

**EFFECTS OF PAPAYA LEAVES IN
COMBINATION WITH MEDICINAL HERBS
AND PROBIOTIC AS FUNCTIONAL FEED
ADDITIVES ON GROWTH PERFORMANCES
AND HEALTH OF HYBRID TILAPIA
(*Oreochromis niloticus* × *O. mossambicus*)**

SOMDARE PEACE ONAS

UNIVERSITI SAINS MALAYSIA

2025

**EFFECTS OF PAPAYA LEAVES IN
COMBINATION WITH MEDICINAL HERBS
AND PROBIOTIC AS FUNCTIONAL FEED
ADDITIVES ON GROWTH PERFORMANCES
AND HEALTH OF HYBRID TILAPIA
(*Oreochromis niloticus* × *O. mossambicus*)**

by

SOMDARE PEACE ONAS

**Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy**

August 2025

ACKNOWLEDGEMENT

First and foremost, I extend my sincere appreciation to my main supervisor, Dr Noor Khalidah Abdul Hamid, whose patience, guidance, expertise, and unwavering commitment to excellence have shaped and elevated my research. Your mentorship has been a beacon, illuminating the path to academic achievement. I am especially grateful for the financial support provided, which eased the challenges associated with my studies. I am deeply grateful to my co-supervisor, Dr Kamarul Zarkasi for his guidance and patience during the course of my research.

I would like to express my gratitude to my sponsor, Tertiary Education Trust Fund (TETFund). Your support has been pivotal, and I am truly thankful for the opportunities it afforded me. To my employer, Federal University Lokoja, Nigeria, thank you for the opportunity granted to pursue further studies.

A special acknowledgment goes to all the technical staff of the School of Biological Sciences, especially Mr Ahmad Rizal, Mr Faizul Hasmadi, Mrs Santhini, Mr Nizam, Mrs Madiha, Mr Masrul and Mr Syed Mohd Edzam. Thank you for providing all the necessary support and conducive environment for research.

I am grateful to Dr Faisal Ahmad, Nadia, Kroesna, Khadijah, Arif, and others, for their friendship, shared experiences, and collaborative spirit.

My deepest appreciation goes to my husband Christian Uchendu, whose unwavering love, encouragement, sacrifices and understanding sustained me through the highs and lows of this academic journey. To my mother and siblings, your sacrifices and believe in my capabilities are deeply cherished. Finally, I acknowledge the divine guidance and grace of God Almighty. In moments of uncertainty and challenge, His providence has been a source of strength and resilience.

TABLE OF CONTENTS

ACKNOWLEDGEMENT.....	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	ix
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS	xiv
ABSTRAK	xvi
ABSTRACT.....	xix
CHAPTER 1 INTRODUCTION	1
1.1 Background	1
1.2 Statement of Research Problem	8
1.3 Objectives of the Study	10
CHAPTER 2 LITERATURE REVIEW	11
2.1 Overview of Tilapia Aquaculture	11
2.1.1 Fishmeal in Aquafeed Production.....	14
2.1.2 Non-Conventional Protein Source for Aquafeed	16
2.1.2(a) Black soldier fly larvae: An Excellent Non- Conventional Alternative to Fishmeal	21
2.1.3 Non-conventional Plant-based Aquafeed.....	28
2.1.3(a) Soybean meal and Non-conventional Plant-based Ingredients in Aquafeed Production	31
2.1.3(b) Azolla: An Emerging plant-based ingredient as replacement for soybean in aquafeed.....	32
2.1.3(c) Challenges associated with the use of azolla in aquafeed	36
2.2 Role of Functional Feed Additives Supplementation in Aquafeed	37
2.2.1 Papaya leaf as functional feed additive in aquaculture	38
2.2.2 Medicinal herbs as functional feed additive in aquaculture.....	40

2.2.3	Potential of Probiotic Supplementation in Aquaculture	44
2.3	Synergistic Effects of Medicinal Herbs and Probiotic Supplementation on Aquaculture Species.....	45
2.3.1	Potential synergistic effects of papaya leaf, medicinal herbs and probiotic addition	46
CHAPTER 3 GENERAL METHODOLOGY		48
3.1	Introduction.....	48
3.2	Culture and Processing of Azolla	48
3.3	Black Soldier Fly Larvae	49
3.4	Experimental Diets.....	50
3.5	Experimental Fish and Husbandry	50
3.6	Weighing and Feeding	51
3.7	Sampling Procedures	52
3.7.1	Experimental diet sampling	52
3.7.2	Experimental fish sampling	52
3.8	Growth Performance, Feed Utilization, Body Indices and Survival Determination	52
3.9	Proximate Analysis of Experimental Diets and Fish Carcasses	53
3.9.1	Moisture content analysis	54
3.9.2	Crude protein	54
3.9.3	Crude lipid	56
3.9.4	Crude fibre	56
3.9.5	Ash	57
3.10	Digestive Enzyme Activities.....	58
3.10.1	Tissue collection	58
3.10.2	Enzyme assays	58
3.10.2(a)	Protease activity.....	59
3.10.2(b)	Amylase activity	60
3.10.2(c)	Lipase activities	61

3.11	Haematological Parameters	61
3.12	Histological Examination of Mid-intestine.....	63
3.13	Cost-Benefit Analysis	64
CHAPTER 4 EFFECTS OF PAPAYA LEAF EXTRACT SUPPLEMENTATION IN PRACTICAL DIET FOR HYBRID TILAPIA.....		65
4.1	Introduction.....	65
4.2	Materials and methods	66
4.2.1	Diet formulation.....	66
4.2.2	Fish husbandry	68
4.2.3	Sampling procedure	69
4.2.4	Growth, feed utilization and somatic indices.....	69
4.2.5	Digestive enzyme analyses	69
4.2.6	Haematological parameters.....	70
4.2.7	Analytical procedure	70
4.2.8	Cost-benefit analysis.....	70
4.2.9	Statistical analysis	70
4.3	Results.....	70
4.3.1	Growth, feed utilization, somatic indices, and survival rate.....	70
4.3.2	Carcass position of hybrid tilapia fed diet supplemented with PLE	73
4.3.3	Digestive enzyme activities	73
4.3.4	Biochemical parameters.....	74
4.3.5	Haematological parameters.....	76
4.3.6	Cost-benefit analysis	78
4.4	Discussion	78
4.5	Conclusion	84

CHAPTER 5	EVALUATION OF THE IMPACT OF VARIOUS PROCESSING FORMS OF PAPAYA LEAVES AND THEIR EFFECT ON HYBRID TILAPIA.....	86
5.1	Introduction.....	86
5.2	Materials and methods	88
5.2.1	Collection and processing of oven-dried papaya leaf powder	88
5.2.2	Collection and processing of freeze-dried papaya leaf extract	89
5.2.3	Experimental design and feed formulation	89
5.2.4	Experimental fish and feeding trial	90
5.2.5	Sample collection and analysis	91
5.2.6	Cost-benefit analysis	91
5.2.7	Statistical analysis	91
5.3	Results.....	91
5.3.1	Growth performance, feed utilization, and survival rate	91
5.3.2	Whole-body composition hybrid tilapia after twelve weeks of the feeding trial	92
5.3.3	Biochemical parameters.....	93
5.3.4	Haematological parameters.....	93
5.3.5	Cost-benefit analysis of different papaya leaf processing methods in hybrid tilapia diets.....	94
5.4	Discussion	95
5.5	Conclusion	98
CHAPTER 6	EFFECT OF COMBINATION OF PAPAYA LEAF POWDER AND MEDICINAL HERBS IN DIETS FOR HYBRID TILAPIA.....	99
6.1	Introduction.....	99
6.2	Materials and Methods.....	100
6.2.1	Preparation of papaya leaf powder.....	100
6.2.2	Preparation of herbal (A, B and C)	100
6.2.3	Experimental design.....	103

6.2.4	Experimental diets	104
6.2.5	Fish husbandry	107
6.2.6	Sample collection.....	107
6.2.7	Growth, feed utilization, body indices and survival	107
6.2.8	Digestive enzyme analyses	107
6.2.9	Haematological investigations	107
6.2.10	Histological examination of mid-intestine	108
6.2.11	Cost-benefit analysis	108
6.2.12	Statistical analysis	108
6.3	Results.....	108
6.3.1	Growth performance and survival rate.....	108
6.3.2	Body indices.....	111
6.3.3	Whole-body composition.....	112
6.3.4	Digestive enzyme activities	114
6.3.5	Haematological parameters.....	115
6.3.6	Biochemical parameters	116
6.3.7	Intestinal and morphometry and morphology	118
6.3.8	Cost-benefit analysis	125
6.4	Discussion	126
6.5	Conclusion	139
CHAPTER 7 EFFECT OF THE COMBINATION OF PAPAYA LEAF POWDER AND MEDICINAL HERBS IN DIETS OF HYBRID TILAPIA REARED IN CULTURE MEDIUM CONTAINING PROBIOTIC SOLUTION.....		140
7.1	Introduction.....	140
7.2	Materials and methods	143
7.2.1	Experimental design.....	143
7.2.2	Experimental diets	144
7.2.3	Fish husbandry	146

7.2.4	Sample collection.....	146
7.2.5	Growth performance assessments.....	146
7.2.6	Proximate analyses.....	147
7.2.7	Digestive enzyme assays.....	147
7.2.8	Blood haematological parameters.....	147
7.2.9	Intestinal morphology examinations.....	147
7.2.10	Cost-benefit analysis.....	147
7.2.11	Statistical analysis.....	147
7.3	Results.....	148
7.3.1	Growth performance and survival rate.....	148
7.3.2	Body indices.....	152
7.3.3	Whole-body composition.....	153
7.3.4	Digestive enzyme activities.....	154
7.3.5	Blood haematology.....	156
7.3.6	Blood biochemistry.....	158
7.3.7	Intestinal morphometry.....	160
7.3.8	Cost-benefit analysis.....	166
7.4	Discussion.....	167
7.5	Conclusion.....	175
CHAPTER 8 CONCLUSION AND FUTURE RECOMMENDATIONS.....		176
8.1	Conclusion.....	176
8.2	Recommendations for Future Research.....	177
REFERENCES.....		178
LIST OF PUBLICATIONS		

LIST OF TABLES

	Page
Table 2.1 Summary of some studies that investigated the effects of non-conventional animal-based protein sources as substitutes for fishmeal in fish diets.	18
Table 2.2 Nutritional value of BSFL in comparison to fishmeal (g kg ⁻¹ , dry matter basis).....	23
Table 2.3 Non-conventional plant feedstuffs as alternatives to fishmeal in aquaculture species	29
Table 2.4 Proximate composition of Azolla species (dry weight basis).....	33
Table 4.1 Ingredients and proximate composition of experimental diets (% dry matter)	67
Table 4.2 Growth, feed utilization, somatic indices and survival rate of hybrid tilapia fed experimental diets for twelve weeks.	72
Table 4.3 Carcass composition of hybrid tilapia after twelve weeks of the feeding trial	73
Table 4.4 Activities of digestive enzymes in the gastrointestinal tract of hybrid tilapia after twelve weeks of the feeding trial.....	73
Table 4.5 Blood biochemical parameters of hybrid tilapia fed different levels of dietary supplementation of papaya leaf extract for twelve weeks.....	75
Table 4.6 Haematological parameters of hybrid tilapia fed diets containing different levels of papaya leaf extract inclusion for twelve weeks.....	76
Table 4.7 Cost-benefit analysis of feeding hybrid tilapia with papaya leaf extract.....	78
Table 5.1 Ingredients and proximate composition of experimental diets (% dry matter)	90
Table 5.2 Growth, feed utilization, body indices and survival rate of hybrid tilapia fed experimental diets for twelve weeks.	92
Table 5.3 Whole-body composition of experimental fish after twelve weeks of the feeding trial.	92

Table 5.4	Blood biochemical parameters of hybrid tilapia fed different levels of dietary supplementation of papaya leaf extract for twelve weeks.....	93
Table 5.5	Haematological parameters of hybrid tilapia fed diets containing different levels of papaya leaf extract inclusion for twelve weeks.....	94
Table 5.6	Cost-benefit analysis of hybrid tilapia diets supplemented with different papaya leaf processing methods.....	95
Table 6.1	Medicinal herbs (A) used for the study.....	101
Table 6.2	Medicinal herbs (B) used for the study.....	101
Table 6.3	Medicinal herbs (C) used for the study.....	102
Table 6.4	The number of treatments for the 4 x 2 factorial design in this study.....	104
Table 6.5	Experimental diet formulation and proximate composition (% dry matter).....	106
Table 6.6	Growth performance and survival of experimental fish fed diets containing papaya leaf powder and different herbal mixtures for twelve weeks.....	110
Table 6.7	Body indices of fish fed combined addition of papaya leaf powder and herbal mixtures for twelve weeks.	111
Table 6.8	Whole-body composition of the fish after twelve weeks of the experiment.....	113
Table 6.9	Activities of digestive enzyme in gastrointestinal tract of hybrid tilapia fed papaya leaf powder and medicinal herbs supplemented diets.....	114
Table 6.10	Haematological parameters of hybrid tilapia after the feeding trial	115
Table 6.11	Blood biochemical parameters of hybrid tilapia after twelve weeks of the feeding trial	117
Table 6.12	Intestinal morphology of fish fed experimental diets for twelve weeks.....	121
Table 6.13	Cost-benefit analysis	126
Table 7.1	Medicinal herbs used for the study.	142
Table 7.2	The number of treatments for the 4 x 2 factorial design in this study.....	143

Table 7.3	Ingredients and proximate composition (% dry matter) of experimental diets.	145
Table 7.4	Growth performance and survival of hybrid tilapia fed experimental diets for twelve weeks.	151
Table 7.5	Body indices (%) of hybrid tilapia after twelve weeks of the feeding trial.	152
Table 7.6	Proximate composition of hybrid tilapia fed experimental diets for twelve weeks.	153
Table 7.7	Activities of digestive enzymes (U/mg protein) in hybrid tilapia after twelve weeks of the feeding trial.	155
Table 7.8	Haematological parameters of hybrid tilapia after twelve weeks of the feeding trial.	157
Table 7.9	Blood biochemistry of hybrid tilapia after twelve weeks of the feeding trial	159
Table 7.10	Intestinal morphometry parameters of hybrid tilapia after twelve weeks of the feeding trial	163
Table 7.11	Cost-benefit analysis	166

LIST OF FIGURES

	Page
Figure 2.1	Hybrid tilapia (<i>Oreochromis niloticus</i> x <i>O. mossambicus</i>) 13
Figure 3.1	Freshly harvested azolla..... 49
Figure 3.2	Dried black soldier fly larvae meal 49
Figure 3.3	Experimental set up..... 51
Figure 4.1	Papaya leaf extract 67
Figure 6.1	A-H. A: 0% PLP, (B: medicinal herbs A-, C: medicinal herbs B-, D: medicinal herbs C-, E: 2% PLP, F: medicinal herbs A+, G: medicinal herbs B+, H: medicinal herbs C+. Photomicrograph of the mid-intestine of hybrid tilapia showing distinct layers; muscle thickness (MT), External diameter (ED), lumen diameter (LD), length of villi (LV), base width villi (BWV). Scale is 200 µm..... 122
Figure 6.2	Magnification x10. A, E: 0% PLP, B, F: medicinal herbs A-, C, G: medicinal herbs B-, D, H: medicinal herbs C-, I, M: 2% PLE, J, N: medicinal herbs A+, K, O: medicinal herbs B+, L, P: medicinal herbs C+. Scale A, B, C, D, I, J, K, L 200 µm. Scale E, F, G, H, M, N, O, P 100 µm. 123
Figure 6.3	Magnification x40. A, E: 0% PLP, B, F: medicinal herbs A-, C, G: medicinal herbs B-, D, H: medicinal herbs C-, I, M: 2% PLP, J, N: medicinal herbs A+, K, O: medicinal herbs B+, L, P: medicinal herbs C+. Abbreviations: lumen (L), lamina propria (LP), enterocytes (E), intestinal villi (black arrow), nerves (blue arrow), M cell thickness of muscularis propria and serosa (with red arrow showing the distance of muscle. Scale A, B, C, D, I, J, K, L 50 µm. Scale E, F, G, H, M, N, O, P 20 µm. 124
Figure 7.1	Photomicrograph of the midgut intestinal structure of hybrid tilapia (<i>Oreochromis mossambicus</i> x <i>Oreochromis niloticus</i>) at 4x and 10x magnification : fish fed with 0% PLP (A,E); 2% papaya leaf powder, PLP (B,F); 1% medicinal herbs – MHs (C,G); 1% probiotic solution, PRO (D,H); 2% papaya leaf powder, PLP + medicinal herbs, PLP + MHs (I,M); 1% probiotic + 2% papaya leaf powder, PRO + PLP (J,N); 1% probiotic + 1% medicinal herbs, PRO + MHs (K,O); and 1% probiotic + 2% papaya leaf powder + medicinal herbs, PRO + PLP + MHs (L,P). 164

Figure 7.2 Photomicrograph of the midgut intestinal structure of hybrid tilapia (*Oreochromis mossambicus* x *Oreochromis niloticus*) at 20x and 40x magnification : fish fed with 0% PLP (A,E); 2% papaya leaf powder, PLP (B,F); medicinal herbs, MHs (C,G); 1% probiotic, PRO (D,H); 2% papaya leaf powder + medicinal herbs, PLP+MHs (I,M); 1% probiotic + 2% papaya leaf , PRO+PLP (J,N); 1% probiotic and 1% medicinal herbs, MHs (PRO+MHs (K,O); 1% probiotic + 2% papaya leaf powder + 1% medicinal herbs C, PRO+DPL+MHs (L,P). Abbreviations: L lumen, LP lamina propria, E enterocytes, intestinal villi (black arrow), nerves (blue arrow), M cell thickness of muscularis propria and serosa (with red arrow showing the distance of muscle. Scale A, B, C, D, I, J, K, L 50 µm. Scale E, F, G, H, M, N, O, P 20 µm. 165

LIST OF ABBREVIATIONS

ALB	Albumin
ALKP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	One-Way Analysis Of Variance
BUN	Blood Urea Nitrogen
CA	Calcium
CBC	Complete Blood Count
CHOL	Cholesterol
CPLE	Commercially Processed Papaya Leaf Extract
CRE	Creatinine
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FBW	Final Body Weight
FCR	Feed Conversion Ratio
FDPLE	Freeze-Dried Papaya Leaf Extract
FI	Feed Intake
Hb	Haemoglobin
HCT	Concentration, Haematocrit
HSI	Hepato-Somatic Index
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
ODPLP	Oven-Dried Papaya Leaf Powder
PER	Protein Efficiency Ratio
PLE	Papaya Leaf Extract

PLP	Papaya Leaf Powder
PLT	Platelet
RBC	Red Blood Cell
SBM	Soybean Meal
SGR	Specific Growth Rate
SPC	Soy Protein Concentrate
SPI	Soy Protein Isolate
TP	Total Protein
VSI	Viscero-Somatic Index
WBC	White Blood Cell
WG	Weight Gain

**KESAN DAUN BETIK DENGAN CAMPURAN HERBA UBATAN DAN
PROBIOTIK SEBAGAI ADITIF MAKANAN BERFUNGSI TERHADAP
PRESTASI PERTUMBUHAN DAN KESIHATAN TILAPIA HIBRID**

(*Oreochromis niloticus* × *O. mossambicus*)

ABSTRAK

Siasatan ini dijalankan untuk menilai kesan daun betik dalam kombinasi herba perubatan dan probiotik sebagai bahan aditif makanan berfungsi terhadap prestasi pertumbuhan, aktiviti enzim pencernaan, morfologi usus, dan parameter haemato-biokimia diet makanan tilapia kacukan merah. Empat percubaan pemakanan bersiri telah dijalankan. Percubaan pemakanan pertama dijalankan untuk menentukan tahap kemasukan optimal ekstrak daun betik (PLE). Selepas dua belas minggu, ikan yang diberi diet makanan tambahan; 2% PLE mempunyai berat badan akhir tertinggi (FBW), diikuti dengan 1% PLE diet ikan tambahan. Berat badan (WG), kadar pertumbuhan khusus (SGR) dan nisbah penukaran makanan (FCR) telah meningkat dengan ketara pada ikan yang diberi dengan 2% dan 1% PLE. Penambahan PLE tidak mempunyai kesan buruk terhadap indeks badan, kadar kecekapan protein, aktiviti enzim pencernaan dan parameter hematologi, kecuali nitrogen urea darah. Ikan yang diberi makan dengan diet 2.0% ekstrak daun betik mencapai kos insiden terendah dan indek untung tertinggi, menunjukkan prestasi ekonomi terbaik. Percubaan pemakanan kedua bertujuan untuk menilai kesan cara pemprosesan daun yang berbeza terhadap tilapia kacukan. Selepas dua belas minggu, daun betik yang dikeringkan dengan oven meningkatkan pertumbuhan ikan setara dengan ekstrak daun betik yang diproses secara komersial dan dibeku-kering dan ini digalakkan kerana ianya mudah dan kos yang berpatutan. Kumpulan yang diberi makan dengan serbuk daun betik yang

dikering oven mempunyai indeks profit tertinggi dan kos incident terendah, mencadangkan ianya pilihan ekonomi yang paling untung kepada penternak ikan. Percubaan pemakanan ketiga dijalankan untuk menilai kesan kombinasi daun betik (PLP) dan herba perubatan (MHS); A, B, dan C terhadap prestasi pertumbuhan, aktiviti enzim, morfologi usus, dan parameter hematologi tilapia kacukan merah. Ikan yang diberi makan tambahan makanan gabungan PLP dan MHS C mempunyai FBW, WG, SGR, pengambilan makanan (FI) dan FCR tertinggi. Aktiviti lipase telah meningkat dengan ketara pada ikan yang diberi makan dengan kombinasi diet daun betik, tanpa mengira MHS manakala aktiviti amilase dan protease tidak terjejas. Parameter haematologi tidak berubah, kecuali haematokrit dan kreatin. Penambahan PLP dan MHS, dengan ketaranya meningkatkan parameter morfologi usus yang dinilai, kecuali ketebalan otot. Kumpulan yang diberi makan MHs C+ mempunyai indeks keuntungan tertinggi dan kos insiden terendah, menunjukkan keseimbangan terbaik diantara kos dan kebaikan. Percubaan pemakanan keempat telah dijalankan untuk menilai impak gabungan PLP dan MHs dalam diet tilapia kacukan didalam media kultur yang mengandungi larutan probiotik. Selepas dua belas minggu, ikan yang diberi makan gabungan kemasukan diet PLP dan MHS mempunyai FBW, WG, SGR dan FI tertinggi, tanpa mengira penambahan probiotik, manakala ikan yang dibesarkan dalam medium kultur yang mengandungi larutan probiotik dan diberi diet gabungan tambahan PLP dan MHS mempunyai peningkatan FCR dengan ketara. Aktiviti lipase meningkat dengan ketara pada kumpulan yang dibesarkan dalam medium kultur yang mengandungi larutan probiotik. Parameter haemato-biokimia tidak terjejas, kecuali haematokrit dan serum fosforus, masing-masing. Morfometrik usus dipengaruhi oleh diet serbuk daun betik, herba perubatan dan probiotik kecuali diameter lumen dan ketebalan otot. Kumpulan yang di beri makan dengan kombinasi serbuk daun betik,

herba perubatan dan pribiotik mempunyai prestasi kecekapan ekonomi terbaik. Kajian ini menunjukkan daun betik sebagai aditif makanan berfungsi yang boleh digunakan untuk meningkatkan prestasi pertumbuhan tilapia kacukan merah tanpa sebarang kesan sampingan.

**EFFECTS OF PAPAYA LEAVES IN COMBINATION WITH MEDICINAL
HERBS AND PROBIOTIC AS FUNCTIONAL FEED ADDITIVES ON
GROWTH PERFORMANCES AND HEALTH OF HYBRID TILAPIA**

(*Oreochromis niloticus* × *O. mossambicus*)

ABSTRACT

The present investigation was conducted to evaluate the effect of papaya leaves in combination with medicinal herbs and probiotic as functional feed additives on growth performances, activities of digestive enzymes, intestinal morphology, and haemato-biochemical parameters of hybrid tilapia. A series of four feeding trials were carried out. The first feeding trial was conducted to determine the optimal dietary inclusion level of papaya leaf extract (PLE). After twelve weeks, fish fed diet supplemented with 2% PLE had the highest final body weight (FBW), followed by fish diet supplemented with 1% PLE. Weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) were significantly improved in fish fed diet supplemented with 2% and 1% PLE. Supplementation of PLE had no adverse effect on body indices, protein efficiency ratio, digestive enzyme activities, and haematological parameters, except for blood urea nitrogen. Fish fed the 2.0% papaya leaf extract diet achieved the lowest incidence cost and the highest profit index, indicating superior economic performance. The second feeding trial aimed to evaluate the effects of different processing methods of papaya leaves on hybrid tilapia. After twelve weeks, oven-dried papaya leaves enhanced fish growth comparable to commercially processed and freeze-dried papaya leaf extracts and it is recommended for its simplicity and cost-effectiveness. Group fed oven-dried papaya leaf powder had the highest profit index and lowest incidence cost, suggesting it is the most

economically beneficial option for fish farmers. The third feeding trial was conducted to evaluate the effects of the combination of papaya leaf powder (PLP) and medicinal herbs (MHs); A, B, and C on growth performance, enzyme activities, intestinal morphology, and haematological parameters of hybrid tilapia. Fish fed combined dietary addition of PLP and MHs C had the highest FBW, WG, SGR, feed intake (FI) and FCR. Lipase activities were significantly elevated in fish fed dietary combination of papaya leaf, regardless of the MHs while amylase and protease activities were unaffected. Haematological parameters were not altered, except for haematocrit and creatine. Supplementation of PLP and MHs significantly improved intestinal morphological parameters evaluated, except for muscle thickness. Group fed MHs C+ had the highest profit index and lowest incidence cost, indicating a superior balance between cost and benefit. The fourth feeding trial was carried out to assess the impact of the combination of PLP and MHs in diets of hybrid tilapia raised in culture medium containing probiotic solution. After twelve weeks, fish fed combined dietary inclusion of PLP and MHs had the highest FBW, WG, SGR and FI, regardless of probiotic addition, while those raised in culture medium containing probiotic solution and fed combined dietary supplementation of PLP and MHs had significantly improved FCR. Lipase activities were significantly improved in group raised in culture medium containing probiotic solution. Haemato-biochemical parameters were unaffected, except for haematocrit and serum phosphorus, respectively. Intestinal morphometrics were affected by dietary papaya leaf powder, medicinal herbs and probiotic except for lumen diameter and muscle thickness. Group fed with the combination of papaya leaf powder, medicinal herbs, and probiotics had the best performance in terms of economic efficiency. This study demonstrates that papaya leaf is a functional feed

additive that can be used to improve growth performance of hybrid tilapia without any adverse effect.

CHAPTER 1

INTRODUCTION

1.1 Background

Aquaculture plays a crucial role in addressing global food security by contributing significantly to meeting the growing demand for high-quality animal protein. The sector provides aquatic animal products, including fish and fish products, to millions of people globally, thereby making a substantial contribution to eliminating hunger and malnutrition, particularly in underdeveloped and developing countries where seafood is a valuable source of protein as access to high-quality protein-rich food is limited. In addition, aquaculture contributes tremendously to economic growth by providing income and employment opportunities for millions of people globally (FAO, 2022; Subasinghe *et al.*, 2009)

Tilapia stands out as one of the most commonly farmed finfish species among the numerous species farmed globally (FAO, 2022), and aquaculture is the primary source of tilapia, producing 6.2 million tonnes annually, valued at USD 12 billion (Arumugam *et al.*, 2023). Though native to Africa, the fish has been introduced to many countries, and it is farmed in several tropical and subtropical regions of the world. The success of tilapia introduction globally is due to its several desirable characteristics, such as rapid growth, hardiness, multiple spawning, and adaptability to a wide range of environmental conditions, including biotic and abiotic conditions (Canonico *et al.*, 2005; Dawood *et al.*, 2022; Ng & Romano, 2013). In addition, with optimum environmental conditions, tilapia matures within a short period of time, and the parents are known to exhibit a high level of parental care to their offspring (Coward & Bromage, 2000; Russell *et al.*, 2012; Stauffer *et al.*, 2022).

The ability to thrive in various environmental conditions, feed on a variety of food sources and require low maintenance are some of the outstanding characteristics of tilapia that make them excellent aquaculture candidates. Hybrid tilapia is a cross between *Oreochromis niloticus* and *Oreochromis mossambicus*. The species has a good taste, high fecundity, and large market value, making it attractive for aquaculture. The expansion, sustainability, and economic viability of hybrid tilapia production across the globe are dependent on the availability and cost-effectiveness of suitable feed ingredients for tilapia feed production.

With the current global financial crises, especially in developing countries, aquaculture researchers are intensively seeking ways to reduce costs. Feed is the largest variable cost, accounting for over 50% of the total operating cost in intensive and semi-intensive aquaculture systems, with protein as the most expensive component of the feed (El-Sayed, 1999; Magbanua & Ragaza, 2022). Traditionally, fishmeal has been extensively used as the primary protein source in aquaculture feed formulations, and it has been the most preferred over other protein sources due to its nutritional value, such as high crude protein content and excellent amino acid profile, which meets the requirements for the optimum growth and development of cultured fish species (Magbanua & Ragaza, 2022; Olsen & Hasan, 2012). In addition, the high palatability and excellent digestibility of fishmeal have also made it the most reliable and valuable feed ingredient in aquafeed production (Jannathulla *et al.*, 2019; Takakuwa *et al.*, 2022). However, the sustainability of aquaculture is increasingly being challenged by the overreliance on fishmeal. This is owing to the fact that fishmeal has become increasingly expensive and scarce due to increased demand as a result of the global increase in aquaculture production and unstable fishmeal production as a result of depletion in wild catches (FAO, 2018; Naseem *et al.*, 2021;

Tacon & Metian, 2008). Furthermore, the environmental impact associated with the use of fishmeal as a feed ingredient has raised concerns about its sustainability as the primary protein source in aquafeed formulations. These challenges have prompted researchers to explore cost-effective, readily available, and sustainable alternative protein sources to develop aquafeed formulations with comparable nutritional value to fishmeal while reducing the use of fishmeal to a minimum inclusion level. A potential alternative to fishmeal must possess attributes such as high nutritional value, affordability and preferably non-conventional to avoid competition. In addition, the feedstuff should be acceptable, palatable, and digestible to the species (Chakrabarti *et al.*, 2018; Naseem *et al.*, 2021).

In this context, numerous studies have been conducted to evaluate the use of alternative protein sources as a substitute for fishmeal in practical diets for farmed fish (Gómez-Requeni *et al.*, 2004; Hua *et al.*, 2019; Nguyen *et al.*, 2009). For example, terrestrial plants such as soybean meal (Howlader *et al.*, 2023; Ye *et al.*, 2011), corn gluten meal (Lewis & Kohler, 2008; Wang *et al.*, 2020), insect meals such as cricket meal (Fan *et al.*, 2023), black soldier fly larva (Kroeckel *et al.*, 2012; Mapanao *et al.*, 2023), microbial single-cell protein (Al-Hafedh & Alam, 2013; Yang *et al.*, 2023), and many more, have been tested and used as substitutes to partially or completely replace fishmeal in several aquaculture species with mixed but promising results on the growth performance and overall health status of fish. Black soldier fly larva, *Hermetia illucens* have shown promising potential as alternative to fishmeal in several fish species due to their high protein content and sustainable production characteristics (Linh *et al.*, 2024; Tippayadara *et al.*, 2021).

Black soldier fly larva are rich in protein and lipid and unlike plant-based protein sources, they are poor in fibre and other antinutritional factors. The high nutritional value and ease of rearing of BSFL larva has made it a promising sustainable alternative protein source. While they are not a perfect replacement for fishmeal, they can be used as partial substitutes to fishmeal, at up to 30% level of replacement without any adverse effects on the growth performance and overall health condition of the fish (Tran *et al.*, 2015).

Another important conventional feed ingredient is soybean meal. In recent years, the prices of soybean meal have been subjected to fluctuation due to various factors such as market demand, and crop yields (Magbanua & Ragaza, 2024). This unpredictability in pricing poses challenges for aquaculture producers, especially small-scale farmers in developing countries. The high cost of soybean meal contributes to the financial burden on aquaculture operations, ultimately affecting profitability and sustainability. Moreover, the growing demand for soybean, driven by both human consumption and animal feed purposes, is leading to increased competition between food and feed industries (Riel *et al.*, 2023). These further emphasizes the need for alternative substitutes to soybean meal in aquafeed formulations. In many developing countries like Nigeria for instance, where food security and malnutrition are pressing issues, diverting soybean for feed production can intensify existing challenges related to food availability and affordability.

In response to these challenges, researchers are actively exploring alternative ingredients that can effectively replace soybean meal in aquaculture feed formulations. These alternatives include locally available and cost-effective sources of protein and essential nutrients, insect meal, microbial-derived proteins, plant-based by-products, and aquatic macrophytes. Amongst these plethora of choices, Azolla, a free-floating

aquatic fern is being explored as a potential alternative to soybean meal in aquafeed formulations (Sudaryono, 2006). Azolla is among the alternative protein sources explored as sustainable alternative protein sources for aquafeed formulations. It is a potential feed ingredient due to its ease of cultivation, productivity and relatively high nutritional value (Das *et al.*, 2018; Prabina & Kumar, 2010). At optimum growth conditions, azolla contains 20 - 30% crude protein, on a dry matter basis. It is rich in amino acids, carotenoids, minerals and vitamins (Anitha *et al.*, 2016; Basak *et al.*, 2002). Thus, azolla is recognised as a promising alternative protein source for animal feed production. However, there are limitations associated with the use of plant-based alternative protein sources. For instance, in comparison with fishmeal, plant protein feedstuffs have unbalanced amino acid profile, high fibre, lower palatability and digestibility issues due to the presence of antinutritional factors (Ma *et al.*, 2022). These limitations can affect the performance of fish species.

While previous studies have investigated the utilization of azolla (Abou *et al.*, 2007; Lumsangkul *et al.*, 2022; Magouz *et al.*, 2020) and black soldier fly larva (Devic *et al.*, 2018; Tippayadara *et al.*, 2021; Yildirim-Aksoy *et al.*, 2020) in tilapia diets, a review of related literature showed that the presence of antinutritional factors such as high fibre in plant feedstuffs (Tejeda & Kim., 2021) and chitin in insect meals (Gasco *et al.*, 2021) and can limit their use in aquafeed production. Diets incorporated with functional feed additives can overcome problems caused by anti-nutritional factors, amino acid imbalances, and digestibility associated with some alternative aquafeed ingredients.

Functional feed additives include a diverse range of substances that are incorporated into aquafeed formulations with the specific purpose of improving various aspects of animal health, nutrition, and performance (Chen *et al.*, 2023). The

use of functional feed additives in aquafeed has emerged as a promising strategy to enhance feed efficiency and promote the overall performance of aquaculture systems. Functional feed additives include probiotics, prebiotics, enzymes, immunostimulants, antioxidants, acidifiers and medicinal herbs (Dawood *et al.*, 2018). These additives are incorporated into aquafeed formulations to optimize feed efficiency in aquaculture production systems (Chen *et al.*, 2024). The utilization of such additives represents a proactive approach towards achieving sustainable aquaculture practices by enhancing animal welfare, reducing reliance on antibiotics, improving feed conversion ratios, and minimizing environmental impacts. Papaya leaf and herbal mixtures are promising functional feed additives in aquaculture. However, the incorporation to improve the nutritional quality, growth parameters and health of aquaculture species has not been largely explored.

Papaya leaf contains numerous bioactive compounds with growth and health-promoting properties. For example, it contains exogenous enzymes which facilitate digestion, enhance nutrient absorption and utilization (Choi *et al.*, 2016; Hamid *et al.*, 2022; Kirimi *et al.*, 2023). The incorporation of papaya leaf as additive may enhance the nutritional quality of the diets and improve growth and health of cultured fish. Therefore, it is essential to evaluate the promising potential of utilizing papaya leaf as functional feed additive in formulating practical diets for hybrid tilapia using azolla and black soldier fly larvae as alternative protein sources to reduce the dependence on fishmeal, offering a sustainable solution for aquaculture industry.

The utilization of herbal mixtures as functional feed additives in aquaculture represents a promising approach to enhance the health, growth, and performance of farmed aquatic species (Kamble *et al.*, 2024). Comprising various plant-derived compounds, herbal mixtures offer a holistic solution to improving feed efficiency and

mitigating health challenges in aquaculture systems. Unlike single herb additives, which may have limited efficacy or narrow spectrum of action, herbal mixtures harness the synergistic interactions between multiple bioactive compounds, thereby exerting a broader range of beneficial effects (Güroy *et al.*, 2024).

Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, have emerged as valuable water (El-Kady *et al.*, 2022) and feed (Shahzad *et al.*, 2023) additives in aquaculture. Probiotics are added into water serving as the culture medium to improve and maintain a healthy water quality (El-Kady *et al.*, 2022). Additionally, the addition of probiotics can aid in the digestion of food as they contain beneficial bacteria that can enhance the gut microbiota of fish thus, helping in breaking down nutrients efficiently leading to improved digestion and nutrient absorption (Assan *et al.*, 2022). Probiotics can also improve haematological parameters and boost the immune system of fish (El-Kady *et al.*, 2022). By promoting the growth of beneficial bacteria in the gut, probiotics help to competitively exclude harmful pathogens, reducing the risk of infections and diseases in fish. Furthermore, some probiotics have been found to have growth-promoting effects on fish (El-Kady *et al.*, 2022). By enhancing nutrient utilization and improving overall health, probiotics can contribute to faster growth rates and better weight gain in fish, ultimately leading to improved production efficiency in fish farming operations. Studies have also shown that probiotics improve the intestinal histology in fish (El-Kady *et al.*, 2022). With the increasing demand for sustainable and environmentally-friendly practices in aquaculture, the use of probiotics represents a promising approach to enhance the health, performance, and disease resistance of farmed aquatic species.

While individual studies have explored the effects of papaya leaf extracts, medicinal herbs, and probiotics on fish health and performance, the potential interactions among these supplements remain largely unexplored. Additionally, no study has been conducted to investigate the impact of combined dietary incorporation of papaya leaf, medicinal herbs, and the addition of probiotics into water of fish diet formulated using non-conventional protein sources such as black soldier fly larvae and azolla as substitutes for fishmeal and soybean meal, respectively. Given that papaya leaf, medicinal herbs and probiotics may exert their effects through overlapping or complementary mechanisms, there is a need to investigate their combined effects on fish health and productivity. Understanding the synergistic interactions between papaya leaf extracts, medicinal herbs, and probiotics holds immense implications for optimizing dietary formulations in aquaculture practices.

1.2 Statement of Research Problem

The use of alternative plant-based feed additives in aquaculture, such as papaya leaf extract, has gained attention due to their potential to improve fish health and growth performance. However, the optimum inclusion level of papaya leaf extract in diets formulated with sustainable ingredients like black soldier fly larvae and azolla remains underexplored, particularly for red hybrid tilapia. Without clear guidelines on the appropriate inclusion level, there is a risk of suboptimal growth, health benefits, or nutrient imbalances in the fish. Therefore, determining the optimal inclusion level of papaya leaf extract is critical for maximizing both the growth performance and health benefits of red hybrid tilapia, while also ensuring the sustainability and nutritional balance of the diet.

Additionally, papaya leaves are known to contain bioactive compounds that may enhance growth and health in tilapia; however, the impact of different processing methods on the bioavailability and effectiveness of these compounds remains poorly understood. The methods of preparing papaya leaves: whether fresh, dried, powdered, or extracted could significantly alter their nutritional content and medicinal properties. Understanding how these different processing forms influence the growth performance and overall well-being of red hybrid tilapia is essential for determining the most effective application of papaya leaves as a functional feed additive. Without such knowledge, the full potential of papaya leaves in sustainable aquaculture diets cannot be realized.

Furthermore, herbal supplementation in aquaculture diets has shown promise for improving fish health and performance, but there is limited research on the synergistic effects of combining papaya leaf powder with other medicinal herbs. While papaya leaf powder alone may offer beneficial properties, the combined effects of papaya leaves and Chinese medicinal herbs on hybrid tilapia's growth and health remain unexplored. Understanding how single and combined herbal supplementation influences tilapia's growth performance and overall health is critical for optimizing dietary formulations. Inadequate knowledge about the interactions between these feed additives may hinder their effective use in commercial aquaculture.

Finally, the role of probiotics in aquaculture is well-documented for enhancing gut health and immunity in fish, but the combined effects of probiotics with medicinal herbs, particularly papaya leaf powder, on tilapia health and performance have not been adequately studied. Probiotic solutions are typically used to improve microbial balance in aquaculture systems, but their interaction with herbal additives like papaya leaf powder and medicinal herbs may produce synergistic effects that enhance growth,

immunity, and disease resistance in tilapia. Therefore, there is a need to assess the impact of probiotic supplementation in combination with papaya leaves and medicinal herbs on the health and growth of red hybrid tilapia. Without understanding these interactions, the full potential of probiotics and herbs in improving tilapia culture may not be realized.

1.3 Objectives of the Study

The study was aimed at achieving the following objectives:

1. To determine the optimum inclusion level of papaya leaf extract in diets for hybrid tilapia.
2. To determine the effect of different processing forms of papaya leaves on growth performance and health status of hybrid tilapia.
3. To investigate the effects of the combination of papaya leaf powder and medicinal herbs in diets for hybrid tilapia.
4. To assess the impact of the combination of papaya leaf powder and medicinal herbs in diet of hybrid tilapia raised in culture medium containing probiotic solution.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Tilapia Aquaculture

Tilapia belongs to the *Cichlidae* family that inhabits mainly freshwater streams, ponds, rivers, and lakes (Prabu *et al.*, 2019; Tabasian *et al.*, 2023). Although native to Africa, tilapia has been introduced to several countries and tilapia aquaculture has transcended borders and extended its influence globally, and it has become one of the most important and most consumed aquaculture species in the world. Tilapia contributes significantly to global aquaculture production as they are the second most important and commonly farmed fish after carps (Prabu *et al.*, 2019; Thompson *et al.*, 2023).

Tilapia is considered an excellent aquaculture species due to its ability to feed on a variety of feeds, its ability to convert inexpensive low protein feed into high-quality protein. In addition, its hardiness, resistance to disease, multiple spawning, high parental care and exceptional ability to thrive in various aquatic conditions has made tilapia an ideal candidate for aquaculture (Lal *et al.*, 2023; Prabu *et al.*, 2019). Furthermore, the increasing awareness of health benefits and the growing demand for protein-rich diets contribute to the rising popularity of tilapia as it contains vitamin B, iron, vitamin D, selenium, and omega-3 fatty acids. Advancements in genetic technology including selective breeding is also responsible for the growth of tilapia market.

The total tilapia production was over 6.1 million tons, with an associated value of USD 12.342 billion in 2019 (FAO, 2021). The global tilapia production is expected to reach 7.3 million tons by 2030 (WorldBank, 2013). The significant increase in

tilapia production over the past four decades is attributed to ease of aquaculture, marketability, and stable market prices. Tilapia is majorly farmed in Africa, Asia and the Americas (El-Sayed & Fitzsimmons, 2023; Prabu *et al.*, 2019). In 2018, the global tilapia production accounted for 10.2% of the overall production of finfish. China is the leading producer of tilapia with an annual production of 1.8 million tons, followed by Indonesia with an annual production of about 1.2 million tons. This is followed by Africa and Brazil. These two countries also contributed significantly to the total tilapia production (FAO, 2021).

Today, tilapia aquaculture provides valuable protein source, income, and employment to millions of people globally, especially in developing countries (El-Sayed & Fitzsimmons, 2023; Prabu *et al.*, 2019), and with advancement in aquaculture practices and a growing demand for seafood, tilapia production is expected to increase.

Hybrid tilapia is a crossbreed of *Oreochromis niloticus* and *Oreochromis mossambicus*, known for its rapid growth and distinctive reddish or pinkish coloration (Basavaraja & Raghavendra, 2017; Méndez-Martínez *et al.*, 2023). This color is often more intense in certain areas of the body, such as the scales, fins, and sometimes the eyes. The intensity of the color can be influenced by factors like diet, water quality, and overall health (Ng *et al.*, 2019). The coloration of hybrid tilapia is often considered aesthetically pleasing, making it attractive to consumers in addition to its economic value.

Tilapia has an elongated streamlined body shape, common to many tilapia species. The body is laterally compressed, and they have a relatively deep body compared to other freshwater fish. Like other tilapia species, hybrid tilapia generally possess well-developed fins (Ukenye *et al.*, 2019). The dorsal fin, located on the back

is continuous and may have a slightly pointed or rounded shape. The anal fin, on the ventral side, is also long and may have shape similar to the dorsal fin.

The size of hybrid tilapia can vary but are generally medium-sized fish. Their size can be influenced by factors such as age, genetics, and environmental factors. Hybrid tilapia, like other tilapia species, typically have small, subterminal mouths. They are equipped with numerous small, comb-like teeth on the jaws, pharynx, and sometimes on the roof of the mouth. These teeth are adapted for grazing on algae and detritus. The scales of hybrid tilapia are usually small and cycloid (rounded) and cover their bodies, providing support and contributing to their overall appearance. The coloration of the scales contributes to the overall appearance of the fish.



Figure 2.1 Hybrid tilapia (*Oreochromis niloticus* x *O. mossambicus*)

Hybrid tilapia is native to Africa and has been introduced to tropical and subtropical regions of the world due to its adaptability to diverse environmental conditions. It is commonly cultured in ponds, tanks, and other aquaculture systems and thrives in water temperature ranging from 25 to 32°C while the optimal pH level ranges from 6.5 to 8.5 (Hamid *et al.*, 2022; Ismail *et al.*, 2016).

The fish is omnivorous in nature as it feeds on various feed resources including algae, plankton, small vertebrates, and exhibits efficient feed conversion, making it economically viable. The species typically exhibits a high reproductive potential, making them well suited for aquaculture, like most tilapia (Prabu *et al.*, 2019). They are often preferred for their ability to spawn multiple times a year under controlled condition. Hybrid tilapia aquaculture is favoured for its economic viability, high growth rates, and adaptability, making it a lucrative choice for sustainable fish farming.

2.1.1 Fishmeal in Aquafeed Production

Traditionally, fishmeal has been regarded as an important source of protein in aquatic animal feeds making. The choice of fishmeal as a primary source in aquafeed formulation is attributed to several factors such as high level of protein (72%) (NRC, 1993), balanced amino and fatty acid profiles, palatability, high digestibility of nutrients and the absence of antinutritional factors (Akter *et al.*, 2023; NRC, 2011). These qualities make fishmeal an ideal and most preferred ingredients in aquafeed production as it provides a complete source of protein, which is essential for the growth and development of aquaculture species. Fishmeal also contains all the essential amino acids necessary for optimal growth thus making it an ideal choice for carnivorous and omnivorous aquatic animals (Cai *et al.*, 2022). In addition, the presence of essential nutrients, including vitamins such as B vitamins and minerals such as phosphorus in fishmeal support the overall health, immune function, and reproductive success in cultured aquatic species. Fishmeal-based feeds are highly palatable thus, encourage feed intake and minimize feed wastage. The presence of omega-3-fatty acid in fish such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are critical for fish health and also contribute to the nutritional quality of seafood for human

consumption. Overall, the excellent nutritional content of fishmeal contributes to faster growth and good feed conversion ratio (FCR) in aquaculture species, leading to more efficient and sustainable aquaculture production.

Despite the beneficial properties associated with fishmeal, it is usually sourced from marine fisheries and the stagnation in global landing of wild caught fisheries has raised concerns about the sustainability of fishmeal as an important protein source in aquaculture feed production (Carral & Sáez-Royuela, 2022). In addition, the aquafeed industry also competes with the poultry and animal feed industries, which also use fishmeal in feed formulations. Competition between these industries increases the market demand for fishmeal, and the stagnation in wild caught fish leading to rising costs of fishmeal are additional constraints that will permanently affect the supply of fishmeal for aquafeed production and thus affect the overall cost of aquaculture production (Asche *et al.*, 2022). Thus, there is need to identify, develop and utilise alternative ingredients in aquafeed production to reduce fishmeal inclusion in aquafeed.

Among various aquaculture species, tilapia can grow well with low inclusion of fishmeal in the diet. For instance, studies have shown that complete replacement of fishmeal with black soldier fly larva meal had no adverse effects on growth performance of Nile tilapia, *Oreochromis niloticus* (Agbohessou *et al.*, 2021). Similarly, replacement of fishmeal with black soldier fly larvae meal (0-100%) (Tippayadara *et al.*, 2021) and defatted black soldier fly larva meal (0-100%) had no adverse effects on the growth, feed efficiency and haematological parameters in Nile tilapia (Kishawy *et al.*, 2022). Recent study has shown that the replacement of fishmeal with up to 75% black soldier fly larva meal significantly improved growth performance of Nile tilapia (Limbu *et al.*, 2022), implying that tilapia farming could

potentially support sustainable aquaculture practices and can reduce the strain on marine ecosystem by reducing the amount of fishmeal used in tilapia feed production. The findings of these studies show that tilapia can tolerate low fishmeal inclusion in their diets. Despite the tolerance of tilapia to dietary low fishmeal inclusion, fishmeal are still being incorporated in fish feed, at least 10% level of inclusion due to its excellent characteristics mentioned earlier.

2.1.2 Non-Conventional Protein Source for Aquafeed

Over the years, studies have been conducted to identify and evaluate various non-conventional protein sources (Table 2.1). In recent years, animal-based materials are being explored as sustainable solutions to address the growing demand for fish feed while reducing the pressure on marine raw materials. To be a viable alternative, the potential feed ingredient must possess characteristics such as availability, affordability, ease of handling, transport, storage and use for use in aquafeed production. Additionally, they should contain relatively high protein, favourable amino acid composition, high digestibility, and excellent palatability (Jannathulla *et al.*, 2019). Other factors such as low fibre content, low starch, and little to no antinutritional factors should also be put into consideration when evaluating a potential viable alternative.

In view of the characteristics of viable alternative feedstuff mentioned above, insect meals are considered as promising non-conventional ingredients for aquafeed. This is because insects are rich in proteins, essential amino acids, fats, and micronutrients, making them a valuable nutritional source for cultured fish species. For instance, studies have shown that insect meal has a protein content similar to fishmeal and is increasingly being used in aquafeed formulations (Barragan- Fonseca

et al., 2017; Oteri *et al.*, 2021). In addition, insects are abundant and can be sustainably produced as they require less land, water, and resources, thus, offering potential solution to the depletion of marine resources. Furthermore, insects can be reared on organic waste, thus, contributing to the circular economy by recycling nutrients and reducing environmental pollution. Studies have shown that many aquaculture species readily accept insect-based diets, indicating that growth performance and feed conversion ratios are comparable to or better than traditional diets (Mapanao *et al.*, 2023). The acceptance of insect-based meal by farmed fish species is crucial for the successful utilization of insect meals in aquafeed formulations. Therefore, the use of insect-based meals for aquafeed production is essential for the continued growth and sustainability of aquaculture industry as they provide essential nutrients for aquaculture species, while reducing the reliance on traditional fishmeal.

Table 2.1 Summary of some studies that investigated the effects of non-conventional animal-based protein sources as substitutes for fishmeal in fish diets.

Protein sources replacing fishmeal	Fish	Recommended level of inclusion	Feeding trial duration (weeks)	Major findings	References
Black soldier fly larva meal (<i>Hermetia illucens</i>)	Nile tilapia (<i>Oreochromis niloticus</i>)	Up to 100%	12	No significant differences on growth, feed utilization, feed intake, survival and haematological parameters compared to the control group	(Tippayadara <i>et al.</i> , 2021)
Field cricket (<i>Gryllus bimaculatus</i>)	Hybrid tilapia (<i>Oreochromis spp</i>)	up to 50%	14	Up to 50 % level of inclusion had no significant effect on growth performance and feed utilization. Reduced growth performance was observed beyond 50 % inclusion level	(Hanan <i>et al.</i> , 2022)
Field cricket meal (<i>Gryllus bimaculatus</i>)	Channel catfish (<i>Ictalurus punctatus</i>)	50 -75% level of inclusion	10	Significantly improved weight gain and SGR at 50 and 75 % levels of inclusion compared with the control. Improved haematological parameters.	(Fan <i>et al.</i> , 2023)
Cricket meal (<i>Gryllus bimaculatus</i>)	African catfish (<i>Clarias gariepinus</i>)	75 -100% level of inclusion	7	Significantly improved final body weight and SGR at 75 -100 % level of inclusion compared to control. No significant difference in FCR, PER and blood parameters between the treatments.	(Taufek <i>et al.</i> , 2016)
Short-horned grasshopper (<i>Oxya hyla hyla</i>) meal	Rohu, <i>Labeo rohita</i>	Up to 50% replacement of FM	14	Up to 50% level of replacement had no adverse effects on the growth performance of the fish. No adverse effect on hepatosomatic index and whole-body composition of the fish, regardless of the level of fishmeal replacement in the diet	(Ghosh & Mandal, 2019)
Red wigglers (<i>Eisenia fetida</i>) worm meal	Nile tilapia (<i>Oreochromis niloticus</i>)	Up to 20%	12	Improved growth and feed utilization (P< 0.05). Increased crude protein and ash content of the fish fed on 10-25 % level of inclusion. Elevated lipid content of the fish fed on 5-15 % level of replacement.	(El-Ouny <i>et al.</i> , 2023)

Table 2.1 (Continued)

Protein sources replacing fishmeal	Fish	Recommended level of inclusion	Feeding trial duration (weeks)	Major findings	References
Mopane (Imbrasia belina) worm	Tilapia (<i>Oreochromis mossambicus</i>)	Not applicable	7	Increased growth performance was observed with increasing (10-60 %) levels of mopane worm addition. Increased protease and amylase activities at high inclusion level. No histological alterations were observed in the intestine of fish fed mopane worm.	(Rapatsa & Moyo, 2017)
Mopane (Imbrasia belina) worm meal	Dusky kob (<i>Argyrosomus japonicus</i>)	11.3%	7	Increased weight gain and SGR with increasing level of inclusion and improved FCR. Substituting FM with mopane worm meal did not alter ($p > 0.05$) digestive enzyme activities, haemato-biochemical parameters, except for increased urea.	(Nyuliwe et al., 2022)
Mopane (Imbrasia belina) worm meal	African catfish (<i>Clarias gariepinus</i>)	Not applicable	7	Decline in growth performance indices and PER with increasing (0-60 %) mopane worm meal inclusion. Increased protease and amylase activities in the stomach with increasing level of mopane worm meal	(Rapatsa & Moyo, 2019)
Defatted Superworm (<i>Zophobas morio</i>) larval meal	Asian sea bass (<i>Lates calcarifer</i>)	Not applicable	12	No significant differences were observed in weight gain, SGR, FCR, HSI, VSI and the haemato-biochemical parameters among fish fed different levels (0-44.44 %) of Superworm larval meal dietary treatments.	(Prachom et al., 2021)
Housefly (<i>Musca domestica</i>) maggot meal	Nile tilapia (<i>Oreochromis niloticus</i>)	18%	10	Replacing fishmeal with maggot meal up to 27 % level of inclusion had no adverse effects on growth performance and feed utilization. Complete replacement (36 %) had significant reduction in weight gain and SGR, and higher FCR.	(Wang et al., 2017)

Table 2.1 (Continued)

Protein sources replacing fishmeal	Fish	Recommended level of inclusion	Feeding trial duration (weeks)	Major findings	References
Earthworm meal (<i>Libyodrilus violaceus</i>)	African catfish (<i>Clarias gariepinus</i>)	Up to 25%	7	The highest final weight, weight gain and SGR was observed in fish fed 25 % FM replacement with earthworm meal. Depressed growth was observed in fish fed on earthworm beyond 25 % level of inclusion in the diet. FCR was highest in fish fed 35 % level of inclusion.	(Dedeke et al., 2013)
Meal worm (<i>Tenebrio molitor</i>) larvae meal	rainbow trout (<i>Oncorhynchus mykiss</i>)	Not applicable	22	The growth performance was not affected by meal worm inclusion, regardless of the level (0-20 %)	(Chemello et al., 2020)
Yellow mealworm (<i>Tenebrio molitor</i>)	Red Seabream (<i>Pagrus major</i>)	Up to 65%	4	Fish fed 65 % defatted mealworm meal had significantly elevated growth performance	(Ido et al., 2019)
Yellow mealworm (<i>Tenebrio molitor</i>) larvae meal	Blackspot sea bream (<i>Pagellus bogaraveo</i>)	Not applicable	18	No detrimental effects on growth performance among the treatments, regardless of mealworm inclusion (0-50 %)	(Iaconisi et al., 2017)

2.1.2(a) Black soldier fly larvae: An Excellent Non-Conventional Alternative to Fishmeal

The black soldier fly larva (BSFL) (*Hermetia illucens*) is an insect that belongs to the order: Diptera and family: Stratiomyidae. The species is a saprophyte that feeds primarily on organic waste including plant residues, animal manure, food waste, and agricultural by-products. Black soldier fly larvae have emerged as an important non-conventional protein source in aquafeed productions due to its nutritional profile, production cost, economic viability, and environmental sustainability. Studies have shown that BSFL contain high protein, an important component for the growth and development of aquatic animal species, making them a valuable protein source and one of the most promising candidates for aquafeed formulations (Mlambo *et al.*, 2023). In addition, BSFL provide an excellent source of essential amino acids, such as lysine, methionine, and threonine, which are essential for optimal growth and immune function in fish, making them a promising candidate for aquafeed (Barragan-Fonseca *et al.*, 2017). It has been reported that the amino acid composition of BSF larvae is comparable to or even higher than that of fishmeal. Furthermore, the presence of beneficial fatty acids such as lauric acid (C:12) and palmitic acid (C:16) in BSFL are crucial for fish growth and health and have been reported to improve the overall nutritional quality of feed thus, enhancing the growth performance and health status of fish (Kroeckel *et al.*, 2012).

In terms of economic viability, the farming of BSFL is cost-effective as it can be reared on various organic substrates, including agricultural by-products and food wastes thus, contributing to cost-effectiveness (Opoku *et al.*, 2023; Spranghers *et al.*, 2017b). In addition, by utilising BSF larvae in aquafeed, the aquaculture industry can

reduce the dependency on fluctuating fishmeal prices thus, providing a more stable and potentially sustainable raw material for aquaculture feed formulations.

Furthermore, the use of BSFL also contributes to environmental sustainability. For instance, the rearing of BSFL on organic wastes mitigates the environmental impacts of wastes such as pollution and also contributes to circular economy (Diener *et al.*, 2011; Rehman *et al.*, 2023; Sayed *et al.*, 2023). The farming of BSFL can significantly lower environmental footprints (Gebiola *et al.*, 2023; Oonincx *et al.*, 2019), as the cultivation of BSFL requires less land and water, thus, making them environmentally friendly alternatives for protein production for aquaculture feed production. Additionally, one of the primary environmental advantages of using BSFL is the reduced reliance on wild-caught fish for fishmeal production. This reduces the pressure on wild fish stock and preserves the marine ecosystems.

Though black soldier fly originates from the South American savannah, they are widely distributed across temperate, subtropical, and tropical regions (Kaya *et al.*, 2021). The species thrive within an optimal temperature of 30 °C (Chia *et al.*, 2018), allowing for their widespread presence in diverse climates. However, they cannot survive in regions with temperature below 5 °C (Spranghers *et al.*, 2017a).

Black soldier fly is a large, slender, and black insect whose body is divided into three segments: head, thorax, and abdomen, with brownish wings and tentacles extending from the head. The abdomen is made of five segments marked with distinctive white spots (Singh & Kumari, 2019). Males, although longer than females, possess smaller genitals and wings. Females typically measure between 12 and 20 mm in body length and exhibit wings spanning 8 to 14.8 mm (Lu *et al.*, 2022).

The life cycle of BSF has five stages: egg, larva, pre-pupa, pupa, and adult. Among these, the larval and pupal stages are particularly nutrient-rich. Their nutrient composition is influenced by the quality of food they consume. Black soldier fly has a lifespan of around 20-22 days, with the pupal stage lasting the initial 6-8 days and the subsequent adult metamorphosis extending over the last 14 days (Tomberlin *et al.*, 2002).

Remarkably, adult BSF lack a mouth, digestive system, or stinger, rendering them harmless to other organisms (Mlambo *et al.*, 2023). They also exhibit no affinity for human beings or fresh food, thus, eliminating any potential role as disease vectors.

Black soldier fly larvae processed for animal feed production are categorized into defatted and full fat BSFL, depending on the fat and saturated fatty acid content. The nutritional composition of BSFL is dependent on its form (Table 2.2).

Table 2.2 Nutritional value of BSFL in comparison to fishmeal
(g kg⁻¹, dry matter basis)

Form	BSFL Defatted	Full fat	Fishmeal
Crude protein	655.00	431.00	675.30
Crude fat	46.00	386.00	103.60
Ash	93.00	27.00	171.50
References	(Spranghers <i>et al.</i> , 2017b)	(Schiavone <i>et al.</i> , 2017)	(Onsongo <i>et al.</i> , 2018)

Numerous investigations have been conducted to ascertain the feasibility of using BSFL as a viable and efficient substitute for protein source in the formulation of fish diets. For instance, Muin and Taufek (2024) fed (initial weight, 30 g) two experimental diets containing fishmeal as a reference diet and BSFL meal as a test diet for six weeks and found that fish fed BSFL had significantly elevated growth performance (final weight, weight gain, SGR) and significantly improved FCR compared with those fed the control diet. Similarly, Limbu *et al.* (2022) included BSFL

meal in Nile tilapia fry (0.001 g) diet as fishmeal replacement at 0%, 25%, 50%, 75% and 100% for twelve weeks and reported significantly increased WG, SGR and enhanced feed efficiency without any adverse effects on the intestinal morphology in fish fed BSFL at 75% level of inclusion while those fed 50% BSFL had significantly elevated HSI. The authors recommended 81-84% level of BSFL inclusion in Nile tilapia fry as the optimum level required for growth of the fish. An eight-week studies by Muin *et al.* (2017) on the replacement of fishmeal with different levels (0%, 25%, 50%, 75%, 100%) of BSFL found that BSFL could effectively replace up to 50% of fishmeal in diet for Nile tilapia (initial weight, 3.0 g) as fish fed 50% BSFL had the highest weight gain, SGR, PER and significantly improved FCR compared with the other treatments.

Despite the beneficial effects of BSFL reported in these studies, a few studies reported no significant effects. For instance, Devic *et al.* (2018) added BSFL meal at 0, 30, 50 and 80 g/kg in commercially formulated diet and fed Nile tilapia (initial weight, 5.7 g) for 32 days and reported that growth performance (FBW, WG, SGR), feed utilization (FCR and PER) feed intake, whole-body composition (moisture, protein, lipid, ash, fibre) were unaffected by the treatments. Similarly, a 12-week experiment by Tippayadara *et al.* (2021) on replacement of fishmeal by BSFL meal at 0, 20, 40, 60, 80 and 100% in diet of Nile tilapia (initial weight, 14.77 g) showed that growth performance, feed utilization indices, feed intake, survival and haemato-biochemical parameters were not significantly different between the treatment.

Studies involving the effects of BSFL inclusion in diets of other fish species have been reported. According to Mapanao *et al.* (2023), the replacement (0, 25, 50, 75, 100%) of fishmeal with BSFL meal in diets for Thai climbing perch (*Anabas testudineus*) for eight weeks had significant effect on the fish as those fed 75% BSFL