an antihyperlipidemic, anticoagulant, antiviral, anticholesterol and antitumor agent (Zhang *et al*., 2012). However, Abudabos *et al*. (2013) reported contrasting results, where dietary inclusion of *Ulva lactuca* (10 and 30 g/kg) in broiler diets had no effect on electrolytes and serum enzymes, except for alanine transaminase, which was lower for the *U. lactuca* fed birds, which indicated that the birds did not suffer from any liver damage.

2.6.4 Effect of seaweeds on gut microbiota and health

The functionality and health of a chicken's GIT are essential elements in determining animal performance. For sustainable poultry production, the gastrointestinal system must work optimally (Celi *et al*., 2017). Jha *et al*. (2019) noted that improving or maintaining gut health is crucial for monogastric animals' overall health, feed efficiency and growth performance. Currently, there is evidence that seaweeds contain bioactive chemicals with antiviral and antimicrobial properties, as well as immunomodulatory effects, which could improve poultry productivity and health (Michalak & Mahrose, 2020). Indeed, when *Ulva rigida* meal was added to broiler diets (20, 40 and 60 g/kg), the villus measurements (height, width and contour length) were higher than in the control group (Cañedo-Castro *et al*., 2019). Awad *et al*. (2008) reported that the increase in the height and width of the villi in chickens' GIT is linked to the increase in the digestion and absorption function due to the larger absorption area.

Choi *et al*. (2014) reported that the cecal microbiome was altered when brown seaweed derivative polymannuronate was incorporated into broiler diets at levels of 1, 2, 3, and 4 g/kg. This resulted in higher acetic and lactic acid concentrations in the caeca, as well as enhanced antioxidant capacity, FCR, ADG and immune status of the birds. Furthermore, adding 20 g/kg of *Chondrus crispus* and *Sarcodiotheca gaudichaudii* to laying hens diets improved the comparative number of beneficial microorganisms (*Streptococcus salivarius* and *Bifidobacterium longum*) than the control group while reducing the pathogenic microbes (*Clostridium perfringens)* in the ileal fillings (Kulshreshtha *et al*., 2014). The reported findings, therefore, show that the inclusion of seaweeds in poultry diets can improve the health and productivity of the birds. Nonetheless, several studies have focused on the use of seaweeds as a feed ingredient in broiler chickens than their utility in indigenous chickens.

2.6.5 Limitations on the use of seaweeds in poultry production

Literature shows that the use of different seaweeds at 30 to 100 g/kg inclusion levels is suitable as a feed ingredient in poultry diets without any major adverse effects on broilers (EL-Deek *et al*., 2011). Indeed, Makkar *et al*. (2016) concluded that seaweeds should normally be incorporated at lower levels (50 - 60 g/kg) in the diets of growing poultry, not exceeding 100 g/kg due to high fibre levels that might have adverse effects on the growth performance. Jha & Mishra (2021) stated that fibre constituents such as β-glucans, arabinoxylans and pectins have negative effects on poultry production because they increase the gastrointestinal digesta viscosity, which in turn reduces feed intake and the rate of feed passage or nutrient absorption, which may negatively impact growth performance.

Earlier research showed that inclusion levels higher than 100 g/kg of green seaweed *U. lactuca* in three-week-old broiler chickens resulted in lower feed intake and reduced growth rate (Ventura *et al*., 1994). Furthermore, El-Deek *et al*. (2011) stated that the inclusion of brown seaweed *Sargassum* at 20, 40 and 60 g/kg depressed the growth of broiler chickens but enhanced polyunsaturated fatty acids and n-3 fatty acids, which eventually improved meat quality. Recently, Matshogo *et al*. (2020) found that the inclusion of graded levels of dietary green seaweed up to 35 g/kg compromised feed conversion efficiency in broiler chickens. This effect could be attributed to the presence of NSPs (ulvans, xylans, hemicellulose and cellulose) in green seaweed (Gullón *et al*., 2020). Therefore, it is important that biological treatments such as white-rot fungi are identified and explored to enhance the feed value of brown seaweeds for poultry production, more especially indigenous chicken production.

2.7 Oyster mushrooms

Mushrooms are heterotrophic eukaryotic organisms that belong to the fungi kingdom (Falandysz & Borovička, 2013) that are classified as mutualists-ectomycorrhizal symbionts, saprotrophs and parasites (Gadd, 2008). Mushrooms are regarded as saprotrophic microorganisms because they grow on agricultural residues, and organic matter of vegetative origin and can utilize nearly all forest material as substrates, resulting in the breakdown of fibre constituents (Adejoye *et al*., 2006). In contrast to saprotrophic fungi, parasitic fungi form symbiotic relationships by invading living organisms, penetrating their outer defence and obtaining nourishment from their cytoplasm, which then causes diseases and in some cases host death (Gerphagnon *et al*., 2013).

Nongthombam *et al*. (2021) reported that the absence of chlorophyll in mushrooms prevents them from synthesizing their nutrients, hence they rely on dead and decay as saprophytes. Mushrooms have the ability to grow or be cultivated on agricultural wastes such as straw, rice hull and sawdust (Khan *et al*., 2012). Additionally, Nongthombam *et al*. (2021) revealed that mushroom cultivation has potential to solve many global problems such as food shortages, unemployment, and environmental pollution, among others.

The three most-grown mushroom species include button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* spp.) and shiitake mushroom (*Lentinula edodes*). In 2018, the global market value of fresh mushrooms surpassed US$ 38 billion, with China being the largest mushroom producer in Asia, accounting for around 35% of the global mushroom industry (Mahari *et al*., 2020). Among the three most cultivated species, the genus *Pleurotus* is regarded as the second most widely distributed and cultivated edible fungus in the entire globe, after button mushroom (*Agaricus bisporus*) due to its adaptability (El-Ramady *et al*., 2022).

According to Van Peer *et al*. (2009), the environment has an important role in the production of oyster mushrooms (OYM), with other genera reported to be sensitive to various major climatic conditions such as humidity, temperature and fresh air in mushroom production. Maniruzzaman (2004) stated that OYM cultivation is more effective because it requires a small plot of land, low production inputs and shorter growth cycles. The OYM mycelial development is optimal at a relative humidity range of 70 – 90% and a temperature ranging between 28°C and 32°C (Cikarge & Arifin, 2018). Additionally, Al Mamum *et al*. (2021) revealed that storage of mushroom spawn-inoculated substrates in absolute darkness during vegetative development is a major factor for high-quality mushroom cultivation.

2.7.1 Oyster mushrooms as a source of food

Mushrooms have been used as a source of food since ancient times and the Romans considered them as "Food of the gods" (Rahi & Malik, 2016). Edible mushrooms are recently recognized as one of the sources of various functional ingredients due to their therapeutic and nutraceutical properties (Rangel-Vargas *et al*., 2021). Mushrooms are classified as a healthy food source because they contain low fat and calories but are high in proteins, minerals and dietary fibre (mannans, chitin, β-glucans and hemicellulose) (Zhang *et al*., 2021). Yadav & Negi (2021) reported that mushrooms contain all essential amino acids and a larger proportion of polyunsaturated fatty acids compared to saturated fatty acids.

Furthermore, OYMs are a rich source of nutritional components and contain various bioactive chemicals such as nucleotides, terpenoids, steroids, alkaloids, lectins, and phenols (Lindequist *et al*., 2005), which can boost immunological function while also exhibiting antimicrobial, anti-inflammatory, hypocholesterolemic, antitumor, antioxidant and hypoglycemic effects (Rangel-Vargas *et al*., 2021). The consumption of OYMs can also be a viable alternative for alleviating energy and protein malnutrition in impoverished and emerging countries.

Indeed, Eswaran & Ramabadran (2000) revealed that mushrooms have been identified as a good source of food in reducing malnutrition in developing nations due to their texture, flavour, and nutritional content, and high output per square metre. Mushrooms are also considered a potential source of income for landless poor people. The food industry has developed a huge interest on using mushrooms as potential substitutes for a variety of additives and ingredients in processed foods, especially in meat and bakery products. This is because they are nutritious, provide texture and flavour, and have antioxidant properties that promote good health (Patinho *et al*., 2021).

2.7.2 Solid-state fermentation

White-rot fungi are regarded as the most efficient in the delignification of fibrous material because they produce extracellular ligninolytic oxidative enzymes (Abdel-Hamid *et al*., 2013). Langer *et al*. (2021) identified two subtypes of white-rot fungi: subtype 1, which can degrade all fibrous constituents simultaneously; and subtype 2, which exhibits selective delignification. Tuyen *et al*. (2013) reported that white-rot fungi such as *Pleurotus ostreatus* (falls under subtype 2) produces enzymes that can preferentially degrade lignin more readily and later cellulose and hemicellulose.

According to Lombard *et al*. (2014), a group of enzymes called carbohydrate-active enzymes are responsible for the breakdown and consumption of lignocellulosic biomass by fungus. Enzymes such as peroxidases (lignin (LiP) and manganese (MnP)), laccases (Lac), oxidoreductases, and copper radical and oxidases aryl alkyl oxidases degrade lignin, while enzymes such glycoside hydrolases, carbohydrate esterases, pectate lyases and copper-dependent lytic polysaccharide monooxygenases degrade carbohydrates (Yoav *et al*., 2018). However, Cui *et al*. (2021) stated that LiP, MnP and Lac are the most extensively studied ligninolytic enzymes responsible for the lignin degradation mechanisms.

In addition, Bilal & Asgher (2016) reported that the lignin peroxide enzyme oxidizes various aromatic compounds while manganese peroxidase oxidizes Mn^{+2} to Mn^{+3} almost exclusively, which results in the degradation of phenolic compounds. Sanchez (2010) further stated that aromatic radicals are produced when ligninolytic peroxidases or laccases oxidize the lignin polymer, which results in different non-enzymatic reactions evolving into the C-4-ether breakdown, aromatic ring cleavage, carbon alpha and beta (Cα-Cβ) breakdown as well as the demethoxylation of the substrate. The biodegradation process of the white-rot fungi would proceed through the process of hydrolysis of beta-1,4-glycosidic linkage bonds until the cellulose and the hemicellulose matrix are degraded by the cellulase enzyme (Valadares *et al*., 2016).

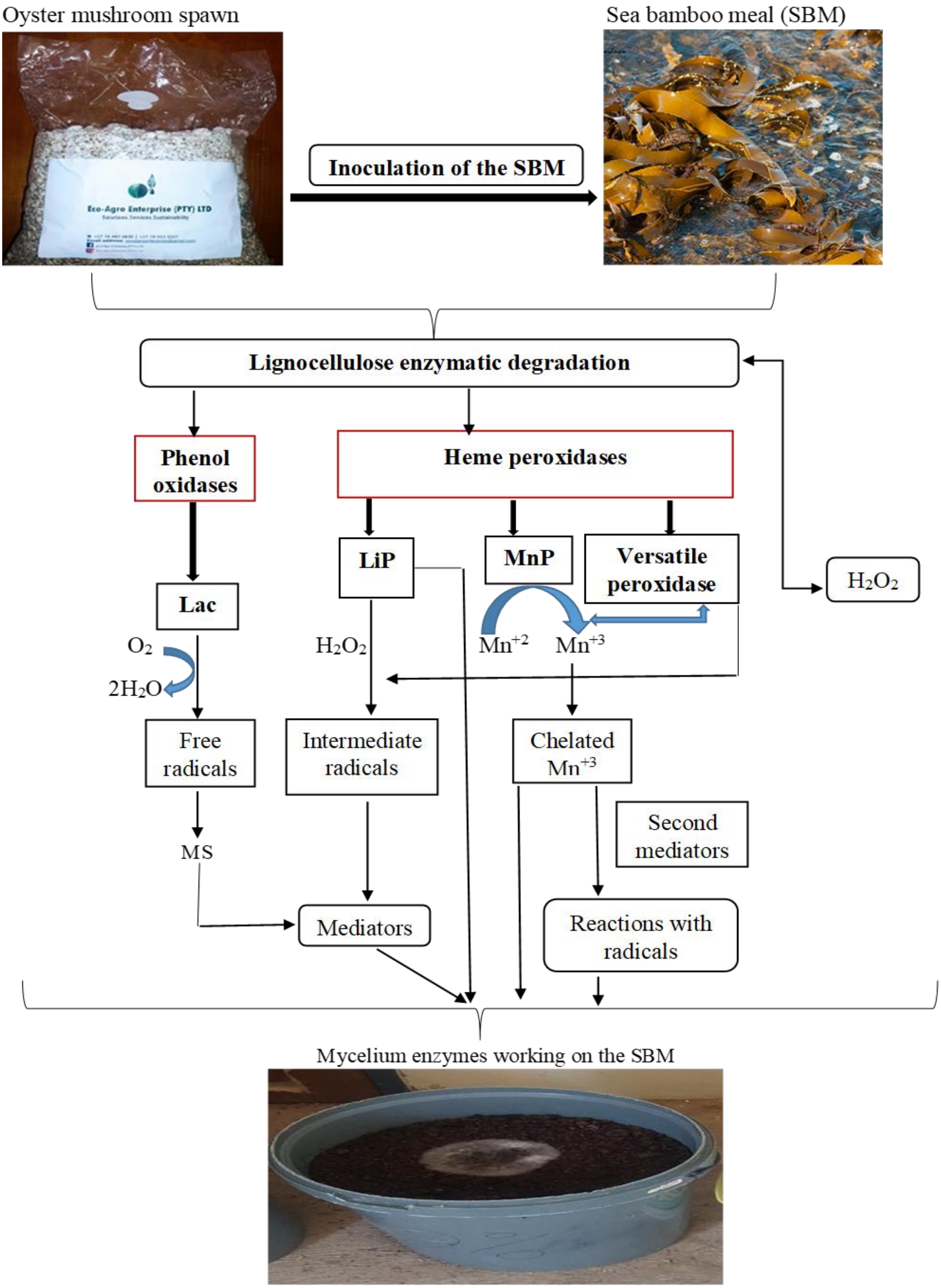

Figure 2.1. A schematic diagram showing the solid-state fermentation of lignin by white-rot enzymes (Adapted from: Javaid *et al*., 2019)

2.7.3 Effect of spent mushroom substrates in poultry production

According to Dewi *et al*. (2019), brown seaweeds contain a high content of alginate that may have a negative effect on poultry performance. Alginates are natural polymers that widely exist in brown seaweeds (genera: *Ecklonia*, *Ascophyllum*, *Durvillaea*, *Laminaria*, *Lessonia*, *Turbinaria*, *Sargassum*, and *Macrocystis*) consisting of linear co-polymers of β-(1–4) linked D-mannuronic acid and β-(1–4)-linked l-guluronic acid units (Qin, 2008), which are known to bind nutrients and inhibit their absorption in the GIT of chickens. Choi *et al*. (2018) and Dewi *et al*. (2019) proposed that the fermentation of feedstuff with microorganisms (*Bacillus subtilis*, *Aspergillus oryzae* and *Bacillus megaterium*) might improve the nutritional quality and increase poultry meat shelf life. Machado & Ferraz (2016) stated that the use of white-rot fungi is an economically and environmentally sustainable biological method to improve the feed value of fibrous feeds, which could be ideal for chickens.

Some studies have been conducted by several researchers using spent mushroom substrates (SMS) in poultry production. For instance, Santos *et al*. (2014) noted that supplementing the diets with 100 and 200 g/kg of spent *Pleurotus ostreatus* substrate in 21-day-old broiler chicken diets improved body weight gain by reducing feed conversion ratio. Foluke *et al*. (2014) reported a higher feed intake and lower body weight gain when spent mushroom (*Pleurotus ostreatus*) substrates (25, 50, 75, and 100%) were used in replacing wheat bran in broiler chicken diets. However, no research has been reported on the effect of sea bamboo SMS on the growth performance, health and meat quality of indigenous chickens.

2.8 Summary

Globally, the poultry industry is currently striving to increase production to meet the demand for animal protein in response to human population growth and high rate of urbanisation. This calls for the identification of strategies that would deliver sustainable production of indigenous chickens using natural resources. Indigenous birds like the Boschveld chicken can be used as a reliable source of protein in many communities in South Africa. However, these chickens are naturally slow-growing with a low feed conversion efficiency compared to conventional broiler chickens, posing a major setback in their sustainable intensification. Their advanced slaughter weights and late sexual maturity results in higher production cost. Thus, the use of sea bamboo meal (SBM) as a feed ingredient has the potential to contribute to sustainable poultry production. Sea bamboo contains bioactive compounds with antimicrobial, antioxidant and growth-stimulating effects that can enhance growth performance, meat quality and health status of indigenous chickens. However, the use of SBM at higher inclusion levels in poultry diets may be limited by high fibre levels. It is, therefore, imperative to employ biological treatments such as the use of OYM spawn to reduce the high fibre content and improve the utilization of SBM as a feed ingredient in indigenous chicken diets. Theoretically, oyster mushroom spawn (OMS) contains lignin peroxide, manganese peroxidase and laccases, which can oxidise various aromatic compounds and break down phenolic structures. The breakdown of fibre is claimed to be achieved by ligninolytic laccases and peroxidases oxidizing and cleaving aromatic structures, producing lignin polymer aromatic radicals. Additionally, the hydrolysis of different glycosidic linkage bonds further explains the breakdown of hemicellulose and cellulose matrix by the fungal enzymes. Unfortunately, there is no reported information on inoculating SBM with OMS to improve its feed value for Boschveld cockerels. Thus, the first experiment (Chapters Three and Four) sought to determine the effect of feeding graded levels of SBM-containing diets on Boschveld cockerel's growth metrices, physiological responses, and carcass and breast meat quality traits. While the second experiment (Chapters Five and Six) assessed the effect of feeding spent OMS-containing diets on growth metrices, physiological responses and carcass and meat quality attributes of Boschveld cockerels.

2.9 References

Abdel-Hamid, A.M., Solbiati, J.O. & Cann, I.K.O., 2013. Insights into lignin degradation and its potential industrial applications. Adv. Appl. Microbiol. 82, 1–28.

Abdul Khalil, H.P.S., Tye, Y.Y., Saurabh, C.K., Leh C.P., Lai, T.K., Chong, E.W.N., Fazita, M.N., Hafiidz, J.M, Banerjee, A. & Syakir, M.I., 2017. Biodegradable polymer films from seaweed polysaccharides: A review on cellulose as a reinforcement material. Express Polym. Lett. 11(4), 244–265.

Abka-Khajouei, R., Tounsi, L., Shahabi, N., Patel, A.K., Abdelkafi, S. & Michaud, P., 2022. Structures, Properties and Applications of Alginates. Mar. Drugs. 20(6), 364.

Abudabos, A.M., Okab, A.B., Aljumaah, R.S., Samara, E.M., Abdoun, K.A. & Al-Haidary, A.A., 2013. Nutritional value of green seaweed (*Ulva lactuca*) for broiler chickens. Ital. J. Anim. Sci. 1, 177–181.

Adejoye, O.D., Adebayo-Tayo, B.C., Ogunjobi, A.A. & Olaoye, O.A., 2006. Effect of carbon, nitrogen and mineral sources on growth of *Pleurotus florida* Nigeria edible mushroom. Afr. J. Biotechnol. 5, 1355–1359.

Adl, S.M., Simpson, A.G., Farmer, M.A., Andersen, R.A., Anderson, O.R., Barta, J.R., Bowser, S.S., Brugerolle, G.U.Y., Fensome, R.A., Fredericq, S. & James, T.Y., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protests. J. Eukaryot. Microbiol. 52, 399–451.

Afrikelp. 2020. Product information. https://afrikelp.com/production/. Accessed 14 April 2021.

AgriOrbit. 2022. Boschveld chickens tick all the right boxes. https://agriorbit.com/boschveld-chicken-ticks-all-the-boxes/. Accessed 06 September 2022.

Ahmed, A.B.A., Adel, M., Karimi, P. & Peidayesh, M., 2014. Pharmaceutical, cosmeceutical, and traditional applications of marine carbohydrates. Adv. Food Nutr. Res. 73, 197–220.

Akinyemi, F. & Adewole, D., 2022. Effects of brown seaweed products on growth performance, plasma biochemistry, immune response, and antioxidant capacity of broiler chickens challenged with heat stress. Poult. Sci. 101(12), 102215.

Al Mamun, M.R., Deb, I., Hridoy, T., Soeb, M.J.A. & Shammi, S., 2021. Effects of different lighting conditions on growth, yield and nutrient content of white oyster mushroom in vertical farm**.** European J. Agric. Food Sci. 3(6), 61–67.

Alagawany, M., Elnesr, S.S., Farag, M.R., Tiwari, R., Yatoo, M.I., Karthik, K., Michalak, I. & Dhama, K., 2021. Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health–a comprehensive review. Vet. Q. 41(1), 1-29.

Alders, R.G., Dumas, S.E., Rukambile, E., Magoke, G., Maulaga, W., Jong, J. & Costa, R., 2018. Family poultry: Multiple roles, systems, challenges, and options for sustainable contributions to household nutrition security through a planetary health lens. Matern. Child Nutr. 14 (3), e12668.

Al-Harthi, M.A. & El-Deek, A.A., 2012. Effect of different dietary concentrations of brown marine algae (*Sargassum dentifebium*) prepared by different methods on plasma and yolk lipid profiles, yolk total carotene and lutein plus zeaxanthin of laying hens. Ital. J. Anim. Sci. 11, 347–353.

Anderson, R.J., Bolton, J.J., Molloy, F.J. & Rotmann, K.W.G, 2003. Commercial seaweed production and research in southern Africa. In: Chapman ARO, Anderson RJ, Vreeland

VJ, Davison IR (Eds) Proceedings of the 17th International Seaweed Symposium. Oxford University Press, Oxford, England. pp. 1–12.

Anderson, R.J., Rand, A., Rothman, M.D. & Bolton, J.J., 2007. Mapping and quantifying the South African kelp resource. Afr. J. Mar. Sci. 29, 369–378.

Arumugam, N., Chelliapan, S., Kamyab, H., Thirugnana, S., Othman, N. & Nasri, N.S., 2018. Treatment of wastewater using seaweed: a review. Int. J. Environ. Res. Public Health. 15(12), 2851.

Ashkenazi, D.Y., Israel, A. & Abelson, A., 2019. A novel two-stage seaweed integrated multi-trophic aquaculture. Rev. Aquac. 11, 246–262.

Athis Kumar, K., 2018. Effect of *Sargassum wightii* on growth, carcass and serum qualities of broiler chickens. Vet. Sci. Res. 3, 000156.

Awad, W.A., Ghareeb, K., Nitch, S., Pasteiner, S., Raheem, S.A. & Bohm, J., 2008. Effect of dietary inclusion of probiotic, prebiotic and symbiotic on intestinal glucose absorption on broiler chickens. Int. J. Poult. Sci. 7(7), 688–691.

Bai, J., Wang, R., Yan, L. & Feng, J., 2019. Co-Supplementation of dietary seaweed powder and antibacterial peptides improves broiler growth performance and immune function. Braz. J. Poult. Sci. 21, 1–9.

Baião, N.C. & Lara, L.J.C., 2005. Oil and fat in broiler nutrition. Braz. J. Poult. Sci. 7, 129–141.

Baweja, P., Kumar, S., Sahoo, D. & Levine, I.A., 2016. Biology of seaweeds. In Seaweed in health and disease prevention. Eds. Fleurence, J., Levine, I., Academic Press: London, UK. pp. 41–106.

Benjama, O. & Masniyom, P., 2012. Biochemical composition and physicochemical properties of two red seaweeds (*Gracilaria fisheri* and *G. tenuistipitata*) from the Pattani Bay in Southern Thailand. Sonklanakarin J. Sci. Technol. 34, 223–230.

Beski, S.S.M., Swick, R.A. & Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: A review. Anim. Nutr. 1, 47–53.

Biesek, J., Kuźniacka, J., Banaszak, M., Kaczmarek, S., Adamski, M., Rutkowski, A., Zmudzińska, A., Perz, K. & Hejdysz, M., 2020. Growth performance and carcass quality in broiler chickens fed on legume seeds and rapeseed meal. Animals, 10(5), 846.

Bikker, P., Stokvis, L., van Krimpen, M.M., van Wikselaar, P.G. & Cone, J.W., 2020. Evaluation of seaweeds from marine waters in North-Western Europe for application in animal nutrition. Anim. Feed Sci. Technol. 263, 114460.

Bilal, M. & Asgher, M., 2016. Biodegradation of agro-waste by lignocellulolytic activity of an oyster mushroom, *Pleurotus sapidus*. J. Nat. Sci. 44(4), 399–407.

Bindu, M.S. & Sobha, V., 2004. Conversion efficiency and nutrient digestibility of certain seaweed diets by laboratory reared *Labeo rohita* (Hamilton). Indian J. Exp. Biol. 42, 1239–1244.

Bolton, T., 2015. The state of the South African poultry industry. Super Market & Retailer. pp. 17–23.

Boulom, S., Robertson, J., Hamid, N., Ma, Q. & Lu, J., 2014. Seasonal changes in lipid, fatty acid, α-tocopherol and phytosterol contents of seaweed, *Undaria pinnatifida*, in the Marlborough Sounds, New Zealand. Food Chem. 161, 261–269.

Cañedo-Castro, B., Piñón-Gimate, A., Carrillo, S., Ramos, D. & Casas-Valdez, M., 2019. Prebiotic effect of *Ulva rigida* meal on the intestinal integrity and serum cholesterol and triglyceride content in broilers. J. Appl. Phycol. 31, 3265–3273.

Carrillo, S., López, E., Casas, M.M., Avila, E., Castillo, R.M., Carranco, M.E., Calvo, C. & Pérez-Gil, F., 2009. Potential use of seaweeds in the laying hen ration to improve the quality of n-3 fatty acid enriched eggs. J. Appl. Phycol. 2, 271–278.

Carrillo, S., Rios, V.H., Calvo, C., Carranco, M.E., Casas, M. & Perez-Gil, F., 2012. n-3 fatty acid content in eggs laid by hens fed with marine algae and sardine oil and stored at different times and temperatures. J. Appl. Phycol. 24(3), 593–599.

Catarino, M.D., Silva, A. & Cardoso, S.M., 2018. Phycochemical constituents and biological activities of *Fucus* spp. Mar. Drugs. 16, 249.

Catarino, M.D., Silva-Reis, R., Chouh, A., Silva, S., Braga, S.S., Silva, A.M. & Cardoso, S.M., 2023. Applications of antioxidant secondary metabolites of *Sargassum* spp. Mar. Drugs. 21(3), 172.

Celebi, S. & Utlu, N., 2004. Laying performance, serum lipoproteins, cholesterol and triglyceride of hens as influenced by dietary fat sources. J. Appl. Anim. Res. 25, 121–124.

Celi, P., Cowieson, A.J., Fru-Nji, F., Steinert, R.E., Kluenter, A.M. & Verlhac, V., 2017. Gastrointestinal functionality in animal nutrition and health: new opportunities for sustainable animal production. Anim. Feed Sci. Technol. 234, 88–100.

Choi, Y., Lee, E., Na, Y. & Lee, S., 2018. Effects of dietary supplementation with fermented and non-fermented brown algae by-products on laying performance, egg quality, and blood profile in laying hens. Asian-Australas. J. Anim. Sci. 31, 1654–1659.

Choi, Y.J., Lee, S.R. & Oh, J.W., 2014. Effects of dietary fermented seaweed and seaweed fusiforme on growth performance, carcass parameters and immunoglobulin concentration in broiler chicks. Asian-Australas. J. Anim. Sci. 27, 862–870.

Cikarge, G.P. & Arifin, F., 2018, December. Oyster mushrooms humidity control based on fuzzy logic by using Arduino ATMega238 microcontroller. J. Phys. Conf. Ser. 1140(1), 012002.

Corino, C., Modina, S., Di Giancamillo, A., Chiapparini, S. & Rossi, R., 2019. Seaweeds in pig nutrition. Animals. 9, 1126.

Cotas, J., Leandro, A., Monteiro, P., Pacheco, D., Figueirinha, A., Gonçalves, A.M., da Silva, G.J. & Pereira, L., 2020. Seaweed phenolics: From extraction to applications. Mar. Drugs. 18(8), 384.

Cruz-Suarez, L.E., Tapia-Salazar, M., Nieto-Lopez, M.G., Guajardo-Barbosa, C. & Ricque-Marie, D., 2008. Comparison of Ulva clathrata and the kelps Macrocystis pyrifera and *Ascophyllum nodosum* as ingredients in shrimp feeds. Aquacult. Nutr. 15, 421–430.

Cui, T., Yuan, B., Guo, H., Tian, H., Wang, W., Ma, Y., Li, C. & Fei, Q., 2021. Enhanced lignin biodegradation by consortium of white rot fungi: microbial synergistic effects and product mapping. Biotechnol. Biofuels. 14(1), 1–11.

Dang, T.T., Bowyer, M.C., Van Altena, I.A. & Scarlett, C.J., 2018. Comparison of chemical profile and antioxidant properties of the brown algae. Int. J. Food Sci. Technol. 53, 174–181.

Daub, C.D., Mabate, B., Malgas, S. & Pletschke, B.I., 2020. Fucoidan from *Ecklonia maxima* is a powerful inhibitor of the diabetes-related enzyme, α-glucosidase. Int. J. Biol. Macromol. 151, 412–420.

Delany, M.E., 2003. Genetic diversity and conservation of poultry. In: Muir W.M. & Aggrey S.E. (Eds), Poultry genetics, breeding and biotechnology. CABI Publishing, CAB International, Trowbridge, England.

Déléris, P., Nazih, H. & Bard, J.M., 2016; Seaweeds in human health. In seaweed in health and disease prevention, 1st ed., Fleurence, J., Levine, I., Eds. Academic Press: London, England. pp. 319–367.

Denis, C., Morançais, M., Li, M., Deniaud, E., Gaudin, P., Wielgosz-Collin, G., Barnathan, G., Jaouen, P. & Fleurence, J., 2010. Study of the chemical composition of edible red macroalgae *Grateloupia turuturu* from Brittany (France). Food Chem. 119, 913–917.

Dewi, Y.L., Yuniza, A., Sayuti, K., Mahata, M.E. & Nuraini, N., 2019. Fermentation of *Sargassum binderi* seaweed for lowering alginate content of feed in laying hens. J. World Poult. Res. 9, 147–153.

El-Deek, A., Bri, A.M. & Brikaa, A.M., 2009. Effect of different levels of seaweed in starter and finisher diets in pellet and mash form on performance and carcass quality of ducks. Int. J. Poult. Sci. 8, 1014–1021.

El-Deek, A.A., Al-Harthi, M.A., Abdalla, A.A. & Elbanoby, M.M., 2011. The use of brown algae meal in finisher broiler diets. Egypt Poul. Sci. 1(31), 767–781.

El-naga, M.A. & Megahed, M.M., 2018. Impact of brown algae supplementation in drinking water on growth performance and intestine histological changes of broilers. Egypt J. Nutr. Feeds. 21(2), 495–507.

El-Ramady, H., Abdalla, N., Fawzy, Z., Badgar, K., Llanaj, X., Törős, G., Hajdú, P., Eid, Y. & Prokisch, J., 2022. Green biotechnology of oyster mushroom (*Pleurotus ostreatus* L.): A sustainable strategy for myco-remediation and bio-fermentation. Sustainability. 14(6), 3667.

El-Said, G. & El-Sikaily, A., 2013. Chemical composition of some seaweeds from Mediterranean Sea coast, Egypt. Environ. Monit. Assess. 185, 6089–6099.

Erickson, P.S., Marston, S.P., Gemmel, M., Deming, J., Cabral, R.G., Murphy, M.R. & Marden, J.I., 2012. Short communication: Kelp taste preferences by dairy calves. J. Dairy Sci. 95, 856–858.

Erum, T., Frias G.G. & Cocal, C.J., 2017. *Sargassum muticum* as feed substitute for broiler. Asia pacific. J. Educ. Arts Sci. 4, 6–9.

Eswaran, A. & Ramabadran, R., 2000. Studies on some physiological, cultural and post-harvest aspects of oyster mushroom, *Pleurotus eous*. Trop. Agric. Res. 12, 360–374.

Evans, F.D. & Critchley, A.T., 2014. Seaweeds for animal production use. J. Appl. Phycol. 26, 891–899.

Falandysz, J. & Borovička, J., 2013. Macro and trace mineral constituents and radionuclides in mushrooms: health benefits and risks. Appl. Microbiol. Biotechnol. 97, 477–501.

FAO. 2018. The global status of seaweed production, trade and utilization. Globefish research programme volume 124. Rome, Italy. pp. 120.

FAO. 2021. The State of World Fisheries and Aquaculture 2021 (SOFIA). Rome, Italy.

FAOSTAT. 2010. Statistical database of Food and Agricultural Organization of the United Nations, Rome, Italy. pp. 1–10.

Fernando, I.P.S., Lee, W., Han, E.J. & Ahn, G., 2020. Alginate-based nanomaterials: fabrication techniques, properties, and applications. Chem. Eng. J. 391, 123823.

Ferreira, J., 2017. Farming chickens ethically and profitably. Farmer's weekly. Johannesburg, South Africa, https://www.farmersweekly.co.za/animals/poultry/farming-chickens-ethically-and-profitably/. Accessed 06 September 2019.

Ferreres, F., Lopes, G., Gil-Izquierdo, A., Andrade, P.B., Sousa, C., Mouga, T. & Valentão, P., 2012. Phlorotannin extracts from fucales characterized by HPLC-DAD-ESI-MS n:

Approaches to hyaluronidase inhibitory capacity and antioxidant properties. Mar. Drugs. 10(12), 2766–2781.

Fidai, Y.A., Dash, J., Tompkins, E.L. & Tonon, T., 2020. A systematic review of floating and beach landing records of *Sargassum* beyond the Sargasso Sea. Environ. Res. Commun. 2(12), .122001.

Fleurence, J., 2004. Seaweed proteins. In Yada, R.Y. (Ed.), Proteins in food processing. Woodhead Publishing Limited, Cambridge, England. pp. 197–213.

Fleurence, J., Morançais, M., Dumay, J., Decottignies, P., Turpin, V., Munier, M., Garcia-Bueno, N. & Jaouen, P., 2012. What are the prospects for using seaweed in human nutri¬tion and for marine animals raised through aquaculture? Trends Food Sci. Technol. 27, 57–61.

Foluke, A., Olutayo, A. & Olufemi, A., 2014. Assessing spent mushroom substrate as a replacement to wheat bran in the diet of broilers. Am. Int. J. Contemp. Res. 4 (4), 178–183.

Forbes, C.J., 2009. Analysis of protein content of two kelps, *Ecklonia maxima* and *Laminaria pallida* for feed in abalone aquaculture. BSc dissertation. University of Cape Town Cape town, South Africa.

Frasiska, N., Suprijatna, E. & Susanti, S., 2016. Effect of diet containing *Gracilaria* sp. waste and multi-enzyme additives on blood lipid profile of local duck. Anim. Prod. 18(1), 22–29.

Gadd, G.M., 2008. Fungi and their role in the biosphere. In: Jorgensen SE, Fath B, Eds. Encyclopedia of ecology. Elsevier, Amsterdam, Netherlands. pp. 1709–1717.

Ganesan, A.R., Tiwari, U. & Rajauria, G., 2019. Seaweed nutraceuticals and their therapeutic role in disease prevention. Food Sci. Hum. Wellness. 8(3), 252–263.

Gerphagnon, M., Latour, D., Colombet, J. & Sime-Ngando, T., 2013. Fungal parasitism: life cycle, dynamics and impact on cyanobacterial blooms. PLoS ONE. 8(4), e60894.

Ghosh, R., Banerjee, K. & Mitra, A., 2012. Eco-biochemical studies of common seaweeds in the lower Gangetic Delta. In: Kim, S.K. (Ed.), Handbook of Marine Macroalgae; Biotechnology and Applied Phycology. John Wiley & Sons, Ltd, New York.

Gomez-Zavaglia, A., Lage, M.A.P., Cecilia Jimenez-Lopez, C., Mejuto, J.C. & Simal-Gandara, J., 2019. The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. Antioxidants. 8(9), 406.

Gullón, B., Gagaoua, M.F.J., Barba, F.J.P., Gullón, P., Zhang, W.J.M. & Lorenzo, J.M., 2020. Seaweeds as a promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products. Trends Food Sci. Technol. 100, 1–18.

Hecht, H. & Srebnik, S., 2016. Structural characterization of sodium alginate and calcium alginate. Biomacromolecules. 17, 2160–2167.

Hobbs, R.P. & Bernstein, P.S., 2014. Nutrient supplementation for age-related macular degeneration, cataract, and dry eye. J. Ophthalmic Vis. Res. 9(4), 487–93.

Holdt, S.L. & Kraan, S., 2011. Bioactive compounds in seaweed: Functional food applications and legislation. J. Appl. Phycol. 23, 543–597.

Hussain, M., Mahmud, A., Hussain, J. & Qaisrani, S.N., 2018. Effect of dietary lysine regimens on growth performance and meat composition in Aseel chicken. Braz. J. Poult. Sci. 20, 203–210.

Islam, M.N., Ishita, I.J., Jin, S.E., Choi, R.J., Lee, C.M., Kim, Y.S., Jung, H.A. & Choi, J.S., 2013. Anti-inflammatory activity of edible brown alga *Saccharina japonica* and its constituents pheophorbide a and pheophytin a in LPS-stimulated RAW 264.7 macrophage cells. Food Chem. Toxicol. 55, 541–548.

Jamal, P., Olorunnisola, K.S., Jaswir, I., Tijani, I.D.R. & Ansari, A.H., 2017. Bioprocessing of seaweed into protein enriched feedstock: Process optimization and validation in reactor. Int. Food Res. J. 24, 382–386.

Javaid, R., Sabir, A., Sheikh, N. & Ferhan, M., 2019. Recent advances in applications of acidophilic fungi to produce chemicals. Molecules. 24(4), 786.

Jha, R. & Mishra, P., 2021. Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: a review. J. Anim. Sci. Biotechnol. 12, 51.

Jha, R., Fouhse, J.M., Tiwari, U.P., Li, L. & Willing, B.P., 2019. Dietary fiber and intestinal health of monogastric animals. Front. Vet. Sci. 6, 48.

Jiménez-Escrig, A., Gómez-Ordóñez, E. & Rupérez, P., 2012. Brown and red seaweeds as potential sources of antioxidant nutraceuticals. J. Appl. Phycol. 24, 1123–1132.

Kadam, S.U., O'Donnell, C.P., Rai, D.K., Hossain, M.B., Burgess, C.M., Walsh, D. & Tiwari, B.K., 2015. Laminarin from Irish brown Seaweeds *Ascophyllum nodosum* and *Laminaria hyperborea*: Ultrasound assisted extraction, characterization and bioactivity. Mar. Drugs. 13(7), 4270–80.

Kadam, S.U., Tiwari, B.K. & O’Donnell, C.P., 2014. Extraction, structure and biofunctional activities of laminarin from brown algae. Int. J. Food Sci. Technol. 50, 24–31.

Karuppusamy, S., Rajauria, G., Fitzpatrick, S., Lyons, H., McMahon, H., Curtin, J., Tiwari, B.K. & O'Donnell, C., 2022. Biological properties and health-promoting functions of laminarin: A comprehensive review of preclinical and clinical studies. Mar. Drugs. 20(12), 772.

Khajouei R.A., Keramat J., Hamdami N., Ursu A.V., Delattre C., Laroche C., Gardarin C., Lecerf, D., Desbrières J., Djelveh G. & Michaud, P., 2018. Extraction and characterization of an alginate from the Iranian brown seaweed *Nizimuddinia zanardini*. Int. J. Biol. Macromol. 118, 1073–1081.

Khan, S.M., Nawaz, A., Ali, M.A., Ahmad, T., Khan, N.A. & Rehman, A.U., 2012. The response of oyster mushroom on different agricultural wastes of Southern Punjab. Pak. J. Agric. Sci. 49, 127–130.

Kim, S.K., 2019. Genetic diversity and DNA markers in fish. In: Kim, S.K. (Ed). In: Essentials of maarine biotechnology. Springer International Publishing, Cham, Switzerland. pp. 109–144.

Kingori, A.M., Tuitoek, J.K., Muiruri, H.K. & Wachira, A.M., 2003. Protein requirements of growing indigenous chickens during the 14-21 weeks growing period. S. Afr. J. Anim. Sci. 33(2), 78–82.

Kraan, S., 2012. Algal Polysaccharides, Novel applications and outlook. Pages 489–532 in Carbohydrates – Comprehensive studies on Glycobiology and Glycotechnology. C. Chang, ed. InTech, Rijeka, Croatia.

Kulshreshtha, G., Hincke, M.T., Prithiviraj, B. & Critchley, A.T., 2020. A Review of the varied uses of macroalgae as dietary supplements in selected poultry with special reference to laying hen and broiler chickens. J. Mar. Sci. Eng. 8, 536.

Kulshreshtha, G., Rathgeber, B., MacIsaac, J., Boulianne, M., Brigitte, L., Stratton, G., Thomas, N.A., Critchley, A.T., Hafting, J. & Prithiviraj, B., 2017. Feed supplementation with red seaweeds, *Chondrus crispus* and *Sarcodiotheca gaudichaudii*, reduce *Salmonella* Enteritidis in laying hens. Front. Microbiol. 8, 567.

Kulshreshtha, G., Rathgeber, B., Stratton, G., Thomas, N., Evans, F., Critchley, A., Hafting, J. & Prithiviraj, B., 2014. Feed supplementation with red seaweeds, *Chondrus crispus* and *Sarcodiotheca gaudichaudii*, affects performance, egg quality, and gut microbiota of layer hens. Poult. Sci. 93(12), 2991–3001.

Kumar, A.K., 2018. Effect of *Sargassum wighti*i on growth, carcass and serum qualities of broiler chickens. Vet. Sci. Res. 3, 000156.

Kumar, L.R., Paul, P.T., Anas, K.K., Tejpal, C.S., Chatterjee, N.S., Anupama, T.K., Mathew, S. & Ravishankar, C.N., 2022. Phlorotannins–bioactivity and extraction perspectives. J. Appl. Phycol. 34(4), 2173–2185.

Kumar, Y., Tarafdar, A. & Badgujar, P.C., 2021. Seaweed as a source of natural antioxidants: Therapeutic activity and food applications. J. Food Quality. 1–17.

Langer, G.J., Bußkamp, J., Terhonen, E. & Blumenstein, K., 2021. Fungi inhabiting woody tree tissues. In Forest Microbiology. Ed. Asiegbu, F.O., Academic Press: San Diego, USA. pp. 175–205.

Langin, K., 2018. Seaweed masses assault Caribbean islands. Science**.** 360, 1157–11578.

Le Blé, S. & Morice, C., 2013. Algae in pig feeds: an ingredient from the future. http://www.wattagnet.com. Accessed 15. November 2021.

Leandro, A., Pacheco, D., Cotas, J., Marques, J.C., Pereira, L. & Goncalves, A.M.M., 2020. Seaweed's bioactive candidate compound to the food industry and global food security. Life. 10, 140.

Leeson, S. & Summers, J. D., 2010. Broiler Breeder Production. Nottingham University Press: Nottingham, England. pp, 139 – 143.

Leyton, A., Pezoa-Conte, R., Barriga, A., Buschmann, A.H., Mäki-Arvela, P., Mikkola, J.P. & Lienqueo, M.E., 2016. Identification and efficient extraction method of phlorotannins from the brown seaweed *Macrocystis pyrifera* using an orthogonal experimental design. Algal Res. 16, 201–208.

Li, B., Lu, F. & Wei, X. & Zhao, R., 2008. Fucoidan: Structure and Bioactivity. Molecules. 13, 1671–1695.

Li, L., Abouelezz, K.F.M., Cheng, Z., Gad-Elkareem, A.E.G., Fan, Q., Ding, F., Gao, J., Jiang, S. & Jiang, Z., 2020. Modelling methionine requirements of fast-and slow-growing Chinese yellow-feathered chickens during the starter phase. Animals, 10(3), 443.

Li, Y., Fu, X., Duan, D., Liu, X., Xu, J. & Gao, X., 2017. Extraction and identification of phlorotannins from the brown alga, *Sargassum fusiforme* (Harvey) Setchell. Mar. Drugs. 15(2), 49.

Lindequist, U., Niedermeyer, T.H. & Jülich, W.D., 2005. The pharmacological potential of mushrooms. Evid. Based Complement. Alternat. Med. 2(3), 285–99.

Liu, J., Yang, S., Li, X., Yan, Q., Reaney, M.J.T. & Jiang, Z., 2019. Alginate Oligosaccharides: Production, Biological Activities, and Potential Applications. Compr. Rev. Food Sci. Food Saf. 18, 1859–1881.

Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M. & Henrissat, B., 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 42(1), 490–495.

Mac Monagail, M., Cornish, L., Morrison, L., Araújo, R. & Critchley, A.T., 2017. Sustainable harvesting of wild seaweed resources. Eur. J. Phycol. 52(4), 371–390.

Machado, A.D. & Ferraz, A., 2016. Biological pretreatment of sugarcane bagasse with basidiomycetes producing varied patterns of biodegradation. Bioresour. Technol. 225, 17–22.

Mahari, W.A.W., Peng, W., Nam, W.L., Yang, H., Lee, X.Y., Lee, Y.K., Liew, R.K., Ma, N.L., Mohammad, A., Sonne, C. & Van Le, Q., 2020. A review on valorization of oyster mushroom and waste generated in the mushroom cultivation industry. J. Hazard. Mater. 400, 123156.

Mahendra, K.P., 2016. Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance. Scientifica. 9, 1–10.

Makkar, H.P.S., Tran, G., Heuze, V., Giger-Reverdin, S., Lessire, M., Lebas, F. & Ankers, P., 2016. Seaweeds for livestock diets: A review. Anim. Feed Sci. Technol. 212, 1–17.

Mamata, J., Abdul, A., Anadamoy, M., Shiv Mohan, M., Satyanarayana, B., Bhogeshwar, C. & Bhagchand, C., 2018. Effect of (*Azolla pinnata*) feed on the growth of broiler chicken. J. Entomol. Zool. Stud. 6, 391–393.

Maniruzzaman, M., 2004. Influence of media composition and growth regulators on mycelial growth and spawn production of three mushroom species. MSc dissertation. Department of Biotechnology, BAU, Mymensingh, Bangladesh.

Manyelo, T.G., Selaledi, L., Hassan, Z.M. & Mabelebele, M., 2020. Local chicken breeds of Africa: Their description, uses and conservation methods. Animals. 10, 2257.

Maoka, T., 2020. Carotenoids as natural functional pigments. J. Nat. Med. 74(1), 1–16.

Martínez, Y., Ayala, L., Hurtado, C., Más, D. & Rodríguez, R., 2019. Efects of dietary supplementation with red algae powder (*Chondrus crispus*) on growth performance, carcass traits, lymphoid organ weights and intestinal pH in broilers. Braz. J. Poult. Sci. 21, 1–7.

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.

Maurer, A.S., De Neef, E. & Stapleton, S., 2015 *Sargassum* accumulation may spell trouble for nesting sea turtles Front. Ecol. Environ. 13, 394–5.

McAinsh, C.V., Kusina, J., Madsen, J. & Nyoni, O., 2004. Traditional chicken production in Zimbabwe. Worlds Poult. Sci. J. 60, 233–246.

McCullough, C., 2017. Chickens can provide a nice boost to income. https://www.poultryworld.net/Home/General/2017/10/Chickens-can-provide-a-nice-boost-to-income-204536E/. Accessed 06 September 2022.

McGlathery, K.J., 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. J. Phycol. 37(4), 453–456.

McHugh, D.J., 2003. A guide to the seaweed industry. FAO Fisheries Technical Paper, No. 441. FAO, Rome, Italy. pp. 105.

Miao, H.Q., Ishai-Michaeli, R., Peretz, T. & Vlodavsky I., 1995. Laminarin sulfate mimics the effects of heparin on smooth muscle cell proliferation and basic fibroblast growth factor-receptor binding and mitogenic activity. J. Cell. Physiol. 164, 482–490.

Michalak, I. & Chojnacka, K., 2015. Algae as production systems of bioactive compounds. Eng. Life Sci. 15, 160–176.

Michalak, I. & Mahrose, K., 2020. Seaweeds, intact and processed, as a valuable component of poultry feeds. J. Mar. Sci. Eng. 8(8), 620.

Mikami, K. & Hosokawa, M., 2013. Biosynthetic pathway and health benefits of fucoxanthin, an algae-specific xanthophyll in Brown seaweeds. Int. J. Mol. Sci. 14(7), 13763–13781.

Misurcova, L., Skrovankova, S. & Samek. D., 2012. Health benefits of algal polysaccharides in human nutrition. Adv. Food Nutr. Res. 66, 75–145.

Miyashita, K., Beppu, F., Hosokawa, M., Liu, X. & Wang, S., 2020. Nutraceutical characteristics of the brown seaweed carotenoid fucoxanthin. Arch. Biochem. Biophys. 686, 1083642.

Miyashita, K., Nishikawa, S., Beppu, F., Tsukui, T., Abe, M. & Hosokawa, M., 2011. The allenic carotenoid fucoxanthin, a novel marine nutraceutical from brown seaweeds. J. Sci. Food Agric. 91(7), 1166–1174.

Mlambo, V., Mnisi, C.M., Matshogo, T.B. & Mhlongo, G., 2022. Prospects of dietary seaweeds and their bioactive compounds in sustainable poultry production systems: A symphony of good things?. Front. Anim. Sci. 3, 998042.

Montero, L., Sanchez-Camargo, A.P., Garcia-Canas, V., Tanniou, A., Stiger-Pouvreau, V., Russo, M., Rastrelli, L., Cifuentes, A., Herrero, M. & Ibanez, E., 2016. Anti-proliferative activity and chemical characterization by comprehensive two-dimensional liquid chromatography coupled to mass spectrometry of phlorotannins from the brown macroalga *Sargassum muticum* collected on North-Atlantic coasts. J. Chromatogr. A. 1428, 115–125.

Moreira, R., Sineiro, J., Chenlo, F., Arufe, S. & Díaz-Varela, D., 2017. Aqueous extracts of *Ascophyllum nodosum* obtained by ultrasound-assisted extraction: effects of drying temperature of seaweed on the properties of extracts. J. Appl. Phycol. 29, 3191–3200.

Mtileni, B.J., Muchadeyi, F.C., Maiwashe, A., Phitsane, P.M., Halimani, T.E., Chimonyo, M. & Dzama, K., 2009. Characterisation of production systems for indigenous chicken genetic resources of South Africa. Appl. Anim. Husb. Rural Dev. 2, 18–22.

Mulders, K., Lamers, P. P., Martens, D. E. & Wijffels, R.H., 2014. Phototrophic pigment production with microalgae: Biological constraints andopportunities. J. Phycol. 50, 229–242.

Murty, U.S. & Banerjee, A.K., 2012. Seaweeds: the wealth of oceans. In: Kim, S.K. (Ed.), Handbook of Marine Macroalgae: Biotechnology and Applied Phycology. Oxford, England, pp. 36–44.

Mwalugha, H.M., Wakibia, J.G., Kenji, G.M. & Mwasaru, M.A., 2015. Chemical composition of common seaweeds from the Kenya coast. J. Food Res. 4, 28–38.

Myers, S.P., Mulder, A.M., Baker, D.G., Robinson, S.R., Rolfe, M.I., Brooks, L. & Fitton, J.H., 2016. Effects of fucoidan from Fucus vesiculosus in reducing symptoms of osteoarthritis: A randomized placebo-controlled trial. Biol. Targets Ther. 10, 81–88.

Nhlane, L.T., 2020. Physiological and meat quality parameters of indigenous chickens in response to dietary seaweed. MSc dissertation. North-West University, Mafikeng, South Africa.

Nkukwana, T.T., 2018. Global poultry production: Current impact and future outlook on the South African poultry industry. S. Afr. J. Anim. Sci. 48 (5), 1–16.

Nongthombam, J., Kumar, A., Ladli, B.G.V.., Madhushekhar, M. & Patidar, S., 2021. A review on study of growth and cultivation of oyster mushroom. Plant Cell Biotechnol. Mol. Biol. 22(5&6), 55–65.

NRC, 1994. Nutrient Requirements of Poultry (9th ed.). National Research Council; National Academy Press, Washington DC, USA.

Obluchinskaya, E.D., Pozharitskaya, O.N., Zakharov, D.V., Flisyuk, E.V., Terninko, I.I., Generalova, Y.E., Smekhova, I.E. & Shikov, A.N., 2022. The Biochemical composition and antioxidant properties of *Fucus vesiculosus* from the Arctic region. Mar. Drugs. 20(3), 193.

Okoro, V.M.O., Ravhuhali, K.E., Mapholi, T.H., Mbajiorgu, E.F. & Mbajiorgu, C.A., 2017. Effect of age on production characteristics of Boschveld indigenous chickens of South Africa reared intensively. S. Afr. J. Anim. Sci. 47(2), 157–167.

Olasehinde, T.A., Olaniran, A.O. & Okoh, A.I., 2019. Macroalgae as a valuable source of naturally occurring bioactive compounds for the treatment of Alzheimer's disease. Mar. Drugs. 17, 609.

Patinho, I., Selani, M.M., Saldaña E., Bortoluzzi A.C.T., Rios-Mera J.D., da Silva C.M., Kushida M.M. & Contreras-Castillo, C.J., 2021. *Agaricus bisporus* mushroom as partial fat replacer improves the sensory quality maintaining the instrumental characteristics of beef burger. Meat Sci. 172, 108307.

Pereira, L., 2012. Cytological and cytochemical aspects in selected carrageenophyte (Gigartinales, Rhodophyta). In Advances in Algal Cell Biology, 1st ed. Heimann, K., Katsaros, C., Eds.; De Gruyter: Berlin, Germany, pp. 81–104,

Pereira, L., 2016. Edible Seaweeds of the World. CRC Press: Boca Raton, FL, USA, pp. 463.

Pereira, L., 2021. Macroalgae. Encyclopedia, 1(1), 177-188.

Ponce, N. & Stortz, C.A., 2020. A comprehensive and comparative analysis of the fucoidan compositional data across the *phaeophyceae*. Front. Plant Sci. 1844.

Poorghasemi, M., Seidavi, A., Qotbi, A.A.A., Laudadio, V. & Tufarelli, V., 2013. Influence of dietary fat source on growth performance responses and carcass traits of broiler chicks. Asian-Australas. J Anim. Sci. 26(5), 705.

Priyadarshani, I. & Biswajit, R., 2012. Commercial and industrial applications of micro algae – a review. J. Algal Biomass. Util. 3(4), 89–100.

Qadri, S.S.N., Biswas, A., Mandal, A.B., Kumawat, M., Saxena, R. & Nasir, A.M., 2018. Production performance, immune response and carcass traits of broiler chickens fed diet incorporated with *Kappaphycus Alvarezii*. J. Appl. Phycol. 31, 753–760.

Qin, Y., 2008. Alginate fibres: an overview of the production processes and applications in wound management. Polym. Int. 57 (2), 80–171.

Qin, Y., 2018. Production of seaweed-derived food hydrocolloids. In Bioactive seaweeds for food applications Academic Press. pp. 53–69.

Rahi, D.K. & Malik, D., 2016. Diversity of mushrooms and their metabolites of nutraceutical and therapeutic significance. J. Mycol. 1–18.

Rajauria, G., 2015. Seaweeds: a sustainable feed source for livestock and aquaculture. Seaweed Sustainability, 389–420.

Rajauria, G., Foley, B. & Abu-Ghannam, N., 2016. Identification and characterization of phenolic antioxidant compounds from brown Irish seaweed *Himanthalia elongata* using LC-DAD–ESI-MS/MS. Innov. Food Sci. Emerg. Technol. 37, 261–268.

Rangel-Vargas, E., Rodriguez, J.A., Domínguez, R., Lorenzo, J.M., Sosa, M.E., Andrés, S.C., Rosmini, M., Pérez-Alvarez, J.A., Teixeira, A. & Santos, E.M., 2021. Edible mushrooms as a natural source of food ingredient/additive replacer. Foods. 10(11), 2687.

Rathore, S.S., Chaudhary, D.R., Boricha, G.N., Ghosh, A., Bhatt, B.P., Zodape, S.T. & Patolia, J.S., 2009. Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. S. Afr. J. Bot. 75(2), 351–355.

Ravindran, V., 2013. Poultry feed availability and nutrition in developing countries. Poultry development review. FAO Publication: Rome, Italy. pp. 60–63.

Rebours, C., Marinho-Soriano, E., Zertuche-Gonzalez, J.A.,Hayashi, L., Vásquez, J.A., Kradolfer, P., Soriano, G., Ugarte, R., Abreu, M.H., Bay-Larsen, I., Hovelsrud, G.,Rodven, R. & Robledo, D., 2014. Seaweeds: an opportunity for wealth and sustainable livelihood for coastal communities. J. Appl. Phycol. 26, 1939–1951.

Resiere, D., Kallel, H., Florentin, J., Banydeen, R., Compton, K., Gueye, P., Mehdaoui, H. & Neviere, R., 2023. Sargassum seaweed in the Caribbean: A major public health problem still unsolved. J. Glob. Health. 13.

Rioux, L.E., Turgeon, S.L. & Beaulieu, M., 2007. Characterization of polysaccharides extracted from brown seaweeds. Carbohydr. Polym. 69, 530–537.

Rodic, V., Peric, L., Bukic-Stojcic, M. & Natasa, V., 2011. The environmental impact of poultry production. Biotech. Anim. Husb. 27 (4), 1673–1679.

Rodríguez-Martínez, R.E., Medina-Valmaseda, A.E., Blanchon, P., Monroy-Velázquez, L.V., Almazán-Becerril, A., Delgado-Pech, B., Vásquez-Yeomans, L., Francisco, V. & García-Rivas, M.C., 2019. Faunal mortality associated with massive beaching and decomposition of pelagic Sargassum. Mar. Pollut. Bull. 146, 201–205.

Rosenfeld, L., 2000. Discovery and early uses of iodine. J. Chem. Educ. 77(8), 984.

Rothman, M.D., Mattio, L., Wernberg, T., Anderson, R.J., Uwai, S., Mohring, M.B. & Bolton, J.J., 2015. A molecular investigation of the genus *Ecklonia* (*Phaeophycae, Laminariales*) with special focus on the southern hemisphere. J. Phycol. 51, 236–246.

Rybak, A., 2018. Species of *Ulva* (*Ulvophyceae, Chlorophyta*) as indicators of salinity. Ecol. Indic. 85, 253–261.

Sanchez, C., 2010. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Appl. Microbiol. Biotechnol. 85, 1321–1337.

Santos, M., Alberton, L., Valle, J., Linde, G & Colauto, N., 2014. Effect of *Pleurotus ostreatus* colonized substrate on broiler chicken growth. Proceedings of the 8th international conference on mushroom biology and mushroom products, New Delhi, India, 19–22 November 2014. pp. 323–324.

SAPA, 2016. South African Poultry Association 2016, Industry Profile. http://www.sapoultry.co.za/. Accessed 20 May 2021.

SAPA, 2019. South Africa Poultry Association 2019, http://www.sapoultry.co.za/pdf-docs/sapa-industry-profile.pdf . Accessed 22 May 2021.

SAPA, 2021. South African Poultry Association, Annual Report 2021. https://www.sapoultry.co.za/wp-content/uploads/2022/06/SAPA-ANNUAL-REPORT-2021.pdf. Accessed 14 October 2023.

Sherwood, A.R., 2016. Green algae (*Chlorophyta* and *Streptophyta*) in rivers. River Algae. Springer, Cham. pp. 35–63.

Shin, Y.S., Kim, K.J., Park, H., Lee, M.G., Cho, S., Choi, S.I., Heo, H.J., Kim, D.O. & Kim, G.H., 2021. Effects of *Ecklonia cava* extract on neuronal damage and apoptosis in PC-12 cells against oxidative stress. J. Microbiol. Biotechnol. 31(4), 584–591.

Shukla, P.S., Mantin, E.G., Adil, M., Bajpai, S., Critchley, A.T. & Prithiviraj, B., 2019. *Ascophyllum nodosum*-based biostimulants: Sustainable applications in agriculture for the stimulation of plant growth, stress tolerance, and disease management. Front. Plant Sci. 10, 655.

Skinner, J.T., Waldroup, A.L. & Waldroup, P.W., 1992. Effects of removal of vitamin and trace mineral supplements from grower and finisher diets on live performance and carcass composition of broilers. J. Appl. Poult. Res. 1, 280–286.

Solarin, B.B., Bolaji, D.A., Fakayode, O.S. & Akinnigbagbe, R.O., 2014. Impacts of an invasive seaweed *Sargassum* hystrix var. fluitans (Børgesen, 1914) on the fisheries and other economic implications for the Nigerian coastal waters. IOSR J. Agric. Vet. Sci. 7(7), 1-6.

Sultana, F., Wahab, M.A., Nahiduzzaman, M., Mohiuddin, M., Iqbal, M.Z., Shakil, A., Mamun, A.A., Khan, M.S.R., Wong, L. & Asaduzzaman, M., 2022. Seaweed farming for food and nutritional security, climate change mitigation and adaptation, and women empowerment: A review. Aquac. Fish. 8(5), 463–480.

Tarwireyi, L. & Fanadzo, M., 2013. Production of indigenous chickens for household food security in rural KwaZulu-Natal, South Africa: A situation analysis. Afr. J. Agric. Res. 8(46), 5832–5840.

Tayyab, U., Novoa-Garridob, M., Roledab, M.Y., Lind, V. & Weisbjerg, M.R., 2016. Ruminal and intestinal protein degradability of various seaweed species measured *in situ* in dairy cows. Anim. Feed Sci. Technol. 213, 44–54.

Teas, J. Vena, S., Cone, D.L. & Irhimeh, M., 2013. The consumption of seaweed as a protective factor in the etiology of breast cancer: proof of principle. J. Appl. Phycol. 25(3), 771–779.

Tseng, C., 2001. Algal biotechnology industries and research activities in China. J. Appl. Phycol. 13, 375–380.

Tuyen, D.V., Phuong H.N., Cone, J.W., Baars J.J.P., Sonnenberg, A.S.M. & Hendriks, W.H., 2013. Effect of fungal treatments of fibrous agricultural by-products on chemical composition and *in vitro* rumen fermentation and methane production. Bioresour. Technol. 129, 256–263.

Valadares, F., Goncalves, T.A., Goncalves, D.S.P.O., Segato, F., Romanel, E., Milagres, A.M.F., Squina, F.M. & Ferraz, A., 2016. Exploring glycoside hydrolases and accessory proteins from wood decay fungi to enhance sugarcane bagasse saccharification. Biotechnol. Biofuels. 9, 1–11.

Valente, L.M.P., Gouveia, A., Rema, P., Matos, J., Gomes, E.F. & Pinto, I.S., 2006. Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary in-gredients in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture. 252, 85–91.

Van Alstyne, K.L., Wolfe, G.V., Freidenburg, T.L., Neill, A. & Hicken, C., 2001. Activated defense systems in marine macroalgae: evidence for an ecological role for DMSP cleavage. Marine Ecol. Prog. 213, 53–65.

Van den Hoek, C., Mann, D. & Jahns, H.M., 1996. Algae: An introduction to phycology. Cambridge University Press. Cambridge, UK.

Van Peer, A.F., Muller, W.H., Boekhout, T., Lugones, L.G. & Wosten, H.A., 2009. Cytoplasmic continuity revisited: closure of septa of the filamentous fungus *Schizophyllum commune* in response to environmental conditions. PLoS ONE 4(6), 5977.

Vasconcelos, M.T.S.D. & Leal, M.F.C., 2001. Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. Mar. Chem. 74, 65–85.

Ventura, M.R., Castanon, J.I.R. & McNab, J.M., 1994. Nutritional value of seaweed (*Ulva rigida*) for poultry. Anim. Feed Sci. Technol. 49(1), 87–92.

Viana, E.D.F, de Carvalho Mello, H.H., Carvalho, F.B., Café, M.B., Leandro, N.S.M., Arnhold, E. & Stringhini, J.H., 2022. Blood biochemical parameters and organ development of brown layers fed reduced dietary protein levels in two rearing systems. Anim. Biosci. 35(3), 444.

Vijayalingam, T.A., Rajesh, N.V., Vairamuthu, S., Raja, M.B. & Kumar, N.S., 2023. Effect of dietary supplementation of seaweeds on growth and blood profiles of TANUVAS Aseel chicken: Seaweed feed trails in poultry. J. Hellenic. Vet. Med. Soc. 74(3), 6085–6092.

VijaySankar, N.P., Jagtap, A.S., Baghel, R.S., Imchen, T. & Manohar, C.S., 2023. Elucidation of the antioxidant potential of marine macroalgal biomolecules for healthcare applications: Current status and future prospects. Se-Kwon Kim, S.K., Shin, KH. & Venkatesan, J. (Eds.), Marine Antioxidants: Preparations, Syntheses, and Applications. Elsevier, Amsterdam, Netherlands.

Wang, S., Shi, X., Zhou, C. & Lin, Y., 2013. *Entermorpha prolifera*: Effects on performance, carcass quality and small intestinal digestive enzyme activities of broilers. Chin. J. Anim. Nutr. 25, 1332–1337.

Wan-Loy, C. & Siew-Moi, P., 2016. Marine algae as a potential source for anti-obesity agents. Mar. Drugs. 14(12), 222.

Wassef, E.A., El Masry, M.H. & Mikhail, F.R., 2001. Growth enhancement and muscle structure of striped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal-based diets. Aqua. Cult. Res. 32, 315–322.

Whitehead, C.C., 2002. Nutrition and poultry welfare. Worlds Poult. Sci. J. 58(3), 349–356.

Wickramasuriya, S.S., Young-Joo, Y., Kim, J.C., Yoo, J., Kang, N.K. & Heo, J.M., 2015. A review of canola meal as an alternative feed ingredient for ducks. J. Anim. Sci. Technol. 57, 29.

Wong, K.H. & Cheung, P.C., 2001. Nutritional evaluation of some subtropical red and green seaweeds Part II. *In vitro* protein digestibility and amino acid profiles of protein concentrates. Food Chem. 72(1), 11–17.

Xing, M., Cao, Q., Wang, Y., Xiao, H., Zhao, J., Zhang, Q., Ji, A. & Song, S., 2020. Advances in research on the bioactivity of alginate oligosaccharides. Mar. Drugs. 18, 144.

Yabe, T., Ishii, Y., Amano, Y., Koga, T., Hayashi, S., Nohara, S. & Tatsumoto, H., 2008. Green tide formed by free-floating *Ulva* spp. at Yatsu tidal flat, Japan. Limnology. 10, 239–245.

Yadav, D. & Negi, P.S., 2021. Bioactive components of mushrooms: Processing effects and health benefits. Food Res. Int. 148, 110599.

Yoav, S., Salame, T.M., Feldman, D., Levinson, D., Ioelovich, M., Morag, E., Yarden, O., Bayer, E.A. and Hadar, Y., 2018. Effects of cre1 modification in the white-rot fungus *Pleurotus ostreatus* PC9: altering substrate preference during biological pretreatment. Biotechnol. Biofuels. 11, 1–16.

Yokus, B., Cakir, D.U., Kanay, Z., Gulten, T. & Uysal, E., 2006. Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. J. Vet. Med. A. 53(6), 271–276.

Zhang X., Border A., Goosen N. & Thomsen, M., 2021. Environmental life cycle assessment of cascade valorisation strategies of South African macroalga *Ecklonia maxima* using green extraction technologies. Algal Res. 58, 102348.

Zhang, Q., Li, N., Liu, X., Zhao, Z., Li, Z. & Xu, Z. 2012. The structure of a sulfated galactan from *Porphyra haitanensis* and its *in vivo* antioxidant activity. Carbohydr. Res. 339, 105–111.

Zhang, Y., Wang, D., Chen, Y., Liu, T., Zhang, S., Fan, H., Liu, H. & Li Y., 2021. Healthy function and high valued utilization of edible fungi. Food Sci. Hum. Wellness. 10, 408–420.

3 CHAPTER THREE – EFFECT OF SEA BAMBOO MEAL INCLUSION IN A DIET OF BOSCHVELD COCKERELS ON GROWTH PERFORMANCE, APPARENT RETENTION OF PROXIMATE COMPONENTS AND BLOOD PARAMETERS

Some parts of this chapter were published as: **Mhlongo, G.** & Mnisi, C.M., 2023. Effect of seaweed (*Ecklonia maxima*) on apparent nutrient digestibility, growth performance, and physiological and meat quality parameters in Boschveld cockerels. Poult. Sci. 102(2), 102361; https://doi.org/10.1016/j.psj.2022.102361 (Appendix 1)

Abstract

Seaweeds are renowned as rich sources of nutrients and bioactive chemicals. However, the amount of sea bamboo (*Ecklonia maxima*) meal (SBM) that can be incorporated into the diets of Boschveld indigenous chickens is currently unknown. This study, therefore, investigated the growth performance, apparent retention (AR) of proximate components and blood parameters in Boschveld cockerels fed a diet containing graded levels of SBM. A total of 225, five-week-old Boschveld cockerels (316.4 ± 23.01 g live weight) were reared on five isoenergetic and isonitrogenous experimental diets formulated by treating a standard grower diet with SBM at a concentration of 0 (SBM0), 20 (SBM20), 40 (SBM40), 60 (SBM60), and 80 g/kg (SBM80). At 5 weeks of age, the cockerel's initial body weights were measured followed by weekly live weights measurements to determine the weekly body weight gain (BWG). Daily feed intake measurements were done to calculate average weekly feed intake per bird (FI = the difference between the feed offered and feed refused divided by the total number of chickens in a pen). Blood was drawn from two randomly selected birds per pen at 15 weeks and 6 days of age for haematology and serum biochemistry examination. At 11 weeks of age, two cockerels were randomly selected for measurements of AR of proximate components, while the rest of the birds were slaughtered at a local abattoir. There were neither quadratic nor linear effects (*P*

>0.05) induced by feeding graded levels of dietary SBM for overall BWG and G:F, AR of proximate components and haematological parameters in Boschveld cockerels. However, overall FI (R^2 = 0.397; P = 0.021) linearly increased, whereas alanine aminotransferase (R^2 = 0.530; P = 0.001) resulted in a positive quadratic response as SBM levels increased. The cockerels reared on diet SBM0 had higher serum calcium levels (2.68 mmol/L) than those reared on diet SBM60 (1.83 mmol/L). In conclusion, the inclusion of dietary SBM in the diets of Boschveld cockerels improved overall feed intake, altered alanine transaminase and increased the blood calcium levels but had no effect on growth performance, AR of proximate components and blood indices. The absence of dietary improvement on performance metrics necessitates the pre-treatment of SBM using oyster mushroom spawn to improve its utilisation by Boschveld cockerels.

Keywords: Bioactive compounds, Chickens, seaweed, Growth performance, Poultry production

3.1 Introduction

The overall per capita consumption of poultry products (meat and eggs) in South Africa is estimated to be approximately 48.22 kg (SAPA, 2020), with chicken products being the most consumed source of animal protein. However, the poultry industry is under pressure to boost production to fulfil the rising demand for animal protein caused by the burgeoning human population (Nhlane *et al*., 2021). This shows that measures need to be put in place to enable the sustainable intensification of native chickens (*Gallus gallus domesticus*) as the main source of animal protein for people in rural South Africa. Indigenous chicken farming contributes significantly to the nutritional and socioeconomic well-being of people in rural communities by providing employment, income and vital micro- and macro-nutrients (Magothe *et al*., 2012; Manyelo *et al*., 2020). These birds have desirable characteristics such as hard eggshells, succulent meat, thermotolerance, firm texture, high fertility and hatchability, and disease resistance (Dessie, 2011; Atela *et al*., 2019). However, their poor feed conversion ratios and low growth rates limit their large-scale intensification due to the high feeding costs (Larbi *et al*., 2013; Hussain *et al*., 2018).

From a nutritional perspective, the inclusion of functional feed components such as seaweeds can enhance indigenous chicken production, which would enable the sustainable intensification of these birds. This is because the growth of seaweeds, also known as marine macro-algae, does not require fresh water, land, fertilizers, machinery, pesticides or fertilizers. Furthermore, seaweeds can be regarded as environmentally friendly ingredients whose nutrients and bioactive compounds have health-promoting, gut-modulating, growth-boosting and meat-enhancing properties (Michalak *et al*., 2022). Indeed, Nhlane *et al*. (2020) stated that seaweeds contain nutraceuticals, that when incorporated into poultry diets can provide nutritional and subtherapeutic benefits. Seaweeds also contain bioactive compounds such as carotenoids, fucoidan, polyphenols, carrageenan, chlorophyll and phlorotannins, to name a few, that can reduce stressors brought by large-scale farming (Michalak *et al*., 2022).

Michalak & Mahrose (2020) revealed that incorporating seaweeds into poultry diets can enhance the health and performance of the birds while also enriching poultry products with naturally bioactive compounds. Additionally, Nhlane *et al*. (2020) revealed that the addition of green seaweed (*Ulva* spp.) at 25 and 30 g/kg had no effect on nutrient digestibility but increased mean corpuscular concentration, mean corpuscular volume and mean corpuscular haemoglobin concentration. Notably, seaweed bioactive compounds have antimicrobial, anti-inflammatory, antioxidant, antiallergic, antitumoral, hypoglycemic, antithrombotic, neuroprotective and hypocholesterolemic properties that could improve the performance of indigenous chickens, while also ensuring that these birds continue to contribute to the nutritional and food security of households (Michalak & Mahrose, 2020; Nhlane *et al*., 2021). Resiere *et al*. (2020) reported that despite having desirable nutraceutical properties, large amounts of seaweeds are frequently washed into beach inshore areas, releasing non-volatile and volatile compounds caused by anaerobic decomposition. Heaps of decomposition seaweed emit toxic ammonia and hydrogen sulphide that have detrimental effects on humans, animals and environmental health (Resiere *et al*., 2020).

Thus, incorporating seaweeds such as the sea bamboo (*Ecklonia maxima*) meal (SBM) in indigenous chicken diets can help protect the environment while also allowing for sustainable indigenous poultry production. However, no studies have been conducted to assess the effect of including SBM in a diet of Boschveld cockerels. Consequently, the amount that can be safely incorporated into their diets is unknown. This study, therefore, evaluated the effect of feeding a diet containing graded levels of SBM on growth performance, blood parameters and apparent nutrient digestibility of Boschveld cockerels. The study tested the hypothesis that the inclusion of SBM will improve the growth performance, AR of proximate components and blood parameters in Boschveld cockerels.

3.2 Materials and method

3.2.1 Ethics statement

The protocols used in this chapter were approved by the Animal Production Research Ethics Committee of the North West University, South Africa (approval no. NWU-00809-21-A5; Appendix 2).

3.2.2 Study area and sources of ingredients

The research was conducted at North-West University Experimental Farm in Molelwane, South Africa (25°86′00″ S, 25°64′52″ E). The 11-week feeding trial was conducted in summer (December 2021 – February 2022) with ambient temperatures ranging from 17°C to 35°C. The sea bamboo was hand-collected on Sunset Beach, along the Sea Point Promenade (33°54′55″ S, 18°23′33″ E) in Cape Town, South Africa. The sea bamboo was sun-dried and then milled to pass through a 2-mm sieve (Retsch Cutting Mill SM 100, Retsch, Germany) to produce the meal. SimpleGrow (PTY) LTD (Pretoria, South Africa) and Nutrifeeds (PTY) LTD (Lichtenburg, South Africa) supplied all the other feed ingredients.

3.2.3 Analyses and diet formulation

Triplicate samples of SBM were analysed for their nutritional composition following the Association of Official Analytical Chemist methods (AOAC, 2005) before diet formulations. Laboratory dry matter (DM) was determined (AOAC, 2005: method no. 930.15) by oven-drying pre-weighed samples (1 g) at 105°C for 12 h. Organic matter (OM) content was determined (AOAC, 2005: method no.924.05) by ashing the dried samples in a muffle furnace set at 600°C for 12 hours. The loss in weight was measured as OM content and the residue as ash. Crude protein was determined by the standard macro-Kjeldahl method (AOAC, 2005: method no. 984.13).). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using the ANKOM^{2000} Fiber analyzer (ANKOM Technology, New York, NY) following the detergent methods by van Soest *et al*. (1991). A heat-stable amylase was used to analyze the NDF, and the results were as expressed inclusive of residual ash. The cellulose in ADF residue bags was dissolved for 3 h in 72% H_2SO_4 to determine the acid detergent lignin (ADL). The Waters Acquity Ultra Performance Liquid Chromatograph equipped with a photodiode array detector (UPLC-PDA) was used for amino acid determination. The guidelines by the Agri-Laboratory Association of Southern Africa were used for mineral content determination (AgriLASA, 1998). Metabolizable energy (ME) of the SBM was calculated using the Carpenter & Clegg (1956) formula [ME (kcal/kg) = (35.5 × CP %) + (79.5 × EE %) + (40.6 × NFE %) + 199.0] as shown in Table 3.1.

Table 3.1. Proximate composition (g/kg DM, unless shown otherwise) and calculated metabolizable energy (MJ/Kg) of sea bamboo meal

Proximate composition	Levels
Dry matter	871.0
Crude protein	101.3
ME (MJ/kg)	7.40
Ash	262.3
Organic matter	608.7
Methionine (g/100g DM)	0.36
Lysine (g/100g DM)	1.01
Histidine (g/100g DM)	0.29
Threonine (g/100g DM)	1.01
Phenylalanine (g/100g DM)	0.79
Leucine (g/100g DM)	1.25
Isoleucine (g/100g DM)	0.67
Tryptophan (g/100g DM)	0.60
Valine (g/100g DM)	1.01
Neutral detergent fibre	486.8
Acid detergent fibre	226.8
Acid detergent lignin	40.65
Ether extract	370.2
Calcium	0.9
Phosphorus	0.45

A feed formulation software (Format®) was used to formulate five dietary treatments (in a mash form) as follows: 1) SBM0 = standard chicken grower meal without sea bamboo meal; 2) SBM20 = standard chicken grower meal containing 20 g/kg of sea bamboo meal; 3) SBM40 = standard chicken grower meal containing 40 g/kg of sea bamboo meal; 4) SBM60 = standard chicken grower meal containing 60 g/kg of sea bamboo meal; and 5) SBM80 = standard chicken grower meal containing 80 g/kg of SBM, as shown in Table 3.2.

Table 3.2. Gross ingredient composition (g/kg as fed basis) of experimental diets containing sea bamboo meal

	[1]Diets				
Ingredients	SBM0	SBM20	SBM40	SBM60	SBM80
Limestone	11.10	11.20	11.20	11.20	11.20
Palm oil	48.10	54.20	60.30	67.50	68.60
Choline Cl 70	0.50	0.50	0.50	0.50	0.50
Common salt	4.60	2.60	0.50	0.00	0.00
Soybean meal (44%)	360.6	361.9	363.3	365.3	366.0
Brown seaweed meal	0	20	40	60	80
[2]Premix	2.00	2.00	2.00	2.00	2.00
DL-methionine	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	14.70	14.50	14.40	14.30	14.20
Maize yellow	556.4	531.1	505.8	477.2	455.5
Total	1000	1000	1000	1000	1000

[1]Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal.

[2]Premix: Zinc sulphate (79 mg), ferrous sulphate (80 mg), Copper sulphate (8.0 mg), magnesium sulphate (100 mg), niacin (30 mg), vitamin D3 (2500 IU) and sodium selenite (0.25 mg), pantothenic acid (10 mg), potassium iodide (0.34 mg), folic acid (0.7 mg), vitamin A (11000 IU), vitamin B_1 (2.5 mg), vitamin B_2 (4.5 mg), vitamin B_6 (5.1 mg), vitamin K_3 (2.0 mg), vitamin E (25 IU), and biotin (0.12 g).

3.2.4 Diets' composition

Table 3.3 displays the calculated values of the experimental diets. The diets were analysed for dry matter, ash, crude protein, organic matter, metabolizable energy, crude fat, amino acids (methionine and lysine) and minerals (phosphorus and calcium), NDF, ADF and ADL.

Table 3.3. Analysed and calculated nutritional values (g/kg, unless shown otherwise) of experimental diets containing sea bamboo meal

	[1]Diets				
Components	SBM0	SBM20	SBM40	SBM60	SBM80
Calculated values (g/kg as is, unless shown otherwise)					
[2]ME (MJ/kg)	12.98	12.98	12.98	12.98	12.98
Methionine (g/100g)	0.54	0.54	0.54	0.55	0.55
Lysine (g/100g)	1.21	1.22	1.22	1.23	1.23
Phosphorus	4.5	4.5	4.5	4.5	4.5
Calcium	9.0	9.0	9.0	9.0	9.0
Analysed values (g/kg DM)					
Dry matter	918.5	907.6	909.3	901.4	905.3
Crude protein	205.1	205.0	205.1	205.1	205.2
Ash	57.00	54.84	58.94	63.70	67.40
Organic matter	861.3	852.8	850.4	837.7	839.2
Neutral detergent fibre	142.1	191.6	192.3	193.4	206.6
Acid detergent fibre	131.7	141.6	149.5	152.1	158.1
Acid detergent lignin	23.49	32.76	33.00	34.98	37.44
Crude fat	657.5	619.5	886.9	838.1	894.6

[1]Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal.
[2]ME = metabolizable energy.

3.2.5 Experimental design and feeding

A total of 225, four-week-old Boschveld male chicks were purchased from Boschveld Ranching (PTY) LTD (Bela-Bela, Limpopo, South Africa). Upon arrival, electrolytes and vitamins were offered via drinking water for the first three days to alleviate transportation stress and help chicks acclimatize. Before commencement of measurements at 5 weeks of age, the birds were adapted to the experimental diets for 7 days. The chicks were then weighed and randomly distributed to 25 replicate pens (experimental units) in a completely randomised design, with each pen (9 birds/pen) measuring 0.51 m length × 0.49 m breath × 0.36 m height. The diets were assigned to the pens to produce five replicates per diet. The experimental diets and clean water were given *ad libitum* using Poltek poultry tube feeders and drinkers, respectively. For proper ventilation, the house curtains were opened in the morning (07h00) and closed in the evenings (16h00). A Thermo-Hygrometer (IN-OUT, Alla France Automatic Control Equipment Co., Ltd, PRC. Paris) was used to monitor the average house temperature and humidity, which was averaged to be 32°C and 60%, respectively. The birds were raised under natural lighting from the morning to the evening (12 hours of daylight). During the 11-week feeding trial, all experimental pens were constantly observed for mortalities and sicknesses, and no mortalities were recorded in this experiment.

3.2.6 Measurements of growth performance

The bird's initial body weights (316.4 ± 23.01 g) were measured at 5 weeks of age and thereafter weighed at weekly intervals to determine average weekly body weight gain (BWG). From week 5 to week 16, feed intake (FI) was measured daily and calculated as feed offered – feed refusals. The BWG and FI data were used to calculate the gain-to-feed ratio (G:F).

3.2.7 Blood collection and assays

At 15 weeks and 5 days of age, fresh blood samples (4 mL) were collected in the morning from two cockerels randomly selected per pen (10 birds/treatment). The blood samples were collected from the brachial vein using 23-gauge needles and sterilized 5 mL syringes. Approximatelty 1 mL of the blood was instantly transferred into whole blood tubes (with an anticoagulant (EDTA)) and the remaining 3 mL into serum plain coated (without anticoagulant) tubes (Jiangsu Huida Medical Instruments Co., Ltd, Jaingsu, China). The whole blood tubes were kept in a cooler and the blood was analysed within 48 hours of collection (Washington & van Hoosier, 2012) for white cell count (WCC), haematocrit, heterophils, platelets, monocytes and lymphocytes using an automated IDEXX LaserCyte Haematology Analyser (model no. 93−30001−01, IDEXX Laboratories (Pty) Ltd., Gauteng, South Africa). Blood collected into serum tubes was centrifuged at 14989 relative centrifugal force (rgf) for 5 minutes (Cryste Varispin 4 Multi-purpose centrifuge, Cryste CO., LTD, South Korea) to produce serum (Washington & van Hoosier, 2012). Serum calcium, phosphorus, symmetric dimethylarginine (SDMA), glucose, urea, globulin, total protein, amylase, total bilirubin, albumin concetrations, albumin/globulin ratio and alanine aminotransferase (ALT), alkaline phosphatase (ALKP), cholesterol, and lipase were determined using an automated IDEXX Catalyst One Chemistry Analyser (model no. 89−92525−00, IDEXX Laboratories (Pty) Ltd., Gauteng, South Africa).

3.2.8 Apparent retention of proximate components

At 16 weeks of age, two cockerels per replicate pen (10 cockerels/treatment) were randomly chosen for the nutrient digestibility trial and housed in metabolic cages (0.51 m L × 0.49 m B × 0.36 m H) for measurements of apparent retention (AR) of proximate components. The cockerels were given a 3-day adaptation period before a 5-day excreta collection period. Feed refusals were collected daily and used to determine feed intake, whereas the excreta was collected, weighed, stored in polythene bags and frozen (at -15ºC) to prevent fermentation pending proximate analyses (as described in Section 3.2.3). At the end of the collection period, samples were thawed, weighed, and homogenized. The AR of DM, OM, NDF, ADF and CP were calculated using the following formula:

$$AR\ of\ proximate\ component\ (g/kg) = \frac{component\ intake - excreta\ component}{component\ intake} \times 1000$$

3.2.9 Statistical analyses

Polynomial contrasts were used to evaluate the data for linear and quadratic effects. The response surface regression analysis (PROC RSREG; SAS, 2010) was applied to determine the response of all tested parameters to the inclusion levels of SBM in diets of Boschveld cockerels, according to the following quadratic model:

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

Where y = response variable; a and b are the coefficients of the quadratic equation; c is intercept; x is SBM level (g/kg) and $-b/2a$ is the x value for optimal response.

The GLM procedure (SAS, 2010) of SAS was used to analyze the overall FI, BWG, G:F, and AR of proximate components and blood parameters. For all statistical tests, significance was considered at $P < 0.05$. Least square means were compared by using the probability of difference option in SAS.

3.3 Results

3.3.1 Mortality and growth performance

No mortalities were observed in this study. Repeated measures analysis showed no diet × week interaction effect on average weekly BWG (P = 0.995), FI (P = 0.765) and G:F (P = 0.998). Table 3.4 shows that there were neither linear nor quadratic effects (P >0.05) for overall BWG and G:F, except for overall FI which linearly increased [$y = 44.21 (\pm 88.02) x + 5964.6 (\pm 148.6)$; $R^2 = 0.397$; P = 0.021] as dietary SBM levels increased. Cockerels fed with diet SBM80 had the highest (P >0.05) overall FI (6684.9 g/bird) compared to those fed on diet SBM0 (5993.2 g/bird). Diet SBM0 had a similar (P >0.05) overall FI as diets SBM20, SBM40 and SBM60.

Table 3.4. Effects of incremental levels of sea bamboo meal on overall feed intake (g/bird), overall body weight gain (g/bird) and overall feed conversion ratio in Boschveld indigenous cockerels

	[1]Diets						Significance		
[2]Parameters	SBM0	SBM20	SBM40	SBM60	SBM80	[3]SEM	P-GLM	P-Linear	P-Quadratic
Overall BWG	1827.6	1814.0	1889.7	1888.7	1840.1	45.73	0.673	0.490	0.392
Overall FI	5993.2[a]	6022.1[a]	6236.3[a]	6514.4[a]	6684.9[b]	162.3	0.032	0.001	0.367
Overall G:F	0.307	0.300	0.303	0.290455	0.278621	0.008	0.295	0.159	0.204

[a.b] Means, in a row, with different superscripts denote statistical differences (P <0.05).
[1]Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 520g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal.
[2]Parameters: BWG = body weight gain; FI = feed intake; G:F = gain-to-feed ratio.
[3]SEM = standard error of the mean.

3.3.2 Blood parameters

Table 3.5 shows that there were no quadratic and linear responses (*P* >0.05) observed for haematological parameters in response to incremental levels of dietary SBM. Similarly, there were no significant dietary effects observed on the measured haematological parameters of the cockerels.

Table 3.5. The haematological parameters of Boschveld cockerels fed with diets containing incremental levels of sea bamboo meal

	[1]Diets						Significance			
Parameters	SBM0	SBM20	SBM40	SBM60	SBM80	[3]SEM	P-$_{GLM}$	P-$_{Linear}$	P-$_{Quadratic}$	Reference values
Haematocrits (%)	36.30	34.60	35.30	35.90	35.00	1.037	0.782	0.997	0.630	6.74 – 46.10[1,2,3,4,5,6]
[2]WBC ($\times 10^9$/L)	16.99	18.31	16.49	15.05	16.85	2.067	0.861	0.294	0.319	12.89 – 22.65[3,4,7]
Platelets ($\times 10^9$/L)	46.30	41.90	42.20	45.10	49.70	4.429	0.718	0.208	0.301	9.00 – 95.50[2,4]
Heterophils ($\times 10^9$/L)	9.77	11.06	10.27	8.38	9.79	1.044	0.536	0.274	0.670	1.52 – 22.41[1,2,8]
Lymphocytes (%)	26.70	23.90	25.00	25.50	24.60	2.963	0.972	0.666	0.495	10.35 – 84.57[3,6,7]
Monocytes (%)	14.50	13.40	14.00	18.20	15.90	1.861	0.403	0.163	0.600	3.90 – 7.42[6,7]

[1]Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal.
[2]WCC = white cell count.
[3]SEM = standard error of the mean.
Sources: [1]Jonathan *et al*. (2021); [2]Nhlane *et al*. (2020); [3]Matshogo *et al*. (2018); [4]Duah *et al*. (2020); [5]Odunitan-Wayas *et al*. (2018); [6]Abdi-Hachesoo *et al*. (2011); [7]Manyeula *et al*. (2019); [8]Atela *et al*. (2019).

Table 3.6 indicates the effect of seaweed meal-containing diets on serum biochemical parameters of Boschveld cockerels. There were neither quadratic nor linear effects for serum biochemical parameters, except for alanine aminotransferase (ALT). A positive quadratic response was observed for ALT [$y = 1.479\ (\pm 0.361)\ x^2 - 12.94\ (\pm 3.221)\ x + 54.91(\pm 6.361)$; $R^2 = 0.530$; $P = 0.0009$], which was optimized at 44 g SBM/kg diet. With the exception of calcium, the dietary SBM levels had no effects on all serum biochemical parameters. Birds reared on diet SBM0 had higher calcium levels (2.675 mmol/L) compared to those reared on diet SBM60 (1.834 mmol/L). However, the birds fed with diet SBM60 had the same (P >0.05) calcium levels as those fed with diets SBM40 and SBM80. The control group had similar (P >0.05) calcium levels as the SBM20, SBM40 and SBM80 groups.

Table 3.6. Effects of sea bamboo meal-containing diets on the serum biochemical parameters of Boschveld cockerels

	[1]Diets						Significance			
[2]Parameters	SBM0	SBM20	SBM40	SBM60	SBM80	[3]SEM	P-GLM	P-Linear	P-Quadratic	Reference values
Glucose (mmol/L)	3.65	4.655	4.242	4.359	3.962	0.752	0.900	0.805	0.958	1.950 – 16.64[1,2]
SDMA (µg/dL)	41.50	26.88	37.90	35.40	30.30	7.734	0.720	0.828	0.628	55.00 – 85.63[1,2]
Urea (mmol/L)	0.760	0.840	0.700	0.840	0.750	0.075	0.629	0.348	0.259	0.690 – 0.840[1,2,8]
Phosphorus (mmol/L)	3.254	3.952	3.454	2.924	3.106	0.393	0.427	0.250	0.910	2.630 – 4.330[1,2]
Calcium (mmol/L)	2.675[b]	2.628[b]	2.311[ab]	1.834[a]	2.546[ab]	0.194	0.036	0.555	0.162	2.050 – 3.340[1,2,3,4]
Total protein (g/L)	32.25	30.13	37.50	35.30	32.80	6.925	0.942	0.986	0.256	37.20 – 69.20[1,2,3,4,6,7]
Albumin (g/L)	17.60	16.90	16.90	17.60	17.90	0.822	0.870	0.684	0.528	15.00 – 38.47[1,2,3,4,7,8]
Globulin (g/L)	19.00	21.00	22.20	23.63	20.30	3.898	0.804	0.804	0.371	16.67 - 52.38[1,2,4]
Albumin/globulin ratio	0.525	2.500	0.610	1.630	0.440	0.904	0.5381	0.498	0.577	0.480 – 0.600[2]
ALT (U/L)	43.30	41.50	26.20	28.50	41.00	6.157	0.185	0.772	0.000	0.400 – 43.32[1,2,5,6,7]
ALKP (U/L)	150.9	258.0	153.1	202.6	167.3	29.18	0.086	0.360	0.508	259.8 – 266.8[1,2]
Total bilirubin (umol/L)	5.10	3.90	4.20	5.40	5.40	0.988	0.731	0.683	0.763	0.28 – 25.10[1,2,3,6]
Cholesterol (mmol/L)	2.389	2.342	2.520	2.213	2.531	0.337	0.959	0.551	0.776	3.18 – 4.77[1,2]
Amylase (U/L)	248.4	267.2	250.3	281.7	270.1	48.73	0.987	0.991	0.931	900.3[1]
Lipase (U/L)	135.0	162.9	143.1	108.4	138.6	23.09	0.590	0.583	0.949	-

[a.b] Means, in a row, with different superscripts denote statistical differences ($P < 0.05$).

[1]Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal.
[2]Parameters: SDMA= symmetric dimethylarginine; ALT = alanine aminotransferase; ALKP = alkaline phosphatase.
[3]SEM = standard error of the mean.
Sources: [1]Jonathan *et al*. (2021); [2]Nhlane *et al*. (2020); [3]Matshogo *et al*. (2018); [4]Duah *et al*. (2020); [5]Odunitan-Wayas *et al*. (2018); [6]Manyeula *et al*. (2019); [7]Atela *et al*. (2019).

3.3.3 Apparent retention of proximate components

Table 3.7 displays the AR of proximate components in Boschveld cockerels-fed diets containing SBM. There were no significant linear or quadratic effects for AR of dry matter, organic matter, neutral detergent fibre, acid detergent fibre and crude protein in response to the SBM levels. Likewise, there were no significant dietary effects on AR of all the proximate components.

Table 3.7. Apparent retention of proximate components (g/kg DM, unless stated otherwise) in Boschveld cockerels fed sea bamboo meal-containing diets

	[1]Diets						Significance		
[2]Parameters	SBM0	SBM20	SBM40	SBM60	SBM80	[3]SEM	P-GLM	P-Linear	P-Quadratic
DM	632.9	588.8	602.3	618.6	543.4	44.12	0.883	0.883	0.673
OM	631.3	625.6	641.4	630.0	589.4	37.64	0.913	0.913	0.640
NDF	397.3	359.9	351.5	446.7	366.0	68.54	0.611	0.611	0.414
ADF	235.8	299.5	294.2	358.3	226.4	90.46	0.941	0.941	0.738
CP	307.4	281.0	174.4	308.2	277.1	79.20	0.586	0.667	0.910

[1]Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal.
[2]Parameters: DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre.

3.4 Discussion

3.4.1 Growth performance

The use of seaweed additives has been shown to improve growth performance, immunity, meat yield and quality in livestock (Bendary *et al*., 2013). In the current study, repeated-measures analysis showed no significant interaction effect between diet and week (bird age) on average weekly FI, BWG and G:F, indicating the cockerel's ability to utilise the diets was not influenced by their age. Dietary supplementation of incremental SBM levels significantly affected overall FI, where cockerels on SBM80 resulted in the highest overall FI compared to the control group. This aligns with Nhlane *et al*. (2020), who attributed the increase in FI in Boschveld hens to extra dietary fibre in seaweed meal-containing diets, which may have stimulated compensatory feeding. The linear increment could be due to the negative dilution effect of fibre diluting the nutrient density of the diet. Thus, the fibre restricted voluntary absorption and digestion of nutrients resulting in increased feed intake to extract more nutrients from the diets.

Similarly, Balasubramanian *et al*. (2021) reported a linear increase in feed intake in broilers fed diets containing up to 2 g/kg of seaweed supplementation. However, including SBM up to 80 g/kg did not impact the overall BWG and G:F of the Boschveld cockerels, suggesting that supplementing the bird's diets with SBM did not compromise or improve their performance. Indeed, neither linear nor quadratic effects were observed for overall BWG and G:F. This aligns with findings from other researchers who found no differences on growth metrices of various poultry species fed seaweed meal-containing diets (El-Deek & Brikaa, 2009; Abudabos *et al*., 2013; Karu *et al*., 2018).

3.4.2 Blood parameters

Haematological and biochemical parameters serve as crucial and reliable indicators for assessing an animal's physiological and health status by examining various indices (Egbe-Nwiyi *et al*., 2000; Onasanya *et al*., 2015). Doyle (2006) emphasised that blood analysis enables the clinical investigation of various metabolites and other constituents, which play a pivotal role in evaluating an organism's nutritional, physiological and pathological status (Etim *et al*., 2014; Onasanya *et al*., 2015). Dietary supplementation with SBM showed no significant effects on haematological values, aligning with findings by Lokaewmanee *et al*. (2013) and Matshogo *et al*. (2020), who reported that incorporating fermented brown seaweed and untreated green seaweed in broiler diets had no negative effect on the nutritional and health status of chickens. All the observed haematological values fell within the normal reference range for healthy chickens (Matshogo *et al*., 2020; Matshogo *et al*., 2021), suggesting that feeding diets containing up to 80 g/kg SBM did not compromise the birds' health

The significant increase in serum calcium levels was anticipated due to the high amount of micro and macro-minerals present in seaweeds, potentially influencing calcium utilisation. This suggests that adequate blood calcium was available to the birds for bone formation, muscle contraction, blood clotting and transmission of nerve impulses (Dijkslag *et al*., 2021). Alanine transaminase (ALT) and aspartate aminotransferase (AST) serve as standard biomarkers for diagnosing hepatocellular injury. When the integrity of the hepatocellular membrane is compromised, the ALT and AST enzymes, which are present in the cytoplasm of hepatocytes, are released into the bloodstream (Schomaker *et al*., 2020). Lala *et al*. (2021) stated that elevated ALT and AST levels that are out of proportion to ALKP and bilirubin are used to diagnose hepatocellular disease. A quadratic decline in ALT was observed with incremental dietary SBM levels, the SBM40 and SBM60-fed birds had lower ALT levels when compared to SBM0, SBM20 and SBM80-fed birds, suggesting that the hepatocellular membrane integrity of the latter groups might have been compromised.

3.4.3 Apparent retention of proximate components

Dietary supplementation with SBM showed no impact on the apparent retention (AR) of dry matter, crude protein, organic matter, and fibre. Despite the anticipation of increased AR due to the bioactive compounds in SBM with growth-stimulating and antimicrobial properties aiding feed utilisation (Schiener *et al*., 2015), the results align with that of Nhlane *et al*. (2020), who reported similar findings that the incorporation of green seaweed (*Ulva* spp.; 0, 20, 25, 30, and 35 g/kg) meal in Boschveld hen diets did not alter nutrient utilisation. The lack of dietary effects on AR components indicates that the birds had similar capacity to utilise the diets.. However, contrary findings were reported by Balasubramanian *et al*. (2021), who reported an improvement in the DM utilisation by broilers fed diets containing (0, 0.5, 0.1, 1.5, and 2.5 g/kg) red seaweed (*Halymenia palmata*). These contradictory findings could be attributed to the use of different seaweed species. Indeed, Ortiz *et al*. (2006) and Schiener *et al*. (2015) reported that seaweeds have different chemical compositions which are influenced by age at harvesting stages, water temperatures, growing sites and environmental conditions. Future research should, therefore, investigate the use of seaweed extracts to enhance AR of nutrients in Boschveld chickens.

3.5 Conclusion

The dietary inclusion of sea bamboo meal up to 80 g/kg in Boschveld cockerel’s diets boosted overall feed intake, altered blood calcium and ALT concentration but did not influence growth metrices, blood profiles and apparent retention of proximate components. The inability to optimise growth metrices highlights the necessity to valorise the sea bamboo with oyster mushroom spawn to further improve its feed value for Boschveld cockerels.

3.6 References

Abdi-Hachesoo, B., Talebi, A. & Asri-Rezaei, S., 2011. Comparative study on blood profiles of indigenous and Ross-308 broiler breeders. Glob. Vet. 7(3), 238–241.

Abudabos, A.M., Okab, A.B., Aljumaah, R., Samara, E., Abdoun, K.A. & Al-Haidary, A.A., 2013. Nutritional value of green seaweed (*Ulva lactuca*) for broiler chickens. Ital. J. Anim. Sci. 2, 28.

AgriLASA. 1998. Feed and plant analysis methods. Agri Laboratory Association of Southern Africa. Pretoria, South Africa.

Al-Harthi, M.A. & El-Deek, A.A., 2012. Effect of different dietary concentrations of brown marine algae (*Sargassum dentifebium*) prepared by different methods on plasma and yolk lipid profiles, yolk total carotene and lutein plus zeaxanthin of laying hens. Ital. J. Anim. Sci. 11, e64.

AOAC. 2005. Official Methods of Analysis of AOAC International (16th Ed.). Association of Official Analytical Chemists, Arlington, VA, USA.

Atela, J., Mlambo, V. & Mnisi. C.M., 2019. A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chickens. Anim. Nutr. 5, 179–184.

Balasubramanian, B., Shanmugam, S., Park, S., Recharla, N., Koo, J.S., Andretta, I. & Kim, I.H., 2021. Supplemental impact of marine red seaweed (*Halymenia palmata*) on the growth performance, total tract nutrient digestibility, blood profiles, intestine histomorphology, meat quality, fecal gas emission, and microbial counts in broilers. Animals. 11, 1244.

Bendary, M.M., Bassiouni, M.I., Ali, M.F., Gaafar, H.M. & Shamas, A.S., 2013. Effect of premix and seaweed additives on productive performance of lactating Friesian cows. Int. Res. J. Agric. Sci. Soil Sci. 3, 174–181.

Carpenter, K.J. & Clegg., K.M., 1956. The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. J. Sci. Food Agri. 7, 45–51.

Dessie, T., Taye, T., Dana, N., Ayalew, W. & Hanotte, O., 2011. Current state of knowledge on phenotypic characteristics of indigenous chickens in the tropics. Worlds Poult. Sci. J. 67, 507–516.

Dijkslag, M.A., Kwakkel, R.P., Martin-Chaves, E., Alfonso-Carrillo, C., Walvoort, C. & Navarro-Villa, A., 2021. The effects of dietary calcium and phosphorus level, and feed form during rearing on growth performance, bone traits and egg production in brown egg-type pullets from 0 to 32 weeks of age. Poult. Sci. 100(6), 101130.

Doyle, D., 2006. William Hewson (1739-74): the father of haematology. Br. J. Haematol. 133, 375–381.

Duah, K.K., Essuman, E.K., Boadu, V.G., Olympio, O.S. & Akwetey, W., 2020. Comparative study of indigenous chickens on the basis of their health and performance. Poult. Sci. 99(4), 2286–2292.

Egbe-Nwiyi, T.N., Nwaosu, S.C. & Salami, H. A., 2000. Haematological values of apparently healthy sheep and goats as influenced by age and sex in arid zone in Nigeria. Afr. J. Biomed. Res. (32), 109–115.

El-Deek, A. & Brikaa, M.A., 2009. Nutritional and biological evaluation of marine seaweed as a feedstuff and as a pellet binder in poultry diet. Int. J. Poult. Sci. 8, 875–881.

Etim, N.N., Williams, M.E., Akpabio, U. & Offiong, E.E., 2014. Haematological parameters and factors affecting their values. Agric. Sci. 2(1), 37–47.

Hussain, M., Mahmud, A., Hussain, J., Qaisrani, S.N., Mehmood, S. & Rehman, A., 2018. Subsequent effect of dietary lysine regimens fed in the starter phase on the growth performance, carcass traits and meat chemical composition of aseel chicken in the grower phase. Rev. Bras. Cienc. Avic. 20(3), 455–462.

Jonathan, O., Mnisi, C.M., Kumanda, C. & Mlambo, V., 2021. Effect of dietary red grape pomace on growth performance, hematology, serum biochemistry, and meat quality parameters in Hy-line Silver Brown cockerels. PLoS One, 16(11), .e0259630.

Karu, P., Selvan, S., Gopi, H. & Manobhavan, M., 2018. Effect of macroalgae supplementation on growth performance of Japanese quails. Int. J. Curr. Microbiol. Appl. Sci. 7, 1039–1041.

Lala, V., Goyal, A. & Minter, D.A., 2021. Liver function tests. In StatPearls. StatPearls Publishing.

Larbi, A., Rymkiewicz, P., Vasudev, A., Low, I., Shadan, N.B., Mustafah, S. Ayyadhury, S. & Fulop. T., 2013. The immune system in the elderly: A fair fight against diseases? Aging Health. 9, 35–47.

Lokaewmanee, K., Yamauchi, P.K. & Thongwittaya, N., 2012. Effects of fermented plant product on growth performance, some blood variables, carcase characteristics, and intestinal histology in broilers. Br. Poult. Sci. 53, 215–223.

Magothe, T.M., Okeno, T.O., Muhuyi, W.B. & Kahi. A.K., 2012. Indigenous chicken production in Kenya: II. Prospects for research and development. Worlds Poult. Sci. J. 68, 133–144.

Manyelo, T.G., Selaledi, L. Hassan, Z.M. & Mabelebele. M., 2020. Local chicken breeds of Africa: their description, uses and conservation methods. Animals. 10, 2257.

Manyeula, F., Mlambo, V., Marume, U. & Sebola, N.A., 2019. Nutrient digestibility, haemo-biochemical parameters and growth performance of an indigenous chicken strain fed canola meal–containing diets. Trop. Anim. Health Prod. 2019, 51, 2343–2350.

Matshogo T.B., Mlambo V., Mnisi C.M. & Manyeula F., 2021. Effect of pre-treating dietary green seaweed with fibrolytic enzymes on growth performance, blood indices, and meat quality parameters of Cobb 500 broiler chickens. Livest. Sci. 251, 104652.

Matshogo, T.B., Mlambo, V., Marume, U. & Sebola, N., 2018. Growth performance, blood parameters, carcass characteristics and meat quality traits in Potchefstroom Koekoek chickens fed Lippia javanica leaf meal. Trop. Anim. health Prod. 50, 1787–1795.

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, 547.

Michalak, I. & Mahrose. K., 2020. Seaweeds, intact and processed, as a valuable component of poultry feeds. J. Mar. Sci. Eng. 8, 620.

Michalak, I., Tiwari, R., Dhawan, M., Alagawany, M., Farag, M.R., Sharun, K., Emran, T.B. & Dhama. K., 2022. Antioxidant effects of seaweeds and their active compounds on animal health and production – a review. Vet. Q. 42, 48–67.

Nhlane, L.T., Mnisi, C.M., Madibana, M.J. & Mlambo, V., 2020. Nutrient digestibility, growth performance and blood indices of 24etaboli chickens fed seaweed-containing diets. Animals. 10, 1296.

Nhlane, L.T., Mnisi, C.M., Mlambo, V. & Madibana, M.J., 2021. Effect of seaweed-containing diets on visceral organ sizes, carcass characteristics, and meat quality and stability of Boschveld indigenous hens. Poult. Sci. 100(2), 949–956.

NRC. 2004. Nutrient Requirements of Poultry, 9th ed.; National Academy Press: Washington, DC, USA.

Odunitan-Wayas, F., Kolanisi, U. & Chimonyo, M., 2018. Haematological and serum biochemical responses of Ovambo chickens fed provitamin A biofortified maize. Braz. J. Poultry Sci. 20, 425–434.

Onasanya, G.O., Oke, F.O., Sanni, T.M. & Muhammad, A.I., 2015. Parameters influencing haematological, serum and bio-chemical references in livestock animals under different management systems. Open J. Vet. Med, 5(08), 181.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A. & Rios, A., 2006. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. Food Chem. 99(1), 98–104.

Resiere, D., Mehdaoui, H., Florentin, J., Gueye, P., Lebrun, T., Blateau, A., Viguier, J., Valentino, R., Brouste, Y., Kallel, H., Megarbane, B., Cabie, A., Banydeen, R. & Neviere. R., 2020. Sargassum seaweed health menace in the Caribbean: clinical characteristics of a population exposed to hydrogen sulfide during the 2018 massive stranding. Clin. Toxicol. Phila. 59, 215–223.

SAPA. 2020. South African Poultry Association 2020 industry profile. https://www.sapoultry.co.za/wpcontent/uploads/2022/03/SAPAINDUSTRYPROFILE-2020.pdf (accessed Jul. 2022).

Schiener, P., Black, K.D., Stanley, M.S. & Green, D.H., 2015. The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. J. Appl. Phycol. 27, 363–373.

Schomaker, S., Potter, D., Warner, R., Larkindale, J., King, N., Porter, A.C., Owens, J., Tomlinson, L., Sauer, J.M., Johnson, K. & Aubrecht, J., 2020. Serum glutamate dehydrogenase activity enables early detection of liver injury in subjects with underlying muscle impairments. PLoS ONE. 15(5), e0229753.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Washington, I.M. & van Hoosier, G., 2012. Clinical biochemistry and hematology. University of Washington, Seattle, WA, USA. pp. 59–91.

4 CHAPTER FOUR – EFFECT OF GRADED LEVELS OF SEA BAMBOO MEAL ON CARCASS CHARACTERISTICS, VISCERA MACROMORPHOMETRY, MEAT QUALITY AND STABILITY OF BOSCHVELD COCKERELS

Some parts of this chapter were published as: **Mhlongo, G.** & Mnisi, C.M., 2023. Effect of seaweed (*Ecklonia maxima*) on apparent nutrient digestibility, growth performance, and physiological and meat quality parameters in Boschveld cockerels. Poult. Sci. 102(2), 102361; https://doi.org/10.1016/j.psj.2022.102361 (Appendix 1)

Abstract

The use of sea bamboo meal (SBM) as a source of beneficial nutrients and bioactive compounds can enhance poultry product quality. This study investigated the effect of feeding graded levels of SBM on carcass characteristics, viscera morphometry, and meat quality and stability of Boschveld cockerels. Five-week-old Boschveld cockerels (316.4 ± 23.01 g live weight) were reared on five isoenergetic and isonitrogenous experimental diets formulated by diluting a standard grower diet with SBM at a concentration of 0 (SBM0), 20 (SBM20), 40 (SBM40), 60 (SBM60), and 80 g/kg (SBM80) feed. The cockerels were slaughtered at 16 weeks of age to measure carcass characteristics, viscera masses and lengths and meat quality and stability indicators. Breast meat quality parameters showed neither linear nor quadratic responses in response to graded levels of SBM. However, cold carcass weight [R^2 = 0.323; *P* = 0.039], relative caeca weight [R^2 = 0.417; *P* = 0.013], small intestines length [R^2 = 0.305; *P* = 0.041] and duodenal length [R^2 = 0.537; *P* = 0.04] linearly increased as SBM levels increased. A negative quadratic effect was observed for ileal length [R^2 = 0.457; P = 0.0450] in response to increasing SBM levels. The cockerels reared on the SBM80 diet had larger (*P* <0.05) relative caeca weights compared to those fed with diets SBM0 and SBM20. For meat shelf life, a significant effect was observed on chroma (5.50 – 62.66) on day 2 of storage, the SBM20 (9.83)

diet promoted the highest chroma value than diet SBM0 (5.58). It was, therefore, concluded that the dietary inclusion of SBM improved growth and development of some gastrointestinal tract organs, but did not influence the meat quality attributes of Boschveld cockerels.

Keywords: Carcass characteristics, Chickens, Internal organs, Meat quality, Sea bamboo, Shelf life

4.1 Introduction

Poultry production stands as one of the world's largest and fastest-growing food industries (Nhung *et al*., 2017). According to the Food and Agriculture Organisation (FAO), poultry meat and eggs are highly consumed globally due to their relative low cost, low-fat and high-quality protein content when compared to red meat (FAO, 2020). . Forecasts indicate a projected increase in global demand for poultry eggs and meat by 65% and 121%, respectively, from 2005 to 2050 (Islam *et al*., 2021). Population growth, rising incomes, and urbanisation are key drivers behind this surge in demand for animal protein (Agina *et al*., 2017). In response to these trends, there is a pressing need to increase animal protein production while considering environmental sustainability of the environment. Indigenous chickens are recognised as valuable genetic resources and have gained increased attention as they offer a sustainable approach to agriculture (Lordelo *et al*., 2020; Manyelo *et al*., 2020).

In developing countries, indigenous chickens play a vital role in providing income and protein to resource-constrained communities (Manyelo *et al*., 2020). Their preference over exotic breeds is attributed to firm texture, succulent meat, disease resistance, adaptability to tropical climates, and natural camouflage from predators due to their plumage colour (Padhi, 2016; Manyelo *et al*., 2020). Despite these advantages, the slow growth rates (reaching 2.0 kg for cockerels in 25 weeks), low feed efficiency, late sexual maturity (6-10 months), and lower egg production (averaging 60 eggs annually) limit their sustainable intensification (Kingori, 2004;

Moges *et al*., 2010). Thus, there is a dire need for research focused on low-cost feed ingredients with functional properties tailored for indigenous chickens.

Seaweed and its derivatives, particularly sea bamboo meal (SBM), play a crucial role in the food industry as valuable feed additives and fertilizers (Ganesan *et al*., 2019). Brown seaweeds like SBM are notable for their abundance in bioactive compounds such as antioxidants, minerals, polysaccharides, essential amino acids, vitamins, and fatty acids, making them valuable functional ingredients (Kumar *et al*., 2021). These bioactive compounds and nutrients have demonstrated their ability to modulate the immune system, enhance intestinal integrity, improve nutrient utilisation efficiency, and elevate product quality and stability in poultry production, thereby preventing disease outbreaks (Cañedo-Castro *et al*., 2019; Stokvis *et al*., 2022).

Seaweeds' bioactive compounds exhibit various health beneficial properties, including among mang, antifungal, antibacterial, vermifuge, neuroprotective, antiviral, antitumoral, anti-allergic, anti-inflammatory, hypocholesterolemic, antithrombotic, and hypoglycemic (Matshogo *et al*., 2021). Moreover, seaweeds are rich in n-3 long-chain polyunsaturated fatty acids (LC-PUFA), suggesting that products derived from seaweed-fed birds could provide beneficial LC-PUFA to consumers (Mlambo *et al*., 2022). Indeed, Mlambo *et al*. (2022) reported that the addition of seaweeds in poultry diets could improve growth performance, feed utilization efficiency, bird health, meat stability and quality, and environmental and consumer health. However, there is a gap in literature regarding the impact of SBM on meat quality attributes of Boschveld cockerels when incorporated into their diets. Therefore, this chapter evaluated the effects of incremental levels of SBM on carcass characteristics, viscera morphometry, meat quality, and stability in Boschveld cockerels. The "hypothesis that the inclusion of SBM levels will lead to improvements in carcass characteristics, viscera morphometry, meat quality and shelf life indicators in Boschveld cockerels" was tested.

4.2 Materials and methods

4.2.1 Study area, ingredients, diets and feeding

The study site and ingredient sources align with the details provided in Chapter 3, subheading 3.2.2. The formulation of the experimental diets, the experimental design and the feeding protocols are thoroughly explained in subheadings 3.2.3 to 3.2.5.

4.2.2 Slaughter procedure, measurement of carcass traits and viscera morphometry

At 16 weeks of age, all cockerels were weighed to determine their final slaughter weight. However, only 35 birds per treatment were transported to a local abattoir in Rooigrond (Mafikeng, South Africa). Upon arrival, the cockerels rested for 2 hours before being electrically stunned (for 10 seconds) and slaughtered by cutting the jugular vein with a sharp knife. Following bleeding, feathers were removed using a plucker machine and the carcasses were each eviscerated by hand. Hot carcass weight (HCW) was immediately recorded using a digital scale (Model 330 Weighing, Richter Scale (Pty) Ltd., Gauteng, South Africa). After 24 hours of chilling in a cold room, the carcasses were reweighed to determine the cold carcass weight (CCW). Dressing percentage was calculated based on the proportion of HCW to slaughter weight. Commercial carcass cuts (thigh, wing, drumstick, and breast) were weighed using the above-described scale. Relative weights of cleaned (of residual digesta where necessary) ventriculus, liver, spleen, proventriculus, small intestines, jejunum, duodenum, ileum, colon, caeca, and large intestines were assessed using a digital weighing scale (Explorer EX224, OHAUS Corp, NJ), while intestines, thigh, wing, drumstick and back lengths were measured using a tape measurer.

4.2.3 Breast meat pH and temperature

Meat pH was measured at 1 hour and 24 hours post-slaughter on the central area of the breast muscle using a digital metre fitted with a spear piercing electrode (HI98163 Professional Portable pH Meter, Hanna Instruments (Pty) Ltd, JHB, South Africa). Standard solutions (pH 4, 7 and 10) were used to calibrate the metre at the start of the pH measurements for each experimental unit.

4.2.4 Breast meat colour

A colorimeter (Konica Minolta Chroma Meter CR-400, Narich (Pty) Ltd, Japan) with a 20 mm diameter measurement area, an illuminant D65-daylight, and a 10º observation angle (CIE, 1976) was used to determine the breast meat colour coordinates (L* = lightness, a* = redness and b* = yellowness) at 1 h and 24 h post-mortem. The metre was calibrated for every experimental unit using recommended white calibration plate supplied by the manufacturer. Chroma and hue angle values were calculated according to Priolo *et al*. (2002) using the a* and b* coordinates.

4.2.5 Determination of water holding capacity (WHC) and drip loss

The WHC of breast meat was measured using the filter-paper press method developed by Honikel (1987), whereby a freshly cut slice of the pectoralis major muscle (8 g) was used to determine and express the amount of water held under pressure (60 kg) on the surface for 5 minutes. Water holding capacity was calculated as the amount of water expressed from fresh meat held under pressure (60 kg) in proportion to the initial weight of the meat using following equation:

$$WHC(\%) = [100 - (\frac{Initial\ weight - Weight\ after\ pressing}{Initial\ Weight} \times 100)]$$

A method adapted from Honikel (1998) was used to determine the drip loss measurement, where breast meat samples (~2 g; wet weight, w1) were suspended using wire steel inside an airtight sample bottle and stored in a refrigerator at 4°C for 72 h. The meat samples were reweighed to determine their weights after drip (w2). The difference between each sample before and after drip in proportion to the wet weight was expressed as percentage drip loss and calculated as follows:

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

4.2.6 Determination of cooking loss

Samples of the breast meat were individually weighed to determine the initial weight (w1) and then placed on a foil plate and pre-heated in an oven set at 75°C for 20 min (Honikel, 1998). The samples were then taken out of the oven and allowed to cool for 20 min before being re-weighed to determine the cooked weight (w2), which was then used to calculate the cooking loss as ascribed by the following formula:

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

Where, *w1* is the weight of raw meat and *w2* is the weight after cooking.

4.2.7 Determination of meat shelf life

Two breast meat samples from each experimental unit were randomly chosen to determine shelf life. The samples in labelled foil trays were kept at room temperature at the meat science laboratory of the North-West University. Meat pH and colour (L*, a* and b*) were measured daily for 4 days.

4.2.8 Statistical analyses

To account for dietary differences, the GLM procedure of SAS version 9.3 (SAS, 2010) was used as described in Chapter 3, subheading 3.2.9. Response surface regression was used to determine the linear and quadratic coefficients of SBM levels using the model described in

Chapter 3, subheading 3.2.9. The repeated measures analysis of SAS (2010) was used to determine the interaction effect of storage time and diets on breast meat shelf-life indicators.

4.3 Results

4.3.1 Carcass characteristics

Table 4.1 shows the effects of SBM-containing diets on the carcass characteristics of Boschveld cockerels. There were no linear or quadratic effects (P >0.05) observed for all carcass characteristics, except for CCW. A positive linear response was observed for CCW [$y = 24.72 (\pm 21.16) x + 1141.7 (\pm 31.38)$; $R^2 = 0.323$; $P = 0.039$]. The GLM results showed that the experimental diets had no significant effects on all carcass characteristics of the cockerels.

Table 4.1. Effects of feeding diets containing incremental levels of sea bamboo meal on carcass characteristics (g / 100 g CCW) of Boschveld cockerels

	[1]Diets						Significance		
[2]Parameters	SBM0	SBM20	SBM40	SBM60	SBM80	[3]SEM	P-GLM	P-Linear	P-Quadratic
FBW (g)	1780.3	1753.6	1848.6	1830.9	1846.4	43.53	0.450	0.097	0.987
HCW (g)	1204.6	1183.5	1267.6	1281.5	1252.2	41.10	0.409	0.196	0.939
CCW (g)	1149.2	1150.7	1244.2	1252.4	1222.7	38.92	0.195	0.039	0.560
Dressing (%)	67.70	67.33	68.54	69.98	67.82	1.235	0.84	0.920	0.905
Breast	10.28	10.40	10.20	9.991	10.30	0.402	0.962	0.857	0.661
Drumstick	8.06	7.79	7.95	7.81	7.87	0.202	0.878	0.316	0.432
Thigh	8.04	7.46	7.61	7.08	7.48	0.231	0.100	0.162	0.392
Wing	6.52	6.45	6.34	6.33	6.40	0.150	0.894	0.651	0.802
Drumstick (cm)	14.09	14.15	14.43	14.56	14.60	0.261	0.531	0.329	0.332
Thigh (cm)	9.261	9.667	9.950	10.56	10.55	0.364	0.084	0.140	0.588
Wing (cm)	23.43	23.55	23.248	24.40	24.23	0.405	0.212	0.121	0.601
Back (cm)	20.82	17.89	17.92	16.98	17.13	1.052	0.108	0.067	0.313

[1]Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40

g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal.
[2]Parameters: FBW = Final body weight; HCW = hot carcass weight; CCW = cold carcass weight.
[3]SEM = standard error of the mean.

Table 4.2 shows the effect of SBM-containing diets on the macromorphometry of viscera in Boschveld cockerels. The viscera showed no linear or quadratic responses (*P* >0.05), except for the ceca weight and the lengths of the duodenum, ileum and small intestines. Positive linear responses were observed for relative ceca weight [$y = 0.019 (\pm0.030) x + 0.858 (\pm0.045)$; $R^2 = 0.417$; $P = 0.013$], duodenum length [$y = 0.016 (\pm0.361) x + 28.75 (\pm0.550)$; $R^2 = 0.537$; $P = 0.040$] and small intestines length [$y = 1.237 (\pm3.005) x + 148.9 (\pm4.456)$; $R^2 = 0.305$; $P = 0.041$] as dietary SBM levels increased. However, a negative quadratic effect was observed for ileum length [$y = 0.238 (\pm0.106) x^2 - 1.141 (\pm0.887) x + 62.66 (\pm1.315)$; $R^2 = 0.457$; $P = 0.045$]. Diet SBM0 promoted higher relative proventriculus weights (0.614 g / 100 g CCW) than diet SBM40 (0.530 g / 100 g CCW). Chickens fed diet SBM0 had similar (*P* >0.05) relative proventriculus weights as chickens fed diets SBM20, SBM60 and SBM80. Diet SBM40 (1.384 g / 100 g CCW) promoted least relative ileum weights compared to chickens reared on diet SBM0, SBM20, SBM60 and SBM80, whose relative ileum weights were similar (*P* >0.05). Birds fed the SBM80 (1.076 g / 100 g CCW) diet had the largest relative caeca weights than diets SBM0 and SBM20, whose relative caeca weights were similar (*P* >0.05). Nonetheless, diets SBM40, SBM60 and SBM80 promoted similar (*P* >0.05) relative caeca weight.

Table 4.2. Internal organs of Boschveld cockerels fed diets containing incremental levels of sea bamboo meal

	[1]Diets					[2]SEM	Significance		
	SBM0	SBM20	SBM40	SBM60	SBM80		P-GLM	P-Linear	P-Quadratic
Weights (g / 100 g CCW)									
Liver	2.765	2.725	2.727	2.527	2.833	0.098	0.281	0.656	0.271
Ventriculus	2.543	2.565	2.550	2.533	2.717	0.084	0.520	0.073	0.173
Ventriculus fats	0.816	0.669	0.675	0.785	0.696	0.150	0.949	0.940	0.874
Proventriculus	0.614^{b}	0.609^{ab}	0.530^{a}	0.588^{ab}	0.605^{ab}	0.020	0.040	0.719	0.119
Spleen	0.309	0.284	0.311	0.293	0.317	0.021	0.792	0.455	0.736
Duodenum	1.392	1.430	1.315	1.348	1.451	0.056	0.402	0.142	0.119
Jejunum	2.219	2.256	2.350	2.132	2.071	0.190	0.856	0.844	0.653
Ileum	1.625^{ab}	1.422^{ab}	1.384^{a}	1.539^{ab}	1.839^{b}	0.108	0.051	0.063	0.077
Caeca	0.833^{a}	0.887^{a}	0.943^{ab}	0.964^{ab}	1.076^{b}	0.041	0.007	0.013	0.903
Small intestines	5.279	5.152	5.090	5.059	5.403	0.252	0.860	0.238	0.128
Large intestines	0.216	0.233	0.293	0.190	0.229	0.031	0.239	0.829	0.214
Lengths (cm)									
Duodenum	28.90	29.86	30.64	30.02	31.03	0.608	0.168	0.004	0.383
Jejunum	62.20	63.65	74.10	69.20	62.51	4.060	0.206	0.302	0.445
Ileum	61.18	57.26	59.67	61.51	64.22	2.107	0.248	0.044	0.045
Caeca	18.78	17.78	19.83	18.75	19.18	0.522	0.127	0.674	0.813
Small intestines	151.1	149.9	163.3	160.7	157.8	4.348	0.161	0.041	0.864
Large intestines	5.695	5.178	7.524	5.700	6.007	0.760	0.278	0.700	0.296

[a.b] Means, in a row, with different superscripts denote statistical differences (P <0.05).
[1]Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal.
[2]SEM = standard error of the mean.

4.3.2 Breast meat quality parameters

Table 4.3 indicates that there were no quadratic or linear effects (P >0.05) for all breast meat quality parameters measured 1 h and 24 h after slaughter in response to increasing levels of dietary SBM. Likewise, there were no dietary effects (P >0.05) on the breast meat quality of the birds measured 1 and 24-hour post-mortem.

Table 4.3. Effects of diets containing incremental levels of sea bamboo meal on breast meat pH, temperature, and colour measured 1 hour post-mortem in Boschveld cockerels.

	[1]Diets						Significance		
Parameters	SBM0	SBM20	SBM40	SBM60	SBM80	[2]SEM	P-GLM	P-Linear	P-Quadratic
pH_1	5.85	5.90	5.89	5.94	6.0	0.037	0.090	0.051	0.849
$Temp_1$ (°C)	1.92	2.60	2.24	2.08	2.69	0.403	0.614	0.505	0.513
L^*_1	28.29	28.41	27.94	33.55	25.80	3.494	0.355	0.138	0.827
a^*_1	61.02	59.25	62.23	62.66	61.71	1.236	0.400	0.483	0.673
a^*_1	0.711	1.095	0.861	0.692	0.740	0.162	0.614	0.908	0.559
$Chroma_1$	61.02	59.26	62.24	62.66	61.72	1.236	0.606	0.483	0.674
Hue $angle_1$	0.012	0.018	0.014	0.011	0.012	0.003	0.805	0.876	0.527
pH_{24}	6.046	6.018	6.004	6.064	6.057	0.061	0.133	0.189	0.975
$Temp_{24}$ (°C)	2.052	1.692	2.284	1.496	2.494	0.402	0.409	0.476	0.492
L^*_{24}	15.31	20.30	14.89	14.65	15.01	2.128	0.419	0.538	0.489
a^*_{24}	57.63	61.44	63.05	60.78	62.16	2.186	0.442	0.316	0.504
b^*_{24}	0.596	0.922	0.828	0.687	0.941	0.152	0.409	0.513	0.949
$Chroma_{24}$	57.63	61.45	63.06	60.79	62.17	2.186	0.447	0.316	0.504
Hue $angle_{24}$	0.011	0.015	0.013	0.011	0.015	0.002	0.425	0.723	0.779
[2]WHC	11.40	13.31	10.63	14.20	12.91	1.193	0.245	0.623	0.348
Drip loss	4.978	5.405	5.419	6.462	6.405	0.684	0.469	0.242	0.623

Cooking loss	28.98	29.49	27.92	30.24	29.94	1.542	0.842	0.602	0.884

[1]Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal.

[2]SEM = standard error of the mean.

4.3.3 Shelf life parameters

Figures 4.1 – 4.4 below display the shelf-life indicators of meat from Boschveld cockerels fed with diets containing SBM. Repeated measures analysis showed no time × diet interaction effect on breast meat pH, *L** and *b**, but a significant interaction effect was observed on *a**. There were neither linear nor quadratic effects (P >0.05) observed for all shelf life indicators. Likewise, there were no significant dietary effects on meat pH (5.85 – 7.93), *L** (25.80 – 50.90), *a** (2.88 – 62.66), *b** (0.69 – 8.61) and the hue angle (0.01 – 1.17), except for the chroma (5.50 – 62.66). On day 2 of storage, diet SBM20 (9.83) promoted the highest chroma value than diet SBM0 (5.58), which did not differ (P >0.05) from diets SBM40, SBM60 and SBM80.

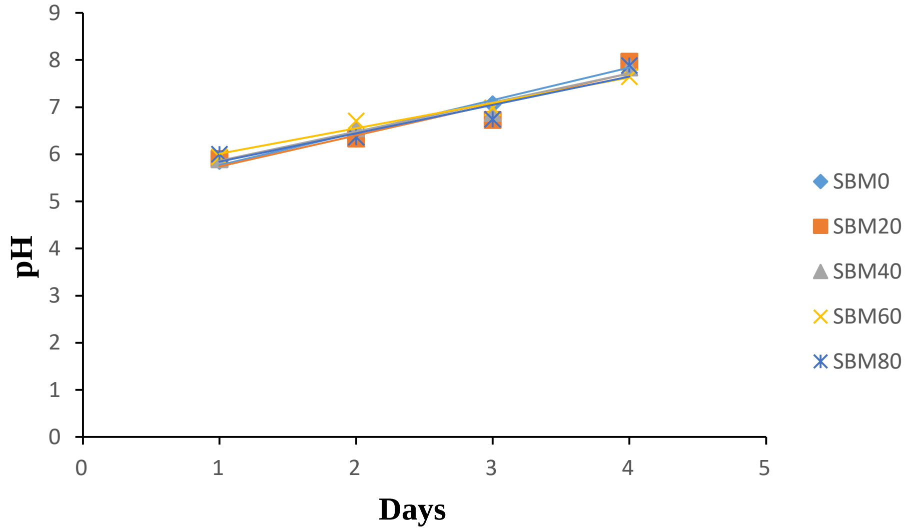

Figure 4.1. Effect of sea bamboo meal on stability of breast meat pH upon storage at room temperature for four days [Diets: SBM0 = standard chicken diet without sea bamboo meal;

SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal]

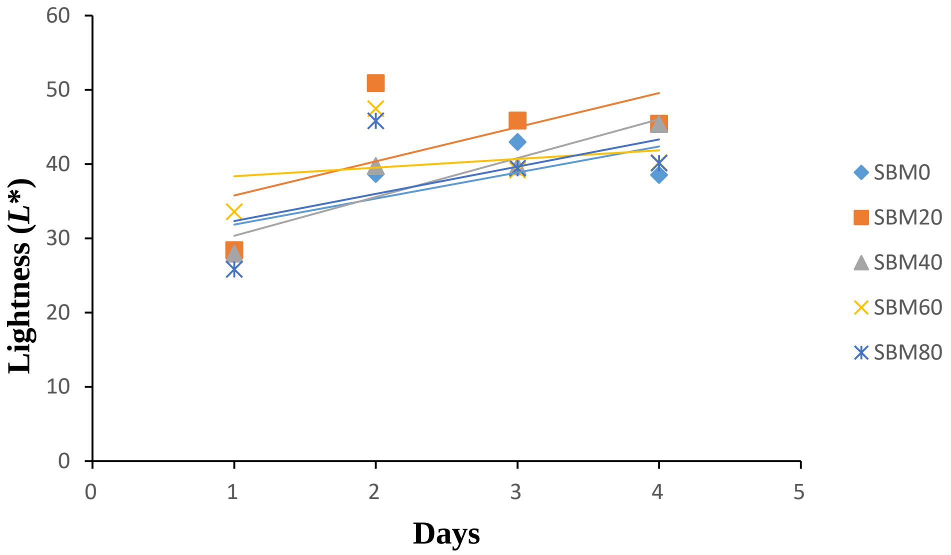

Figure 4.2. Effect of sea bamboo meal on stability of breast meat lightness upon storage at room temperature for four days [Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal]

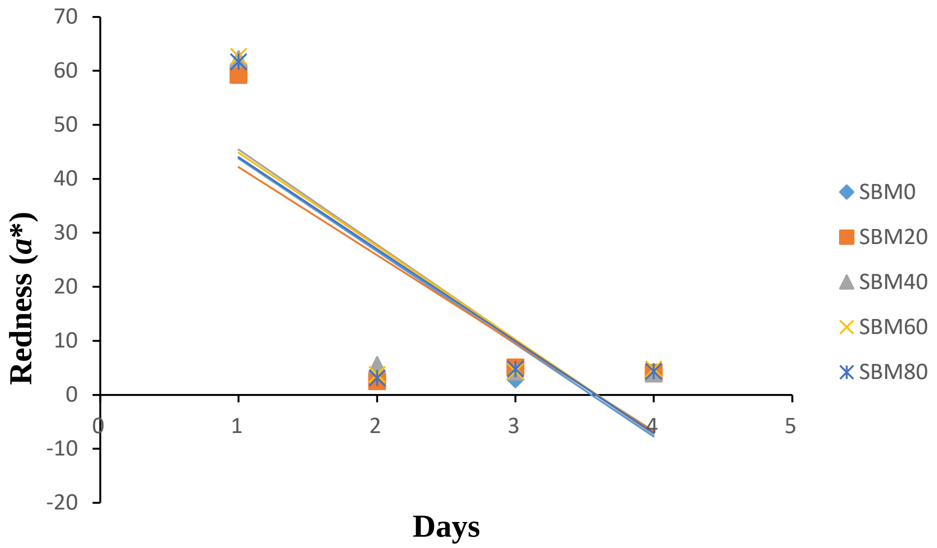

Figure 4.3. Effect of sea bamboo meal on stability of breast meat redness upon storage at room temperature for four days [Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal]

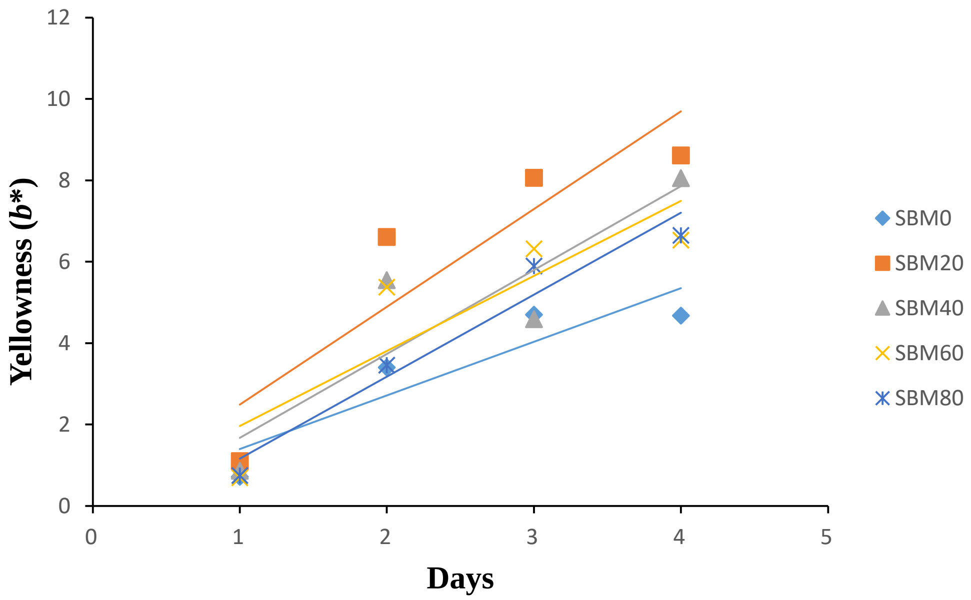

Figure 4.4. Effect of sea bamboo meal on stability of breast meat yellowness upon storage at room temperature for four days [Diets: SBM0 = standard chicken diet without sea bamboo meal; SBM20 = standard chicken diet containing 20 g/kg of sea bamboo meal; SBM40 = standard chicken diet containing 40 g/kg of sea bamboo meal; SBM60 = standard chicken diet containing 60 g/kg of sea bamboo meal; SBM80 = standard chicken diet containing 80 g/kg of sea bamboo meal]

4.4 Discussion

4.4.1 Carcass characteristics and viscera morphometry

Nutrition, genetic material, sex, age at slaughter and body weight are some of the factors that have been implicated to cause considerable variation in carcass composition (Never, 2015). In the current study, dietary supplementation of SBM in Boschveld cockerels did not have an effect on any of the carcass characteristics. Similarly, no linear and quadratic responses were observed for all carcass characteristics, except for CCW which showed a linear increment with SBM levels. However, the linear increment on CCW could have been a storage effect. The current results were consistent with those of Cañedo-Castro *et al*. (2019), who reported that similar carcass weights and yield in Arbor Acres broilers fed a diet with various levels (20, 40 and 60 g/kg) of *Ulva rigida* (air-dried and ground). However, varying results were presented by Abudabos *et al*. (2013), who reported that the inclusion of up to 30 g/kg of green seaweed improved the breast yield and dressing percentage in broiler chickens. Similarly, Martínez *et al*. (2019) reported that feeding 3 g/kg red algae powder (*Chondrus crispus*) to male broiler chickens improved the breast and carcass yield while decreasing abdominal fat yield. Kulshreshtha *et al*. (2020) reported that the inconsistencies in these results could be ascribed to variations in factors such as the amount of seaweed supplemented, seaweed purity, particle size, processing and seaweed species differences.

There were no dietary effects on the visceral organs of the Boschveld cockerels, however, the relative proventriculus weight was reduced while relative caecal weight was increased by increasing SBM levels. The reason for a lower proventriculus weight for the SBM40-fed birds could be explained by the increase in feed intake, which suggests that there was higher a feed outflow rate in the glandular stomach. These findings are in line with those of Kulshreshtha *et al*. (2014) and Nhlane *et al*. (2021), who reported an increase in the caecal weights when red

(5, 10 and 20 g/kg) and green seaweeds (20, 25, 30 and 35 g/kg) were supplemented into laying hens and indigenous chicken diets compared to the control diet, respectively. The increase on caeca weights was expected because the intake of high dietary fibre components such as arabinoxylans, cellulose and lignin is reported to have the potential to modulate caeca size, which is an adaptive mechanism to handle high fibre levels for subsequent fermentation (Tajeda & Kim, 2020).

Indeed, positive quadratic responses were observed for the caecal weights and the lengths of the duodenum and the small intestines with the increase in SBM. The quadratic increase indicated an anatomical adaption mechanism from the SBM80-fed birds to cope with the increase in fibre levels in the diets. However, the weights of the ventriculus, ventriculus fats, liver, spleen, duodenum and jejunum were not affected by the diets, suggesting dietary SBM have low anti-nutritional factors that may negatively affect the GIT and ancillary organs in chickens (Matshogo *et al*., 2020).

4.4.2 Meat quality parameters and shelf life

The appearance, colour, texture, tenderness, flavour, juiciness or water-holding capacity and odour are traditionally the set of sensory properties used to define the quality of meat that is intended for consumption as whole meat rather than as products (Purslow, 2017). Van Laack *et al*. (2000) reported that breast meat quality traits are evaluated to determine biological, chemical and physical characteristics of meat such as colour, pH, storage qualities, texture and tenderness. In the present study, no dietary effects on the meat quality parameters of the birds were observed, which indicated that the inclusion of dietary SBM did not have an effect on the quality of Boschveld meat. This is despite the fact that seaweeds contain amino acids, pigments, minerals, and natural antioxidants such as flavonoids, carotenoids, vitamins A, C and E. (Michalak *et al*., 2022), which reported to have the potential to positvely affect meat quality parameters (Costa *et al*., 2021). These findings supported Nhlane (2020), who reported a lack of dietary seaweed on meat pH, temperature and colour post-slaughter. Nonetheless, the meat pH (5.9 – 6.1) reported in the current study was within the normal meat pH range of poultry (5.7 – 6.1) reported by Zhang and Barbut (2005).

The most important meat quality characteristic is WHC (Bowker & Zhuang, 2015). Protein denaturation and muscle pH are thought to be the primary determining factors of WHC in meat. In the current study, there were no dietary effects observed on the WHC, cooking loss and drip loss. These findings corroborated the results of Matshogo *et al*. (2020), who discovered no dietary responses on WHC, cooking loss and drip loss in broilers. Dietary alterations were expected because seaweed is regarded as a natural resource that is high in polyphenolic antioxidants, protein, minerals and vitamins (Rajauria *et al*., 2017), which have been reported to positively influence some of the meat quality attributes in broilers (Wang *et al*., 2013; Balasubramanian *et al*., 2021). Nonetheless, the absence of dietary differences on WHC, drip loss and cooking loss may be explained by the similar pH readings across the treatment groups, as there is a significant correlation between muscle pH and these meat quality attributes (Fletcher, 2002).

For all shelf life indices measured at room temperature over a period of 4 days, the dietary inclusion of SBM affected chroma only. Diet SBM20 had a higher chroma value than the control, which suggested that the meat might have produced more myoglobin, hence it became darker in colour (Karamucki *et al*., 2013). Nhlane *et al*. (2021) also reported that the inclusion of green seaweed did not improve shelf life indicators over a period of 7 days. Nonetheless, more scientific explorations are needed to fully understand the effect of SBM-containing diets on the shelf life indicators of indigenous chickens. The similarities in shelf life indicators between the control diet and SBM-containing diets suggest that the seaweed used in this study did not slow microbial growth and oxidation reactions in chicken meat.

4.5 Conclusion

Dietary inclusion of sea bamboo meal at levels up to 80 g/kg in diets increased some of the GIT viscera masses and lengths but did not compromise carcass characteristics, meat quality and stability attributes of Boschveld cockerels. This suggests that levels beyond 80 g/kg can be further investigated in poultry to improve the carcass characteristics, meat quality and stability of Boschveld cockerels.

4.6 References

Abudabos, A.M., Okab, A.B., Aljumaah, R., Samara, E., Abdoun, K.A. & Al-Haidary, A.A., 2013. Nutritional value of green seaweed (*Ulva lactuca*) for broiler chickens. Ital. J. Anim. Sci. 2, 28.

Agina, O.A., Ezema, W.S. & Iwuoha, E.M., 2017. The haematology and serum biochemistry profile of adult Japanese quail (*Coturnix coturnix japonica*). Not. Sci. Biol. 9, 67–72.

Balasubramanian, B., Shanmugam, S., Park, S., Recharla, N., Koo, J.S., Andretta, I. & Kim, I.H., 2021. Supplemental impact of marine red seaweed (*Halymenia palmata*) on the growth performance, total tract nutrient digestibility, blood profiles, intestine histomorphology, meat quality, fecal gas emission, and microbial counts in broilers. Animals. 11, 1244.

Bowker, B. & Zhuang, H., 2015. Relationship between water-holding capacity and protein denaturation in broiler breast meat. Poult. Sci. 94(7), 1657–1664.

Cañedo-Castro, B., Piñón-Gimate, A., Carrillo, S., Ramos, D. & Casas-Valdez, M., 2019. Prebiotic effect of *Ulva rigida* meal on the intestinal integrity and serum cholesterol and triglyceride content in broilers. J. Appl. Phycol. 31, 3265–3273.

CIE. 1976. Recommendations on Uniform Color Spaces-Color Difference Equations, Psychometric Color Terms. Commission Internationale de l'Eclairage, Paris, France. Supplement No. 2 to CIE Publication No. 15 (E-1.3.1.) 1978, 1971/(TC-1-3).

Costa, M., Cardoso, C., Afonso, C., Bandarra, N.M. & Prates, J.A.M. 2021. Current knowledge and future perspectives of the use of seaweeds for livestock production and meat quality: a systematic review. J. Anim. Physiol. Anim. Nutr. 105, 1075–1102.

FAO. 2020. Gateway to poultry production and products. http://www.fao.org/poultry-production-prod-ucts/en/

Fletcher, D.L., 2002. Poultry meat quality. Worlds. Poult. Sci. J. 58, 131–145.

Ganesan, A.R., Tiwari, U. & Rajauria, G., 2019. Seaweed nutraceuticals and their therapeutic role in disease prevention. Food Sci. Hum. Wellness. 8(3), 252–263.

Honikel, K.O., 1987. How to measure the water-holding capacity of meat? Recommendation of standardized methods. In Evaluation and Control of Meat Quality in Pigs. P. V. Tarrant, G. Eikelenboom, and G, Monin, eds. Springer; Dordrecht, Netherlands. Pp. 129−142.

Honikel, K.O., 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49, 447–457.

Islam, M.R., Martinez-Soto, C.E., Lin, J.T., Khursigara, C.M., Barbut, S. & Anany, H., 2021. A systematic review from basics to omics on bacteriophage applications in poultry production and processing. Crit. Rev. Food Sci. Nutr. 63(18), 3097–3129.

Karamucki, T., Jakubowska, M., Rybarczyk, A. & Gardzielewska, J., 2013. The influence of myoglobin on the colour of minced pork loin. Meat Sci. 94(2), 234–238.

Kingori, A.M., 2004. The protein and energy requirements of indigenous chickens (*Gallus Domesticus*) of Kenya. Ph.D. thesis. Egerton University, Kenya, pp. 80–93.

Kulshreshtha, G., Hincke, M. T., Prithiviraj, B. & Critchley, A., 2020. A Review of the varied uses of macroalgae as dietary supplements in selected poultry with special reference to laying hen and broiler chickens. J. Mar. Sci. Eng. 8(7), 536.

Kulshreshtha, G., Rathgeber, B., Stratton, G., Thomas, N., Evans, F., Critchley, A., Hafting, J. & Prithiviraj, B., 2014. Feed supplementation with red seaweeds, *Chondrus crispus* and *Sarcodiotheca gaudichaudii*, affects performance, egg quality, and gut microbiota of layer hens. Poult. Sci. J. 933, 2991–3001.

Kumar, Y., Tarafdar, A. & Badgujar, P.C., 2021. Seaweed as a source of natural antioxidants: Therapeutic activity and food applications. J. Food Qual. 5753391.

Lordelo, M., Cid, J., Cordovil, C.M., Alves, S.P., Bessa, R.J. & Carolino, I., 2020. A comparison between the quality of eggs from indigenous chicken breeds and that from commercial layers. Poult. Sci. 99(3), 1768–1776.

Manyelo, T.G., Selaledi, L., Hassan, Z.M. & Mabelebele, M. 2020. Local chicken breeds of Africa: Their description, uses and conservation methods. Animals. 10(12), 2257.

Martínez, Y., Ayala, L., Hurtado, C., Más, D. & Rodríguez, R., 2019. Effects of dietary supplementation with red algae powder (*Chondrus crispus*) on growth performance, carcass traits, lymphoid organ weights and intestinal pH in broilers. Braz. J. Poult. Sci. 21.

Matshogo, T.B., Mlambo, V., Mnisi, C.M. & Manyeula F., 2021. Effect of pre-treating dietary green seaweed with fibrolytic enzymes on growth performance, blood indices, and meat quality parameters of Cobb 500 broiler chickens. Livest. Sci. 251, 104652.

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, 547.

Michalak, I., Tiwari, R., Dhawan, M., Alagawany, M., Farag, M.R., Sharun, K., Bin Emran, T. & Dhama, K., 2022. Antioxidant effects of seaweeds and their active compounds on animal health and production a review. Vet. Q. 42, 48–67.

Mlambo, V., Mnisi, C.M., Matshogo, T.B. & Mhlongo, G., 2022. Prospects of dietary seaweeds and their bioactive compounds in sustainable poultry production systems: A symphony of good things?. Front. Anim. Sci. 3, 998042.

Moges, F., Melesse, A. & Dessie, T., 2010. Assessment of village chicken production system and evaluation of the productive and reproductive performance of local chicken ecotype in Bure District, Northwest Ethiopia. Afr. J. Agric. Res. 5(13), 1739–1748.

Never, A., 2015. Some major factors affecting carcass composition in goats. Sci. J. Anim. Sci. 4(7), 81–88.

Nhlane, L.T., Mnisi, C.M., Madibana, M.J. & Mlambo, V., 2020. Nutrient digestibility, growth performance and blood indices of Boschveld chickens fed seaweed-containing diets. Animals. 10, 1296.

Nhlane, L.T., Mnisi, C.M., Mlambo, V. & Madibana, M.J., 2021. Effect of seaweed-containing diets on visceral organ sizes, carcass characteristics, and meat quality and stability of Boschveld indigenous hens. Poult. Sci. 100(2), 949–956.

Nhung, N.T., Chansiripornchai, N. & Carrique-Mas, J.J., 2017. Antimicrobial resistance in bacterial poultry pathogens: a review. Front. Vet. Sci. 4, 1–17.

Padhi, M.K., 2016. Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance. Scientifica. 1–9.

Priolo, A., Micol, D., Agabriel, J., Prache, S. & Dransfield, E., 2002. Effect of grass or concentrate feeding systems on lamb carcass and meat quality. Meat Sci. 62, 179–185.

Purslow, P.P., 2017. New aspects of meat quality: From genes to ethics. Woodhead Publishing, Cambridge, England. pp. 1–9.

Rajauria, G., Foley, B. & Abu-Ghannam, N., 2017. Characterization of dietary fucoxanthin from *Himanthalia 48etaboli* brown seaweed. Food Res. Int. 99, 995–1001.

Stokvis, L., Kwakkel, R.P., Hendriks, W.H. & Kals, J., 2022. Proteolytic enzyme-treated seaweed co-product (*Ulva laetevirens*) inclusion in corn-soybean and European broiler diets to improve digestibility, health and performance. Poult. Sci. 101(6), 101830.

Tejeda, O.J. & Kim, W.K., 2020. The effects of cellulose and soybean hulls as sources of dietary fiber on the growth performance, organ growth, gut histomorphology, and nutrient digestibility of broiler chickens. Poult. Sci, 99(12), 6828–6836.

Van Laack, R.L., Liu, C.H., Smith, M.O. & Loveday, H.D., 2000. Characteristics of pale, soft, exudative broiler breast meat. Poult. Sci. 79, 1057–1061.

Wang, S., Shi, X., Zhou, C. & Lin, Y. 2013. *Entermorpha prolifera*: Effects on performance, carcass quality and small intestinal digestive enzyme activities of broilers. Chin. J. Anim. Nutr. 25, 1332–1337.

Zhang, L. & Barbut, S., 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. Br. Poult. Sci. 46, 687–693.

5 CHAPTER FIVE – EFFECT OF SEA BAMBOO SPENT OYSTER MUSHROOM SUBSTRATE ON GROWTH PERFORMANCE AND BLOOD PARAMETERS OF BOSCHVELD COCKERELS

Some parts of this chapter are submitted to a peer-reviewed journal: **Mhlongo, G.** & Mnisi, C.M., 2023. Dietary incorporation of brown seaweed spent oyster mushroom substrate alters growth performance, physiological responses and meat quality parameters in Boschveld roosters. Scientific reports. (**Under review**).

Abstract

In Chapters 3 and 4, the dietary inclusion level of 80 g/kg sea bamboo meal (*Ecklonia maxima*; SBM) did not improve growth metrices. Therefore, this chapter explores a higher level (150 g/kg) of SBM substrate inoculated with oyster mushroom (*Pleurotus ostreatus*) spawn (OMS) to offset the negative effects of fibre and improve growth metrices. In that regard, the effect of inoculating SBM with incremental levels of OMS on growth performance and blood parameters in Boschveld cockerels was investigated. Priorly sterilized (autoclave 121°C for 1 hour at 100 kPa) SBM substrates were weighed into five containers and inoculated with the OMS at 0, 20, 30, 40 and 50% of the SBM for 35 days. After the inoculation, the SBM spent mushroom substrates (SMS) were analysed and used to formulate six dietary treatments. The diets were formulated to be isonitrogenous and isoenergetic as follows: a standard grower diet (CON); and CON containing 150 g/kg of SBM substrate after inoculating with 0 (SMS0), 20 (SMS20), 30 (SMS30), 40 (SMS40) and 50% (SMS50) of OMS. Four-week-old Boschveld cockerels (n = 324; 310.8 ± 13.98 g live weight) were randomly distributed in 36 pens (9/birds) that were replicated 6 times per dietary treatment. Inoculating SBM with incremental levels of OMS tended to reduce the crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents of the SMS. Additionally, the SMS treatment levels tended to have similar crude protein across all the treatment groups. Repeated measures analysis revealed significant week × diet interaction effects on feed intake (FI), body weight gain (BWG) and gain-to-feed ratio (G:F). In weeks 7, 8, 12, 14 and 15, both the SMS0 and SMS treatments promoted higher FI (*P* >0.05) than the CON group. However, a negative linear effect was observed for FI in week 13 [R^2 = 0.212; *P* = 0.012]. The BWG showed negative quadratic effects in week 5 [R^2 = 0.377; *P* = 0.015] but increased linearly in week 8 [R^2 = 0.396; *P* = 0.012], week 11 [R^2 = 0.531; *P* = 0.002], week 14 [R^2 = 0.407; *P* = 0.014] and week 15 [R^2 = 0.473; *P* = 0.005] in response to increasing OMS levels. The G:F showed positive and negative quadratic effects in week 5 [R^2 = 0.320; *P* = 0.006] and week 6 [R^2 = 0.383; *P* = 0.025], respectively. Positive linear responses were observed for G:F in week 7 [R^2 = 0.288; *P* = 0.010], week 11 [R^2 = 0.581; *P* = 0.0001] and week 14 [R^2 = 0.389; *P* = 0.004]. Furthermore, quadratic responses were observed for white cell counts [R^2 = 0.483; *P* = 0.008], heterophils [R^2 = 0.191; *P* = 0.024], platelets [R^2 = 0.443; *P* = 0.008], lymphocytes [R^2 = 0.541; *P* = 0.001] and monocytes [R^2 = 0.316; *P* = 0.007] as OMS levels increased. It can be concluded that the inclusion of the SBM spent mushroom substrate improved growth metrices and altered some haematological parameters but had no effect on the serum biochemical parameters of Boschveld cockerels.

Keywords: Blood parameters, Growth performance, Indigenous chickens, Oyster mushroom spawn, Seaweed

5.1 Introduction

The Boschveld chicken is a light red-brown hybrid cross of three indigenous chickens (Ovambo, Matabele, and Venda chicken breeds), and serves as a reliable source of animal protein in rural South Africa (Manyelo *et al*., 2020). This chicken breed is renowned for its firm meat texture, high fertility, disease resistance, thermo-tolerance and strong eggshells (Dessie *et al*., 2011). However, the intensification of this breed is constrained by high feeding costs, poor feed conversion ratios, low growth rates, and delayed attainment of market weight and sexual maturity (Mhlongo *et al*., 2023). To address the high feeding costs, current research explores the utilization of locally available feed resources, such as seaweeds with nutraceutical properties (Shannon & Abu-Ghannam, 2019). Sea bamboo, unlike conventional grains, lacks direct human food value because they have been largely harvested to produce animal feed, fertilizers, plant biostimulants and alginate over the last few years (Miceli *et al*., 2021). Thus, their use in diets of Boschveld chickens can be an efficient strategy to deliver sustainable production of non-conventional chickens (Mlambo *et al*., 2022).

Rich in bioactive metabolites like polyphenols, proteins, polysaccharides (alginate, agar, and carrageenan) and polyunsaturated fatty acids, sea bamboo exhibits various biological functionalities such as antiviral, antibacterial, antifungal, prebiotic, immunomodulatory, anti-inflammatory and cholesterol-lowering effects (Holdt & Kraan, 2011; Balasubramanian *et al*., 2021). These biomolecules have the potential to stimulate growth performance, promote immunological responses, and safeguard the chickens against debilitating pathogenic infections (Cañedo-Castro *et al*. 2019; Mlambo *et al*., 2022). However, the presence of non-starch polysaccharides (NSPs), such as cellulose and hemicellulose (Gullón *et al*., 2020), may limit the utilization of sea bamboo at higher dietary levels. These NSPs hinder feed digestion, which affects the bioavailability of beneficial bioactive components resulting in poor growth performance (Matshogo *et al*., 2020). Therefore, strategies to degrade NSPs and lignin, such as the use of white-rot fungi or oyster (*Pleurotus ostreatus*) mushroom mycelium can be employed to reduce the high fibre levels of sea bamboo and enhance the utility of the spent mushroom substrate (SMS) in diets of Boschveld chickens.

Solid-state fermentation with fungi is widely recognized as an environmentally and economically feasible technique for bioconverting NSP- and lignin-rich substrates (Machado & Ferraz, 2016). White-rot fungi produce ligninolytic enzymes such as laccases, manganese-dependent peroxidases, lignin peroxidases and versatile peroxidases (Andlar *et al*., 2018). These enzymes catalyse the oxidation of various aromatic substrates, generating aromatic radicals and altering the structure of raw materials containing lignocellulose (Andlar *et al*., 2018). Thus, the inoculation of seaweeds with OMS could facilitate the breakdown of its lignocellulolytic matrix, enhancing the feed value of SMS. The valorisation of sea bamboo using OMS could improve the performance of Boschveld chickens while addressing environmental challenges associated with the accumulation of seaweed heaps along offshore areas (Resiere *et al*., 2020). Thus, this chapter investigated the effect of including incremental levels of sea bamboo spent oyster mushroom substrates on growth performance and blood parameters of Boschveld cockerels. The study tested the hypothesis that the inclusion of SMS in a diet of Boschveld cockerels would improve growth metrices and blood parameters.

5.2 Material and methods

5.2.1 Study area description and ingredient sources

The feeding trial took place in the summer (November 2022 − January 2023) at Rooigrond Commercial Farm (25°55′00″ S; 25°48′25″ E; North West, South Africa), with ambient temperatures ranging between 20°C and 37°C. The sea bamboo (*E. maxima*) was harvested and milled as described in Chapter 3, subheading 3.2.2. The oyster mushroom spawn was bought from Eco-Agro Enterprise (PTY) LTD (Nelspruit, South Africa). The other feed ingredients were purchased from Simple Grow (PTY) LTD (Gauteng, South Africa) and Nutroteq (PTY) LTD (Gauteng, South Africa).

5.2.2 Inoculation of sea bamboo with oyster mushroom spawn

Exactly 52 kg of SBM per treatment was weighed into five containers, each measuring 7000 cm^3, and then moistened with distilled water to increase its moisture content without allowing the leaching of nutrients (Shah *et al*., 2004). Following the protocol outlined by Tuyen *et al*. (2012), the weighed samples were sterilized in an autoclave (121°C for 1 hour at 100 kPa) and subsequently cooled at room temperature. The SBM was then inoculated with OMS at 0, 20, 30, 40 and 50%. The containers were covered with black plastic bags and stored in a dark room to conserve moisture, a prerequisite for the reproduction of the mushroom spores. All substrates in the containers were moistened occasionally to keep the surface moist and humid (Chang & Wasser, 2017). The containers were kept at room temperature and the relative humidity (50 – 70%) was recorded on a two-day interval from day 1 to day 35 of inoculation using a Thermo-Hygrometer (IN-OUT, Alla France Automatic Control Equipment Co., Ltd, PRC. Paris). At day 35, the remaining mycelial development was removed by hand from the SMS and then air-dried at room temperature (27°C) to constant weight. The dried SMS was milled (2 mm sieve;

Polymix PX-MFC 90 D, Laufenburg, Switzerland) before being blended with a standard grower diet.

5.2.3 Analyses and diet formulation

The proximate composition values of the substrates were determined as described in Chapter 3, subheading 3.2.3, while metabolizable energy (ME) was calculated using the formula [ME (kcal/kg) = 2778–66 (Crude Fibre × 88% DM)] adapted from Masenya *et al*. (2021) as presented in Table 5.1.

Table 5.1. Proximate composition (g/kg DM, unless shown otherwise) and ME values of untreated and oyster mushroom spawn-treated sea bamboo substrate (SMS).

	[1]Substrates				
	S0	S20	S30	S40	S50
Dry matter (g/kg)	858.2	878.2	884.9	872.6	871.5
Ash	227.8	203.9	200.5	231.5	214.2
Organic matter	630.5	674.3	684.3	659.2	657.3
[2]ME (Kcal/Kg)	2.754	2.757	2.755	2.758	2.757
Crude fibre	421.6	359.9	396.5	346.7	366.8
Neutral detergent fibre	340.7	322.1	321.3	307.9	304.0
Acid detergent fibre	288.3	247.0	246.91	237.2	234.0
Acid detergent lignin	14.07	11.45	11.43	10.44	8.065
Crude protein	111.7	115.7	115.0	115.5	111.8
Ether extract	285.1	279.3	263.8	286.0	261.7
Calcium	27.89	26.14	28.80	32.27	29.42
Potassium	23.73	20.86	20.31	21.99	22.09
Magnesium	9.24	7.78	7.78	8.44	8.43
Sodium	27.45	23.71	23.32	24.64	25.07
Phosphorus	1.59	1.68	1.62	1.75	1.68
Arginine (g/100g DM)	0.28	0.34	0.38	0.39	0.35
Methionine (g/100g DM)	0.21	0.21	0.21	0.26	0.22
Threonine (g/100g DM)	0.60	0.57	0.60	0.59	0.58

Lysine (g/100g DM)	0.70	0.61	0.66	0.60	0.73
Leucine (g/100g DM)	0.54	0.53	0.55	0.51	0.55

[1]Substrates: S0 = SBM without oyster mushroom spawn, S20 = SBM inoculated with 20% oyster mushroom spawn, S30 = SBM inoculated with 30% oyster mushroom spawn, S40 = SBM inoculated with 40% oyster mushroom spawn, and S50 = SBM inoculated with 50% oyster mushroom spawn.

[2]ME = Metabolizable energy

Six experimental diets were formulated (Table 5.2) to meet the nutritional requirements of Boschveld chickens (Nhlane *et al*., 2020) using a Spesfeed Express nutritional software. The diets were as follows: CON = a standard grower diet; SMS0 = CON containing 150 g/kg of SBM without OMS inoculation; SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS; SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS; SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS; and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS. The diets were in a mash form and were formulated by hand mixing. The diets' ingredient composition was presented in Table 5.2. The proximate composition of the diets were also determined as described in Chapter 3, subheading 3.2.3.

Table 5.2. Ingredient composition (g/kg *as fed basis*, unless stated otherwise) of diets containing sea bamboo spent oyster mushroom substrate

	[1]Diets					
Ingredients	CON	SMS0	SMS20	SMS30	SMS40	SMS50
SMS	0.00	150.0	150.0	150.0	150.0	150.0
Yellow maize	645.05	476.89	476.89	476.89	476.89	476.89
Soya oilcake (46%)	311.74	314.07	314.07	314.07	314.07	314.07
Soya crude oil	10.0	40.14	40.14	40.14	40.14	40.14
Limestone powder	14.09	0.00	0.00	0.00	0.00	0.00
Monocalcium phosphate	7.01	7.64	7.64	7.64	7.64	7.64
Salt (Fine)	3.45	3.53	3.53	3.53	3.53	3.53
DL-Methionine	3.20	3.32	3.32	3.32	3.32	3.32
L-Threonine	0.62	0.49	0.49	0.49	0.49	0.49
Lysine HCL	2.23	1.33	1.33	1.33	1.33	1.33
[2]Premix	2.50	2.50	2.50	2.50	2.50	2.50
Axtra phytase	0.10	0.10	0.10	0.10	0.10	0.10

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.

[2]Premix: 0.25 mg sodium selenite; 0.34 mg potassium iodine; 80 mg ferrous sulphate; 0.7 mg folic acid; 11 000 IU vitamin A; 79 mg zinc sulphate; 30 mg niacin; 100 mg magnesium sulphate; 10 mg pantothenic acid; 0.12 g biotin; 5.1 mg vitamin B_6; 4.5 mg vitamin B_2; 2.5 mg vitamin B_1; 2500 IU vitamin D_3; 25 IU vitamin E; 2.0 mg vitamin K_3; and 8.0 mg copper sulphate.

The nutritional composition of the diets is shown in Table 5.3. The diets were analysed for dry matter (DM), ME, ash, organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre ADF, acid detergent lignin (ADL), crude protein (CP), crude fibre, crude fat, minerals (calcium, phosphorus, chloride, and sodium) and amino acid (arginine, methionine, threonine, lysine and leucine as described in Chapter 3, subheading 3.2.3.

Table 5.3. Nutritional composition (g/kg DM, unless stated otherwise) of the diets containing sea bamboo spent oyster mushroom substrate

	[1]Diets					
Parameters	CON	SMS0	SMS20	SMS30	SMS40	SMS50
Dry matter (g/kg)	894.7	899.5	903.2	898.9	923.2	924.2
ME (Kcal/kg)	2.776	2.774	2.774	2.774	2.774	2.774
Crude fibre	33.14	66.17	63.42	76.57	65.40	67.26
Ash	54.13	66.99	67.59	61.63	56.51	73.80
Organic matter	840.6	832.5	835.7	837.3	866.7	866.7
NDF	112.1	152.9	153.4	151.5	144.8	125.8
ADF	45.70	72.28	72.57	70.75	68.29	62.10
ADL	3.55	10.61	10.68	9.23	7.80	5.23
Crude protein	20.25	21.85	21.84	21.60	23.33	22.03
Crude fat	271.0	459.7	380.0	311.3	345.2	264.2
Calcium	7.37	6.27	7.63	8.07	6.94	6.52
Potassium	8.29	12.94	13.61	12.50	12.12	12.78
Magnesium	1.48	2.55	2.67	2.66	2.54	2.60
Sodium	1.38	5.34	5.66	5.33	4.83	5.37
Phosphorus	4.98	4.72	5.36	5.12	4.54	4.73
Arginine (g/100g DM)	1.68	1.72	1.85	1.73	1.59	1.66
Methionine (g/100g DM)	0.75	0.94	0.69	0.84	0.63	0.85
Threonine (g/100g DM)	0.97	1.04	1.01	1.07	0.93	0.98
Lysine (g/100g DM)	1.23	1.71	1.13	1.16	1.15	1.41
Leucine (g/100g DM)	1.75	1.94	1.91	1.95	1.66	1.76

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.

5.2.4 Feeding trial, bird management and growth metrices

A total of 800, one-week-old Boschveld cockerels were bought from Boschveld Ranching (PTY) LTD in Bela-Bela (Limpopo, South Africa). Upon arrival, the birds were offered a stress pack (Phenix stresspac, Virbac, South Africa) for the first three days and were initially fed a commercial starter diet for 3 weeks. At 4 weeks of age, the birds were sexed, and a total of 324 Boschveld cockerels were acclimatized to the experimental diets for 3 days before the commencement of the feeding trial. In a completely randomized design, the 4-week-old cockerels were weighed and randomly allocated to 36-floor pens (experimental units) such that each of the 6 experimental diets had 6 replicate groups. Each replicate pen (measuring 1.5 m Length × 1.5 m Width × 1.7 m Height) carried 9 birds and the floors were covered with sunflower husks. A Thermo-Hygrometer (IN-OUT, Alla France Automatic Control Equipment Co., Ltd, PRC) was used to monitor the average temperature (30 ± 5°C) and relative humidity (55 – 65%) inside the house. The birds were reared under natural lighting from the morning (06h00) to the evening (18h00), and ventilation was achieved by opening the house curtains in the morning and closing them in the evenings. Without any restrictions, fresh clean water and diets were offered daily using Poltek water fountain drinkers (10 L, NWK, Mafikeng, South Africa) and tube feeders (3/100, NWK, Mafikeng, South Africa), respectively. Initial live weights (310.8 ± 13.98 g) were recorded for all the birds per pen and then weighed weekly (from week 4 to week 15) to determine the average weekly body weight gain (BWG). The FI, BWG and gain-to-feed ratio (G:F) were determined as outlined in Chapter 3, subheading 3.2.6. Throughout the 11-week feeding trial, mortality was recorded and was used to correct the G:F data.

5.2.5 Blood collection and analysis

At 14 weeks and 5 days of age, fresh blood samples (4 mL) were collected in the morning from two randomly selected cockerels per pen as outlined in Chapter 3, subheading 3.2.7. An automated IDEXX LaserCyte Haematology Analyser (model no. 93−30001−01, IDEXX Laboratories (Pty) Ltd., Gauteng, South Africa) was used to analyse haematological indices: haematocrits, monocytes, platelets, white cell counts (WCC), lymphocytes, and heterophils. Additionally, an automated IDEXX Catalyst One Chemistry Analyzer (model no. 89−92525−00, IDEXX Laboratories (Pty) Ltd., Gauteng, South Africa) was used to analyse the serum for glucose, total protein, albumin, symmetric dimethyl arginine (SDMA), creatinine, blood urea nitrogen (BUN), calcium, alanine aminotransferase (ALT), lipase, phosphorus, alkaline phosphatase (ALKP), cholesterol, and amylase.

5.2.6 Statistical analyses

All statistical tests were performed as described in Chapter 3, subheading 3.4. Briefly, repeated measures analysis was conducted using PROC GLM (SAS, 2010) to determine the interaction effect between time and diet on average weekly FI, BWG, and G:F data. One-way analysis of variance was used to account for dietary differences in growth performance, and physiological and meat quality data (PROC GLM; SAS, 2013). The probability of difference option in SAS was used to separate the treatment means. Further, polynomial contrasts were used to evaluate linear and quadratic coefficients in response to the incremental levels of OMS using PROC RSREG in SAS. The CON data was not used for the regression analysis. Pre-planned orthogonal contrast statements were used to compare the performance of CON against SMS0. For all the statistical tests, significance was considered at $P < 0.05$.

5.3 Results

5.3.1 Sea bamboo spent oyster mushroom substrate

Results indicate that inoculating SBM with OMS tended to enhance the DM, OM, calcium, phosphorus and arginine content as the SMS levels increased. Furthermore, higher SMS levels showed a tendency to reduce crude fibre, NDF, ADF and ADL contents in SMS by 17.7, 9.0, 17.6 and 25.75%, respectively. Meanwhile, ME and CP remained consistent across the SMS treatment groups.

5.3.2 Mortality and growth performance

There were no dietary effects on overall mortality (P = 0.183) with the means ranging from 1.70 – 10.19%. Repeated measures analysis revealed significant week × diet interaction effects on FI (P = 0.0001), BWG (P = 0.0001) and G:F (P = 0.0001). Table 5.4 indicates that there were neither linear nor quadratic effects (P >0.05) for the FI, except for week 13 [y = 716.9 (±26.50) – 1.653 (±2.293) x; R^2 = 0.212; P = 0.012] where a linear decrease was observed for FI in response to increasing OMS levels. In weeks 7, 8, 11, 12, 14 and 15, the CON diet promoted the least (P <0.05) FI than the other treatment groups, which were similar (P >0.05). In week 9, diet SMS40 promoted the highest FI (P <0.05), followed by SMS0, SMS20, SMS30 and SMS50, and the lowest FI was from diet CON. In week 11, the CON diet promoted the lowest FI (578.5 g/bird) than the SMS0 and SMS50 diets but were all similar (P >0.05) to those fed the SMS20, SMS30 and SMS40 diets. Orthogonal contrasts showed that SMS0 promoted a higher (P <0.05) FI in weeks 6 – 15 than CON, with the exception of week 13.

Table 5.4. Average weekly feed intake (g/bird) of Boschveld cockerels fed diets containing sea bamboo spent oyster mushroom substrate

	[1]Diets						[2]SEM	Significance		
	CON	SMS0	SMS20	SMS30	SMS40	SMS50		P-GLM	P-Linear	P-Quadratic
Week 5	164.6	192.3	189.6	187.7	172.2	190.0	11.21	0.411	0.513	0.691
Week 6	319.8	372.3	360.6	369.1	359.9	360.4	9.034	0.051	0.366	0.841
Week 7	347.2[a]	417.4[b]	409.1[b]	428.5[b]	417.7[b]	417.5[b]	10.42	0.0001	0.812	0.911
Week 8	387.2[a]	486.0[b]	467.3[b]	493.0[b]	501.0[b]	495.9[b]	11.14	0.0001	0.222	0.429
Week 9	436.3[a]	535.2[b]	517.8[b]	530.0[b]	544.2[c]	530.8[b]	13.65	0.0001	0.798	0.621
Week 10	509.4	561.7	539.4	535.0	548.4	557.3	15.18	0.206	0.828	0.167
Week 11	578.5[a]	646.9[b]	634.4[ab]	628.2[ab]	624.8[ab]	646.2[b]	15.41	0.041	0.723	0.302
Week 12	523.1[a]	602.9[b]	614.4[b]	580.3[ab]	607.6[b]	608.6[b]	17.83	0.009	0.952	0.690
Week 13	667.5	717.0	683.0	658.5	648.6	620.7	26.74	0.224	0.012	0.917
Week 14	596.0[a]	755.3[b]	753.0[b]	850.1[b]	840.3[b]	774.3[b]	27.87	0.0001	0.187	0.198
Week 15	557.9[a]	707.2[b]	662.0[b]	726.4[b]	711.5[b]	718.8[b]	17.03	0.0001	0.348	0.344

[a,b] Means, in a row, with different superscripts denote statistical differences (P <0.05).

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.

[2]SEM = standard error of the mean.

Table 5.5 shows that there were linear increments (P >0.05) for BWG in week 8 [y = 0.396 (±0.549) x + 143.9 (±5.192); R^2 = 0.396; P = 0.012], week 11 [y = 0.769 (±0.806) x + 128.9 (±7.621); R^2 = 0.531; P = 0.002], week 14 [y = 3.819 (±2.192) x + 77.25 (±20.74); R^2 = 0.407; P = 0.014], and week 15 [y = 2.525 (±1.732) x + 133.4 (±16.38); R^2 = 0.473; P = 0.005]. However, a negative linear effect was observed for BWG in week 13 [y = 164.9 (±14.60) – 0.850 (±1.543) x; R^2 = 0.137; P = 0.049] as dietary OMS levels increased. There was a positive quadratic effect for BWG in week 5 [y = 0.05 (±0.018) x^2 – 2.12 (±0.788) x + 83.98 (±7.454); R^2 = 0.377; P = 0.015; optimised weight gain at 22%]. In weeks 6 and 7, birds fed the CON promoted a lower BWG (132.8 g/bird) than the SMS40 (150.2 g/bird) diet, but were all similar (P >0.05) to those fed the SMS0, SMS20, SMS30 and SMS50 diets. In week 9, diet SMS40 (180.0 g/bird) promoted the highest BWG than the CON and SMS20 diets, which were similar (P >0.05) to the SMS0, SMS30, SMS40 and SMS50 diets. In week 10, the CON diet (168.8 g/bird) promoted the highest BWG than all the other treatment groups, which were similar (P >0.05). In week 11, birds fed diets SMS30, SMS40 and SMS50 promoted the highest (P <0.05) BWG than those fed the CON, SMS0 and SMS20 diets. In week 14, the CON diet promoted the lowest BWG (55.88 g/bird) than diets SMS30 and SMS40 but was similar (P >0.0) to diets SMS0 and SMS20. In week 15, birds fed diet CON had the lowest (117.3 g/bird) BWG than diets SMS30, SMS40 and SMS50, which were all similar (P >0.05) to diets SMS0 and SMS20. Relative to CON, SMS0 promoted higher (P <0.05) BWG in weeks 5, 9, 10 and 14.

Table 5.5. Average weekly body weight gain (g/bird) of Boschveld cockerels fed diets containing sea bamboo spent oyster mushroom substrate

	[1]Diets						[2]SEM	Significance		
	CON	SMS0	SMS20	SMS30	SMS40	SMS50		P-$_{GLM}$	P-$_{Linear}$	P-$_{Quadratic}$
Week 5	54.49	81.31	70.42	68.24	65.46	94.70	8.404	0.054	0.906	0.015
Week 6	132.8[a]	133.8[ab]	141.9[ab]	147.8[ab]	150.2[b]	135.6[ab]	4.077	0.011	0.125	0.176
Week 7	143.6[a]	147.1[ab]	147.2[ab]	156.7[ab]	161.0[b]	151.3[ab]	4.060	0.044	0.234	0.294
Week 8	153.4	150.1	148.5	160.0	171.9	159.2	4.850	0.084	0.012	0.822
Week 9	162.1[ab]	173.9[bc]	155.7[ab]	178.3[bc]	180.0[c]	174.3[bc]	4.256	0.004	0.919	0.556
Week 10	168.8[b]	143.4[a]	136.1[a]	130.3[a]	134.9[a]	140.2[a]	5.814	0.001	0.082	0.776
Week 11	139.4[a]	133.4[a]	137.0[a]	166.9[b]	178.3[b]	179.1[b]	6.071	0.0001	0.002	0.773
Week 12	110.2	91.22	91.50	83.75	85.21	80.77	10.02	0.475	0.545	0.377
Week 13	159.3	167.5	145.6	154.5	163.7	115.1	15.59	0.217	0.024	0.776
Week 14	55.88[a]	86.12[ab]	114.2[abc]	165.1[c]	168.3[c]	135.0[bc]	14.95	0.0001	0.014	0.402
Week 15	117.3[a]	140.5[ab]	167.3[ab]	190.6[b]	184.9[b]	206.2[b]	16.65	0.007	0.005	0.707

[a,b,c] Means, in a row, with different superscripts denote statistical differences (P <0.05).

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.

.[2]SEM = Standard error of the mean.

Table 5.6 reveals that there were positive and negative quadratic effects (*P* <0.05) observed for G:F in week 5 [y = 0.0002 (±0.0001) x^2 – 0.007 (±0.003) *x* + 0.430 (±0.029); R^2 = 0.320; *P* = 0.006] and week 6 [*y* = 0.346 (±0.012) – 0.004 (±0.001) *x* – 0.0001 (±0.00002) x^2; R^2 = 0.383; *P* = 0.025], respectively. There were linear increases (*P* <0.05) for G:F in week 7 [*y* = 0.001 (±0.001) *x* + 0.351 (±0.006); R^2 = 0.288; *P* = 0.010], week 11 [*y* = 0.002 (±0.001) *x* + 0.201 (±0.011); R^2 = 0.581; *P* = 0.0001] and week 14 [*y* = 0.004 (±0.002) *x* + 0.109 (±0.017); R^2 = 0.389; *P* = 0.004]. However, a linear decline was recorded for G:F in week 12 [*y* = 0.150 (±0.022) – 0.0001 (±0.002) *x*; R^2 = 0.271; *P* = 0.015]. In week 5, diet SMS50 (0.550) promoted a higher G:F than CON, SMS20 and SMS30 diets, which were all similar (*P* >0.05) to diets SMS0 and SMS40. In week 6, diet SMS40 promoted the highest G:F (0.418) while diet SMS0 resulted in the least G:F value (0.347). However, all the treatment groups were similar (*P* >0.05) to the CON group. In week 7, birds fed the CON and SMS40 diets promoted a higher G:F than those fed the SMS0, SMS20 and SMS50 diets, which were all similar (*P* >0.05) to diet SMS30. In weeks 8 and 10, the CON diet promoted a higher G:F than all the other treatment groups, which was all similar (*P* >0.05). In week 9, the CON and SMS30 diets promoted the highest (*P* <0.05) G:F than SMS0, SMS20 and SMS50 diets. In week 11, diets SMS40 promoted the highest (*P* <0.05) G:F than the SMS0 and SMS20 diets, which were similar (*P* >0.05) to the CON. In week 12, birds fed the CON had the higher G:F (0.211) than diets SMS30, SMS40 and SMS50, but had similar (*P* >0.05) G:F as birds fed diets SMS0 and SMS20. In week 14, the CON diet promoted the least G:F than diets SMS30 and SMS40. Relative to CON, SMS0 promoted higher (*P* <0.05) G:F in weeks 6 – 12.

Table 5.6. Average weekly gain-to-feed ratio of Boschveld cockerels fed diets containing spent oyster mushroom sea bamboo substrate

	[1]Diets						[2]SEM	Significance		
	CON	SMS0	SMS20	SMS30	SMS40	SMS50		P-GLM	P-Linear	P-Quadratic
Week 5	0.353[a]	0.426[ab]	0.370[a]	0.367[a]	0.397[ab]	0.503[b]	0.032	0.042	0.421	0.006
Week 6	0.400[abc]	0.347[a]	0.395[abc]	0.401[bc]	0.418[c]	0.364[ab]	0.013	0.006	0.014	0.025
Week 7	0.388[b]	0.352[a]	0.361[a]	0.366[ab]	0.385[b]	0.362[a]	0.005	0.0004	0.010	0.499
Week 8	0.387[b]	0.308[a]	0.319[a]	0.323[a]	0.343[a]	0.321[a]	0.009	0.0001	0.110	0.501
Week 9	0.372[b]	0.326[a]	0.318[a]	0.344[b]	0.331[a]	0.328[a]	0.008	0.0009	0.466	0.974
Week 10	0.332[b]	0.255[a]	0.253[a]	0.244[a]	0.245[a]	0.245[a]	0.009	0.0001	0.315	0.578
Week 11	0.241[ab]	0.206[a]	0.217[a]	0.266[bc]	0.286[c]	0.278[bc]	0.011	0.0001	0.0001	0.880
Week 12	0.211[b]	0.152[ab]	0.127[ab]	0.103[a]	0.09[a]	0.088[a]	0.023	0.007	0.015	0.404
Week 13	0.239	0.233	0.212	0.230	0.234	0.232	0.020	0.865	0.500	0.068
Week 14	0.094[a]	0.114[ab]	0.150[abc]	0.193[c]	0.199[c]	0.171[bc]	0.015	0.0001	0.004	0.118
Week 15	0.210	0.198	0.254	0.262	0.261	0.288	0.024	0.099	0.052	0.064

[a,b,c] Means, in a row, with different superscripts denote statistical differences (P <0.05).

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.

[2]SEM = standard error of the mean.

5.3.3 Haematological and serum biochemical parameters

There were positive quadratic effects (*P* <0.05) for white blood cell (WBC) count and lymphocytes. However, negative quadratic effects were observed for heterophils, platelets and monocytes in response to the increasing dietary OMS levels (Table 5.7).

Table 5.7. Regression equations for haematological parameters of 15-week-old Boschveld cockerels fed with diets containing graded levels of sea bamboo spent oyster mushroom substrate

Parameters	Quadratic	R^2	Optimum
[1]WBC ($\times 10^9$/L)	y = 11.66 (±0.904) – 0.117 (±0.078) x + 0.004 (±0.002) x^2	0.483	15%
Heterophils ($\times 10^9$/L)	y = 7.232 (±0.690) + 0.122 (±0.060) x – 0.003 (±0,001) x^2	0.191	20%
Platelets ($\times 10^9$/L)	y = 28.85 (±2.109) + 0.300 (±2.183) x – 0.010 (±0,004) x^2	0.443	15%
Lymphocytes %	y = 21.50 (±4.77) – 1.007 (±0.413) x + 0.031 (±0,008) x^2	0.541	16%
Monocytes (%)	y = 11.11 (±1.156) + 0.215 (±0.100) x – 0.006 (±0,002) x^2	0.316	18%

WBC = white blood cell.

Dietary treatments had an effect (*P* <0.05) on WBC, heterophils, platelets, lymphocytes and monocytes only (Table 5.7). Birds fed with diet SMS50 had higher (*P* <0.05) WBC than those fed with diets SMS0 and SMS20, which did not differ (*P* >0.05). Birds fed with CON had similar (*P* >0.05) WBC levels as those fed with diets SMS30, SMS40 and SMS50. SMS30 promoted higher (*P* <0.05) heterophils than CON but were similar to the other treatment groups. The CON and SMS40 diets resulted in the least platelets compared to all the other dietary treatment groups, which did not differ (*P* >0.05). Birds reared on CON and SMS50 diets had higher (*P* <0.05) lymphocytes than those reared on the other treatment groups. Diet SMS20 promoted higher (*P* <0.05) monocytes than diet SMS50, which were similar (*P* >0.05) to the other treatment groups. SMS0 resulted in lower WBC (*P* = 0.014) and lymphocytes (*P*

= 0.0001) relative to CON. However, SMS0 produced higher platelets (*P* = 0.003) and heterophils (*P* = 0.0001) concentrations than the CON

Table 5.8. Haematological parameters of Boschveld cockerels fed with diets containing sea bamboo spent oyster mushroom substrate

	[1]Diets							Significance			
Parameters	CON	SMS0	SMS20	SMS30	SMS40	SMS50	[3]SEM	P-GLM	P-Linear	P-Quadratic	Reference values
Haematocrit (%)	34.83	33.25	34.75	35.42	33.33	33.58	0.823	0.313	0.989	0.068	6.74 – 46.10[1,2,3,4,5,6]
[2]WBC (×10⁹/L)	15.23[bc]	11.50[a]	11.33[a]	12.92[ab]	12.33[ab]	17.45[c]	0.975	0.0002	0.0003	0.008	12.89 – 22.65[3,4,7]
Platelets (×10⁹/L)	21.83[a]	39.50[b]	38.67[b]	38.50[b]	37.92[b]	26.56[a]	2.222	0.0001	0.001	0.008	9.00 – 95.50[2,4]
Heterophils (×10⁹/L)	5.621[a]	7.416[ab]	7.987[ab]	8.271[b]	7.700[ab]	5.867[ab]	0.769	0.031	0.435	0.024	1.52 – 22.41[1,2,8]
Lymphocytes (%)	54.17[b]	20.00[a]	17.08[a]	24.17[a]	18.25[a]	54.25[b]	3.879	0.0001	0.0003	0.0006	10.35 – 84.57[3,6,7]
Monocytes (%)	9.75[ab]	11.00[ab]	13.83[b]	11.50[ab]	11.17[ab]	7.75[a]	1.194	0.032	0.054	0.007	3.90 – 7.42[6,7]

[a,b,c] Means, in a row, with different superscripts denote statistical differences (P <0.05).

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.

[2]WBC = white blood cell.

[3]SEM = standard error of the mean.

Sources: [1]Jonathan *et al*. (2021); [2]Nhlane *et al*. (2020); [3]Matshogo *et al*. (2018); [4]Duah *et al*. (2020); [5]Odunitan-Wayas *et al*. (2018); [6]Abdi-Hachesoo *et al*. (2011); [7]Manyeula *et al*. (2019); [8]Atela *et al*. (2019).

Table 5.9 indicates that there were neither linear nor quadratic effects for serum biochemical parameters, except for albumin which linearly increased [y = 0.010 (±0.005) x + 5.816 (±0.056); R^2 = 0.259; P = 0.043] with dietary OMS levels. Relative to CON, SMS0 resulted in lower blood urea nitrogen (P = 0.038) and amylase (P = 0.031) concentrations.

Table 5.9. Serum biochemical parameters of Boschveld cockerels fed with diets containing sea bamboo spent oyster mushroom substrate

[2]Paramaters	[1]Diets						[3]SEM	Significance			Reference values
	CON	SMS0	SMS20	SMS30	SMS40	SMS50		*P*-GLM	*P*-Linear	*P*-Quadratic	
Glucose (mmol/L)	131.8	122.1	127.3	116.8	127.5	138.6	5.279	0.099	0.642	0.501	1.950 – 16.64[1,2]
SDMA (µg/dL)	28.00	21.50	25.42	23.42	25.58	27.17	3.219	0.733	0.347	0.899	55.00 – 85.63[1,2]
Creatinine (µmol/L)	0.100	0.100	0.100	0.100	0.133	0.100	0.014	0.435	0.297	0.848	12.13 – 22.70[1,2,3,6,7]
BUN	4.75	3.54	4.33	3.92	4.33	4.42	0.446	0.492	0.379	0.635	–
Phosphorus (mmol/L)	16.10	16.10	16.10	15.59	15.45	15.76	0.321	0.544	0.297	0.848	2.630 – 4.330[1,2]
Calcium (mmol/L)	5.08	5.15	5.30	5.29	5.21	5.31	0.153	0.868	0.377	0.328	2.050 – 3.340[1,2,3,4]
Albumin (g/L)	6.0	5.81	6.0	5.68	5.86	6.0	0.132	0.439	0.043	0.228	15.00 – 38.47[1,2,3,4,7,8]
Cholesterol (mmol/L)	33.58	34.75	29.17	24.58	33.00	27.92	7.556	0.926	0.501	0.637	3.18 – 4.77[1,2]
Amylase (U/L)	566.1	455.8	504.5	487.0	484.8	513.6	37.01	0.431	0.984	0.123	890.5 – 922.9[2]
ATL (U/L)	104.1	76.83	93.33	74.50	75.63	88.50	12.25	0.602	0.914	0.413	0.400 – 43.32[1,2,5,6,7]
ALKP (U/L)	9.167	10.00	8.333	9.00	8.00	9.167	0.863	0.681	0.139	0.724	259.8 – 266.8[1,2]
Lipase (U/L)	53.50	65.00	50.58	51.50	44.75	53.00	8.745	0.718	0.124	0.931	–

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.
[2]Parameter: SDMA = symmetric dimethylarginine; BUN = blood urea nitrogen; ALT = alanine aminotransferase; ALKP = alkaline phosphatase.
[3]SEM = standard error of the mean.
Sources: [1]Jonathan *et al*. (2021); [2]Nhlane *et al*. (2020); [3]Matshogo *et al*. (2018); [4]Duah *et al*. (2020); [5]Odunitan-Wayas *et al*. (2018); [6]Manyeula *et al*. (2019); [7]Atela *et al*. (2019); [8]Maliwan *et al*. (2017).

5.4 Discussion

5.4.1 Sea bamboo spent oyster mushroom substrate

Inoculating SBM with OMS tended to reduce the crude fibre, NDF, ADF and ADL contents of the SMS by 17.7, 9.0, 17.6 and 25.75%, respectively, as the OMS levels increased, indicating the effectiveness of this innovative strategy to breakdown the lignocellulytic matrix of SBM. This enhancement in nutrient composition suggests that incorporating SMS into diets will enable birds to absorb sufficient nutrients. Additionally, the ME, CP, minerals and amino acid content of the SMS treatment groups are expected to meet the bird's energy, bone strength and feed utilisation needs, aligning with the nutrient requirements for growing chickens as outlined by Kingori *et al*. (2003) and NRC (1994).

5.4.2 Growth performance

Brown seaweeds, such as sea bamboo, can supplement diets of Boschveld cockerels with nutrients and bioactive substances required for optimum production (Mlambo *et al*., 2022). However, higher fibre levels restrict its efficient utilisation. Thus, the inoculation of sea bamboo with *Pleurotus ostreatus* fungi can potentially reduce its fibre levels and allow optimal utilisation. The absence of dietary effects on mortality indicates that the addition of SBM as well as the SMS did not cause any deaths. The significant interaction effects between week (rooster age) and diet on FI, BWG and G:F, demonstrates that the performance of the birds changed in response to their age, explaining why the inclusion of the SMS in the diets positively influenced FI in weeks 7, 8, 9, 11, 12, 14 and 15. The improvement in FI shows that the use of *Pleurotus ostreatus* reduced the lignocellulolytic matrix of the seaweed substrate (Shrivastava *et al*., 2014), thereby allowing the birds to consume more feed. This could be deduced from the reduced NDF, ADF and ADL levels of the substrates. Feeding SMS-containing diets resulted in a negative linear response for FI in week 13, and the reason for this was not clear.

Previous research has shown that white-rot fungus (*Phanerochaete chrysoporium*) treatment enhances the nutrient composition of agricultural by-products (El-Rahman *et al*., 2014), which could explain the significant linear increase in weight gain in weeks 8, 11, 14 and 15. Treatment SMS40 promoted highest BWG than CON treatment in weeks 6, 7, 9, 11 and 14, indicating the efficacy of OMS to bolster feed utilisation into body mass. This could also explain the observed positive quadratic response for BWG in week 5, which showed that the birds fed the CON diet had lower BWG than SMS0 and SMS treatment groups. These results corroborate those of Shang *et al*. (2014), who reported an improvement in average daily weight gain of broilers fed diets supplemented with 6, 12 and 18 g/kg of *Hericium caput-medusae* (Bull.:Fr.) Pers. fungus powder for 42 days. However, the reason for the linear decline for BWG in week 13 is unknown, which could have been a result of other external factors.

Feeding the SMS40 and SMS50-containing diets improved G:F in weeks 5, 11 and 13, which further confirms its ability to enhance feed utilisation efficiency. This could be attributed to the reduced fibre levels of the diets, further indicating that the birds might have had access to the beneficial bioactive compounds in the seaweed (Gullón *et al*., 2020). Indeed, Sweeney *et al*. (2017) identified that laminarin promotes nutrient digestibility by interacting with the surface layer of the small intestines, hence positively influencing the broiler chickens' growth metrics. Similarly, Zhang *et al*. (2012) reported that the supplementation of broiler diets with 3.5 and 7 g/kg of *Aspergillus niger*-fermented *Ginkgo biloba*-leaves improved the feed/gain ratio of the chickens. However, it is not clear why G:F showed a linear decline in week 12, and this was not consistent with the decline in FI and BWG observed in week 13. Nonetheless, the inclusion of the SMS has been shown to enhance the overall growth performance metrices of the Boschveld cockerels.

5.4.3 Haematological and serum biochemical indices

Blood parameters play key roles in the prognosis, diagnosis and treatment of diseases and the assaying of various factors impacting haematological and serum biochemical indices (Yokus *et al*., 2006). A positive quadratic response was noted for WBC, showing that the SMS-containing diets initially decreased at SMS0 but increased at SMS50. According to Bhatia *et al*. (2023), WBCs are a diverse group of nucleated cells that play a critical role in immunity and phagocytosis and protecting the birds against infections. The WBC counts normally increase in response to stress or infection, however, a reduction might occur due to bone marrow disease, severe acute disease or other causes (Mahdavi & Borumand, 2021). In the current study, SMS50 and CON-fed birds produced higher WBC levels than SMS0 and SMS20-fed birds, indicating that the latter groups might have suffered from the negative activities of fibre and antinutrients in sea bamboo.

Feeding the SMS-containing diets induced a negative quadratic effect on blood platelets, which participate in immune defence and haemostasis in lower vertebrates such as birds and fish and also play a vital role in reducing blood loss by sealing and repairing damaged blood vessels (Yau *et al*., 2015). Platelets are at the balance of bleeding or clotting events, such that excessive bleeding can occur in the case of low platelet levels (thrombocytopenia), whereas thrombosis occurs when platelet levels are high (thrombocytosis) (Brummel-Ziedins & Mann, 2018). In the current study, the SMS0 and SMS treatments increased platelet circulation in the blood relative to the CON, which could imply that these groups might have suffered from the antinutritional activities of secondary metabolites in SBM. Indeed, the presence of anti-nutritional factors such as lectins, phytic acids, high levels of trypsin inhibitors and alpha-amylase inhibitors in seaweeds (Oliveira *et al*., 2009) might have affected the blood indices of the roosters.

Lymphocytes (L) and heterophils (H) play important roles in adaptive and innate immune defence, respectively (Minias, 2019). Campbell (2007) reported that healthy birds should have a higher proportion of L than H in their blood, which influences the primary indicator of physiological stress, the H/L ratio (Fard *et al*., 2014). Emadi & Kermanshahi (2007) reported that stress is measured using the H/L ratio in chickens: 0.2 (low), 0.5 (normal) and 0.8 (high). In this study, birds fed the SMS20 (5.687) had a higher H/L ratio than the CON (0.657), indicating that the SMS0, SMS20, SMS30, SMS40 and SMS50 groups suffered from physiological stress. Contrary to the current findings, Sugiharto *et al*. (2020) reported that supplementing broiler finisher diets with 50, 100 and 150 g/kg of *Chrysonilia crassa* and *Bacillus subtilis* two-stage fermented banana peels affected mean corpuscular haemoglobin concentration only and not other haematological indices such as WBC, platelets, heterophils, lymphocytes and monocytes.

Monocytes are the innate immune system phagocytic cells responsible for eliminating pathogens and dead cells (Parihar *et al*., 2010). They play a crucial role in the initiation and cessation of inflammation, primarily through phagocytosis (Auffray *et al*., 2009). In the case of bacterial infections or inflammation, monocytes-macrophages are released into the bloodstream to regulate homeostasis. Thus, the observed negative quadratic response of monocytes after feeding SMS-containing diets could be explained by the gradual enlargement of the spleen weight, which is regarded as an immediate monocyte reservoir that mobilizes a significant number of monocytes in response to injury (Swirski *et al*., 2009).

Serum albumin is one of the main sources of serum protein that serves as the most favourable source of amino acids for the synthesis of tissue proteins during the phase of rapid somatic growth, particularly during feed restriction conditions (Tóthová *et al*., 2019). The observed linear increase in albumin could be due to the presence of phlorotannins in sea bamboo, which might have interfered with the protein metabolism because of its binding affinity to protein (Belanche *et al*., 2016), thus resulting in high serum albumin. Nonetheless, no differences were observed among the treatments on liver enzymes (ALKP and ALP), indicating that the birds did not suffer from any liver injuries.

5.5 Conclusion

Generally, feeding diets containing sea bamboo spent oyster mushroom substrate improved feed intake, body weight and gain-to-feed ratio. The inclusion of the oyster mushroom spawn-inoculated sea bamboo meal had an effect on certain blood parameters and altered serum albumen without compromising the overall health status of the cockerels. It can, therefore, be concluded that oyster mushroom spawn improved the utility of sea bamboo meal for Boschveld cockerels.

5.6 References

Abdi-Hachesoo, B., Talebi, A. & Asri-Rezaei, S., 2011. Comparative study on blood profiles of indigenous and Ross-308 broiler breeders. Glob. Vet. 7(3), 238–241.

Andlar, M., Rezić, T., Marđetko, N., Kracher, D., Ludwig, R. & Šantek, B., 2018. Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation. Eng. Life Sci. 18(11), 768–778.

Auffray, C., Sieweke, M. H. & Geissmann, F., 2009. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. Annu. Rev. Immunol., 27, 669–692.

Balasubramanian, B., Shanmugam, S., Park, S., Recharla, N., Koo, J.S., Andretta, I. & Kim, I.H., 2021. Supplemental impact of marine red seaweed (*Halymenia palmata*) on the growth performance, total tract nutrient digestibility, blood profiles, intestine histomorphology, meat, quality, fecal gas emission, and microbial counts in broilers. Animals. 11(5), 1244.

Belanche, A., Jones, E., Parveen, I. & Newbold, C.J., 2016. A metagenomics approach to evaluate the impact of dietary supplementation with *Ascophyllum nodosum* or *Laminaria digitata* on rumen function in rusitec fermenters. Front. Microbiol. 7, 299.

Bhatia, K., Dhalla, S., Mittal, A., Gupta, S., Gupta, A. & Jindal, A., 2023. Integrating explainability into deep learning-based models for white blood cells classification. Comput. Electr. Eng. 110, 108913.

Brummel-Ziedins, K. & Mann, K.G. 2018. Molecular basis of blood coagulation. R Hoffman (Ed.), Hematology: Basic principles and practice, Elsevier, Philadelphia, PA. pp, 1885–1905.

Campbell, T.W. & Ellis C.K., 2007. Avian and exotic animal hematology and cytology. 3rd ed. Wiley-Blackwell Pub. Iowa, USA.

Cañedo-Castro, B., Piñón-Gimate, A., Carrillo, S., Ramos, D. & Casas-Valdez, M., 2019. Prebiotic effect of *Ulva rigida* meal on the intestinal integrity and serum cholesterol and triglyceride content in broilers. J. Appl. Phycol. 31, 3265–3273.

Chang, S. & Wasser, S., 2017. The cultivation and environmental impact of mushrooms. Oxford Research Encyclopedia of environmental impact of mushrooms. Oxford University Press, Oxford, England. pp. 1–37.

Dessie, T., Taye, T., Dana, N., Ayalew, W. & Hanotte, O., 2011. Current state of knowledge on phenotypic characteristics of indigenous chickens in the tropics. Worlds Poult. Sci. J. 67, 507–516.

Duah, K.K., Essuman, E.K., Boadu, V.G., Olympio, O.S. & Akwetey, W., 2020. Comparative study of indigenous chickens on the basis of their health and performance. Poult. Sci. 99(4), 2286–2292.

El-Rahman, H.H., Abedo, A.A., El-Nomeary, Y.A.A., Abdel-Magid, S.S. & Mohamed, M.I., 2014. Effect of biological treatments of rice straw on growth performance, digestion and economic efficiency for growing calves. Glob. Vet. 13(1), 47–54.

Emadi, M. & Kermanshahi, H., 2007. Effect of turmeric rhizome powder on the activity of some blood enzymes in broiler chickens. Int. J. Poult. Sci. 6(1), 48–51.

Fard, S.H., Toghyani, M. & Tabeidian, S.A., 2014. Effect of oyster mushroom wastes on performance, immune responses and intestinal morphology of broiler chickens. Int. J. Recycl. Org. Waste Agric. 3, 141–146.

Gullón, B., Gagaoua, M., Barba, F.J., Gullón, P., Zhang, W. & Lorenzo, J.M., 2020. Seaweeds as promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products. Trends Food Sci. Technol. 100, 1–18.

Holdt, S.L. & Kraan, S., 2011. Bioactive compounds in seaweed: Functional food applications and legislation. J. Appl. Phycol. 23, 543–597.

Jonathan, O., Mnisi, C.M., Kumanda, C. & Mlambo, V., 2021. Effect of dietary red grape pomace on growth performance, hematology, serum biochemistry, and meat quality parameters in Hy-line Silver Brown cockerels. PLoS One, 16(11), .e0259630.

Kingori, A.M., Tuitoek, J.K., Muiruri, H.K. & Wachira, A.M., 2003. Protein requirements of growing indigenous chickens during the 14-21 weeks growing period. S. Afr. J. Anim. Sci. 33(2), 78–82.

Machado, A.D. & Ferraz, A., 2016. Biological pretreatment of sugarcane bagasse with basidiomycetes producing varied patterns of biodegradation. Bioresour. Technol. 225, 17–22.

Mahdavi, S.A.H. & Borumand Poor, R., 2021. Detection, and counting of white blood cells in broiler chickens using image processing. J. Anim. Sci. Res. 31(1), 27–36.

Maliwan, P., Khempaka, S. & Molee, W., 2017. Evaluation of various feeding programmes on growth performance, carcass and meat qualities of Thai indigenous crossbred chickens. S. Afr. J. Anim. Sci. 47(1), 16–25.

Manyelo, T.G., Selaledi, L., Hassan, Z.M. & Mabelebele, M., 2020. Local chicken breeds of Africa: Their description, uses and conservation methods. Animals. 10, 2257.

Manyeula, F., Mlambo, V., Marume, U. & Sebola, N.A., 2019. Nutrient digestibility, haemo-biochemical parameters and growth performance of an indigenous chicken strain fed canola meal–containing diets. Trop. Anim. Health Prod. 2019, 51, 2343–2350.

Masenya, T.I., Mlambo, V. & Mnisi, C.M., 2021. Complete replacement of maize grain with sorghum and pearl millet grains in Jumbo quail diets: Feed intake, physiological parameters, and meat quality traits. PLoS ONE, 16(3), e0249371.

Matshogo, T.B., Mlambo, V., Marume, U. & Sebola, N., 2018. Growth performance, blood parameters, carcass characteristics and meat quality traits in Potchefstroom Koekoek chickens fed Lippia javanica leaf meal. Trop. Anim. health Prod. 50, 1787–1795.

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, 547.

Mhlongo, G. & Mnisi, C.M., 2023. Effect of seaweed (*Ecklonia maxima*) on apparent nutrient digestibility, growth performance, and physiological and meat quality parameters in Boschveld cockerels. Poult. Sci. 102(2), 102361.

Miceli, A., Vetrano, F. & Moncada, A., 2021. Influence of *Ecklonia maxima* extracts on growth, yield, and postharvest quality of hydroponic leaf lettuce. Horticulturae. 7(11), 440.

Minias, P., 2019. Evolution of heterophil/lymphocyte ratios in response to ecological and life-history traits: A comparative analysis across the avian tree of life. J. Anim. Ecol. 88, 554–565.

Mlambo, V., Mnisi, C.M., Matshogo, T.B. & Mhlongo, G., 2022. Prospects of dietary seaweeds and their bioactive compounds in sustainable poultry production systems: A symphony of good things?. Front. Anim. Sci. 3, 998042.

Nhlane, L.T., Mnisi, C.M., Madibana, M.J. & Mlambo, V., 2020. Nutrient digestibility, growth performance and blood indices of Boschveld chickens fed seaweed-containing diets. Animals. 10, 1296.

NRC, 1994. Nutrient Requirements of Poultry (9th ed.). National Research Council; National Academy Press, Washington DC, USA.

Odunitan-Wayas, F., Kolanisi, U. & Chimonyo, M., 2018. Haematological and serum biochemical responses of Ovambo chickens fed provitamin A biofortified maize. Braz. J. Poultry Sci. 20, 425–434.

Oliveira, D.M.N., Freitas, A.L.P., Carvalho, A.F.U., Sampaio, T.M.T., Farias, D.F., Teixeira, D.I.A., Gouveia, S.T. & Pereira, J.G., 2009. Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. Food Chem. 115(1), 254–259.

Parihar, A., Eubank, T.D. & Doseff, A.I., 2010. Monocytes and macrophages regulate immunity through dynamic networks of survival and cell death. J. Innate Immun. 2(3), 204–15.

Resiere, D., Mehdaoui, H., Florentin, J., Gueye, P., Lebrun, T., Blateau, A., Viguier, J., Valentino, R., Brouste, Y., Kallel, H., Megarbane, B., Cabie, A., Banydeen, R. & Neviere. R., 2020. Sargassum seaweed health menace in the Caribbean: clinical characteristics of a population exposed to hydrogen sulfide during the 2018 massive stranding. Clin. Toxicol. Phila. 59, 215–223.

Shah, Z.A., Ashraf, M. & Ishtiaq M., 2004. Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (wheat straw, leaves, sawdust). Pak. J. Nutr. 3, 158–160.

Shang, H.M., Song, H., Jiang, Y.Y., Ding, G.D., Xing, Y.L., Niu, S.L., Wu, B. & Wang, L.N., 2014. Influence of fermentation concentrate of *Hericium caput-medusae* (Bull.: Fr.) Pers. on performance, antioxidant status, and meat quality in broilers. Anim. Feed Sci. Technol. 198, 166–175.

Shannon, E. & Abu-Ghannam, N., 2019. Seaweeds as nutraceuticals for health and nutrition. Phycologia. 58, 563–577.

Shrivastava, B., Jain, K.K., Kalra, A. & Kuhad, R.C., 2014; Bioprocessing of wheat straw into nutritionally rich and digested cattle feed. Sci. Rep. 4, 1–9.

Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H.I. & Sartono, T.A., 2020. Growth performance, haematological responses, intestinal microbiology and carcass traits of broiler chickens fed finisher diets containing two-stage fermented banana peel meal. Trop. Anim. Health Prod. 52, 1425–1433.

Sweeney, T., Meredith, H., Ryan, M.T., Gath, V., Thornton, K. & O'Doherty, J.V., 2016. Effects of *Ascophyllum nodosum* supplementation on *Campylobacter jejuni* colonisation, performance and gut health following an experimental challenge in 10 day old chicks. Innov. Food Sci. Emerg. Technol. 37, 247–252.

Swirski, F.K., Nahrendorf, M., Etzrodt, M., Wildgruber, M., Cortez-Retamozo, V., Panizzi, P., Figueiredo, J.L., Kohler, R.H., Chudnovskiy, A., Waterman, P. & Aikawa, E., 2009. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science. 325(5940), 612–616.

Tóthová, C., Sesztáková, E., Bielik, B. & Nagy, O., 2019. Changes of total protein and protein fractions in broiler chickens during the fattening period. Vet. World. 12(4), 598–604.

Tuyen, V.D., Cone, J.W., Baars, J.J.P., Sonnenberg, A.S.M. & Hendriks, W.H., 2012. Fungal strain and incubation period affect the chemical composition and nutrient availability of wheat straw for rumen fermentation. Bioresour. Technol. 111, 336–342.

Yau, J.W., Teoh, H. & Verma, S., 2015. Endothelial cell control of thrombosis. BMC Cardiovasc. Disord. 15, 130.

Yokus, B., Cakir, D.U., Kanay, Z., Gulten, T. & Uysal, E., 2006. Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. J. Vet. Med. 53, 271–276.

Zhang, X., Cao, F., Sun, Z., Yu, W., Zhao, L., Wang, G., & Wang, T., 2012. Effect of feeding *Aspergillus niger*-fermented *Ginkgo biloba*-leaves on growth, small intestinal structure and function of broiler chicks. Livest. Sci. 147(1-3), 170–180.

6 CHAPTER SIX – EFFECT OF SEA BAMBOO SPENT OYSTER MUSHROOM SUBSTRATE ON CARCASS CHARACTERISTICS, VISCERAL MACROMORPHOMETRY, MEAT QUALITY AND STABILITY OF BOSCHVELD COCKERELS

Some parts of this chapter are submitted to a peer-review journal: **Mhlongo, G.** & Mnisi, C.M., 2023. Dietary incorporation of brown seaweed spent oyster mushroom substrate alters growth performance, physiological responses and meat quality parameters in Boschveld roosters. Scientific reports. (**Under review**).

Abstract

High fibre levels in sea bamboo meal (*Ecklonia maxima*; SBM) reduce the chickens' ability to utilise functional bioactive compounds that are known to improve carcass yield and meat quality. Pre-treatment of SBM with oyster (*Pleurotus ostreatus*) mushroom spawn (OMS) could reduce the negative effects of fibre and enhance the feed value of the spent mushroom substrate (SMS). Thus, this experiment investigated the effect of inoculating SBM with different levels of OMS on visceral macromorphometry, carcass characteristics, meat quality and shelf life indicators in Boschveld cockerels. Four-week-old Boschveld cockerels (n = 324; 310.8 ± 13.98 g live weight) were raised on six isonitrogenous and isoenergetic experimental diets formulated as follows: a standard grower diet (CON); and CON containing 150 g/kg of SBM spent oyster mushroom substrate after being inoculated with 0 (SMS0), 20 (SMS20), 30 (SMS30), 40 (SMS40) and 50% (SMS50) of the spawn. At 15 weeks of age, the cockerels were slaughtered to determine viscera macromorphometry, carcass characteristics, and meat quality characteristics. Feeding graded levels of OMS inoculated SBM induced neither linear nor quadratic effects (P >0.05) on carcass characteristics and meat quality parameters. However, slaughter weight [R^2 = 0.197; P = 0.017] and breast weight [R^2 = 0.197; P = 0.020] linearly increased as OMS levels increased. Birds reared on diet SMS40 had the largest slaughter weight (1722.2 g/bird) than those reared with the CON, SMS0 and SMS20 diets, which did not differ (*P* >0.05). Relative caecal weights showed a linear decline [R^2 = 0.176; *P* = 0.035], while relative spleen weights [R^2 = 0.238; *P* = 0.023] and large intestine lengths [R^2 = 0.217; *P* = 0.015] showed positive and negative quadratic effects to dietary OMS levels, respectively. Birds reared on SMS40 diet had longer caeca (21.12 cm) than those reared on CON, SMS0, SMS20, SMS30 and SMS50 diets. For shelf-life indicators, a time × diet interaction effect was observed on meat yellowness (*b**). It was concluded that inoculation of SBM with OMS improved slaughter, breast weights, and altered viscera macromorphometry but had no influence on the breast meat quality of Boschveld cockerels.

Keywords: Chickens, Sea bamboo, Carcass characteristics, Meat quality, Visceral morphometry

6.1 Introduction

The global consumption of animal-based protein is projected to increase by 14% in 2030 due to factors such as income and population growth, as well as urbanisation (FAO, 2021). Poultry meat is preferred over red meat because of its lower saturated fat, high protein, essential amino acids content, lower caloric value and relative affordability compared to red meats costs (Kralik *et al*., 2018; Chivandi *et al*., 2020). These is the reason poultry meat is predicted to account for 41% of all meat protein sources in 2030 (FAO, 2021). Despite this, the poultry industry relies heavily on high-performing conventional broiler breeds to increase product output, leading to a gap in the large-scale intensification of indigenous chickens. Indigenous chickens have received limited genetic progress hence they continue to be excluded from high-input production systems. These chickens are characterised by low growth rates, poor feed conversion ratios, and late sexual maturity (Hussain *et al*., 2018; Atela *et al*., 2019). Thus, this has necessitated the need to explore functional feed components that are readily accessible and locally available for use in indigenous chicken diets.

Currently, sea bamboo is a promising natural resource with various biologically active components that offer beneficial health benefits (Kim & Joo, 2008; Liu *et al*., 2012). These marine plants are rich in active pigments, polyphenols, polysaccharides, and polyunsaturated fatty acids, which are bioactive compounds with antioxidant, antiviral, and antifungal properties that can enhance the quality of poultry products (Balasubramanian *et al*., 2021). The potential benefits of sea bamboo include the ability to boost the avian immune system, reduce pathogenic microorganisms, and minimise oxidation reactions in meat products (Burgin, 2016; Roohinejad *et al*., 2017).

However, sea bamboo contains high dietary fibre levels (486.8 g neutral detergent fibre /kg DM, 226.6 g acid detergent fibre /kg DM and 40.65 g acid detergent lignin /kg DM), primarily composed of non-starch polysaccharides (NSPs) such as cellulose, xylan, ulvan and pectin (Mlambo *et al*., 2022). Additionally, the rigidity of seaweed cell walls, as reported by Wells *et al*. (2017), can hinder the release and bioavailability of bioactive compounds, potentially reducing nutrient utilisation. Indeed, Jha & Mishra (2021) observed a negative relationship between dietary fibre concentration and the digestibility of protein and fats. Therefore, employing oyster mushroom spawn (OMS) to degrade NSP and lignin in sea bamboo for Boschveld cockerels represents a sustainable innovative strategy.

Andlar *et al*. (2018) reported that the mushroom mycelia or white-rot fungi produce a variety of ligninolytic enzymes such as manganese-dependent peroxidases, lignin peroxidases, laccases and versatile peroxidases. These enzymes catalyse the oxidation of a wide range of aromatic substrates, producing aromatic radicals and modifying the structure of lignocellulose-containing raw materials (Andlar *et al*., 2018). However, no studies have explored the potential use of OMS to enhance the nutritive value of SBM for Boschveld cockerels. Therefore, this study evaluated the effect of inoculating SBM with incremental levels of OMS on viscera morphometry, carcass characteristics, meat quality and stability of Boschveld cockerels. The study tested the hypothesis that including the sea bamboo SMS in Boschveld cockerel diets, will improve viscera macromorphometry, carcass characteristics, and breast meat quality parameters and shelf life indicators of the birds.

6.2 Materials and methods

6.2.1 Study area, ingredients, substrate preparation, diet formulation and analyses

The study site and ingredient sources were as described in Chapter 5, subheading 5.2.1, whereas the inoculation of sea bamboo with OMS, diet formulation and analyses of the SMS were outlined in Chapter 5, subheading 5.2.2 and 5.2.3, respectively.

6.2.2 Feeding trial and cockerels management

The feeding trial, experimental design and the cockerel's management were as outlined in Chapter 5, subheading 5.2.4.

6.2.3 Slaughter, carcass traits and internal organs

At 15 weeks of age, all the cockerels were weighed to determine slaughter weights (final body weight). A total of 229 cockerels were slaughtered at the local abattoir as described in Chapter 4, subheading 4.2.2. Hot carcass (HCW) and cold carcass (CCW) weights, dressing percentage, carcass cuts and internal organs were measured as described in Chapter 4, subheading 4.2.2.

6.2.4 Statistical analyes

To account for dietary variations, the general linear model (GLM) procedure of SAS version 9.3 (SAS, 2010) was employed as described in Chapter 3, subheading 3.2.9. Response surface regression was used to determine the linear and quadratic coefficients of OMS levels using the model described in Chapter 3, subheading 3.2.9. The CON data was not included in the regression analysis. The repeated measures analysis was used to analyse breast meat shelf-life indicators as described in Chapter 4, subheading 4.2.8.

6.3 Results

6.3.1 Carcass characteristics and internal organs

Table 6.1 shows that there were linear increases for slaughter weight [y = 2.76 (±2.649) x + 1641.8 (±30.61); R^2 = 0.197; P = 0.017] and breast weight [y = 0.048 (±0.034) x + 8.477 (±0.395); R^2 = 0.197; P = 0.020] as OMS levels increased. Relative weights of drumstick, thigh and wing did not exhibit either linear or quadratic effects (P >0.05) in response to the spent oyster mushroom substrate inclusion in the diets. The SMS-containing diets had a significant effect on the slaughter weight only, where CON, SMS0 and SMS20 diets promoted the least (P <0.05) slaughter weights than the SMS30, SMS40 and SMS50 diets. The orthogonal contrasts also showed that diet CON and SMS0 promoted similar (P > 0.05) carcass characteristics.

Table 6.1. Carcass characteristics (g /100 g CCW, unless stated otherwise) of Boschveld cockerels fed diets containing sea bamboo spent oyster mushroom substrate

[2]Parameters	[1]Experimental diets						[3]SEM	Significance		
	CON	SMS0	SMS20	SMS30	SMS40	SMS50		P-GLM	P-Linear	P-Quadratic
Slaughter weight (g)	1640.8[a]	1653.0[a]	1646.4[a]	1734.9[b]	1762.7[b]	1722.2[ab]	28.69	0.015	0.017	0.784
HCW (g)	1208.0	1210.0	1197.1	1256.9	1202.9	1234.1	33.16	0.794	0.621	0.972
CCW (g)	1172.7	1173.0	1163.4	1214.5	1179.0	1195.1	31.22	0.873	0.556	0.927
Dressing (%)	73.60	73.23	72.64	72.42	69.96	71.70	1.562	0.630	0.259	0.909
Breast	9.94	8.47	9.19	9.78	9.38	9.86	0.442	0.197	0.020	0.498
Drumstick	7.38	7.64	7.41	7.64	7.60	7.45	0.140	0.626	0.630	0.983
Thigh	6.09	6.41	6.31	6.23	6.41	6.24	0.139	0.570	0.391	0.720
Wing	7.95	7.75	7.92	7.59	8.01	7.82	0.204	0.719	0.719	0.990
Drumstick (cm)	18.51	13.75	14.16	14.30	14.05	13.89	1.820	0.423	0.629	0.094
Thigh (cm)	9.64	9.31	9.74	9.62	10.00	9.58	0.290	0.940	0.962	0.476
Wing (cm)	23.27	23.05	23.66	23.09	23.47	22.99	0.536	0.690	0.307	0.346
Back (cm)	17.08	18.46	17.84	19.08	17.46	17.42	0.438	0.023	0.308	0.181

[a,b] Means, in a row, with different superscripts denote statistical differences (P <0.05).

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.

[3]SEM = standard error of the mean

Table 6.2 indicates that there were neither linear nor quadratic responses for viscera morphometry, except for relative caecal and spleen weights, as well as large intestine weight and length. Relative caecal weight linearly declined [y = 1.048 (±0.036) – 0.005 (±0.003) x; R^2 = 0.176; P = 0.035] with increasing OMS levels. A positive quadratic effect was observed for relative spleen weight [y = 0.0001 (±0.0001) x^2 – 0.008 (±0.003) x + 0.350 (±0.033); R^2 = 0.238; P = 0.023], whereas a negative quadratic effect was observed for relative large intestine weight [y = 0.151 (±0.014) + 0.003 (±0.001) x – 0.0001 (±0.00002) x^2; R^2 = 0.210; P = 0.015] and length [y = 4.294 (±0.255) + 0.060 (±0.022) x – 0.001 (±0.0004) x^2; R^2 = 0.217; P = 0.015]. Diet SMS0 promoted a higher relative caeca weight (1.045 % CCW) than CON (0.864 % CCW) but was similar (P >0.05) to SMS20, SMS40 and SMS50 diets. Birds fed the SMS40 diet had a higher caeca length (21.12 % CCW) than the CON (18.05 % CCW), but were all similar (P >0.05) to the other treatment groups. Relative to CON, SMS0 promoted a larger relative gizzard (P = 0.036) and caeca weights (P = 0.009) but the rest of the internal organs were not different (P >0.05).

Table 6.2. Sizes of visceral organs (g /100 g CCW, unless stated otherwise) of 15-week-old Boschveld cockerels fed diets containing sea bamboo spent oyster mushroom substrate

Parameters	[1]Experimntal diets						[2]SEM	Significance		
	CON	SMS0	SMS20	SMS30	SMS40	SMS50		P-GLM	P-Linear	P-Quadratic
Liver	2.053	2.230	2.048	2.088	1.992	2.137	0.066	0.196	0.170	0.075
Gizzard	3.063	3.651	3.744	3.650	3.440	3.609	0.156	0.052	0.444	0.767
Spleen	0.271	0.352	0.231	0.241	0.240	0.282	0.033	0.135	0.117	0.023
Proventriculus	0.456	0.482	0.532	0.504	0.462	0.534	0.022	0.059	0.573	0.921
Duodenum	1.049	1.063	1.083	1.099	1.117	1.120	0.039	0.734	0.201	0.992
Jejunum	1.467	1.577	1.614	1.501	1.503	1.542	0.059	0.527	0.372	0.917
Ileum	1.178	1.256	1.298	1.250	1.359	1.309	0.060	0.397	0.325	0.993
Caeca	0.864[a]	1.045[c]	1.001[bc]	0.901[ab]	0.981[abc]	0.936[abc]	0.033	0.005	0.035	0.368
Small intestines	3.694	3.896	3.995	3.851	3.979	3.972	0.121	0.495	0.682	0.955
Large intestines	0.165	0.149	0.191	0.179	0.151	0.145	0.014	0.166	0.548	0.015
Duodenum (cm)	27.12	26.38	26.84	27.69	26.11	27.19	0.677	0.601	0.618	0.625
Jejunum (cm)	52.98	52.31	54.30	53.49	56.12	56.52	1.815	0.373	0.941	0.403
Ileum (cm)	52.13	51.62	54.48	52.53	56.87	55.90	2.851	0.504	0.061	0.801
Caeca (cm)	18.05[a]	19.84[ab]	20.04[ab]	19.75[ab]	21.12[b]	20.07[ab]	0.571	0.025	0.403	0.898
Small intestines (cm)	129.7	130.2	131.4	148.4	131.6	135.5	6.883	0.396	0.586	0.420
Large intestines (cm)	5.203	4.301	4.985	5.245	4.811	4.552	0.297	0.200	0.425	0.015

[a,b,c] Means, in a row, with different superscripts denote statistical differences (P <0.05).

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.

[2]SEM = standard error of the mean.

6.3.2 Meat quality parameters

Table 6.3 shows that there were neither linear nor quadratic trends (*P* >0.05) observed for all breast meat quality parameters measured at 1-h and 24-h post-mortem in response to increasing dietary OMS levels. Likewise, no dietary effects were recorded on breast meat quality parameters of Boschveld cockerels. The orthogonal contrasts also showed that CON and SMS0 promoted similar (*P* >0.05) breast meat quality.

Table 6.3. Breast meat quality parameters of 15-week-old Boschveld cockerels fed diets containing spent oyster mushroom sea bamboo substrate

	[1]Experimntal diets							Significance		
[2]Parameters	CON	SMS0	SMS20	SMS30	SMS40	SMS50	[3]SEM	P-GLM	P-Linear	P-Quadratic
pH_1	5.764	5.774	5.758	5.754	5.716	5.746	0.033	0.87	0.163	0.559
$Temp_1$ (°C)	31.51	30.69	30.35	29.88	29.74	30.74	0.568	0.799	0.687	0.563
L^*_1	59.73	63.35	60.92	64.02	61.42	60.87	1.140	0.099	0.294	0.781
a^*_1	1.076	0.926	0.720	1.230	0.848	0.815	0.152	0.218	0.758	0.638
b^*_1	3.171	2.285	3.295	2.480	3.289	2.868	0.411	0.377	0.424	0.473
$Chroma_1$	3.371	2.520	3.384	2.805	3.430	3.015	0.393	0.497	0.466	0.437
Hue $angle_1$	1.229	1.143	1.349	1.115	1.249	1.287	0.070	0.194	0.387	0.815
pH_{24}	5.823	5.852	5.881	5.861	5.848	5.865	0.489	0.559	0.602	0.33
$Temp_{24}$ (°C)	11.77	12.56	12.31	11.89	11.73	10.50	1.044	0.799	0.294	0.426
L^*_{24}	65.45	65.69	65.66	67.47	66.37	65.91	0.687	0.343	0.872	0.521
a^*_{24}	1.472	1.849	1.207	2.085	1.503	1.579	0.287	0.354	0.599	0.689
b^*_{24}	3.892	2.425	2.916	2.267	2.836	3.116	0.660	0.584	0.658	0.481
WHC	11.34	13.50	11.34	11.75	13.39	14.35	1.683	0.707	0.969	0.066
Drip loss	12.01	12.45	8.67	12.34	10.81	11.98	1.196	0.231	0.865	0.211
Cooking loss	5.177	6.260	5.584	5.384	5.895	6.088	0.624	0.824	0.917	0.318
Shear force (N)	11.57	11.92	9.797	13.04	13.50	11.00	1.388	0.463	0.889	0.865

[1]Diets: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS.

[2]Parameters: *L**, lightness; *a**, redness; *b**, yellowness; WHC, water holding capacity.

[3]SEM = standard error of the mean.

6.3.3 *Shelf life*

Figures 6.1 – 6.4 display the shelf-life indicators of Boschveld cockerels fed diets containing sea bamboo spent oyster mushroom substrate. Repeated measures analysis showed no significant time × diet interaction effects on breast meat pH, *L** and *a**, but on *b**. Figure 6.1 shows that there were neither linear nor quadratic effects (P >0.05) observed for pH at days 1, 4, 5 and 6, except for days 2 and 3. The SBM spent oyster mushroom substrate quadratically decreased the breast pH values on day 2 [y = 5.84 (±0.022) – 0.004 (±0.002) x – 0.0001 (±0.00004) x^2; R^2 = 0.178; P = 0.035]. A negative linear trend was observed for meat pH at day 3 [y = 6.102 (±0.074) – 0.011 (±0.006) x; R^2 = 0.256; P = 0.009] in response to the increasing OMS levels (Figure 6.1). There was no significant dietary effect on meat pH on days 1, 2, 4, 5 and 6, except for day 3. On day 3 of storage, the CON promoted the highest pH value than diets SMS20, SMS30, SMS40 and SMS50, which did not differ (P >0.05) from diet SMS0. The CON had a similar (P >0.05) pH as diet SMS0.

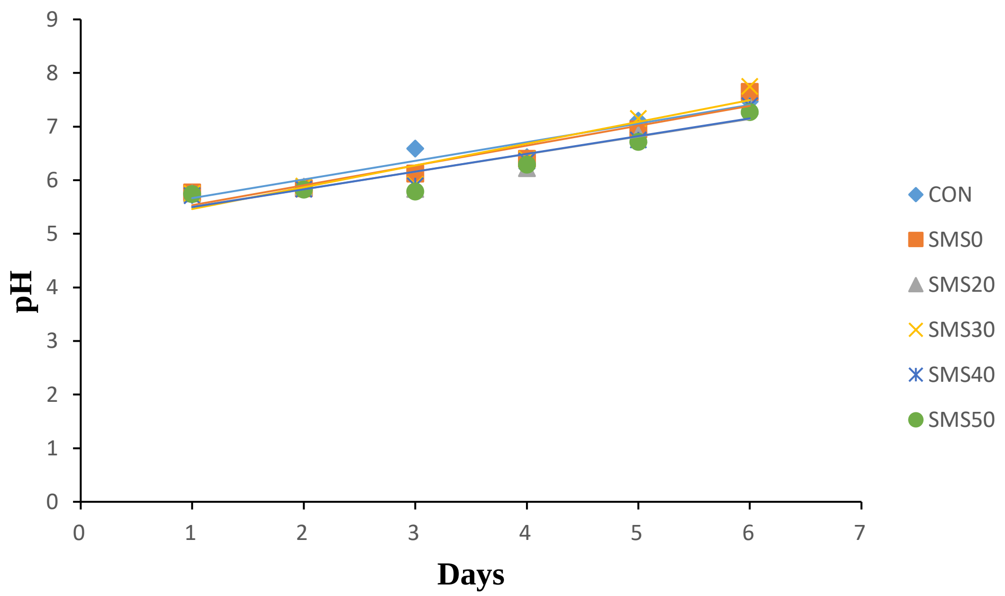

Figure 6.1. Effect of sea bamboo spent oyster mushroom substrate on stability of breast meat pH upon storage at room temperature for 6 days [*Diets*: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150

g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS].

Figure 6.2 indicates that there were no linear effects for breast meat lightness (L^*) for the entire storage duration. However, positive quadratic effects were observed on days 5 [y = 44.34 (±1.163) + 0.204 (±0.096) x – 0.004 (±0.002) x^2; R^2 = 0.167; P = 0.031] and 6 [y = 40.806 (±1.259) + 0.262 (±0.104) x – 0.005 (±0.002) x^2; R^2 = 0.202; P = 0.017] as dietary OMS levels increased. There was no significant dietary effect on meat L^* value on days 1 to 5, except on day 6 of storage. Diet SMS40 (44.18) promoted the highest L^* value than diet SMS50 (40.16), which did not differ (P >0.05) from the CON, SMS0 and SMS20 diets.

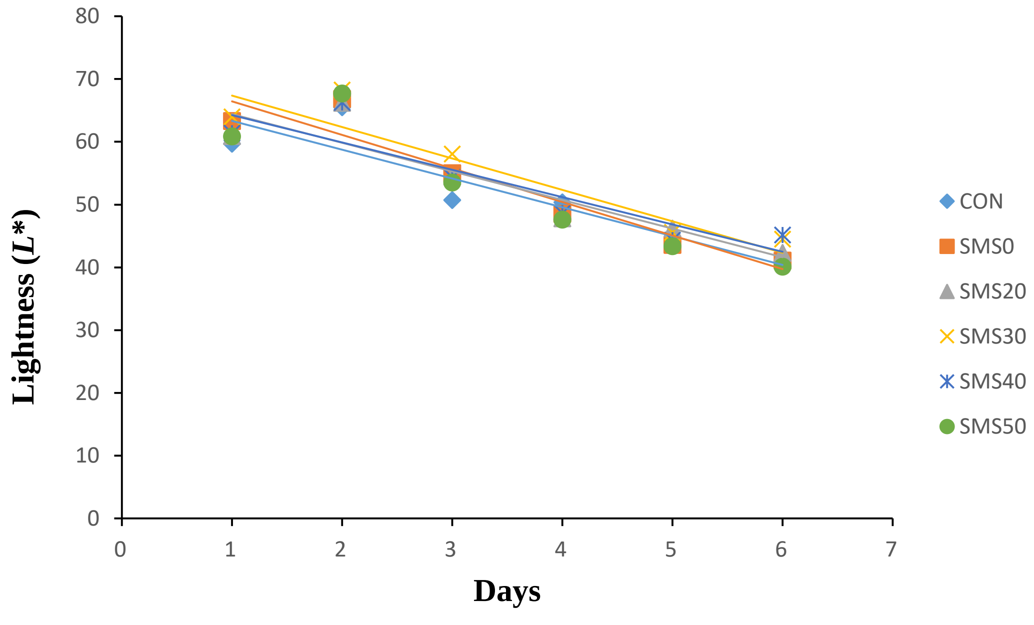

Figure 6.2. Effect of sea bamboo spent oyster mushroom substrate on stability of breast meat lightness upon storage at room temperature for 6 days [*Diets*: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS,

SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS]

Figure 6.3 shows that there were neither linear nor quadratic effects (P >0.05) observed for breast meat redness (a*), except for a negative quadratic trend observed on day 5 [y = 5.301 (±0.499) – 0.098 (±0.041) x + 0.002 (±0.0008) x^2; R^2 = 0.025; P = 0.182]. There were no dietary SMS effects detected on the meat redness of Boschveld cockerels.

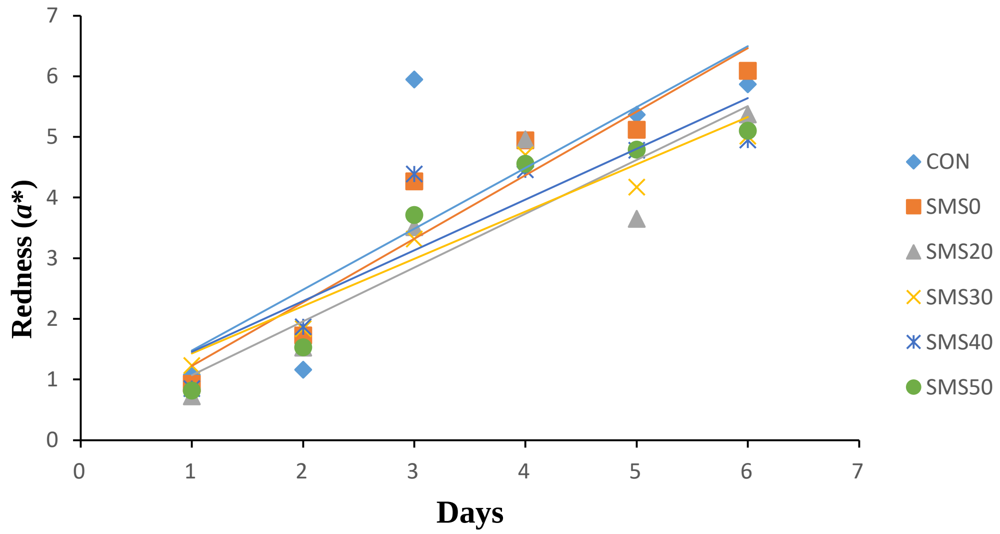

Figure 6.3. Effect of sea bamboo spent oyster mushroom substrate on stability of breast meat redness upon storage at room temperature for 6 days [*Diets*: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS]

Figure 6.4 shows that there were neither linear nor quadratic effects (*P* >0.05) observed for breast meat yellowness (*b**), apart from a negative quadratic response observed on day 4 [y = 2.51 (±0.623) + 0.224 (±0.052) x – 0.004 (±0.001) x^2; R^2 = 0.424; *P* = 0.0005] as dietary OMS levels increased. There was no significant dietary effect on meat *b** value on days 1, 2, 3, 5, and 6, except for day 4 of storage. Diet SMS40 (6.063) promoted the highest *b** value than the CON (1.093), which did not differ (*P* >0.05) from the SMS0 diet. There were no statistical differences between diets SMS30 and SMS40 in terms of the *b** value. Diets SMS20, SMS30 and SMS50 promoted a similar (*P* >0.05) *b** value on day 4.

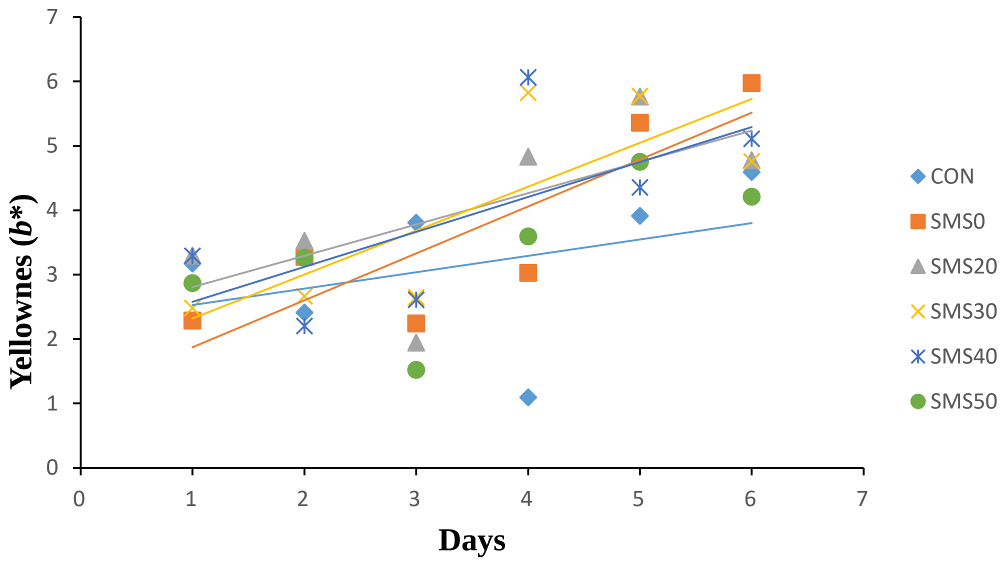

Figure 6.4. Effect of sea bamboo spent oyster mushroom substrate on stability of breast meat yellowness upon storage at room temperature for 6 days [*Diets*: CON = a standard grower diet, SMS0 = CON containing 150 g/kg of SBM without OMS inoculation, SMS20 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 20% OMS, SMS30 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 30% OMS, SMS40 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 40% OMS, and SMS50 = CON containing 150 g/kg of SBM spent substrate after being inoculated with 50% OMS]

6.4 Discussion

6.4.1 Carcass characteristics and internal organs

Higher carcass yield has been closely linked to the profitability and viability of poultry production. In this study, slaughter and breast weights improved upon feeding the sea bamboo SMS treatments, which could be attributed to the improvements observed in growth performance metrices (Chapter 5). However, feeding SMS-containing diets did not affect the weights of breast, drumstick, thigh and wings of birds, which does not align with the findings reported by Sugiharto *et al*. (2020), who found that feeding broiler chickens with finisher diets supplemented with 50, 100 and 150 g/kg of *Chrysonilia crassa* and *Bacillus subtilis* two-stage fermented banana peels reduced the thigh weights relative to the control-fed broiler chickens. Nonetheless, the breast, drumstick, wing and back sizes were also not affected by the two-stage fermentation of the banana peels.

The liver, ventriculus and proventriculus development were not affected by the OMS-inoculated SBM diets. In a case where the liver is injured, the alanine aminotransferase (ALT) and alkaline phosphatase (ALKP) enzymes are released into the blood as standard biomarkers for diagnosing hepatocellular damage (Sherwin, 2003). High concentrations of ALT and ALKP liver enzymes in the blood are linked to hepatocellular injury, which can result from circulatory hypoxia, metabolic abnormalities, inflammation, hepatocyte growth, exposure to toxins and toxaemia (Sherwin, 2003). As a result, the normal levels of ALT and ALKP enzymes produced by cockerels fed SMS-containing diets indicate that SMS was a safe feed ingredient.

The spleen is a vital immunological organ in birds, its size can be utilized as an indicator of immune system responses under different conditions, such as infections, inflammation, increasing growth rate and different breeds in males (Vali *et al*., 2023). Tao *et al*. (2000) reported that birds with enlarged spleen indicate a possibility of an infection or a disease. The observed quadratic increase in the relative spleen weights is indicative that the birds might have suffered from an acute condition, based on the increased production of platelets (thrombocytosis) and a higher H/L ratio observed in Chapter 5 upon feeding SMS-containing diets. The lower NDF, ADF, and ADL levels in SMS treatments could explain the linear decline in relative caeca weights that is said to enlarge as an adaptive mechanism to handle high fibre levels (Manyeula *et al*., 2020). This could also explain the observed negative quadratic response in relative large intestine weight. This was expected considering that the SMS40 and SMS50 diets tended to have lower fibre content (NDF, ADF, and ADL) than the SMS0, SMS20 and SMS30 diets.

6.4.2 Breast meat quality parameters

The overall consumer approval of meat and meat products is influenced by pH, colour, water retention, texture and sensory qualities (Yu *et al*., 2017). Consumers tend to reject meat items because of unusual appearances that deviate from the expected norm (Hlatshwayo *et al*., 2023). However, no variations were observed in meat colour across all treatment groups, indicating that feeding the SMS-containing diets did not influence the meat appearance. Meat quality indicators such as cooking, WHC, drip loss, loss and tenderness depend on the pH value of the meat (Singh & Deshpande, 2018). This is also influenced by glycogen levels in meat muscle before slaughter and the extent to which glycogen is converted to lactic acid postmortem (Moreno *et al*., 2020). Nonetheless, the SMS treatments had no effect on the meat initial (pH_1; 5.72–5.77) and ultimate pH (pH_{24}; 5.82 – 5.88), which were within the normal meat pH range (5.7 – 6.1) for poultry meat (Zhang & Barbut, 2005). This could be the reason no dietary effects were observed on the measured meat quality parameters. The shearing force of meat indicates its tenderness (Pearce *et al*., 2011), thus the absence of dietary effects supports that feeding the SMS treatments does not alter meat tenderness.

The ability of the meat to hold onto water when external forces are applied (pressing, cutting, and grinding) is referred to as WHC (Bowker & Zhuang, 2015). Warner (2017) reported that the tissue's pH plays a major factor in determining how much water is lost or kept; for example, a higher pH leads to less water loss, whereas a lower pH value dictates how much water is retained by the meat. When the pH of chicken meat is low, it causes protein denaturation, which further reduces the meat's WHC (Singh & Deshpande, 2018). The lack of dietary differences in meat pH could explain the lack of variations in the WHC potential of the meat. Indeed, there were no dietary effects on the post-mortem drip and cooking losses, which further indicated that the SBM and SBM spent oyster mushroom substrates did not induce any oxidative stability for meat during storage. Contrary to these findings, Shang *et al*. (2014) reported improvements in the meat colour, drip loss and cooking loss of broiler chickens fed with diets supplemented (6, 12 and 18 g/kg) with *Hericium caput-medusae* (Bull.:Fr.) Pers. fungus powder. These contradictory findings could be attributed to the use of different fungal species, culturing, substrate and treatment methods.

Repeated measures analysis revealed a significant time × diet interaction effect on breast meat yellowness (b^*), indicating that the yellowness of the meat varied with storage duration. On the 4^{th} day of storage, the breast meat b^* values increased, suggesting an increase in metmyoglobin formation in the meat muscles (Seyfert *et al*., 2006). The meat yellowness could be due to the continued exposure of the meat to oxygen during storage, causing oxidative metabolism and producing free radical by-products that eventually induce myoglobin oxidation, resulting in the brown pigment metmyoglobin (Corlett *et al*., 2021).

6.5 Conclusion

The incorporation of sea bamboo spent oyster mushroom substrate in the diets of Boschveld cockerels improved the slaughter weights, breast weights and certain viscera macromorphometry. However, there were no observed improvements in meat quality and stability attributes compared to the control diet. The sea bamboo spent mushroom substrates did not demonstrate inhibitory effects on microbial growth and oxidation reactions on breast meat, as evidenced by similar shelf-life indicators across the treatments.

6.6 References

Andlar, M., Rezić, T., Marđetko, N., Kracher, D., Ludwig, R. & Šantek, B., 2018. Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation. Eng. Life Sci. 18(11), 768–778.

Atela, J., Mlambo, V. & Mnisi, C.M., 2019. A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chickens. Anim. Nutr. 5, 179–184.

Balasubramanian, B., Shanmugam, S., Park, S., Recharla, N., Koo, J.S., Andretta, I. & Kim, I.H., 2021. Supplemental impact of marine red seaweed (*Halymenia palmata*) on the growth performance, total tract nutrient digestibility, blood profiles, intestine histomorphology, meat quality, faecal gas emission, and microbial counts in broilers. Animals. 11, 124.

Bowker, B. & Zhuang, H., 2015. Relationship between water-holding capacity and protein denaturation in broiler breast meat. Poult. Sci. 94(7), 1657–1664.

Burgin, R., 2016. Benefits of seaweed in poultry diets. Poultry world. Doetinchem, Nederland https://www.poultryworld.net/Nutrition/Articles/2016/2/Benefits-of-seaweed-in-poultry-diets–2761921W. Accessed 20 June 2023.

Chivandi, E., Mdoda, B., B.W. & Lembede, B.W., 2020. Red milkwood (*Mimusops zeyheri*) seed meal can replace maize meal in Japanese quail finisher diets without compromising growth performance, feed economy and carcass yield. Vet. Anim. Sci. 10, 100128.

Corlett, M.T., Pethick, D.W., Kelman, K.R., Jacob, R.H. & Gardner, G.E., 2021. Consumer perceptions of meat redness were strongly influenced by storage and display times. Foods. 10, 540.

Evans, F. & Critchley, A.T., 2014. Seaweeds for animal production use. J. Appl. Phycol. 26, 891–899.

FAO. 2021. OECD-FAO Agricultural Outlook2021–2030. Paris, France: OECD Publishing.

Hlatshwayo, I.S., Mnisi, C.M. & Egbu, C.F., 2023. Effect of dietary olive (*Olea europea*) pomace on productive performance, and physiological and meat quality parameters in Jumbo quail. Sci. Rep. 13, 6162.

Hussain, M., Mahmud, A., Hussain, J., Qaisrani, S.N., Mehmood, S. & Rehman. A., 2018. Subsequent effect of dietary lysine regimens fed in the starter phase on the growth performance, carcass traits and meat chemical composition of Aseel chicken in the grower phase. Rev. Bras. Cienc. Avic. 20, 455–462.

Jha, R. & Mishra, P., 2021. Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: a review. J. Anim. Sci. Biotechnol. 12, 51.

Kim, M.H. & Joo, H.G., 2008. Immunomodulatory effects of fucoidan on bone marrow-derived dendritic cells. Immunol. Lett. 115, 138–143.

Kralik, G., Kralik, Z., Grčević, M. & Hanžek, D., 2018. Quality of Chicken Meat. In Animal Husbandry and Nutrition; Yucel, B., Ed.; Intechopen: London, England. pp. 50–63.

Liu, F., Wang, J., Chang, A.K., Liu, B., Yang, L., Li, Q., Wang, P. & Zou, X., 2012. Fucoidan extract derived from *Undaria pinnatifida* inhibits angiogenesis by human umbilical vein endothelial cells. Phytomedicine. 19, 797–803.

Manyeula, F., Mlambo, V., Marume, U. & Sebola, A., 2020. Partial replacement of soybean products with canola meal in indigenous chicken diets: Size of internal organs, carcass characteristics and breast meat quality. Poult. Sci. 99, 256–262.

Mlambo, V., Mnisi, C.M., Matshogo, T.B. & Mhlongo, G., 2022. Prospects of dietary seaweeds and their bioactive compounds in sustainable poultry production systems: A symphony of good things?. Front. Anim. Sci. 3, 998042.

Moreno, I., Lipová, P., Ladero, L., Fernández-García, J.L. & Cava, R., 2020. Glycogen and lactate contents, pH and meat quality and gene expression in muscle *Longissimus dorsi* from iberian pigs under different rearing conditions. Livest. Sci. 240, 104167.

Pearce, K.L., Rosenvold, K., Andersen, H.J. & Hopkins, D.L., 2011. Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes–A review. Meat Sci. 89(2), 111–124.

Roohinejad, S., Koubaa, M., Barba, F.J., Saljoughian, S., Amid, M. & Greiner, R., 2017. Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. Food Res. Int. 99, 1066–1083.

Seyfert, M., Mancini, R.A., Hunt, M.C., Tang, J., Faustman, C. & Garcia, M., 2006. Color stability, reducing activity, and cytochrome coxidase activity of five bovine muscles. J. Agric. Food Chem. 54, 8919–8925.

Shang, H.M., Song, H., Jiang, Y.Y., Ding, G.D., Xing, Y.L., Niu, S.L., Wu, B. & Wang, L.N., 2014. Influence of fermentation concentrate of *Hericium caput-medusae* (Bull.: Fr.) Pers. on performance, antioxidant status, and meat quality in broilers. Anim. Feed Sci. Technol. 198, 166–175.

Sherwin, J.E., 2003. Liver Function. In *Clinical Chemistry: Theory, Analysis, Correlation*, 4th ed. Pages 492–506. Kaplan, L.A., Pesce, A.J., Kazmierczak, S.C., Eds.; Elsevier Science: St. Louis, MO, USA.

Singh, R. & Deshpande, D., 2018. Thermally induced changes in quality of chicken breast meat protein fractions. J. Nutr. Food Sci. 8, 1–5.

Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H.I. & Sartono, T.A., 2020. Growth performance, haematological responses, intestinal microbiology and carcass traits of broiler chickens fed finisher diets containing two-stage fermented banana peel meal. Trop. Anim. Health Prod. 52, 1425–1433.

Tao, K., Shao, J., Skeeles, K. & Chen, Y.R., 2000. Detection of splenomegaly in poultry carcasses by UV and color imaging. Trans. ASAE. 43(2), 469.

Vali, Y., Gumpenberger, M., Konicek, C. & Bagheri, S., 2023. Computed tomography of the spleen in chickens. Front. Vet. Sci. 10, 1153582.

Warner, R.D., 2017. "The eating quality of meat-IV water-holding capacity and juiciness," in Lawrie's meat science (Sawston, United Kingdom: Woodhead Publishing), pp. 419–459.

Wells, M.L., Potin, P., Craigie, J.S., Raven, J.A., Merchant, S.S. & Helliwell, K.E., 2017. Algae as nutritional and functional food sources: Revisiting our understanding. J. Appl. Phycol. 29 (2), 949–982.

Yu, Q.P., Feng, D.Y., Xiao, J., Wu, F., He, X.J., Xia, M.H., Dong, T., Liu, Y.H., Tan, H.Z., Zou, S.G., Zheng, T., Ou, X.H. & Zuo, J.J., 2017. Studies on meat color, myoglobin content, enzyme activities, and genes associated with oxidative potential of pigs slaughtered at different growth stages. Asian-Australas. J. Anim. Sci. 30(12), 1739–1750.

Zhang, L. & Barbut. S., 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. Br. Poult. Sci. 46, 687–693.

7 CHAPTER SEVEN – GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Introduction

This chapter presents a comprehensive discussion and conclusion of the study based on the overarching aim and specific objectives, contextualised within the existing literature. The study, explores an innovative strategy for enhancing the nutritional value of Boschveld cockerel diets, particularly through the integration of sea bamboo meal (SBM) spent oyster mushroom substrate. The study was motivated by the global surge in animal-based protein consumption, which is expected to increase by 14% in 2030, and the need for sustainable poultry production to meet this demand (FAO, 2021). Poultry meat, which offers nutritional benefits and cost-effectiveness, is a dominant source of animal protein for human consumption. However, conventional poultry production relies on high-performing broiler breeds, which marginalises indigenous chickens, such as the Boschveld cockerels, that have suboptimal growth rates and feed conversion ratios (Atela *et al*., 2019; Nhlane *et al*., 2021).

The study focused on sea bamboo, a promising natural resource nutrients that contains biologically active compounds with beneficial effects on poultry production, such as antimicrobial, antioxidant and growth-stimulating properties (Schiener *et al*., 2015). However, sea bamboo also has a high fibre content that limits its digestibility and feed value (Gullón *et al*., 2020). Therefore, the study proposed to use oyster mushroom spawn as a novel strategy to degrade non-starch polysaccharides (NSP) in SBM and improve its utilisation as a feed resource. The study reported notable improvements in growth performance metrices, slaughter weights, and viscera macromorphometry in Boschveld cockerels-fed diets enriched with SBM spent oyster mushroom substrate. These outcomes are significant in addressing the genetic constraints that have hindered the large-scale intensification of indigenous chickens (Hussain *et al*., 2018; Atela *et al*., 2019). Furthermore, the study examined the effects of the dietary interventions on haematological and serum biochemical indices, providing valuable insights into health implications, immune responses and stress factors in Boschveld cockerels.

The study also investigated the carcass characteristics, meat quality parameters, and shelf-life indicators of the Boschveld cockerels fed different diets. The intricate balance between nutrient utilisation, organ development, and meat quality, offering a nuanced perspective on the multifaceted impact of SBM spent oyster mushroom substrate on Boschveld cockerels were explored. This final chapter synthesises research and critically evaluates the implications of the findings within the broader poultry production landscape.

7.2 General discussion

The poultry industry continues to play an important role in providing nutritious, safe and affordable animal protein for the burgeoning human population (Castro *et al*., 2023). Nevertheless, the production of indigenous chickens has been overshadowed by conventional broiler and layer birds in modern-day poultry farming due to their lower feed efficiency and low productivity (Nhlane *et al*., 2020). Furthermore, the current limitations on large-scale intensification of indigenous chickens stem from high feeding costs attributed to their slow growth rates and poor feed conversion ratios (Larbi *et al*., 2013; Hussain *et al*., 2018).

This situation has necessitated the need to explore cost-effective, novel feed ingredients with nutraceutical benefits, such as SBM, to ensure the sustainable intensification of indigenous chickens. Sea bamboo meal can serve as a rich source of natural bioactive chemicals such as proteins and essential amino acids, polysaccharides, polyunsaturated fatty acids, vitamins, minerals, antioxidants and pigments such as carotenoids and chlorophylls (Miceli *et al*., 2021). Given its antioxidative, antifungal, antibacterial, anti-inflammatory and antiviral activities, SBM has the potential to boost poultry production and health (Mahrose & Michalak, 2022). Moreover, unlike conventional feedstock such as soybean and maize, which require land resources for cultivation, SBM can contribute to meeting the growing demand for animal protein as it grows rapidly and can be harvested from the ocean at low cost.

However, the inclusion of SBM in the diets of Boschveld cockerels remains unexplored in terms of its effect on growth performance, blood parameters and meat quality of these chickens. Therefore, the first feeding experiment in this study investigated the growth performance, blood profiles, carcass characteristics, and viscera morphometry, and meat quality indicators of Boschveld cockerels fed diets containing incremental levels of SBM. This experiment tested the hypothesis that growth performance, physiological and meat quality and stability indicators in Boschveld cockerels would exhibit positive responses to increasing dietary SBM levels. Consequently, five-week-old Boschveld cockerels were reared on five isonitrogenous and isoenergetic diets formulated by treating a standard grower diet with SBM at rates of 0 (SBM0), 20 (SBM20), 40 (SBM40), 60 (SBM60), and 80 g/kg (SBM80).

Results from this experiment showed that dietary SBM treatments led to improvements in feed intake and some viscera macromorphometry while altering alanine transaminase levels of Boschveld cockerels. However, growth performance, haematology, apparent retention of proximate components, and carcass and meat quality parameters of the birds were not affected or improved. Consequently, the hypothesis for the first experiment was rejected, as the study could not establish improvements in growth performance, blood parameters and meat quality traits from feeding SBM-containing diets. Thus, the optimum dietary inclusion level could not be determined for this study.

The inability to establish an optimum inclusion from feeding SBM in the first experiment necessitated the use of levels beyond 80 g/kg in the second feeding experiment. However, higher levels (e.g., 150 g/kg) of SBM without prior treatment may negatively suppress the growth performance of the birds due to its high fibre levels, as well as the presence of secondary metabolites in SBM. The presence of secondary metabolites with anti-nutritional factors such as phlorotannins, lectins, trypsin and alpha-amylase inhibitors in SBM (Oliveira *et al*., 2009), might negatively affect simple monogastric animals like chickens. As such, oyster mushroom (*Pleurotus ostreatus*) spawn (OMS) was used to valorise the feeding value of SBM and offset the negative effects of fibre. Therefore, the second experiment tested the hypothesis that inoculating SBM with different levels of OMS would improve growth performance, blood profiles, and carcass and meat quality parameters of Boschveld cockerels. Four-week-old Boschveld cockerels were reared on five isonitrogenous and isoenergetic dietary treatments formulated as follows: a standard grower diet (CON); and CON containing 150 g/kg of spent oyster mushroom substrate after being inoculated with 0 (SMS0), 20 (SMS20), 30 (SMS30), 40 (SMS40) and 50% (SMS50) of the spawn.

Results obtained from the second experiment showed that inoculating SBM with OMS tended to enhance the dry matter, phosphorus, calcium and arginine content of the SMS as the OMS levels increased. The crude fibre, neutral detergent fibre, acid detergent fibre and acid detergent lignin contents of SMS tended to be reduced as OMS levels increased. These improvements in nutrient composition could benefit the birds by boosting feed utilisation to body mass. Feeding OMS-inoculated SBM diets improved overall growth performance and altered the white blood cells, platelets, heterophils, lymphocytes and monocytes without affecting the general health of the cockerels. Further, feeding SMS-containing diets enhanced slaughter and breast weights, and altered the relative weights of proventriculus and caeca.

However, it had no significant effect on the carcass cuts and breast meat quality parameters except for the breast meat yellowness (b^*) on the 4th day of storage for the shelf-life indicators. The hypothesis in the second experiment was accepted based on the improved growth metrices and breast weights, but it was rejected for not improving the meat quality attributes of the Boschveld cockerels. Based on the haematological parameters results, the findings suggest that the blood profile of the birds was optimised between 15 and 20% of dietary OMS. This indicates that SMS should be used with caution in Boschveld cockerel's diets because it might comprise the bird's general health at inclusion levels beyond 20%. Overall, feeding diets containing sea bamboo meal spent oyster mushroom substrates proved to be an effective strategy in optimising the utility of sea bamboo meal as a potential nutraceutical for indigenous chickens.

7.3 Conclusions

Feeding diets containing incremental levels of SBM up to 80 g/kg did not improve growth performance metrices, blood parameters, and carcass and meat quality traits in the first feeding trial. The lack of improvements and the inability to determine an optimum inclusion level prompted the investigation of a higher level (150 g SBM / kg diet) in the second feeding trial. To address the potential negative effect of high fibre levels in SBM, oyster mushroom spawn was utilised to reduce fibre and enhance its utility for Boschveld chickens. The results from the second feeding trial demonstrated that feeding diets containing sea bamboo oyster mushroom substrates improved overall growth metrices, slaughter and breast weights in Boschveld cockerels.

7.4 Recommendations and future studies

The current study laid the foundation for future research into the utilisation of white-rot fungi (*Pleurotus ostreatus*) spawn to offset the high fibre levels of sea bamboo for optimal utilisation in diets of indigenous chickens. Based on the findings and limitations of this study, the following future studies are recommended:

- Future research should be conducted using various strains of mushroom spawn to improve the nutritive value of different seaweed species for inclusion in indigenous chicken diets.

- Future research can be conducted in characterization of the enzymes and their activities in oyster mushroom spawn.
- Exploring sensory panel evaluation, oxidative stability and the fatty acid profile of Boschveld cockerels breast meat fed diets containing sea bamboo meal or spent oyster mushroom-containing diets.
- Investigating the cost-benefit analysis, nutrient digestibility and spawn enzyme activities related to the utilisation of sea bamboo and oyster mushroom-containing diets for Boschveld cockerels.

These recommended studies might further advance our understanding of the potential benefits and challenges associated with incorporating sea bamboo meal and oyster mushroom spawn in indigenous chicken diets.

7.5 References

Atela, J., Mlambo, V. & Mnisi, C.M., 2019. A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chicken. Anim. Nutr. 5, 179–184.

Castro, F.L.S., Chai, L., Arango, J., Owens, C.M., Smith, P.A., Reichelt, S., DuBois, C. & Menconi, A., 2023. Poultry industry paradigms: connecting the dots. J. Appl. Poult. Res. 32(1), 100310.

FAO. 2021. OECD-FAO Agricultural Outlook2021–2030. Paris, France: OECD Publishing.

Gullón, B., Gagaoua, M., Barba, F.J., Gullón, P., Zhang, W. & Lorenzo, J.M., 2020. Seaweeds as promising resource of bioactive compounds: Overview of novel extraction strategies and design of tailored meat products. Trends Food Sci. Technol. 100, 1–18.

Hussain, M., Mahmud, A., Hussain, J., Qaisrani, S.N., Mehmood, S. & Rehman, A., 2018. Subsequent effect of dietary lysine regimens fed in the starter phase on the growth performance, carcass traits and meat chemical composition of aseel chicken in the grower phase. Braz. J. Poult. Sci. 20, 455–462.

Larbi, A., Rymkiewicz, P., Vasudev, A., Low, I., Shadan, N.B., Mustafah, S., Ayyadhury, S. & Fulop, T., 2013. The immune system in the elderly: a fair fight against diseases?. Aging Health. 9(1), 35–47.

Mahrose, K.M. & Michalak, I., 2022. Seaweeds for animal feed, current status, challenges, and opportunities. In: Ranga Rao, A., Ravishankar, G.A., Eds. Sustainable global resources of seaweeds, vol 1. Springer, Cham, pp. 357–379.

Miceli, A., Vetrano, F. & Moncada, A., 2021. Influence of *Ecklonia maxima* extracts on growth, yield, and postharvest quality of hydroponic leaf lettuce. Horticulturae. 7(11), 440.

Nhlane, L.T., Mnisi, C.M., Mlambo, V. & Madibana, M.J., 2020. Effect of seaweed-containing diets on visceral organ sizes, carcass characteristics, and meat quality and stability of Boschveld indigenous hens. Poult. Sci. 100(2), 949–956.

Nhlane, L.T., Mnisi, C.M., Mlambo, V. & Madibana, M.J., 2021. Effect of seaweed-containing diets on visceral organ sizes, carcass characteristics, and meat quality and stability of Boschveld indigenous hens. Poult. Sci. 100(2), 949–956

NRC, 1994. Nutrient Requirements of Poultry (9th ed.). National Research Council; National Academy Press, Washington DC, USA.

Oliveira, D.M.N., Freitas, A.L.P., Carvalho, A.F.U., Sampaio, T.M.T., Farias, D.F., Teixeira, D.I.A., Gouveia, S.T. & Pereira, J.G., 2009. Nutritive and non-nutritive attributes of washed-up seaweeds from the coast of Ceará, Brazil. Food Chem. 115(1), 254–259.

Schiener, P., Black, K.D., Stanley, M.S. & Green, D.H., 2015. The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. J. Appl. Phycol. 27, 363–373.

8 LIST OF APPENDICES

8.1 Appendix 1: Published from Chapters Three and Four

Effect of seaweed (*Ecklonia maxima*) on apparent nutrient digestibility, growth performance, and physiological and meat quality parameters in Boschveld cockerels

G. Mhlongo,[*] and C. M. Mnisi[*,†,1]

[*]*Department of Animal Science, School of Agricultural Science, North-West University, Mafikeng, South Africa; and* [†]*Food Security and Safety Focus Area, Faculty of Natural and Agricultural Science, North-West University, Mafikeng, South Africa*

ABSTRACT Despite being touted as a rich source of nutrients and functional bioactive compounds, the amount of brown seaweed (*Ecklonia maxima*) that can be included in diets of Boschveld indigenous chickens is unknown. This study, therefore, investigated the effect of feeding graded levels of brown seaweed meal (BSM) on apparent nutrient digestibility, growth performance, and physiological and meat quality parameters in Boschveld cockerels. A total of 225, five-wk-old Boschveld cockerels (316.4 ± 23.01 g live weight) were raised on 5 isoenergetic and isonitrogenous experimental diets formulated by incorporating BSM in a standard grower diet at a concentration of 0 (**BSM0**), 20 (**BSM2**), 40 (**BSM4**), 60 (**BSM6**), and 80 g/kg (**BSM8**). Feeding graded levels of dietary BSM induced neither quadratic nor linear effects ($P > 0.05$) on apparent nutrient digestibility, growth performance, hematological parameters, and meat quality characteristics in Boschveld cockerels. However, it resulted in linear increases for overall feed intake ($R^2 = 0.397$; $P = 0.021$), ceca weight ($R^2 = 0.417$; $P = 0.013$), duodenum length ($R^2 = 0.537$; $P = 0.04$), and small intestine length ($R^2 = 0.305$; $P = 0.041$). Negative quadratic responses were recorded for alanine aminotransferase ($R^2 = 0.530$; $P = 0.0009$) and ileum length ($R^2 = 0.457$; $P = 0.045$) as BSM levels increased. In conclusion, dietary inclusion of BSM improved feed intake and some internal organ sizes, altered alanine transaminase levels, but had no significant effect on apparent nutrient digestibility, growth performance, and carcass and meat quality attributes of Boschveld indigenous cockerels.

Key words: blood profile, brown seaweed, growth performance, indigenous chicken, meat quality

2023 Poultry Science 102:102361
https://doi.org/10.1016/j.psj.2022.102361

INTRODUCTION

Poultry products (meat and eggs) are a major source of affordable animal protein in South Africa with an overall per capita consumption of 48.22 kg (SAPA, 2020). However, the poultry industry is under pressure to increase production to meet the high demand for animal protein in response to a rapidly growing human population (Nhlane et al., 2021). This suggests a need to develop strategies that will allow sustainable intensification of indigenous chickens (*Gallus gallus domesticus*) as a main source of animal protein for people in rural South Africa. Indigenous chicken farming plays significant socioeconomic and nutritional roles by providing income, employment, and essential macro- and micronutrients for many rural households (Magothe et al., 2012; Manyelo et al., 2020). These birds possess desirable traits such as disease resistance, thermo-tolerance, firm texture, succulent meat, high fertility and hatchability, and hard eggshells (Dessie, 2011; Atela et al., 2019). However, their large-scale intensification is currently restricted by high feeding costs due to their low growth rates and poor feed conversion ratios (Larbi et al., 2013; Hussain et al., 2018). From a nutritional point of view, the use of functional feed ingredients such as seaweeds can improve indigenous chicken production and thus allow for their sustainable intensification. This is because the production of seaweed, also known as marine macro-algae, does not require land, fresh water, fertilizers, pesticides, or machinery. Moreover, seaweeds can be considered as environmentally friendly ingredients whose bioactive compounds and nutrients have growth boosting, health promoting, gut modulating, and meat enhancing properties (Michalak et al., 2022). Indeed, Nhlane et al. (2020) reported that seaweeds

Received August 23, 2022.
Accepted November 17, 2022.
[1]Corresponding author: 23257539@nwu.ac.za

8.2 Appendix 2: Ethics Certificate

NWU® NORTH-WEST UNIVERSITY

Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Fax: 018 299-4910
Web: http://www.nwu.ac.za

Research Ethics Regulatory Committee
Tel: 018 299-4849
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 29/07/2021, 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: Towards the optimization of sea bamboo (Ecklonia maxima) for indigenous chickens using spent oyster mushrooms

Study Leader/Supervisor (Principal Investigator)/Researcher: Prof K Mnisi

Student: Godfrey Mhlongo

Ethics number: N W U - 0 0 8 0 9 - 2 1 - A 5
Institution Study Number Year Status

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

Application Type: Single Study
Commencement date: 2023/08/10 **Risk Category: 2**
Expiry date: 2024/08/09
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:*

8.3 Appendix 3: Turnin Report

23257539:Mhlongo_G_26735970_PhD_Thesis.docx

ORIGINALITY REPORT

14%	14%	16%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.mdpi.com Internet Source	2%
2	res.mdpi.com Internet Source	2%
3	Sicelo Beauty Dlamini, Caven Mguvane Mnisi, Collins Njie Ateba, Chidozie Freedom Egbu, Victor Mlambo. "In-feed Salmonella-specific phages alter the physiology, intestinal histomorphology, and carcass and meat quality parameters in broiler chickens", Scientific African, 2023 Publication	1%
4	static.frontiersin.org Internet Source	1%
5	Cristina M. Guedes, Mariana Almeida, Maude Closson, Sofia Garcia-Santos et al. "Effect of Total Replacement of Soya Bean Meal by Whole Lupine Seeds and of Gender on the Meat Quality and Fatty Acids Profile of Growing Rabbits", Foods, 2022 Publication	1%

8.4 Appendix 4: Language Editing Certificate

MUSARA LUBOMBO

ACADEMIC EDITOR

CONTACT

Snr Lecturer
North-West University

+27 83 490 1604

apmlubombo@gmail.com

November 15, 2023

EDITORIAL WORK ON A DOCTORAL DISSERTATION: G. MHLONGO

I confirm that I have edited the manuscript "***Towards the optimisation of brown seaweed (Ecklonia maxima) for indigenous chickens using oyster mushrooms***" to ensure effective scientific communication of the research.

Careful attention was given on language and grammar, clarity and structure, consistency and accuracy, academic conventions as well as formating and Harvard referencing style.

In order to preserve the originality and integrity of the work, no ideas, arguments, or conclusions were altered.

Sincerely,

Musara Lubombo, Ph.D

BA (Hons) English & Communication, PGDip Public Relations, MA (summa cumlaude) & Ph.D Cultural & Media Studies