THE NUTRITIONAL EFFECTS OF SELECTED ALGAE, PREBIOTICS AND COMMERCIAL HERBAL FEED ADDITIVES ON THE GROWTH RATE AND HEALTH OF JUVENILE SPOTTED GRUNTER, *POMADASYS COMMERSONNII* (PISCES: HAEMULIDAE)

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DECLARATION

I, Miss Anathi Mbona, solemnly and sincerely declare that this dissertation has been composed entirely by myself, that the work presented in this dissertation is my own except where clearly stated otherwise in the document and that this work has not been submitted to any institution, for any degree or professional qualification.

Signature……. **Date**……

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"His grace is sufficient for me."

"Kuthi mandidwanduluke ndithethe izilwimi ndimbonge ongabonwayo. Zinkulu izinto ondenzele zona, ngoko lingcwele igama Lakho." This is a testimony for my story of triumph through adversity… but let me dedicate it to my loving mother, Zukiswa Mbona-Nompuku "maNyawuza", words fail me. Her support has been amazing.

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ABSTRACT

In the aquaculture of fish and shrimps, diets generally contain high levels of fish meal. Fish meal is a nutrient rich feed that contains essential amino acids and fatty acids, which are required to maintain optimum growth and health of an animal. It is traditionally used in aquaculture to promote feed efficiency, nutrient uptake and feed intake due to its high palatability and digestibility. Overexploitation of fisheries resources and the high growth rate of aquaculture industry, however, continues to put pressure on the supply of fish meal, thus increasing the demand and price of this sought-after product. The use of alternative ingredients to fish meal, therefore, remains a high priority for aquaculture nutrition. Hence, the aim of this was to investigate the physiological and developmental effects on juvenile spotted grunter, *Pomadasys commersonnii*, after supplementing fish meal with different feed additives for 12 weeks. One of the additives was then selected for further feeding at graded levels (increasing levels) for 8 weeks to ascertain whether fish meal could be replaced by higher amounts of the feed additive without negative consequences to the cultured spotted grunter.

P. commersonnii is a marine fish that inhabits the warm waters of the Indian Ocean along the east coast of South Africa. The species is suitable for human consumption and has been identified as a potential finfish for marine aquaculture. Growth rate, feed efficiency and health parameters were investigated after feeding 6 experimental diets to spotted grunter for 12 weeks; the data was compared to those fish that were fed on a non-supplemented control diet of fish meal. The experimental diets were based on fish meal, but supplemented with one of the following additives: algae (*Spirulina platensis* and *Ulva lactuca*), yeasts (*Candida utilis* and *Saccharomyces cerevisiae*), and commercial herbal additives (LIV-UP® and UNP PB-20®).

Growth performance indicators were measured using the body weight, length and specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) that is, the ratio of weight gain to the amount of protein consumed over time. The assimilation of nutrients from the feed was assessed by a proximate composition

analysis in fish body, evaluating hepatosomatic index (HSI) and visceral fat index (VFI), as well as measuring glycogen content in the liver. The health status of the fish was evaluated from histological analyses of the distal intestines, blood glucose concentration and by determining the condition factor (*K*). The condition factor is the relationship between length and fish body weight and was used to evaluate the health condition of fish under changing environmental factors.

After the feeding period of 12 weeks, supplemented diets significantly increased the body weight, length, condition factor and FCR of spotted grunter when compared to the control diet. The growth performance of fish fed on *C. utilis* surpassed all the other treatments. However, fish fed on *Spirulina* supplemented diet had the best FCR when compared to the control. Moreover, there was a significant increase in specific growth rate (SGR), despite individual variation, in fish fed with *C. utilis* (2.14 ± 0.21 %/day). In contrast, the calculated SGR for the control group was 0.95 ± 0.09%/day. Similarly, the condition factor increased from 2.62 ± 0.27 for fish fed the control diet to 3.28 ± 0.34 for fish fed the *C. utilis* containing diet at the end of the feeding trial. Furthermore, the hepatosomatic index (HSI) was significantly affected after 12 weeks of feeding different diets. The HSI decreased from 1.42 ± 0.19 to 1.07 ± 0.10% for fish fed on *U. lactuca* and UNP PB-20® containing diets, respectively. No significant difference was observed in visceral fat index (VFI) after the feeding period of 12 weeks.

The addition of different feed additives in fish diets increased the deposition of visceral fat compared to the control. Similarly, blood glucose and liver glycogen content was affected by the addition of different ingredients in fish diets. Fish fed *C. utilis* included diets mediated the highest blood glucose content (5.59 ± 0.55 mmol/L) when compared with the control group value after 12 weeks of feeding. A significant difference was observed in liver glycogen levels of fish fed the respective diets. The glycogen content of the fish liver significantly decreased from 126.41 ± 6.87 to 65.94 ± 2.94 mg/g for fish fed on *C. utilis* and UNP PB-20® supplemented diet. The addition of *C. utilis*, *U. lactuca* and UNP PB-20® in spotted grunter diets increased the frequency of goblet cells in the distal intestine after 12 weeks of feeding. The *S. platensis* and *S. cerevisiae* fed fish mediated the longest mucosal folds, while *S. platensis* and *C. utilis* fed groups

distinguished themselves with complex mucosal folds. These results suggest that the growth performance and feed utilization of spotted grunter can be enhanced by the addition of feed additives. The slow growth rate of fish fed on diets containing *U. lactuca* and LIV-UP® corresponds with an observed increase in HSI, VFI and well-established signs of intestinal enteritis. The observed increase suggests that spotted grunter was unable to fully utilise the supplemented feed additives, which might explain the reduced growth rate.

The replacement of fish meal with increasing levels of the best performing diet (torula yeast) was evaluated in a second investigation to determine the effects of yeast on growth, feed utilization and health of spotted grunter. Experimental diets with increasing percentages of *C. utilis* (i.e. 0, 4, 8, 12, 16 and 20%) were fed to spotted grunter for 56 days. No significant differences ($p > 0.05$), were observed in all treatments for body weight gain, fish length, SGR and condition factor. There was trend of increase in the VFI of fish fed increasing levels of yeast, ranging from 3.44 ± 1.28 to 5.27 ± 0.92%, although the values were statistically non-significant. The HSI and blood glucose of fish were not affected by increasing percentage of yeast after 56 days of feeding. However, the glycogen storage in the fish liver was significantly affected by increasing levels of yeast after 56 days of feeding. The glycogen storage tended to decrease as the amount of *C. utilis* in the diet was increased, decreasing from 74.19 ± 5.37 to 54.54 ± 1.01 mg/g for fish fed on 8 and 20% yeast containing diet, respectively. The addition of 20% yeast in fish diets resulted in reduced height of intestinal folds, thickening of the submucosa and lamina propria, increased frequency of goblet cells and diminishing absorptive vacuoles while insignificant signs of enteritis were observed in other diets after 56 days of feeding graded levels of yeast. The results suggest that an optimum dietary concentration of 16% *C. utilis* in spotted grunter diets facilitates better growth rate and feed efficiency without any negative health effects.

In conclusion, the results of this study showed that *C. utilis* can be added in formulated diets for spotted grunter to enhance rapid growth rate and an optimum concentration of 16% *C. utilis* can be added in fish diets to improve growth and feed efficiency with no adverse health effects in fish.

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CHAPTER 1

GENERAL INTRODUCTION

As aquaculture, the farming of aquatic plants and animals, continues to grow so does the requirement to include bioactive dietary additives in commercial diets in order to promote fast growth rates, which will result in lowered production costs (Naylor *et al.*, 2000; Tidwell & Allan, 2001). Aquaculture is currently the fastest growing food sector globally, with an annual growth rate of 8-10% (Tacon *et al*., 2011), increasing from 5.2 million tons in 1981 to 62.7 million tons in 2011 (AES, 2013). Asia dominated production in 2011 with 88.9% globally, with China accounting for 35% in 2011 (AES, 2013). Furthermore, it has been projected that in 2020, China will account for more than 37% of the global fish production.

In South Africa, marine aquaculture is still at an infancy stage of development, with production limited to molluscs (i.e. abalone; (Britz, 1995). A number of indigenous fish species have been identified as suitable candidates for marine aquaculture which includes dusky kob, *Argyrosomus japonicas* and yellowtail, *Seriola lalandi* (Deacon & Hecht, 1995). Deacon (1997) also identified the spotted grunter, *Pomadasys commersonnii* as a further candidate for marine aquaculture due to its biological suitability for culturing under captivity and high market demand.

Pomadasys is a genus of grunters and it represents one of 25 marine fish species within the family Haemulidae (Smith & Mckay, 1986). After the grunters are captured from water, they grind their pharyngeal teeth producing the characteristic grunt sound, hence their common name. Only a few species of *Pomadasys* have been studied in aquaculture, namely spotted grunter, *P. commersonnii* and javelin grunter, *P. kaakan* (Bacela, 1998; Hussain & Zakia, 2000; Hecht *et al.*, 2003; Childs, 2005; Rastgoo *et al.*, 2014).

Spotted grunter (Figure 1) is not only valued as a good recreational fish (i.e. sport fishing species) but also for human consumption due to its taste and texture. The species inhabits warm waters of the Western Indian Ocean (Smith & Heemstra, 2003)

extending along the southeast coast of Africa to Seychelles, Madagascar and South Africa. In South Africa, this species is commonly found along the east coast in KwaZulu-Natal with occasional occurrences recorded towards the Southern Cape coast during summer (Day *et al.*, 1981). Spotted grunter occurs in inshore coastal regions, estuaries and in tidal fissures (Smith & Heemstra, 2003).

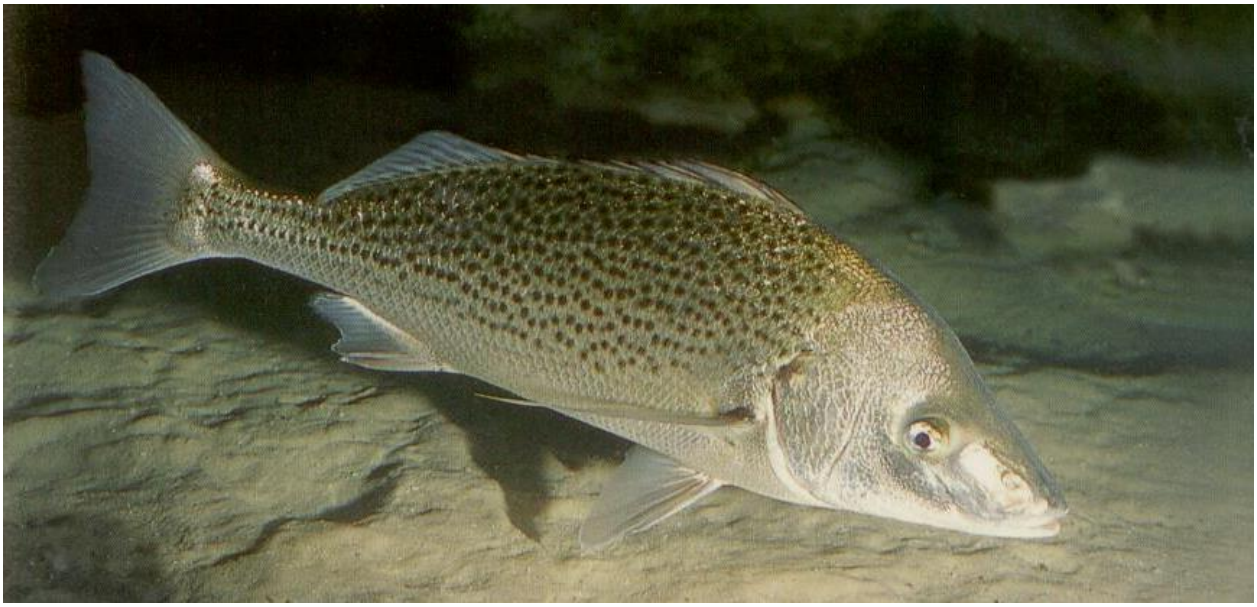

Figure 1: The spotted grunter, *Pomadasys commersonnii* (Photography from Branch *et al.*, 2010).

P. commersonnii is euryhaline (Whitfield, 1980) and has been found to inhabit ecosystems with salinities ranging from 0 to 90‰ (Whitfield *et al.*, 1981) and can survive in low salinities for an extended period (Deacon & Hecht, 1999). From laboratory experiments, it was concluded that the thermal preference for spotted grunter juveniles, under culture conditions, range between 24 and 25ºC (Deacon & Hecht, 1995). Childs *et al.* (2008) reported that temperatures below 16ºC in spring may induce behavioural changes and temperatures below 13ºC in Kosi and St Lucia systems resulted in mass mortalities to the species (Blaber & Whitfield, 1976). The spotted grunter is a benthic carnivorous fish species that is unaffected by turbidity (Hecht & Van de Lingen, 1992; Whitfield, 1998). In fact, Childs (2005) reported that the species can tolerate large variations in turbidity ranging from 4.1 and 567 FTU.

Spotted grunters have a life-span of 15 years with a maximum weight of approximately 10 kg (Day *et al.*, 1981). They reach sexual maturity in their third year of life, with a total length (TL) of approximately 280 mm for males and 350 mm for females (Day *et al.*, 1981). In South Africa, spawning occurs at sea during August and December (Whitfield, 1998; Heemstra & Heemstra, 2004). Newly hatched larvae develop at sea and early juveniles of 20 to 30 mm TL migrate to nutrient-rich estuarine waters during spring and summer for nursery conditions for at least 3 years (Whitfield, 1998). Adults spend most of their lives at sea but post-spawning adults return to estuaries for feeding during spring and summer (Day *et al.*, 1981).

Spotted grunters are pelagic-benthic feeders (Whitfield, 1998) depending on their life stage and area in which they are found. Juveniles between 20 and 40 mm feed on zooplankton, particularly copepods, while larger fish between 50 and 100 mm become benthic feeders, feeding on crustaceans, polychaete worms and small bivalves (Heemstra & Heemstra, 2004). Larger fish occurring in estuaries feed mainly on mud and sand prawns, *Upogebia africana* and *Callianassa kraussi*, respectively (Heemstra & Heemstra, 2004).

Spotted grunter are still under evaluation in aquaculture even though their biology and ecology have been well documented (Bacela, 1998; Whitfield, 1998; Childs, 2005). As mentioned, the optimum rearing temperature for *P. commersonnii* is between 24 and 25ºC (Deacon & Hecht, 1999) and with a photoperiod setting 12L: 12D (Deacon & Hecht, 1996). Furthermore, stocking densities of 6.4 kg/m^3 do not affect growth parameters (Bacela, 1998). An optimal dietary protein and lipid inclusion levels in formulated diets for *P. commersonnii* were found to be at least 45% protein with 12% lipid inclusion (Hecht *et al.*, 2003).

Understanding fish nutrition is regarded as one of the most fundamental biological aspects for the development of aquaculture (De Silva & Anderson, 1994). Feed in aquaculture is considered as the highest recurrent cost and constitutes more than 50% of the total operating expenses of a commercial farm (Davis, 1990). Therefore, it is necessary to compose cost effective diets that can yield optimal fish growth and feed

conversion ratio. The use of feed additives has become increasingly important in the industry to improve production efficiency.

Feed additives in fish diets

The use of antibiotics as feed additives to mitigate disease and improve growth has been banned due to the development of antimicrobial resistance, as well as harmful effects on human health (Pandey *et al.,* 2012). In that context, the single cell proteins (SCP), which include micro algae, bacteria and yeast; and commercial feed additives have been used as an alternative to antibiotics for fish feed ingredients (Li & Gatlin, 2004). The SCP dietary additives consist of various nutritional elements which include proteins, B-vitamins, pigments, complex carbohydrates and glucan (Ravindra, 2000).

Feed additives are supplemented in fish diets in trace amounts to facilitate growth, possibly increase nutrient availability and provide some nourishment to enhance fish growth. In an effort to determine the nutritional value of feed additives, a number of studies have been conducted to evaluate the nutritional composition of different feed additives (Worm *et al.*, 2006; Hasan & Chakrabarti, 2009; Güroy *et al.*, 2010; Rinna *et al.*, 2013; Abdel-Wahab *et al.*, 2016). The use of algae, SCP's (such as yeast and bacteria), as well as the commercial feed additives - have proven to have significantly enhanced the growth performance and health status of fish (Mustafa & Nakagawa, 1995; Balcázar *et al.*, 2006).

The use of commercial feed additives as an alternative to antibiotics in fish feed has been considered in recent years. Herbal extracts have been used as medicine and immune boosters by humans in different parts of the world. According to Pandey *et al.* (2012), selected herbs act as immune-stimulants which trigger early activation of non-specific defence mechanisms in fish and elevate the specific immune response (Yin *et al.*, 2008). Williams (2006) reported that herbs are comprised of a variety of immunological active compounds such as polysaccharides, organic acids, alkaloids, glycosides and some volatile oils which are capable of enhancing immune functions.

The use of herbs as growth promoters is gaining attention because they are cost effective, eco-friendly and have minimal side effects. Many studies have proved that herbal additives play a significant role in livestock and aquaculture (Frankic *et al.,* 2009; Madhuri *et al.*, 2012). The inclusion of herbal feed supplements has the ability to stimulate appetite and digestion, enhance fish growth and protect them from diseases (Ramudu & Dash, 2013).

The effect of dietary supplementation with a herbal growth promoter (Superliv®) was investigated on growth and body composition of *Oreochromis niloticus* (Dada, 2012). The fish that were fed diets supplemented with Superliv® showed higher survival, specific growth rate, feed efficiency, proximate composition and hematological parameters as compared to those that were fed a control diet. The authors recommended the use of Superliv® as a potential herbal supplement to improve sustainable, economical and safe fish production in tilapia.

The potential of incorporating various feed additives in fish diets as partial replacement for fishmeal have been investigated. Most of these evaluations have reported promising results in fish diets (Dada & Olugbemi, 2013; Dada, 2015; Wing-Keong & Chik-Boon, 2016). Of all the alternative dietary ingredients that have been studied, there is no single ingredient that could completely replace fish meal in fish diets hence many ingredients are yet to be investigated. Therefore, it is clear that the growth performance of fish species was positively affected by selected feed additives. Even though the effects of various feed additives are well documented for other fish species, there is no literature on the use of feed additives in the diets of spotted grunter, *P. commersonnii*.

Different algal species have been used for nutritional purposes in aquaculture. Algae are natural photosynthetic organisms that are valuable for both man and animal throughout the world (Nisizawa *et al.*, 1987). According to Amosu *et al.* (2013), South Africa is one of the countries with marine rich flora and high level of endemism. Among 900 seaweed species that are found along the South African coastline, only twelve

marine algal species are currently exploited. The *Ulva sp*. is the most cultivated seaweed species for abalone aquaculture industries and large quantities are grown in paddle-wheel raceway ponds (Chopin *et al*., 2008).

Sea lettuce (*Ulva lactuca*) is a green macro alga that occurs in deep calm waters (approximately up to 10 meters) in oceans and estuaries along sandy and rocky coast. The sea lettuce is a rich source of protein, pigments, minerals and vitamins - especially ascorbic acid (Ortiz *et al.*, 2006). It promotes lipid metabolism, reduces body lipids and increases protein levels. Crude protein content of *Ulva* ranges from 10 to 26% of dry weight and is of high quality (Apaydin *et al.*, 2010).

Seaweed has shown positive effects on the growth performance in some fish species. Investigations on the effect of including small quantities of *U. lactuca* as a possible alternative protein source in specific fish diets have resulted in the improvement of fish growth, feed utilization, physiological activity, disease resistance, carcass quality and reduced stress response (Mustafa & Nakagawa, 1995; El-Tawil, 2010). Other studies proved that using *Ulva* meal as a feed additive for gilthead seabream (*Sparus aurata*) and red tilapia (*Oreochromis sp*.) can improve their growth performance without any adverse effects on feed efficiency and fish survival rate (El-Tawil, 2010; Emre *et al.*, 2013).

Specific microalgae also play a vital role in the rearing of aquatic organisms, which include molluscs, shrimps and fish. Among the microalgae, the genus *Spirulina* is frequently used because of its nutritional composition. *Spirulina platensis* is a cyanobacterium and a source of a SCP (Ghaeni & Matinfar, 2013). It is preferred over the use of most other microorganisms because of its fast proliferation rate, quantity and high quality of proteins (Ghaeni & Matinfar, 2013; Moreira *et al.*, 2013). *S. platensis* has a high protein content ranging from 50 to 70% compared with many other commonly used plant protein sources, such as soybean. It is also valued for its high levels of absorbable irons and other minerals, as well as high levels of B-complex vitamins,

essential fatty acids (linoleic and gamma-linolenic acids) and antioxidants such as carotenoids (Goksan *et al.*, 2007; Moreira *et al.*, 2013).

The *S. platensis* has positive effects on the growth performance, feed utilization, stress and disease tolerance of cultured fish. Several studies have been conducted using dried *Spirulina* as a supplement in the diets of fish. *Spirulina* has been evaluated as a substitute protein source in Nile tilapia (*O. niloticus*), with maximum growth rates, immunity and reduced mortality obtained in diets containing 5 to 10% of algal meal (Abdel-Tawwab *et al.*, 2008; Ibrahem *et al.*, 2013). The fish survival after they were injected with pathogenic *Aeromonas hydrophila* increased with an increase in *Spirulina* level in the diets. There was lowest fish mortality and bacterial counts on the fish that were fed 5 to 10% of *Spirulina*. The authors concluded that the optimum levels of 5-10% of *Spirulina* improves the growth performance and feed efficiency of Nile tilapia as well as its diseases resistance to *A. hydrophila* (Ibrahem *et al.*, 2013; Abdel-Tawwab *et al.*, 2008). The partial replacement of fishmeal with *S. platensis* was evaluated in juveniles of rainbow trout, *Oncorhynchus mykiss* with respect to growth performance and fillet pigmentation, with positive results (Mahdi *et al.,* 2013).

Certain SCP yeasts have the same content of amino acids as soy bean meal (Navarrete & Tovar-Ramirez, 2014). Yeasts are a rich source of protein and B-complex vitamins and their cell walls are selectively composed of chitin, mannan and immunostimulants. In addition, yeasts are also considered a less expensive dietary supplement as they are easily produced on industrial level from a carbon-rich substrate (Schulz & Oslage, 1976).

The brewer's yeast, *Saccharomyces cerevisiae* is a SCP having immunostimulatory properties due to its content of nucleic acid and components of complex carbohydrates (FAO, 2008). It contains 45% proteins, 8% fat, 13% ash, 10% water and 23% of fiber and carbohydrates. It has free amino acids but lacks methionine (FAO, 2008). Ovie and Eze (2014) investigated the response of *Clarias gariepinus* fingerlings fed on diets formulated with varying levels of wet yeast (*S. cerevisiae*). The yeast replaced the highest inclusion level of 40.5% of the fishmeal successfully. The growth rate of *O. niloticus* decreased with an increase in yeast level in the diet (Ozório *et al.*, 2012).

The best growth rate was found at 10% of the inclusion and a linear depression on growth performance was observed when fish were fed diets with more than 10% yeast. Olvera-Novoa *et al.* (2002) fed *Oreochromis mossambicus* fry torula yeast (*Candida utilis*) and in combination with a commercial diet and plant proteins. The authors found that there was no significant difference in the growth parameters but fish fed with 30% yeast diet and 65% plant protein obtained the best growth performance. They concluded that torula yeast can replace 30% of the protein in tilapia fry diets without reducing their growth.

Aim and objectives of the study

The aim of the current study was to investigate the effects of selected feed additives on growth and health status of juvenile spotted grunter, *Pomadasys commersonnii*, a fish species identified for potential marine aquaculture in South Africa. Juvenile *P. commersonnii* were, thus, fed fishmeal with the following dietary inclusions over a 12-week period: macroalgae (*U. lactuca*), microalgae (*S. platensis*), yeast (*C. utilis* and *S. cerevisiae*) and two brands of commercial herbal additives (LIV-UP® and UNP PB-20®). The following specific objectives of the study address various aspects of growth and health of the cultured juvenile *P. commersonnii*:

1. to determine the effects of the dietary inclusion of the above listed ingredients on the growth rate, feed utilization and whole body composition of spotted grunter;

2. to assess the effects of the dietary inclusions on blood glucose, liver glycogen levels, the hepatosomatic index, the visceral fat index and the histology of the gut wall as indicators of the health of the experimental fish.

3. to determine the effects of partial replacement of fishmeal with graded levels of torula yeast (*C. utilis*) on growth performance and health of spotted grunter, *P. commersonnii.*

These specific objectives were tackled through a combination of established morphometric, biochemical and histological techniques. It is hoped that this study will

contribute useful information to the potential of marine culture of *P. commersonnii* in South Africa for research purposes.

CHAPTER 2

GROWTH PERFOMANCE OF SPOTTED GRUNTER, *POMADASYS COMMERSONNII* FED DIFFERENT SUPPLEMENTED DIETS

Introduction

In aquaculture, feed is the primary operating cost and constitutes almost half of the total operating costs of a commercial farm (Davis 1990; FAO 2002). Aquaculture manufactures its key inputs (fish meal and fish oil) from wild caught marine fish species which are not considered for human consumption (Schipp 2008; Tacon & Metian 2008). Fish meal is produced after the process that involves cooking, pressing and drying pelagic fish species and fish oil is obtained during the pressing process of cooked fish (Jensen *et al.*, 1990). However, environmentalists criticize aquaculture industry for further depleting wild fisheries stocks worldwide (Naylor *et al.*, 2000) due to increasing demand of fishmeal. Approximately 30% of the fish harvested each year is converted to fish meal or fish oil (Schipp 2008) to be used in producing animal feed, including aquaculture feed.

The use of fish meal and fish oil in fish diets has increased as culturing of carnivorous fish species has expanded (FAO, 2009a). The reliance on fish meal in aquaculture as an important protein source has affected the cost of aquafeeds and consequently elevated the price of fish meal. This circumstance has led to intensive research within aquaculture, to develop and produce diets that are economically viable and can yield optimum fish growth within the stipulated time frame (Hunter & Roberts, 2000) while ensuring sustainable aquaculture production (FAO, 2009a; Allison, 2011). If growth performance and feed efficiency are substantially increased in commercial aquaculture, the relative cost of production is more likely to be reduced. In order to achieve a significant growth rate output, it is imperative that the diet contains a potential nutritional value. Proteins are the most valuable and essential nutrients required by fish for fast growth. An insufficient supply of proteins in fish diets may result in significant growth reduction and loss of weight (Wilson, 1989).

Generally, fish meal is an important protein source for use in aquaculture diets for carnivorous and omnivorous finfish. It is highly rich in essential amino acids (such as methionine and lysine) and fatty acids (eicosapntanoic acid (EPA) and docosahexanioc acid (DHA)) (De Silva & Anderson, 1994). The research and use of alternatives to fish meal remains a high priority though possibly associated with anticipated risks such as supply consistency, as well as cost and quality fluctuations. Over the past decade, the production of fish meal has been stable but the price has increased (FAO, 2002).

A considerable amount of work has been expended to evaluate a wide range of potential alternatives that are more likely to remain available than limited fish meal resources. Several studies have evaluated a range of ingredients derived from plant and animal based protein. Among plant ingredients that were used, the potential types include soybean meal (Viola *et al.,* 1982; Gallagher, 1994; Fagbenro & Davies, 2001), canola meal (Thiessen *et al.*, 2004; Abbas *et al.*, 2008, Enami, 2011) and lupin meal (Carter & Hauler, 2000; Caruso, 2015; Ranjan & Bavitha, 2015). Animal protein ingredients include meat meal (Lu *et al.,* 2015; Yu *et al*., 2015), blood meal (Nogueira *et al.,* 2012; Aladetohun & Sogbesan, 2013) and poultry meal (Emre *et al.,* 2003; Hernandez *et al.*, 2014).

The use of feed additives as an essential component has been gradually receiving attention from aquaculture researchers. Dietary supplementation of different feed additives (such as, immunostimulants, prebiotics and probiotics) is promising in improving fish growth performance, whilst also contributing nutritionally (Dada & Olugbeni, 2013; Ganguly *et al.,* 2013). The interest in supplementing aquafeeds with acceptable feed additives escalated after public awareness and banned use of antibiotics as growth enhancers in aquaculture, which can result in accumulative unfavourable side effects on human health and aquaculture products (Hao *et al.*, 2014). Many studies have shown positive effects of different feed additives on growth performance and feed utilization of cultured fish (Dada, 2012; Dada & Olugbemi, 2013; Ganguly *et al.,* 2013; Abdel-Wahab *et al.,* 2016).

Hence, the current study aims to examine the growth performance of *P. commersonnii* fed with selected algae, yeast and immunostimulant supplemented diets.

Materials and methods

Experimental fish keeping system

The experiments were carried out at Marine Research Aquarium from the Department of Agriculture, Forestry and Fisheries (DAFF) in Sea Point, South Africa.

An automated Recirculating Aquaculture System (RAS) with a collective tank volume of 9 300 L was used for feeding trials to evaluate the growth performance of spotted grunter fed with selected dietary feed additives for 12 weeks (April to June 2015). The RAS consisted of 20 cylindrical flat-bottom tanks each with an operating volume of 465 L per tank and a maximum depth of 67 cm (Figure 2). The experimental tanks were covered with a green 6 mm mesh (bird eye net) in order to prevent fish from escaping from the tanks. Sea water was drained by gravity from the tanks using a perpendicular up-standing pipe to the drainage pipe. Each tank was supplied with water pumped (750 W Speck Pumps SA Pty Ltd, Aquadrive 1100, 1.1 kW, Johannesburg) from the 4 biological filters (250 L, Kaldnes® biomedia) (Figure 3) at a rate of 9 L/minute which is equivalent to a turnover rate of 540 L per hour per tank. The system water was treated by ultraviolet lights (55W Pro UV, Ultrazap, Johannesburg) and heated to 24.5-25ºC using a heat pump (Aquaheat, SF 060, 17.5 kW, Cape Town). The water from the system was filtered by a drum filter (Fairve®, Figure 3). The filtration system was designed to manage fish densities of 40 kg/m^3, though only 1.5 kg/m^3 were used for this study. Ten percent of the total water volume from the system was replaced daily with sea water from Queens Beach at Sea Point in Cape Town. For aeration, each tank was supplied with an airstone connected to an oil-free air blower. Fish were maintained at a photoperiod of 14L: 10D (Deacon & Hecht, 1996) regime throughout the experiment.

Photoperiod was controlled by an analog timer and with overhead fluorescent lights (400 lux).

Figure 2: Rearing tanks (465 L) for *P. commersonnii* at DAFF Marine Research Aquarium in Sea Point. Note the experimental tanks are covered with green nets to prevent fish from jumping out of the tanks and the (a) perpendicular up-standing pipe that drains water from the tanks to the (b) drainage pipe.

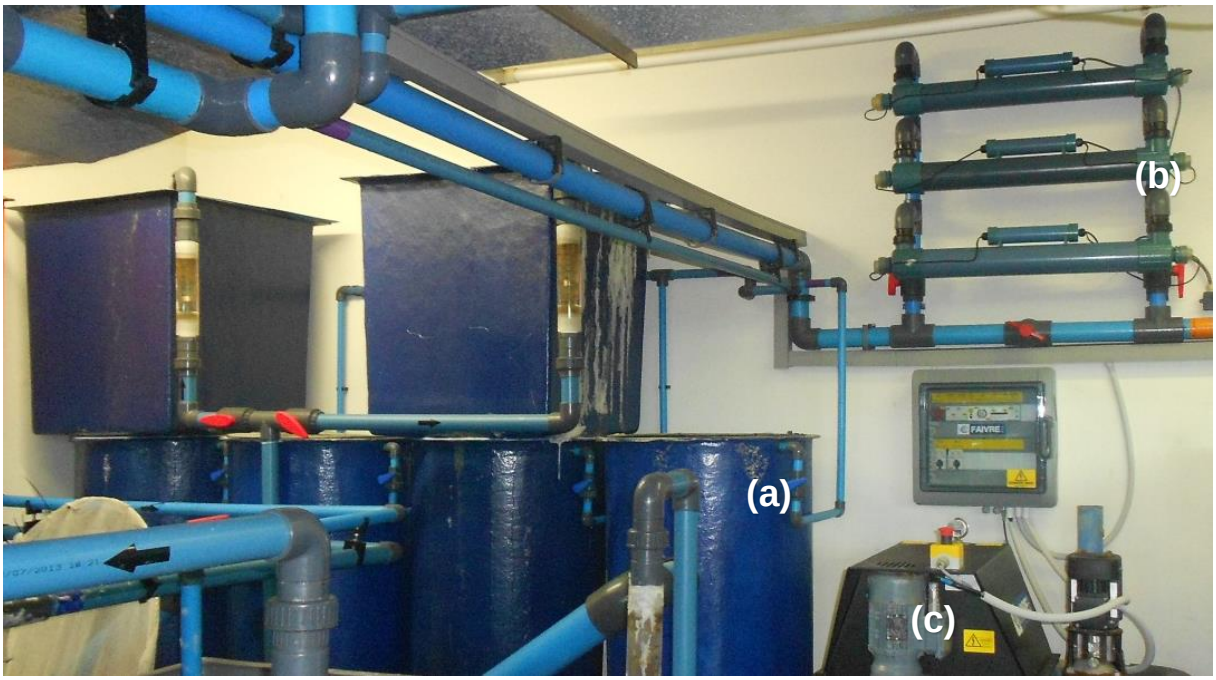

Figure 3: The filtration system in the Marine Research aquarium. The system consists of (a) four biological filters (250 L) to maintain optimum water quality in the culturing

system (b) Ultraviolet lights (55W) for treating water in the system and (c) Drum filter to remove organic compounds from the water system.

Water quality parameters

The fully automated recirculating system incorporated with biological filter and heat pumps was used to control and monitor ammonia and temperature throughout the feeding experiment. Despite this, the system was still manually monitored twice a week to eliminate factors that may contribute to fish mortality. The dissolved oxygen (DO) was measured weekly using a DO meter (YSI 85 DO/SCT meter, Ohio, USA). Total ammonia nitrogen (TAN) (ammonia, nitrite, nitrate and pH) were colorimetrically determined twice a week in the morning before feeding using SERA® test kits (Heinsberg, Germany).

Experimental fish and acclimation

Handling of live fish was conducted in compliance with regulations from DAFF and the Animal Ethics Committee of the Science Faculty at the University of Cape Town Animal Ethics Clearance number: Fish diet (2015/v5/HM) biosecurity protocols that govern animal welfare. One-month old captive bred *P. commersonnii* fry were obtained from the Sea Point Research Aquarium hatchery and were grown-out for three months before the experiment started. During the first month the fry were fed an imported feed: 200-500 micro (µ) pellet (59% protein; 14% lipid; Skretting GEMMA Micro 300, Italy), followed by commercial trout meal (45% protein; 14% lipid; Aquanutro, South Africa) to apparent satiation, four times a day at most.

Two weeks prior to the initiation of the feeding trial, spotted grunter juveniles were fed trout starter pellets twice a day at 09h00 and 15h00 at a maximum of 3.6% of their body weight per day, to acclimate them to the experimental feeding regime. The fish were acclimated in the same tanks that were subsequently used for the experiment.

Starting weight and stocking density

After the acclimation period, fish were purged for 24 hours prior to the feeding trial to ensure stomach evacuation. Fish were then anesthetized with 2-phenoxyethanol (Merck laboratories, Johannesburg) at 0.2 mL/L, weighed to the nearest gram and standard length was measured to the nearest millimeter. According to Deacon *et al.* (1997), 2-phenoxyethanol has no significant effect on the growth rate of spotted grunter. A total of 300 fingerlings of spotted grunter with an average initial weight (mean ± standard error) of 14.38 ± 0.67 g were randomly stocked in 20 tanks (15 fish per tank). All the experimental fish were derived from the same broodstock.

Experimental diets

Feed collection and formulation

All the experimental diets were prepared at DAFF Marine Research Aquarium, feed manufacturing laboratory, Sea Point.

Dietary test ingredients were purchased locally and internationally (Table 1) except for the fresh seaweed (*U. lactuca*) which was collected from an abalone farm in Gaansbaai, Western Cape in South Africa**.** The transportation of the seaweed was made in 50 L polyethylene containers. Upon reaching the Marine Research Aquarium where the study was conducted, the seaweed was washed with sea water, sun-dried and homogenised using a coffee grinder before it was mixed with other ingredients. Formulation and proximate composition of the experimental diets are presented in Table 1. The proximate composition of the experimental diets presented was conducted at the Agricultural Research Council (ARC) in the Department of Aquaculture in Pretoria, South Africa.

The fishmeal was mixed manually with pre-weighed quantities of multi-vitamins. All the diets had therefore a similar basal composition on a dry weight-basis (Table 2). An inclusion level of 12% of the respective test ingredients was supplemented to the basal diet, except for the control. The inclusion level of 12% was arbitrarily decided upon. The

final dry food mixture was kneaded by adding water until a homogeneous dough was obtained. The feed was oven-dried at 38ºC for 16 hours in the laboratory and feed was ground using an adjustable corn kernel hand grinder into desirable particle sizes**.** The feed was packed in sealed plastic bags and kept at -20°C for further use during the experiment.

Table 1: Formulation and proximate composition of experimental diets.

Ingredients (%)	Diets						
	Spirulina	*Ulva*	Torula yeast	Brewer's yeast	LIV-UP® liquid	UNP PB-20®	Control
Fish meal[1]	70	70	70	70	70	70	82
Spirulina platensis[2]	12	-	-	-	-	-	-
Ulva lactuca[3]	-	12					
Torula yeast[2]	-	-	12	-	-	-	-
Brewer's yeast[2]	-	-	-	12	-	-	-
LIV-UP® liquid[4]	-	-	-	-	12	-	-
Ultra-Natural Plus PB-20[5] (UNP PB-20®)	-	-	-	-	-	12	-
Binder	7	7	7	7	7	7	7
Premix[6] (Min/Vit mix)	1	1	1	1	1	1	1
Cod liver oil[7]	10	10	10	10	10	10	10
Diet proximate composition							
Crude protein	49.2	47.5	49.5	49.5	51.2	45.5	56.4
Crude Fat	15.5	14.4	15.6	15.1	14.9	14.5	16.7
Ash	10.1	12.4	10.6	12.8	11.7	10.1	12.5
Moisture	11.9	9.25	11.1	8.10	10.4	12.4	12.5

[1]Aquanutro (Pty) Ltd, 5 Aqua Crescent, Malmesbury, South Africa
[2] Nature's Choice, Meyerton, Cape Town, South Africa
[3]*Ulva lactuca* was freshly collected from Gaanbraai, Cape Town, South Africa
[4] Century Pharmaceuticals Limited, Sayajigunj Vadodara, India
[5] Biologistics Canada, Industrial Chateauguay, Canada
[6]Kyron Laboratories (Pty) Ltd, Benrose, Johannesburg, South Africa
[7]Alpha Pharmaceuticals, Mayville, Durban, South Africa

A predetermined ration of each formulated diet was fed to three tanks of fish (i.e. 3 replicates per treatment), while the control had two replicates. The fish were fed at a ration consistent with a maximum of 3.6% of their body weight per day, but divided into two feeding sessions (09H00 and 15H00).

Data collection

At the start and end of the feeding trial, fish were individually weighed using a Mettler Toleo electronic scale (VP SW 15, Microsep (PTY) Ltd, Sandton, accuracy to 0.01 g) and the standard length (SL) was measured (in mm) using a ruler from the tip of the snout to the posterior end of the last vertebra of the fish. Before the fish were handled, food was withheld from the fish for 24 hours and subsequently anesthetized with 2-phenoxyethanol at 0.2 mL/L whenever handling was necessary. All 10 fish were then returned to their respective tanks. Only ten fish were randomly sampled from each tank to gather morphometric data (body weight and length) after every 7 days of the feeding trial for 12 weeks.

The tanks were daily monitored for any behavioural changes or mortalities and dead fish were removed immediately from the tanks and mortalities recorded.

The fish growth was measured in terms of increase in body weight and length. In addition, specific growth rate (SGR), percentage weight gain, feed conversion ratio (FCR) and condition factor (*K*) were used to assess the effect of different supplemented feed on the growth performance of spotted grunter and were calculated using Equation 1 to 5 respectively:

Specific growth rate (SGR) is the measure of percentage increase in body size over time and is expressed as:

$$SGR\ (\%\ day^{-1}) = [Ln(W_f) - (W_i)] \times D^{-1} \times 100 \quad (1)$$

where W_f represents the final weight of fish in (g), W_i represents the initial fish weight (g) and D represents the number of days.

$$Weight\ gain\ (WG, g) = (W_f - W_i) \quad (2)$$

where W_f represents final weight of fish in (g) and W_i *r*epresents the initial fish weight

Feed conversion ratio (FCR) is a collective mass of the dry feed consumed for the experimental period and wet body weight gained by the fish. It measures the efficiency of the fish in assimilating the nutrients that are contained in a diet. FCR is expressed as follows:

FCR= dry feed consumed* (g) / wet body weight (g) (3)

*Air dry (i.e. 10% moisture). The FCR expresses a ratio factor.

Condition factor (*K*) is the relationship between the length and fish body weight. It can be used to determine health condition of fish under changing environmental factors.

Fulton's condition factor (*K*) is expressed as follows (Fulton, 1902):

$K= 100(W / L^3)$ (4)

where W represents fish wet weight in (g) and L represents the fish length in (mm)

Protein efficiency ratio (PER) is the ratio of the weight gain to the protein consumed over a certain period of time. It is calculated as follows:

PER = Weight gain per fish/protein intake per fish (5)

Proximate analysis

At the end of the 12-week growth experiment, 3 randomly sampled fish from each replicate (thus, 9 fish per supplemented treatment and 6 fish for the control) were pooled. From the pooled material three replicates per treatment were analysed to give a sample number of 21, including the control, hence the reported DF error = 14. A composite sample of 500 g of each experimental diet was selected and used to analyse the chemical composition of each diet. Three replicates of approximately 2 g of each treatment (n = 3) were used to determine total crude protein, lipid, ash and moisture content. The fish were euthanized using an overdose (2.5 mL/ 25 L) of clove oil (Sri Venkatesh Aromas, Dehli, India) and homogenised with a meat mincer. The samples (homogenised fish and experimental diets) were packed in a cooler box with ice and

sent to Agricultural Research Council (ARC) in the Department of Aquaculture for proximate analysis. The crude protein for the pooled material (per replicate) was determined using the Kjeldahl method (AOAC Official Method 2001.11) in a UDK 159 automatic Kjeldahl Analyzer. Lipids were extracted from the samples by solvent petroleum ether (Soxhlet procedure) using Buchi 810 Soxhlet Fat extractor. The lipid percentage was then calculated by gravimetric analysis (AOAC Official Method 948.15). The ash content (AOAC method 942.05) was determined by placing the samples in a furnace for 4 hours at 550°C and for moisture content (AOAC method 934.01), the samples were oven dried for 72 hours at 95°C.

Statistical analysis

All values are represented as mean ± standard error; n = 2 for the control group and n = 3 for each of the supplemented diets. One-way analysis of variance (ANOVA) was used to compare treatment means and a Tukey's multiple range analysis was performed if any significant differences were detected $p < 0.05$. A non-parametric Kruskal-Wallis test was used to compare means between treatments at $p < 0.05$ if the data did not meet the assumptions of ANOVA (Shapiro-Wilk's test for normality of the residuals and Levene's test for homogeneity of variance). Data collected on total body weight gain, SGR, final length and condition factor were analysed using one-way analysis of variance. Tukey's *D*-test was used to compare means between treatments at $p = 0.05$. The statistical analyses were carried out using the software Dell™ STATISTICA™ 13.0 version 13 (Statsoft, US). The data collected for experimental replicates were pooled as no significant difference were found amongst them.

Results

Water quality

All the tested water quality parameters did not differ significantly among the treatments. The mean water temperature and pH were 24.7 ± 0.31ºC and 8.03 ± 0.19, respectively. Similarly, the dissolved oxygen and total ammonia-nitrogen were kept within the range

of 6.2 to 6.8 and 0.01 to 0.25 mg/L, respectively throughout the experiment. The salinity of the water remained consistent at 35 mg/L during the feeding trial.

Juvenile survival

After the seventh week, three and four fish mortalities were recorded on *Ulva* and control diet respectively. Fish samples were immediately collected for autopsy and gill parasites were diagnosed by a fish veterinarian. The outbreak of parasites resulted due to defective UV lights. Apart from this incident, most fish survived until the experiment was completed.

Proximate composition of the experimental fish diets

The proximate composition of the experimental diets varied significantly ($p < 0.05$) among treatments with means (± standard error) of 49.98 ± 1.83% for crude protein ($F_{(6, 14)} = 49.61$, $p=0.001$), 15.1 ± 0.49% for lipids ($F_{(6, 14)} = 9.51$, $p=0.0003$), 10.14 ± 1.67% for moisture ($F_{(6, 14)} = 40.99$, $p=0.001$), 11.3 ± 1.0% for ash ($F_{(6, 14)} = 1579.26$, $p=0.001$) and 0.84 ± 0.17% for fibre (ANOVA: $F_{(6, 14)} = 252.11$, $p = 0.0001$, Table 1).

Growth performance

Acceptability and palatability of the experimental diets were assessed by observing the feeding behaviour of the juvenile *P. commersonnii*. All the diets were well accepted by the spotted grunter but the *U. lactuca* supplemented diet appeared to be less palatable to the fish as the feeding rate was slower than that of other treatments. The fish appeared healthy throughout the experiment.

The initial body weight, length and condition factor of all fish used in the experiment were statistically similar ($p > 0.05$) with mean body weight of 14.38 ± 0.67 g (Kruskal-Wallis: $H_{(0.05,10,10,10,10,10,10,10)} = 4.50$, $p= 0.60$), a mean body length of 8.00 ± 0.33 mm (Kruskal-Wallis: $H_{(0.05,10,10,10,10,10,10,10)} = 8.36$, $p= 0.21$) and mean condition factor of 2.82 ± 0.18 (Kruskal-Wallis: $H_{(0.05,10,10,10,10,10,10,10)} = 7.41$; $p= 0.28$), (Table 2; data shown as mean ± standard error, n = 10).

The growth responses of the juvenile grunter under different treatments are given in Table 2. A significant difference was observed in weight gain after 12 weeks of feeding different diets to spotted grunter juveniles ($F_{(6, 13)} = 8.71$, $p = 0.0006$)**.** In the first four weeks of the feeding trials, however, the growth rate of fish in all treatments was slow; thereafter, a notable growth improvement was evident after feeding the experimental diets (Figure 4 and 5). All fish fed with supplemented diets almost tripled their initial body weight after 12 weeks of feeding, while the fish fed with the control (non-supplemented) diet did not gain so much weight (Table 2; Figure 5)

Juvenile spotted grunter fed on the control diet grew significantly slower after the fourth week of feeding compared to the other groups of fish that were fed the supplemented diets (Figure 4). The control group only attained 23.01 ± 0.6 g of body weight after 12 weeks of feeding, thus an approximate growth rate of 1.92 g per week, while the growth rate of fish fed on torula yeast supplemented diet surpassed all other diets with a body weight gain of 66.4 ± 10.28 g at the end of the experiment, thus an approximate growth rate of 5.53 g per week. There was a successive body weight gain in fish fed other supplemented diets with order of magnitude starting from the lowest average (LIV-UP®) at 44.1± 3.21 g to the succeeding (*Spirulina*) at 58.6 ± 8.27 g. The *Ulva* and UNP PB-20® showed intermediate growth performance than those that fed on LIV-UP®, whilst fish growth with brewer's yeast dietary supplement was performing close to the indicated value for *Spirulina*.

A significant difference in SGR was observed between fish fed with torula containing diet and control while there was no significant variation observed among all other feed additive containing diets ($F_{(6, 13)} = 2.17$, $p = 0.05$). The mean SGR of fish fed torula yeast containing diet was significantly high (2.14 ± 0.21%/day) compared to fish that were fed the other diets (Table 2, Figure 6). The average SGR for fish fed other feed additive containing diets ranged from the lowest 1.56 ± 0.23%/day for UNP PB-20® to 2.10 ± 0.15%/day for *Spirulina* containing diet. The intermediate LIV-UP® (1.68 ± 0.08%/day) performed close to the average SGR value of fish fed *Ulva* supplemented diet (1.70 ± 0.12%/day). It was observed that fish fed with control diet has significantly

slow SGR (0.95 ± 0.09%/day, p = 0.046) than fish fed with supplemented diets (Figure 6).

The condition factor of fish fed different diets over 12 weeks was significantly higher than that of fish fed the control diet ($F_{(6, 13)} = 26.83$, $p < 0.0001$, Table 2, Figure 7). Fish fed on torula supplemented diet mediated the best condition factor (3.28 ± 0.34) among the fish fed other feed additive containing diets, while the control group exhibited the lowest condition factor of 2.62 ± 0.27. The average condition factor of fish that were fed supplemented treatments decreased from 3.20 ± 0.26 for fish fed on brewer's yeast to 2.95 ± 0.15 for fish fed on *Ulva* containing diet. The condition factor of 3.08 ± 0.16, 3.06 ± 0.14 and 2.99 ± 0.11 were observed in fish fed *Spirulina*, LIV-UP® and UNP PB-20® containing diets (Table 2).

A significant difference was observed in the final body length of the fish fed different treatments ($F_{(6, 13)} = 6.13$; $p = 0.003$, Table 2) with a mean of 13.06 ± 0.81 mm. All fish fed with supplemented diets grew to a final length significantly greater than the control fish. The torula yeast supplemented group showed an increase in body length to a maximum measure of 14.05 ± 1.25 mm, compared to body lengths that were attained with the other supplemented treatments. The *Spirulina* containing diet also increased final body length to 13.45 ± 1.12 mm followed by brewer's yeast, UNP PB-20®, *Ulva* and LIV-UP® containing diets with 13.25 ± 1.19, 13.07 ± 1.31, 12.89 ± 1.20 and 12.67 ± 1.26 mm, respectively. The control group attained the lowest final length of 11.4 ± 1.33 mm when compared to other supplemented diets (Table 2).

Table 2: The weight, length, condition factor (CF), weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) of *P. commersonnii* juveniles fed different diets over 12 weeks. Data given as mean ± standard error, n = 3 for each supplemented treatment and n = 2 for the control.

	Spirulina	*Ulva*	Torula yeast	Brewer's yeast	LIV-UP®	U
Initial weight (g)	12.52 ± 3.39	14.42 ± 3.84	13.2 ± 4.00	14.28 ± 4.15	14.16 ± 3.82	13
Initial length (mm)	7.69 ± 0.74	8.04 ± 0.74	7.64 ± 0.75	8.03 ± 0.82	7.94 ± 0.68	8.
Initial CF	2.75 ± 0.11	2.74 ± 0.40	2.81 ± 0.27	2.76 ± 0.05	2.83 ± 0.08	2.
Weight gain (g)	58.6 ± 8.27***	45.1 ± 2.92*	66.4 ± 10.28***	57.9 ± 6.05***	44.1 ± 3.21*	48
Final weight (g)	71.07 ± 15.71***	59.52 ± 12.40*	79.6 ± 20.85***	72.23 ± 11.43*	58.25 ± 12.82	62
Final length (mm)	13.45 ± 1.12	12.89 ± 1.20*	14.05 ± 1.25**	13.25 ± 1.19	12.67 ± 1.26**	13
Final CF	3.08 ± 0.16***	2.95 ± 0.15**	3.28 ± 0.34***	3.20 ± 0.26***	3.06 ± 0.14***	2.
SGR (% body weight per day)	2.10 ± 0.15	1.70 ± 0.12	2.14 ± 0.21*	1.93 ± 0.15	1.68 ± 0.08	1.

ANOVA followed by Tukey's *D*-test was used to compare means between treatments at $p = 0.05$. Footnotes: *significantly different from each other at $p < 0.05$; ** significant at $p < 0.005$; *** significant at $p < 0.0001$.

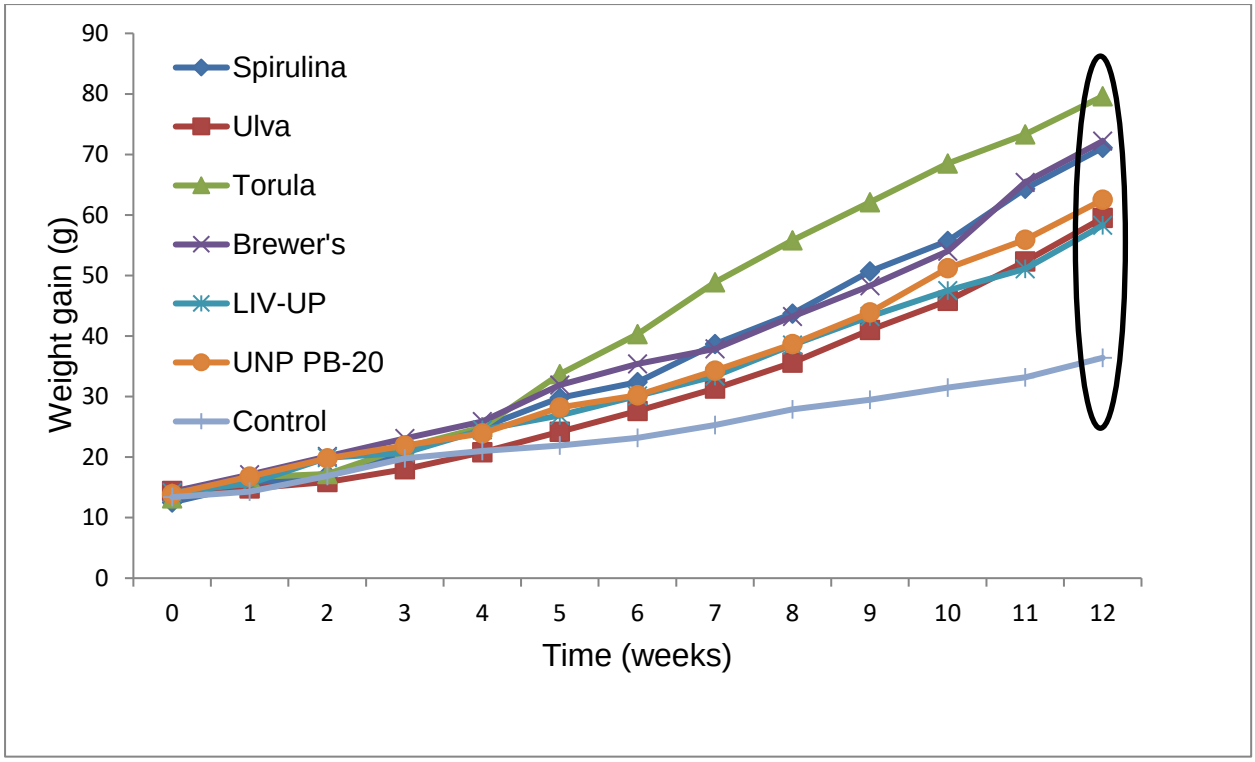

Figure 4: Weekly gains in live mass (g) of *P. commersonnii* fed different diets over a 12 week period. Each data point represents the mean of each treatment (n = 3 for the supplemented diets and n = 2 for the control). Data within the circle is represented in Figure 5.

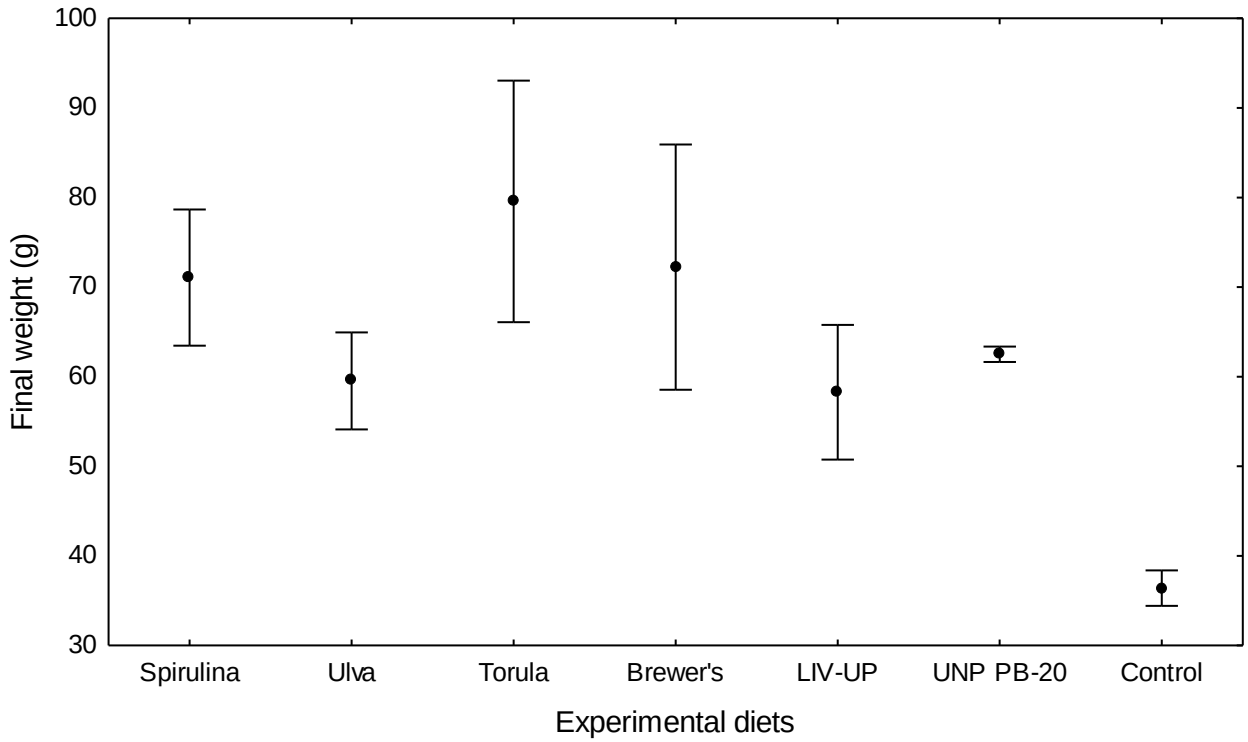

Figure 5: Final weight (g) of *P. commersonnii* after 12 weeks of feeding (n = 3 for the supplemented diets and n = 2 for the control). Data represented as Mean ± S.E.

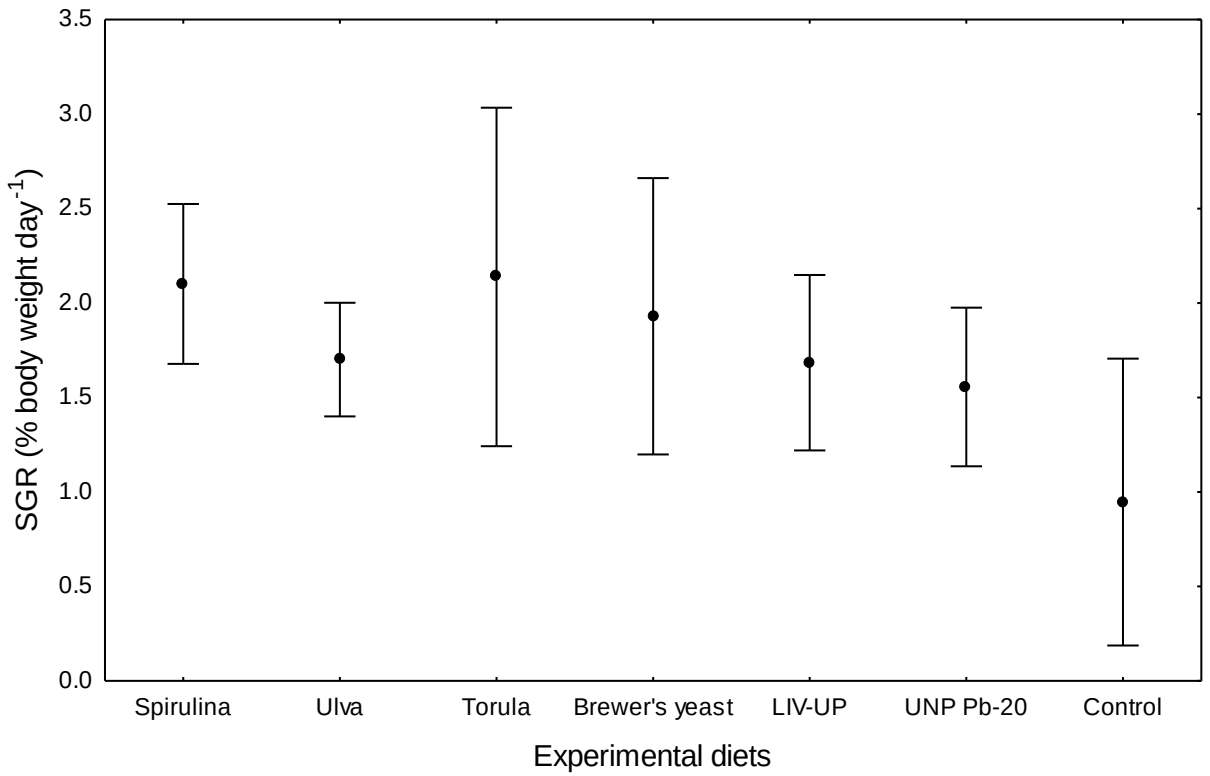

Figure 6: Specific growth rate (%/day) of *P. commersonnii* fed with different diets for a period of 12 weeks (n = 3 for supplemented diets and n = 2 for the control). Data represented as Mean ± S.E.

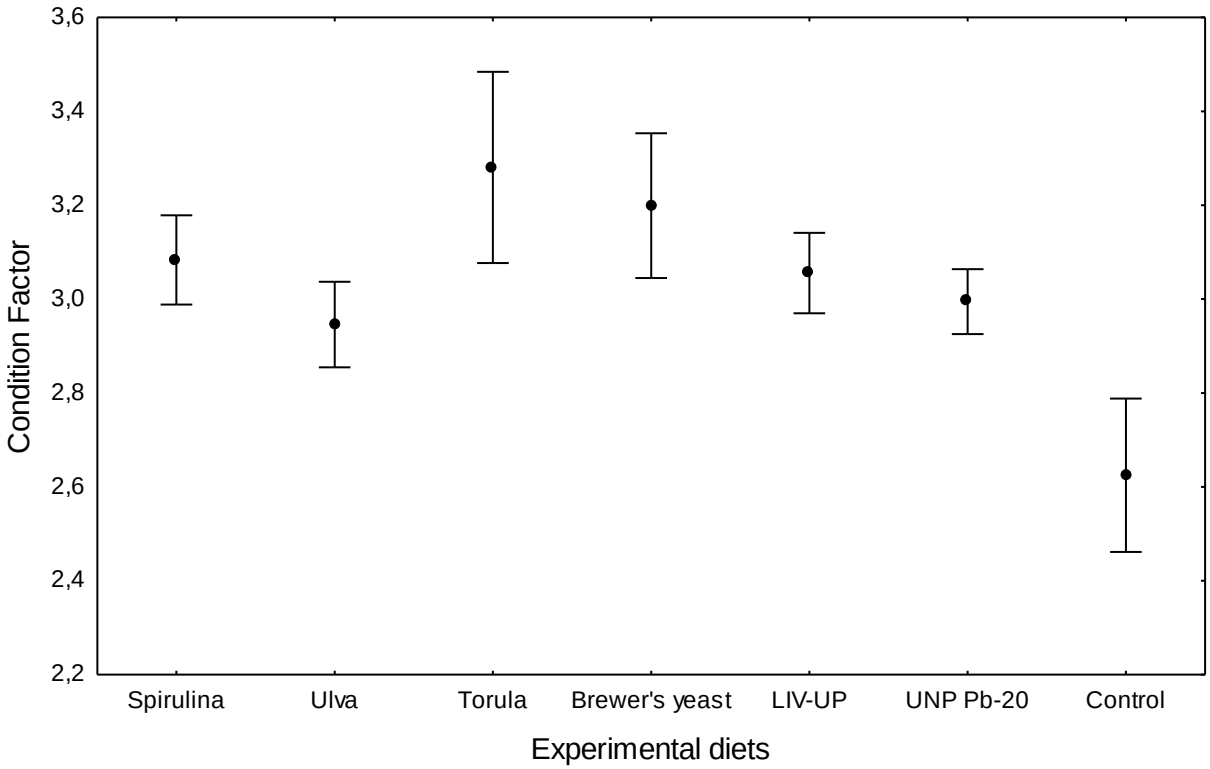

Figure 7: Condition factor of *P. commersonnii* fed with different diets for a period of 12 weeks (n = 3 for supplemented diets and n = 2 for the control). Data represented as Mean ± S.E.

The feed conversion ratio (FCR) was affected by the supplementation of fish diets with different ingredients. Fish fed supplemented diets improved their FCR compared to the control. Moreover, better FCR was observed among the groups where SGR and weight gain were also higher (diet supplemented with torula, brewer's yeast, *Ulva*, LIV-UP® and UNP PB-20® diet, respectively; Table 3). The best FCR (1.03) was mediated in *Spirulina* diet and the worst FCR for the control was determined to be 3.84 (Table 3). The exact same sequence of FCR was observed for protein efficiency ratio (PER). Fish fed on diet (*Spirulina*, torula, brewer's, *Ulva*, LIV-UP® and UNP PB-20®) had higher PER values when compared to the control of 0.45 (Table 3).

Table 3: Feed conversion ratio (FCR) and protein efficiency ratio (PER) of *P. commersonnii* for fish fed with different supplemented diets and the control for 12 weeks.

	Spirulina	*Ulva*	Torula yeast	Brewer's yeast	LIV-UP®	UNP PB-20®	Control
FCR	1.03	1.11	1.08	1.09	1.21	1.35	3.84
PER	1.24	1.07	1.19	1.17	0.95	0.86	0.45

Proximate composition of the experimental fish

At the end of the feeding trial, three fish from each tank were pooled to provide nine replicates per treatment. From the pooled material three replicates per treatment were analysed to give a sample number of 21, including the control, hence the reported DF error = 14. The proximate composition (crude protein and lipid content) of the whole experimental fish is listed in Table 4. The results show that the supplementation of fish diets with dietary ingredients affected protein and lipids: a mean (± standard error) protein content of 19.27 ± 0.23% ($F_{(6; 14)} = 5.68$, $p = 0.004$) and a mean lipid content of 7.10 ± 1.30% ($F_{(6; 14)} = 41.23$, $p = 0.001$) was obtained (Table 4). The whole body fat content was significantly higher ($p < 0.05$) in fish fed torula diet (9.06%) when compared to other treatments. The lipid content of the fish fed on supplemented diets ranged from 6.37 to 9.06% for fish fed on *Ulva* and torula yeast supplemented diets, respectively while the control diet (4.6%) was significantly

lower than all other treatments. The body lipid content of 7.90, 7.82, 7.16 and 6.73 was observed for fish fed on LIV-UP®, *Spirulina*, brewer's yeast and UNP PB-20®, respectively (Table 4). The protein content obtained by the fish that fed on *Ulva*, torula and brewer's yeast was higher (19.5%) than that of the control group (19.1%). Fish fed on *Spirulina* diet obtained the similar protein content as the control group, whilst the body protein content on fish fed with UNP PB-20® was close to an indicated value for the control group. Fish fed on LIV-UP® had a lowest content than the rest of the diets.

Table 4: Whole body proximate composition of *P. commersonnii* fed on different diets for 12 weeks.

Analyte (%)	Diets						
	Spirulina	*Ulva*	Torula yeast	Brewer's yeast	LIV-UP®	UNP PB-20®	Control
Crude Protein	19.1	19.5	19.5	19.5	18.9	19.2	19.1
Crude Fat	7.82	6.37	9.06	7.16	7.90	6.73	4.63

Discussion

The results of the present study suggest that the supplementation of fishmeal with specific ingredients, promote a higher rate of body weight gain, protein efficiency ratio (PER), length, condition factor and lowers feed conversion ratio (FCR) values *P. commersonnii* juveniles after 12 weeks of feeding. The supplemented fish diets, thus, improve nutrient utilization as indicated by enhanced body weight, length, FCR and specific growth ratio (SGR) of spotted grunter fed on supplemented diets when compared to the control group. Such comparative tests have also been documented for other fish species and are indicative of the potential benefits of using supplemented diets in aquaculture. Wassef *et al.* (2005) reported that *Pterocla dia* and *Ulva* inclusions of 10% and 5% respectively in diets for gilthead sea bream (*S. aurata*) produced significantly improved feed utilization, nutrient retention, survival and weight gain of approximately 2.5 to 3 folds to that of the control fed fish. In a similar manner, Atlantic salmon (*Salmo salar*) was observed to have an improved

growth rate, digestibility and nutrient retention when fed 40% *C. utilis* and *Kluyveromyces marxianus* containing diets for 89 days (Øverland *et al.*, 2013). In a study by Dada & Olugbemi (2013), growth performance related parameters on 0.5 g/kg Aqua pro® fed African catfish (*C. gariepinus*) attained higher weight gain, specific growth rate and feed utilization when compared to the non-supplemented diet (control) after the feeding trial. Velasquez *et al.* (2016) also found that 30% of *Arthrospira platensis* improve growth performance of Nile tilapia.

The results of the current study show that, inactivated torula yeast (*C. utilis*) containing diet appeared to be an effective feed supplement for stimulating faster growth rate in juvenile spotted grunter compared to other supplement incorporated diets tested (Figure 3, Table 2). Apparently, *C. utilis* yeast is rich in protein and has an excellent profile of essential amino acids (Athar *et al.*, 2009). The *C. utilis* diet also supported the best growth rate in Atlantic salmon, *S. salar* over other yeast diets (Øverland *et al.*, 2013). Matty & Smith (1978), Mahnken *et al.* (1980) and Olvera-Novoa *et al.* (2002) reported that *Candida* yeast was the best utilized protein source for growth by rainbow trout (*Salmo gairdnerii*) and tilapia (*O. mossambicus*). Conversely, a reduction in the growth rate of grey mullet (*Mugil cephalus*) was observed after 8 weeks of feeding torula yeast diet (Luzzana *et al.*, 2005), while the weight of abalone (*Haliotis midae)* was also negatively affected by the supplementation of torula yeast in its diet as abalone showed no significant growth improvement (Britz, 1996). From the current results, it is assumed that the *C. utilis* yeast portion, supplemented in the diet of the spotted grunter, contributed to the measured growth performance since the yeast strains are highly digestible and also contain prebiotics such as mannan-oligosaccharides (MOS) that stimulate growth of beneficial bacteria in the digestive tract (*Bifidobacterium* and *Lactobacillus*) to enhance digestibility, modulate intestinal microbial communities and concomitant increase gut absorptive surface area for nutrients (Gibson *et al.*, 2004; Dimitroglou *et al.*, 2009).

The selected dietary supplements did not result in any significant variation in the SGR of the fish; instead, the mean SGR followed the same trend as with weight gain. Fish size is one of the factors that may influence fish growth rate (Sumpter, 1992). Hence, fish grow much faster during their early stage. In the present study

fish fed on supplemented diets had a higher SGR compared to the control (Figure 6). The lack of significant difference in SGR observed in our study could be attributed to the larger fish size that was used at the start of the experiment. These results are comparable to the results presented for other fish species such as rainbow trout, *O. mykiss* (Akbulut *et al.*, 2002) who reported that larger fish grew significantly slower than the small fish.

The condition factor (CF) of spotted grunter was affected during the 12 weeks of feeding by the different diet supplements. CF is used to measure the health status of fish based on their weight-length relationship with the assumption that, heavier fish of a given length are healthy. It has been reported that CF is affected by feed availability, stress, sex and water quality parameters (Khallaf *et al.*, 2003). According to Goede & Barton (1990), condition factor decreases when fish are subjected to stocking density stress and might also fluctuate as a reflection of feeding activity and nutrient availability. In the current study, the growth conditions and nutrient availability appeared to be more favourable for almost all other treatments except for fish fed on *Ulva* and the control diet, which exhibited a significantly decreased CF. This might explain the slow growth rate (Figure 3) that was observed in spotter grunter fed these diets.

Like other carnivorous fish species, spotted grunter prefers animal protein diets to plant ingredients and such conclusion is in agreement with our findings showing a decrease in feed intake by fish fed diet containing *U. lactuca* (Table 2). Inclusion of higher plant-based protein in fish diets has been reported to reduce the rate of feed intake (Abdel-Wahab *et al.*, 2016). The apparently slow growth performance observed in *Ulva* diet might be attributed to the presence of anti-nutritional factors (such as saponins, tannins and phytic acids) which can hamper the growth rate of fish due to reduced palatability of the diets (Francis *et al.*, 2001). According to Azaza *et al.* (2008), 10% inclusion of *U. rigida* in fish diets contains 1.13% of saponins, 0.16% tannins and 0.47% phytic acids. Saponins are characterized by bitterness which could cause poor palatability of the diet (Emire *et al.*, 2013). Thiessen *et al.,* (2004) also argued that plant feedstuff contains a considerable amount of fibre which can have adverse effects on nutritional value and palatability. However, lower

inclusion levels of specific plant-based diets may be included in salmonid diets to improve growth and utilization rate (Collins *et al.*, 2013).

Yaich *et al.* (2011) found that seaweed, *U. lactuca*, contains almost 54% of fibre and which might reduce its utilization rate by carnivorous fish. Fibre structures present in fish diets diminishes the accessibility of digestive enzymes to feed nutrients (Abdel-Wahab *et al.*, 2016) making enzymes to be less effective. Therefore, high fibre content present in *Ulva* diet might be the possible reason for reduced palatability, feed intake, growth rate and possibly adverse effects in the intestines (Discussed in Chapter 3) observed in our study.

Different supplemented diets of spotted grunter also affected the efficiency of feed conversion. Generally, better feed conversion ratio values (i.e. 1.03, 1.08, 1.09, 1.11, 1.21 and 1.35) were achieved in all supplemented treatments (*Spirulina*, torula, brewer's yeast, *Ulva*, LIV-UP® and UNP PB-20®, respectively) compared to 3.84 of the non-supplemented feed (control) (Table 2). The best FCR value (1.03) was obtained in the *Spirulina* supplemented diet. These results are consistent with the results reported by Britz, 1996; Palmegiano *et al*., 2005 and Ramakrishman *et al.*, 2008, who observed that *S. platensis* supplemented diets improved the conversion ratio of reed sea bream (*Pagrus major*), abalone (*H. midae*), sturgeon (*Acipencer baerii*) and common carp (*Cyrinus carpio*). Unlike other commonly used plant-protein sources, *Spirulina* cells lack cellulose and makes it easy digestible, thus improving fish appetite, increasing feed intake and nutrient digestibility (FAO, 2008). In addition, Berestov (2001) found that its cell walls are rich in mucopolymer murein which is easily digested by the digestive enzymes secreted by monogastric organisms. The relatively low FCR values found in this study suggest that supplemented ingredients were efficiently utilized by the fish.

Similarly, protein utilization observed in fish fed supplemented diets was evidently more efficient than the control (PER = 0.45), as marked by higher PER values (1.34, 1.19, 1.17,1.07, 0.95 and 0.86) that serially corresponds with *Spirulina*, torula, brewer's, *Ulva*, LIV-UP® and UNP PB-20® as feed supplements. Similar results were observed in abalone (*H. media*) fed different protein sources (Britz, 1996). PER is regarded as a good indicator of protein quality and quantity in fish diet and body. It is often applied to evaluate protein utilization and its conversion into protein growth.

Therefore, low PER values (1.07, 0.95, 0.85 and 0.45) obtained from fish fed all the respective plant-based supplements and the control diets were indicative of poor dietary protein utilization. This might possibly explain the slow growth rates observed for these treatments (Figure 3).

The whole body lipid and protein composition of experimental fish, subjected to the multiple dietary supplements, were variably influenced by the latter (Table 4). The whole body protein content was comparable and in a narrow range between 18.9 and 19.5%. Fish fed on LIV-UP® containing diet had the lowest carcass protein (18.9%) although they were offered the highest dietary protein content (51.2%) when compared to all other treatments. It is evident that protein availability, digestibility and sufficient essential amino acid composition are key to formulating the best performing protein composition of species specific fish diets. The results conform to the findings of Hecht *et al.*, (2003) who reported that the optimum dietary protein level for juvenile spotted grunter varies from 48 to 50%. These results may be due to the fact that each fish species has a certain protein limit after which excess protein could not be utilized efficiently. Therefore, high dietary protein in spotted grunter may affect the growth performance of the fish. However, the PER will be indicative if all the essential amino acids are utilisable and in sufficient amounts.

CHAPTER 3

EFFECTS OF DIFFERENT SUPPLEMENTED FISH DIETS ON THE HEALTH OF SPOTTED GRUNTER, *POMODASYS COMMENSONNII*

Introduction

Proteins are the most expensive and essential nutrients required for fish growth. Therefore, an insufficient supply of protein in the diet will result in growth depression and loss of body weight in animals (Wilson, 1989). Fish require a balanced diet with all the essential and non-essential amino acids for growth. Hecht *et al.*, (2003) reported that at least 48% of dietary protein for spotted grunter is required for optimum growth. Proteins can be sourced from animals, plants and diet formulation permutations must provide for the availability and digestibility of these resources to resemble a complete nutrient profile as required for the fish species to be cultured. It is therefore inevitable that, nutrients for fish grown in captivity will be derived from different protein sources (Craig & Helfrich, 2002). The use of nutritive additives can enhance growth performance but it should be determined if the health of the fish is not affected in the process.

The species subjected to artificial (formulated) diets may be sensitive to these diets in such a way that histology of gastrointestinal tract (GIT) tissues may be altered. The intestinal alterations could have a negative effect on the standard fundamental aspects of a functional gut such as digestion and absorption of nutrients from the feed by the intestinal villi, microbial production and diversity as well as healthy immune system (De Lange *et al.*, 2010). Histological analysis of GIT can be used to indicate the nutritional status of the fish (Green & McCormick, 1999). When supplementing feed with formulated dietary ingredients derived from different raw materials, it is important to evaluate the impacts on gut histology as the digestive system can be adversely affected by feed used during the experiment. The intestines and liver are the most vital organs in digestion and absorption of nutrients, and as a result, progressive evaluation of these organs is considered necessary to monitor feed impacts on fish health (Rašković *et al.*, 2011).

The digestive system of fish is a tube-like structure which differs in complexity based on various feeding habits of the fish species (Merrifield *et al.*, 2011). Its primary role is to digest feed into molecules that are suitable for absorption into the bloodstream via different transport mechanisms of the epithelial border cells of the GIT sections (Merrifield *et al.*, 2011). The digestive tract of fish consists of the oesophagus, stomach and intestine. The digestion process starts from the stomach and the intestine's role is to complete digestion and facilitate absorption of nutrients from the feed (Canan *et al.*, 2012). The exposure of intestinal cells to anti-nutrients is considered a major risk that can result in severe tissue damage or enteritis (Merrifield *et al.* 2011). The anti-nutrients are substances that can hamper nutrient availability when present in diets and consequently induce negative health effects on fish (Soetan & Oyewole, 2009; Emire *et al.*, 2013). It is therefore imperative to progressively evaluate the histological and morphological alterations induced by anti-nutrients.

The wall of the intestine is made up of three distinct layers: the mucosa, the muscular and the outer layer (Figure 8). The mucosa forms the innermost layer of the intestine (the lining of the gut lumen) and consists of three different layers starting with the inner epithelium, then the *lamina propria*, and lastly the *muscularis mucosae* (Canan *et al.*, 2012). The cellular connective tissues consist of the *lamina propria*) and submucosa outer layer. The surface cells of the intestines exposed to the luminal contents are the enterocytes (epithelial layer), with intestinal villi structures which are also termed as the epithelial brush border (Figure 8). The layer of mucus secreted by goblet cells helps during the elimination of gut content and also forms the effective protection barrier against physical and chemical injury induced by the feed, bacteria and microbial products (Kim & Ho, 2010; Nazlic *et al.*, 2014). The submucosa consists of blood vessels and nerve fibres. These blood vessels are the portal for transferred organic molecules as well as minerals via intra- and possibly inter enterocyte passage routes. The submucosa is overlaid by an inner circular and then outer longitudinal muscular layer. The muscular layer is covered by the connective tissue of the outermost layer which is also known as the adventitia in the proximal part of the intestine - and the serosa in the distal part (Nazlic *et al.*, 2014).

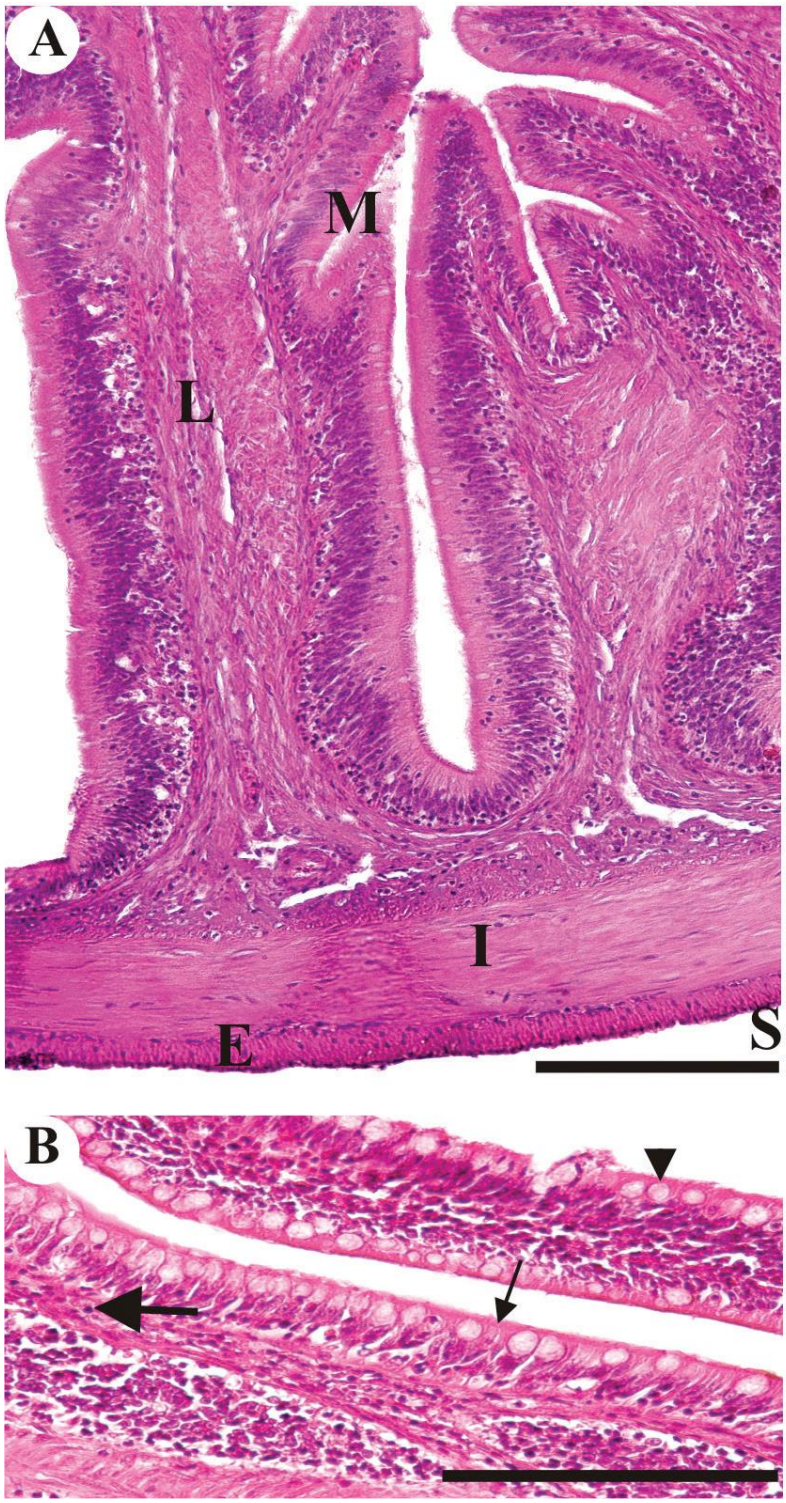

Figure 8: Detailed intestinal wall (Photograph of *Schizodon knerii* from Dos Santos *et al.*, 2015): **(A)** Intestinal wall illustrating the mucosal layers (hematoxylin & eosin, bar = 200 µm): M, lamina propria, L, inner muscular layer, I, outer muscular layer, E, serous membrane (outer protective layer). **(B)** Detailed mucosa (hematoxylin & eosin, bar = 200 µm): arrowhead - goblet cells, thick arrow – lymphocytes and thin arrow – brush border.

Histological features of the fish digestive tract vary in morphology and function depending on the taxonomy, feeding habits and habitat of fish (Abdulhadi, 2005). Typically, the digestive tract of carnivorous fish species is short when compared to herbivorous and omnivorous fish species. Plant materials present in herbivorous fish diets takes longer time to be digested and exposed to digestive enzymes; hence herbivorous fish have longer intestines than carnivores (Ferreira *et al.*, 1998).

As animals continue to grow, body tissues develop and the energy is stored in body tissues in the form of protein, lipids or glycogen. Glycogen is the stored form of glucose and is concentrated in the liver, skeletal muscles and heart tissues (Chia-Hsi *et al*., 2007; Harmon *et al.*, 2011) with varying quantities in different fish species (Enes *et al.*, 2009). Generally, the liver is the major storage site of glycogen. The stored glycogen is readily available as glucose to the body tissues and is reserved in the liver cells (hepatocytes) (Munawar *et al.*, 1989). Glycogen and blood glucose levels are good indicators of stress in fish (Barton, 2002; Martínez-Porchas *et al.*, 2009) and stressed fish have high level of blood glucose and low glycogen content stored in the liver. In fish rearing systems, common stressors associated with confinement, crowding, handling, transport, malnutrition and changes of water quality parameters (for example temperature, salinity and oxygen) are inevitable (Bianca, 2008; Harper & Wolf, 2009). However, severe exposure of fish to these stressors can evoke physiological and behavioural maladaptations resulting in inhibited growth rate, weaken their immune system and resistance to diseases and, eventually result in massive mortalities (Iwama *et al.*, 1997, Bianca, 2008). Glycogen reserves in the hepatocytes (liver cells) serve as a primary source of energy utilized by fish during these emergency stress situations, releasing it to the blood as glucose (Enes *et al.*, 2009; Harper & Wolf, 2009). Concurrently, the amount of glycogen reserves in the liver decreases with increasing plasma glucose concentration (Munawar *et al.*, 1989).

Glycogen storage gets depleted when fish are exposed to toxicants, or subjected to anoxia and starvation (Ince & Thorpe, 1976; Padmavathy & Ramanathan, 2010; Javed & Usmani, 2015). Ince and Thorpe (1976), evaluated the effects of starving and refeeding of the Northern pike (*Esox lucius*) and found a significant reduction in blood glucose concentration, liver and muscle glycogen content after the fish were starved for 3 months. During the starvation period, the metabolic needs of the fish

were served by the hepatic lipid and glycogen reserves. These biochemical indicators completely recovered immediately after the fish were subjected to feed again. Similar results were observed by Blasco *et al.* (1992) and Barcellos *et al.* (2010) in *C. carpio* after a prolonged starvation period of two months and 12 days of refeeding. Barcellos *et al.* (2010) also reported the reduction of liver glycogen content in *Rhamdia quelen* starved for 21 days and an increase after two days of refeeding. Padmavathy & Ramanathan (2010) performed an experiment assessing the effects of anaerobic metabolism on blood glucose, liver and muscle glycogen of *O. mossambicus* under decreasing oxygen concentrations (5.20 to 1.58 mg/L) for two hours. All the tested parameters were significantly reduced under the oxygen level of 5.20 mg/L and further reduced when the fish were exposed to 1.58 mg/L (Padmavathy & Ramanathan, 2010). When African catfish (*C. gariepinus*) was exposed to sublethal levels of paraquat (0.30, 0.15 and 0.00 mg/L) for 8 weeks, there was significant decrease in liver and muscle tissues with increasing levels of the toxicant while the plasma glucose was directly proportional to the increasing concentration of paraquat due to stress (Kori-Siakpere *et al.*, 2007). Similar results were observed in fresh water fish, *Catla catla* that was exposed in heavy metal toxicant, cadmium chloride for 96 hours (Sobha *et al.*, 2007).

Fish respond to stress conditions in different ways and their responses are categorised into primary, secondary and tertiary response (Barton & Iwama, 1991). During the primary response, fish release stress hormones - namely cortisol and catecholamines (adrenaline and epinephrine) into the blood stream (Barton, 2002). The secondary response occurs as a result of the primary response (Barton & Iwama, 1991), causing the metabolic pathway to release a considerable amount of energy which is normally related to glycogen catabolism (Rottmann *et al.*, 1992; Barton & Iwama, 1991) and tissue structural alterations (Begg & Pankhurst, 2004). The tertiary response of fish to stressful conditions is characterised by changes in behaviour and physiology, including stunted growth, lowered resistance to diseases and appetite suppression (Barton, 2002). Consequently, stress in fish causes slow growth rate, susceptibility to diseases and eventually mortalities. Therefore, it is important to evaluate blood parameters which are confirmed indicators of stress and fish health.

Blood analysis can reveal the health status of the fish prior to any apparent signs of stress or health deterioration. Haematological variables (such as leukocytes, erythrocytes and glucose) have been used to monitor physiological changes in fish health (Schutt *et al.*, 1997). Hence the aim of this study is to investigate the effect of unconventional feed additives, such as selected yeast, algae as well as commercial herbal extracts on the health status of *P. commersonnii*.

Materials and methods

Experimental design

See Chapter 2 materials and methods, since tissue and blood samples analysed and discussed here originated from the fish in the feeding experiment described in chapter 2. That is, from juvenile *P. commersonnii* that were fed different diets for a period of 12 weeks (See dietary formulation in Table 1).

Histological preparation and analysis

After 12 weeks of a feeding trial, three fish per tank (i.e. nine fish for supplemented treatments and six fish for the control) were randomly sampled and sacrificed to assess histomorphological alterations induced by the feed. The sampled fish were euthanized with a dose of clove oil, weighed to the nearest gram and measured to the nearest millimetre.

The fish were dissected by cutting the ventral abdomen and the attached visceral fat and connective tissue as well as intestinal content were carefully removed. The distal intestine (DI), was cut longitudinally and fixed in 10% buffered formalin immediately after dissection, for 24 hours before being processed for histology. All the formalin fixed DI tissue samples were routinely dehydrated in ethanol, equilibrated in xylene and embedded in paraffin according to standard histological techniques. Sections of approximately 5–8 µm thickness were cut using microtome instrument (Leica RM2165, Germany) and stained with haematoxylin and eosin (HE). The DI tissue samples were sectioned longitudinally (i.e. perpendicular to the macroscopically visible folds). Processing of the tissues was conducted at the DAFF histology laboratory in Foretrust building (Cape Town, South Africa). Histological examination

was performed with a Zeiss light microscope; tissue morphology was evaluated using a semi-quantitative scoring system proposed by Uran *et al.* (2008). Evaluated tissue parameters consisted of:

- Intestinal fold height and width
- Enterocyte supra nuclear vacuolization
- Lamina propria thickness status of intestinal villi
- Cellular infiltration in the lamina propria and submucosa
- Number of goblet cells.

The criterion for scoring normal and enteritis status is based on assigning a number depending on severity of intestinal pathological alterations. Scores from 1 to 2 were considered as normal histology while scores that ranged from 3 to 5 represented significant, well established and distinguished enteritis, respectively.

Hepatosomatic and visceral fat index

The visceral fat of the euthanized fish was carefully removed, weighed and discarded, while the liver was weighed and later used to determine glycogen analysis content of the fish.

Hepatosomatic index (HSI, %) was determined using Equation 1:

$$HSI\ (\%) = W_L \times (W_F)^{-1} \times 100 \qquad (1)$$

where, W_L stands for the liver weight (grams) and W_F represents the weight of the eviscerated fish (grams).

Visceral fat index (VFI, %) was calculated using Equation 2:

$$VFI\ (\%) = W_{VF} \times (W_F)^{-1} \times 100 \qquad (2)$$

where, W_{VF} stand for the weight of the visceral fats (grams) and W_F represents the weight of the eviscerated fish (grams).

Haematology and Liver Glycogen preparation and analysis

Blood glucose

A blood sample was drawn from the caudal vein of each sampled fish (n = 3 per supplemented treatment and n = 2 for the control) with a 22 gauge, 1.5 inch needle into 3.0 cc syringes and immediately a drop of blood was applied to an Accu-check Active® test strip sensor that was inserted into an Accu-check Active® blood glucose meter (Roche Diagnostic, Germany) to obtain blood glucose concentration reading in millimoles per litre (mmol/L). Blood samples were taken in all the sampled fish, before the liver was removed and weighed.

Liver glycogen

The liver was removed immediately post-euthanasia and frozen in liquid nitrogen (-195ºC) for eventual glycogen analysis which was carried out using methods described by Bennett *et al.* (2007) and Rasouli *et al.* (2014).

Approximately 0,270 g of liver sample was homogenized in 2 mL of 10% perchloric acid ($HClO_4$) at 30 x 1000 rpm using a Polytron® dispensing and mixing machine (PT-MR 2100, Kinematica AG, Switzerland). The samples were allowed to stand on ice for 1 h and thereafter centrifuged at 280 x g for 17 min in a Hettich® centrifuge (D-78532, Germany). Five hundred µL of supernatant (with glycogen in suspension) was transferred to a 1.5 mL micro centrifuge tube and the glycogen present in the supernatant was precipitated by adding 600 µL of 96% ethanol. The supernatant was then centrifuged for 10 minutes at 1700 x g in a Prism R® centrifuge (Labnet International, Inc., NJ USA). The supernatant was decanted and the remaining excess supernatant was carefully removed with a 200 µL pipet. The resulting pellet (sample) was reconstituted in 1 mL of deionized water and incubated at 50ºC for 5 minutes in Accublock™ digital dry bath (Model: D1200, Labnet International Inc., NJ USA).

The phenol-sulphuric acid glycogen analysis method was conducted by adding 10 µL of each respective sample (10 µL of deionised water was used as a blank sample), 40 µL of 6.5% phenol solution and 200 µL of concentrated sulphuric acid to a Uv-star® 96-well polystyrene microplate (Greiner Bio-One®, Germany). The resulting mixture was allowed to stand for 5 minutes before the absorbance of the solution

was recorded using a 96 micro-titre spectrophotometer (Epoch BioTek®, USA) at a wavelength of 492 nm. Absorbance readings were converted to glycogen concentrations using a standard curve prepared with oyster glycogen (Sigma Chemical, USA).

Statistical analysis

All values are represented as mean ± standard error; n = 3 for the supplemented diets and n = 2 for the control group. Treatment means were compared with one-way analysis of variance (ANOVA). If there was any significant difference ($p < 0.05$) detected a Tukey multiple range was performed. A non-parametric Kruskal-Wallis test was used to compare means between treatments at $p < 0.05$ if the data did not meet the assumptions of ANOVA (Shapiro-Wilk's test for normality of the residuals and Levene's test for homogeneity of variance). All statistical analyses were carried out using the software Dell™ STATISTICA™ 13.0 version 13 (Statsoft, 2015).

Results

Hepatosomatic index (HSI)

The mean HSI of fish were significantly different after 12 weeks of feeding different diets (ANOVA: $F_{(6, 53)} = 3.75$, $p = 0.004$). Fish fed with *Ulva* and LIV-UP® containing diets had the higher HSI values of 1.42 ± 0.19 and 1.30 ± 0.12%, respectively, when compared to other diets (Figure 9, Table 5). The UNP PB-20® fed fish had the lowest HSI value (1.07 ± 0.10%) while the HSI values for fish fed on torula, *Spirulina*, brewer's yeast and the control ranged from 1.10 ± 0.13 for control group to 1.18 ± 0.22% for *C. utilis* fed group.

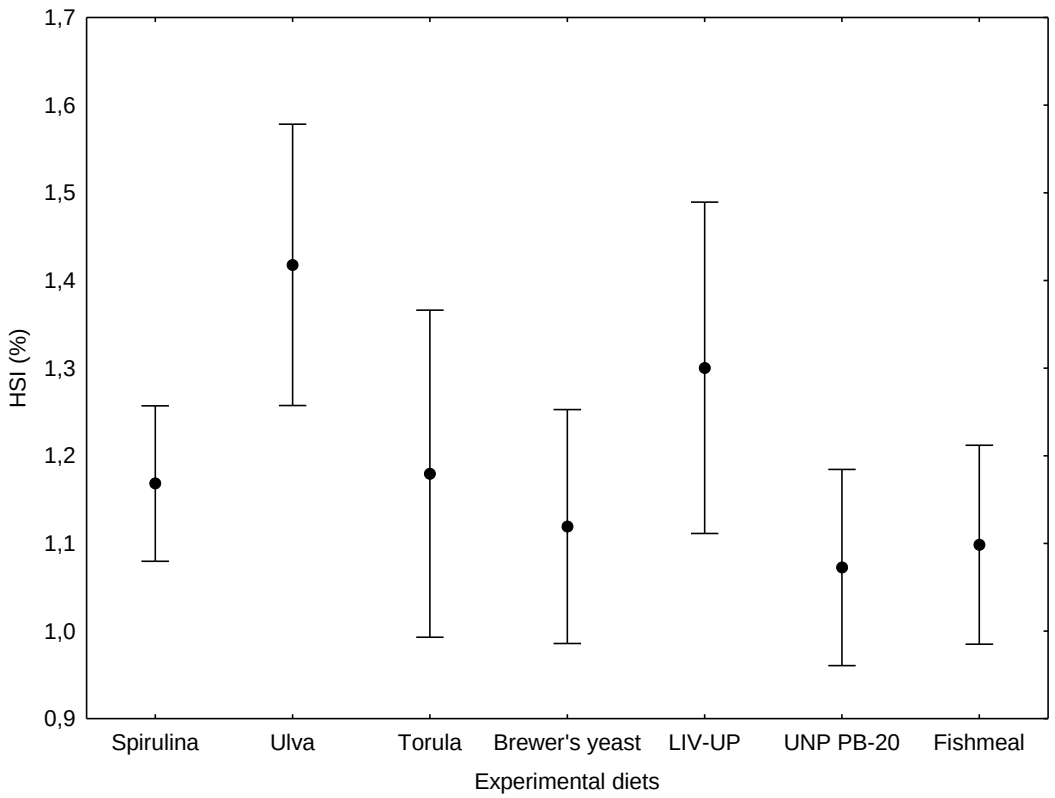

Figure 9: Hepatosomatic index (%) of *P. commersonnii* (n = 3 per supplemented treatment and n = 2 for the control group) fed different diets for a period of 12 weeks. Data represented as Mean ± S.E.

Visceral Fat Index

No significant difference was observed in VFI of spotted grunter juveniles fed with different diets ($F_{(6, 53)} = 1.88$, $p = 0.10$; Figure 10, Table 5). The mean VFI was higher in fish fed on brewer's yeast, *Spirulina*, LIV-UP® and UNP PB-20® containing diets compared to the control. Fish fed on brewer's yeast diet showed the highest VFI value (4.74 ± 0.79%) and the VFI values for *Spirulina*, LIV-UP® and UNP PB-20® fed fish ranged from 4.62 ± 0.78 to 4.72 ± 0.69% for fish fed on UNP PB-20® and *Spirulina*, respectively - with an intermediate value for fish fed on LIV-UP® (Figure 10). Fish fed on *Ulva* and torula had a similar mean VFI value (4.14%) which was fairly close to the lowest VFI value of 3.47 ± 0.85% that was observed on the control (Figure 10, Table 5).

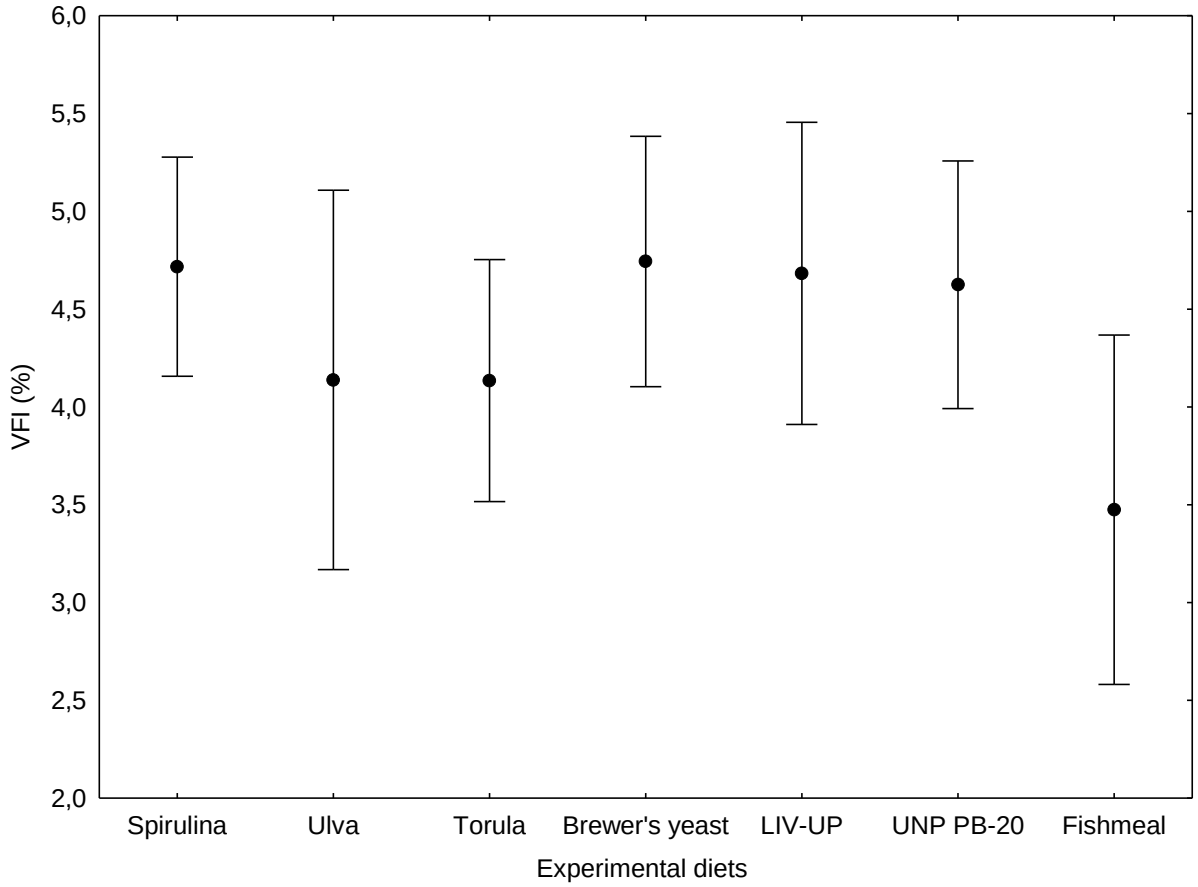

Figure 10: Visceral fat index (VFI, %) of *P. commersonnii* (n = 3 per supplemented treatment and n = 2 for the control group) fed different diets for a period of 12 weeks. Data represented as Mean ± S.E.

Blood glucose

A blood sample was drawn from six of the sampled fish per treatment. Blood glucose concentration of *P. commersonnii* was significantly affected by supplemented diets ($F_{(6, 33)} = 7.63$, $p = 0.00003$; Figure 11, Table 5). Fish fed on torula yeast containing diet was significantly different from all other diets except for *Spirulina* containing diet ($P = 0.94$). The highest mean blood glucose levels (5.59 ± 0.55 mmol/L) was observed for fish fed on torula containing diets. Fish fed on *Spirulina* supplemented diet also attained higher mean blood glucose of 5.20 ± 0.24 mmol/L although it did not differ significantly from that of fish fed on torula yeast. Fish fed on LIV-UP® and UNP PB-20® had a similar mean concentration of blood glucose (3.87 mmol/L). The group of fish fed on brewer's yeast had the lowest mean glucose level of 3.55 ± 0.80

mmol/L. Fish fed on *Ulva* and control diet had mean blood glucose levels of 3.95 ± 0.70 and 3.62 ± 0.74 mmol/L, respectively.

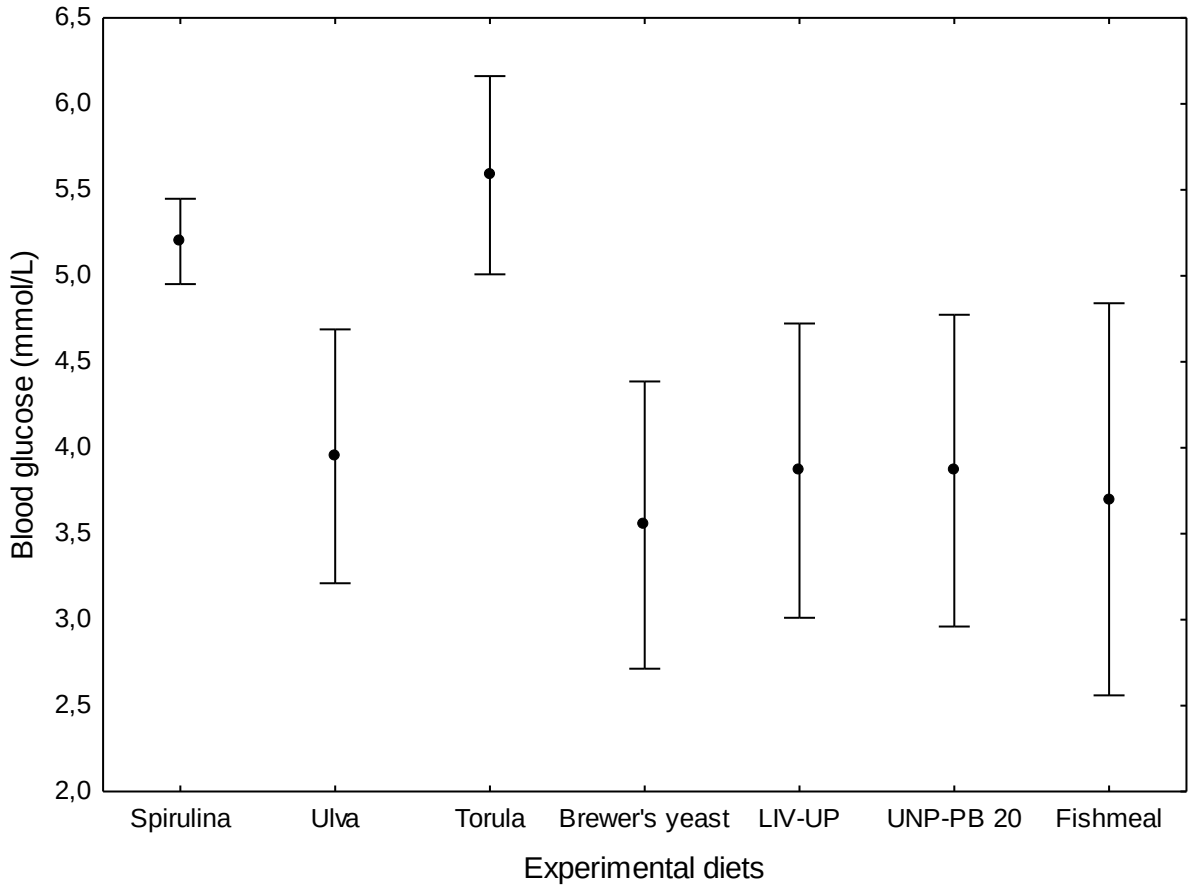

Figure 11: Blood glucose (mmol/L) of *P. commersonnii* (n = 3 per supplemented treatment and n = 2 for the control group) fed different diets for a period of 12 weeks. Data represented as Mean ± S.E.

Liver glycogen

The samples were analysed in triplicate to determine glycogen content. The mean liver glycogen of fish fed on UNP PB-20® was significantly lower (65.94 ± 2.94 mg/g, $p < 0.00001$) when compared to other treatments (ANOVA: $F_{(6, 53)} = 8.78$, $p < 0.00001$; Figure 12, Table 5). The torula yeast containing diet significantly increased glycogen deposition resulting with the highest storage of 126.41 ± 6.87 mg/g which was almost equal to that of fish fed on *Ulva* diet (124.75 ± 9.22 mg/g). The glycogen content of fish fed on the control, brewer's yeast, *Spirulina* and LIV-UP® was found to be 118 ± 6.08, 114.45 ± 13.64, 99.11 ± 8.44 and 95.80 ± 7.92 mg/g, respectively (Figure 12, Table 5).

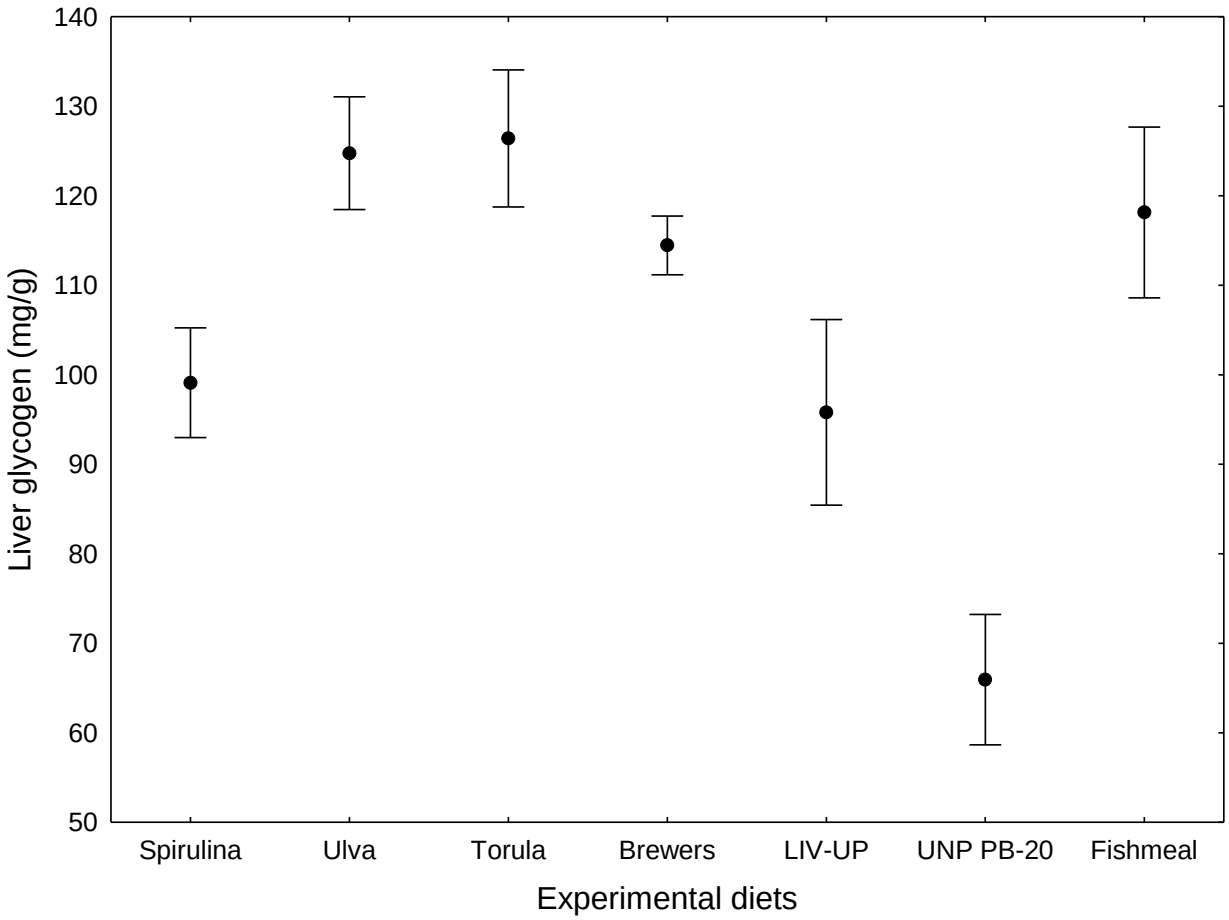

Figure 12: Liver glycogen (mg/g) of *P. commersonnii* (n = 3 per supplemented treatment and n = 2 for the control) fed different diets for a period of 12 weeks. Data represented as Mean ± S.E.

Table 5: Hepatosomatic index, visceral fat index, blood glucose and glycogen content of *P. commersonnii* fed different diets for a period of 12 weeks (n = 3 per supplemented treatment and n = 2 for the control).

Parameters	Diets						
	Spirulina	*Ulva*	Torula yeast	Brewer's yeast	LIV-UP®	UNP PB-20®	Control
HSI (%)	1.17 ± 0.09	1.42 ± 0.19*	1.18 ± 0.22	1.12 ± 0.07*	1.30 ± 0.12	1.07 ± 0.10**	1.10 ± 0.13*
VFI (%)	4.72 ± 0.69	4.14 ± 1.19	4.14 ± 1.29	4.74 ± 0.79	4.68 ± 0.95	4.62 ± 0.78	3.47 ± 0.85
Blood glucose (mmol/L)	5.20 ± 0.24*	3.95 ± 0.70	5.59 ± 0.55*	3.55 ± 0.80**	3.87 ± 0.83	3.87 ± 0.86	3.62 ± 0.74**
Glycogen content (mg/g)	99.11 ± 8.44***	124.75 ± 9.22**	126.41 ± 6.87***	114.45 ± 13.64**	95.80 ± 7.92*	65.94 ± 2.94***	118 ± 6.08**

ANOVA followed by Tukey's *D*-test was used to compare means between treatments at $p = 0.05$. Footnotes: *significantly different from each other at $p < 0.05$; ** significant at $p < 0.005$; *** significant at $p < 0.0001$.

Intestinal Histology

Figure 13 illustrates the detailed normal intestinal mucosa (gut morphology) in spotted grunter (control diet) whereas Figure 14 (*Ulva*) illustrates histological alterations after the feeding trial. The intestinal histology of the control treatment did not show any alterations while for fish fed on *Spirulina*, LIV-UP® and UNP PB-20® distinguished themselves with complex mucosal fold, enteritis and increased number of goblet cells, respectively (Figure 15 A-C).

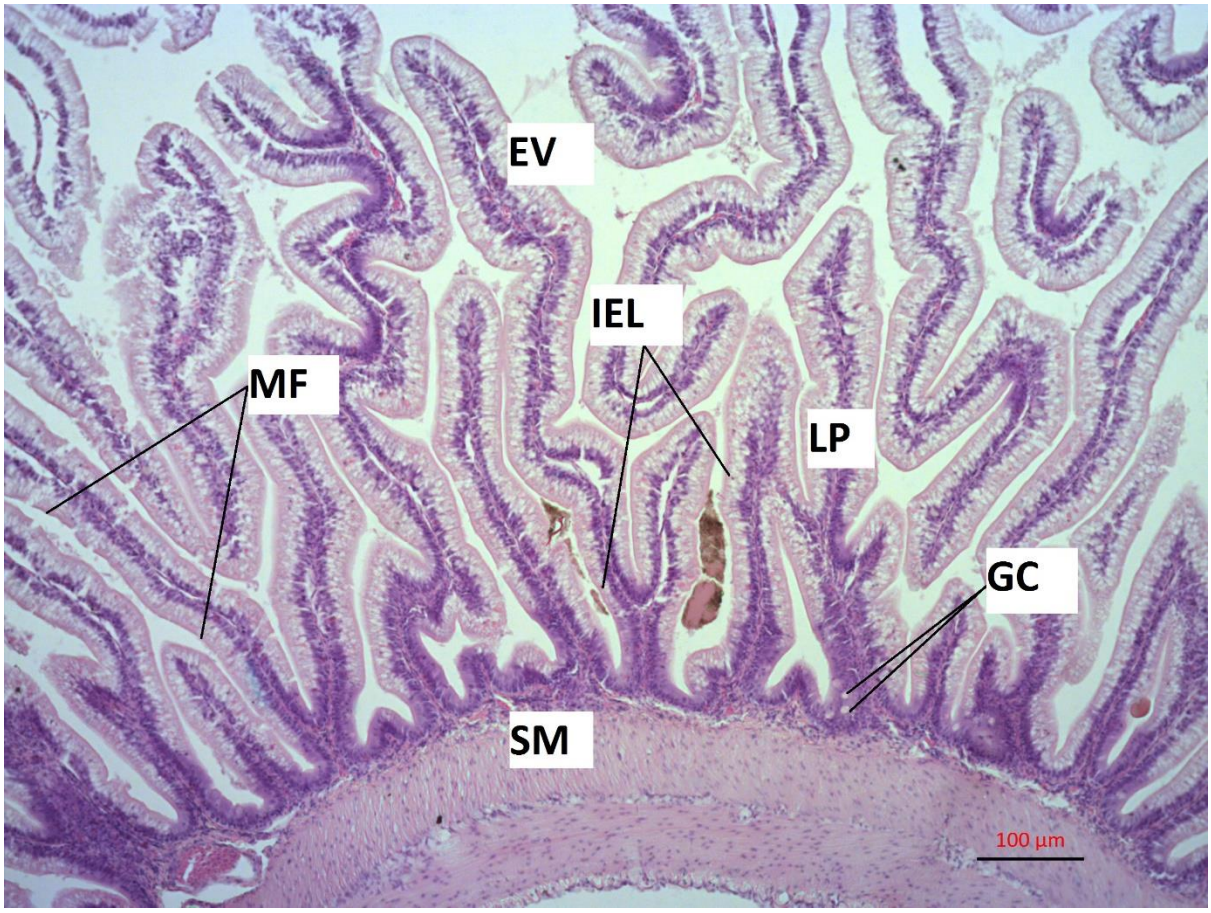

Figure 13: Distal intestinal mucosa in *P. commersonnii* fed on the control diet showing normal intestinal villi: MF, mucosal folds; LP, lamina propria; EV, enterocyte vacuoles; IEL, intraepithelial lymphocytes; GC, goblet cells; SM, submucosa (hematoxylin and eosin, x10).

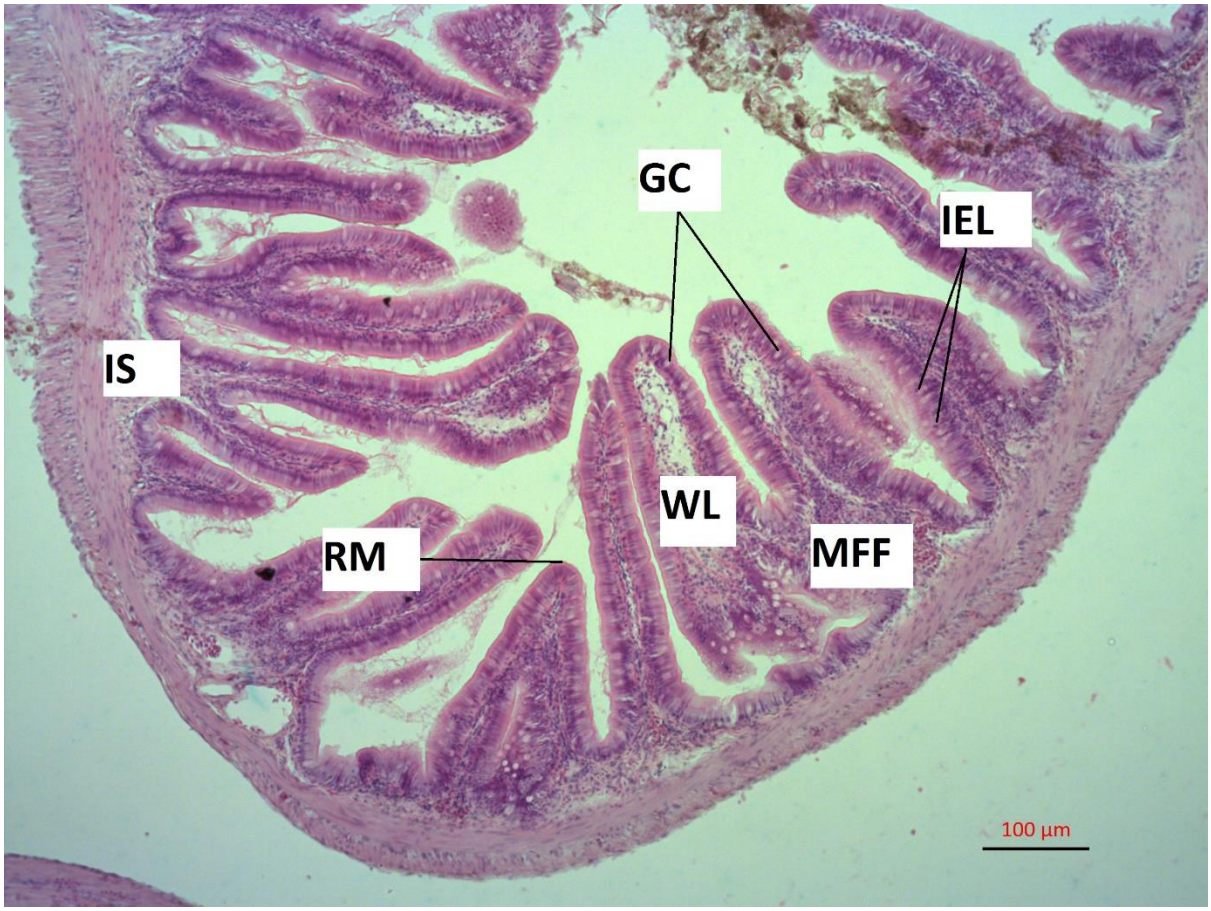

Figure 14: Distal intestinal mucosa in *P. commersonnii* fed on *Ulva* showing some pathological alterations. RM, reduction of mucosal fold height; WL, widened lamina propria; IS, increased submucosa; MFF, intense mucosal folds fusion; IEL, intraepithelial lymphocytes; GC, grouped goblet cells (hematoxylin and eosin, x10)**.**

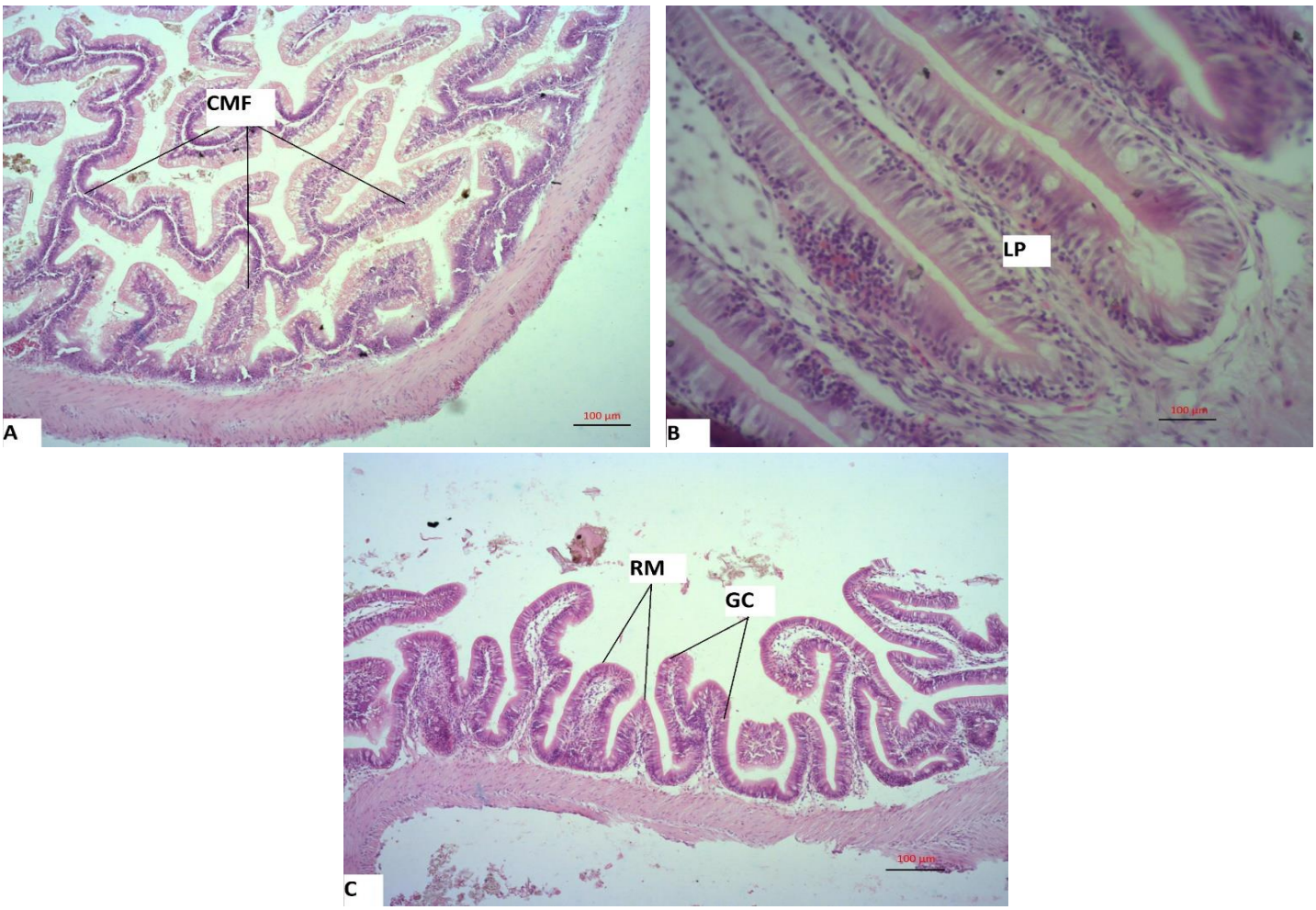

Figure 15: Morphological alteration of distal intestinal mucosa in *P. commersonnii* observed (hamotoxylin and eosin, 10x): **(A)** *Spirulina* supplemented diet: CMF, complex (branched) mucosal folds can be seen; **(B)** LIV-UP® supplemented diet: LP, widened lamina propria can be noted and **(C)** UNP PB-20® supplemented diet: RM, reduction in height of the mucosal folds and GC, increased frequency of goblet cells can be noted.

Distal mucosal fold height and width: The mucosal fold height and width observed in most treatments did not indicate any noteworthy enteritis and were consistent except for fish fed on UNP PB-20® which showed a remarkable reduction of mucosal fold height (Figure 15 C). Spotted grunter juveniles fed on *Spirulina* (Figure 15 A) and *S. cerevisiae* containing diet (Figure 16) clearly distinguished themselves from the rest of the treatment by having complex (branched) and longest intestinal mucosal folds.

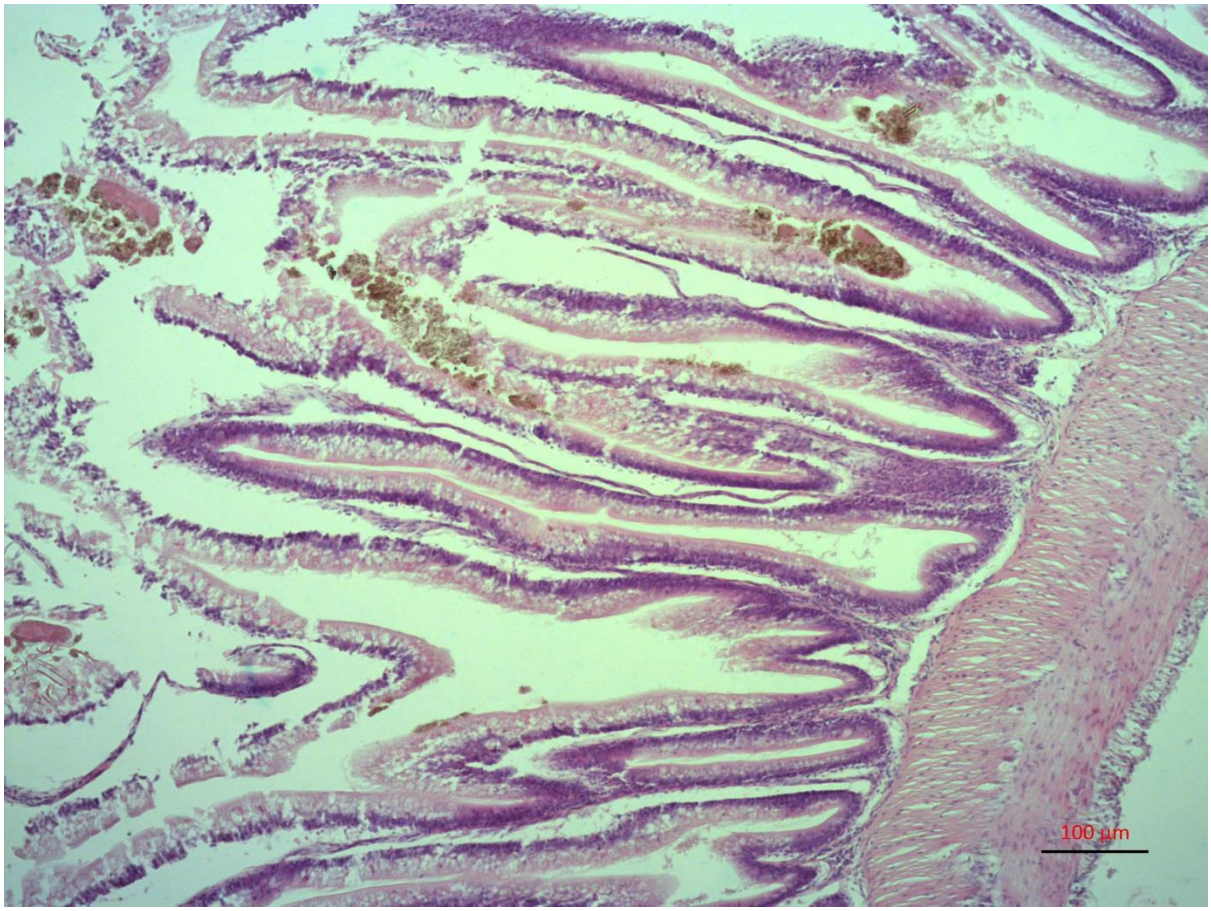

Figure 16: The distal intestinal mucosa that was observed in *P. commersonnii* fed on brewer's yeast (hematoxylin and eosin, x10).

Enterocyte vacuoles: Generally, fish fed on supplemented diets showed high (normal) enterocyte vacuoles except for fish fed on *Ulva*, LIV-UP® (Figure 15 B) and UNP PB-20® (Figure 15 C) where the decreased vacuole size was evident when compared to the control. A similar trend of results was observed for enterocyte nucleus position. In most diets, the nucleus was often pushed towards the base of the enterocyte (centre) as compared to the control diet.

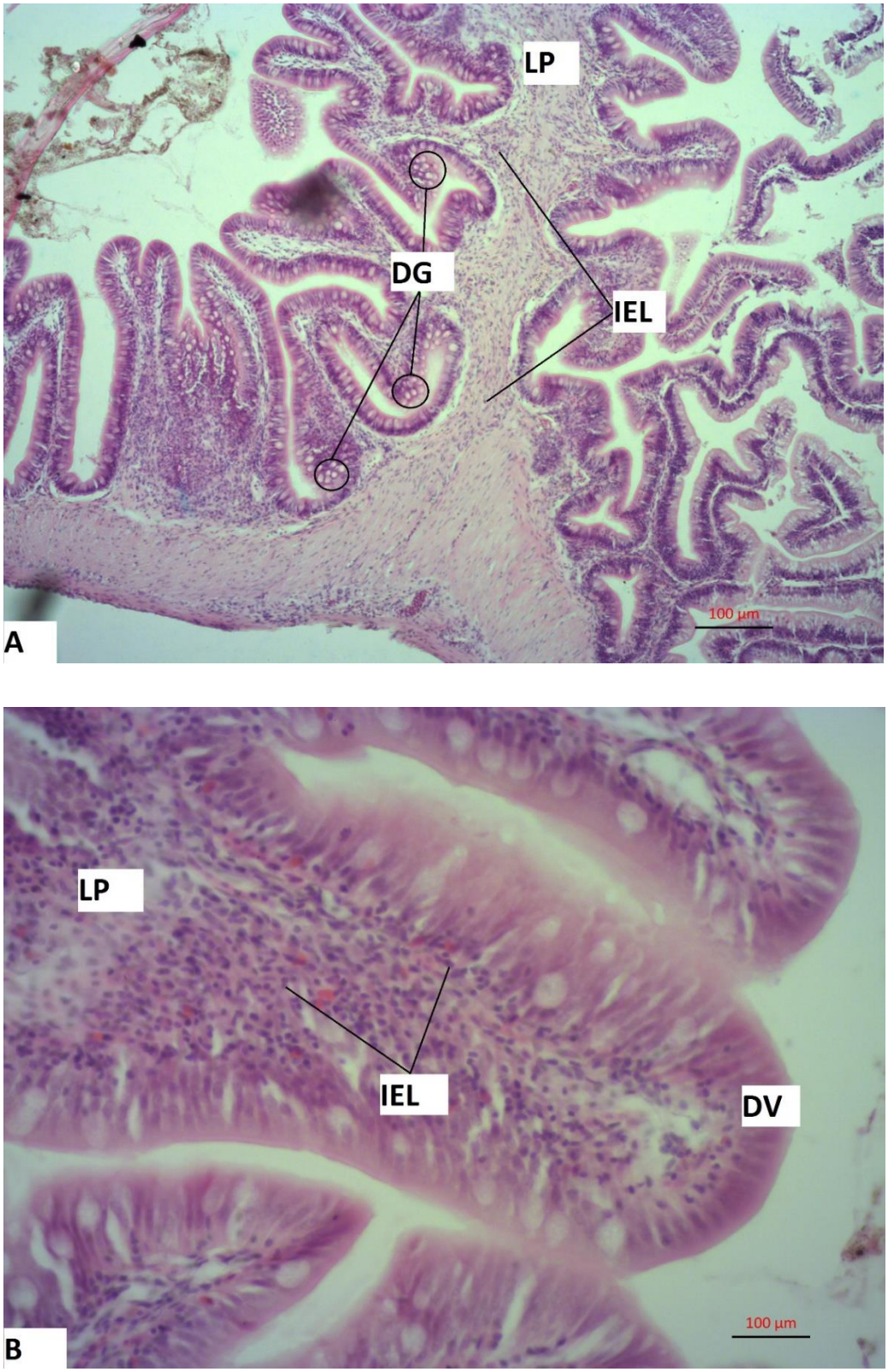

Figure 17: Morphological alteration of distal intestinal mucosa of *P. commersonnii* fed on torula yeast **(A)**: LP, widened lamina propria, DG, densely grouped goblet cells and IEL, intracellular lymphocytes can be noted in fish fed torula yeast diet (hematoxylin and eosin, x10); **(B)**: DV, diminishing absorptive vacuoles, IEL,

intraepithelial lymphocytes and LP widened lamina propria (hematoxylin and eosin, x10).

Lamina propria width: An increased lamina propria width was observed for fish fed on most supplemented treatments while a thin lamina propria made up of single or double cells was observed in response to the control and *Spirulina* containing diets. The distal intestine of fish fed on *Ulva*, brewer's yeast, torula, LIV-UP® and UNP PB-20® containing diets exhibited a distinctive widening of the lamina propria when compared to the control. The lamina propria observed on fish fed *S. cerevisiae* diet had a moderately increased width (Figure 16) when compared to the control.

Submucosa width: A wider submucosa was observed for fish fed on torula yeast and brewer's yeast test diets (Figure 17A and 16) when compared to the other diets. Fish fed on *Ulva* (Figure 18), LIV-UP® and UNP PB-20® (Figure 15C) had a thin submucosa when compared to the control. No effect was observed for fish fed on *Spirulina* containing diet (Figure 15A). The submucosa width of fish that fed on *Spirulina* diet did not deviate from those of the control and exhibited close to normal size of the submucosa width when compared to the rest of the treatments.

Goblet cells: The addition of torula yeast, UNP PB-20® and *Ulva* (Figure 18) in fish diets increased the frequency of goblet cells when compared to the control. A fairly low number of goblet cells was observed on fish that fed on *Spirulina*, brewer's yeast, LIV-UP® and control diets.

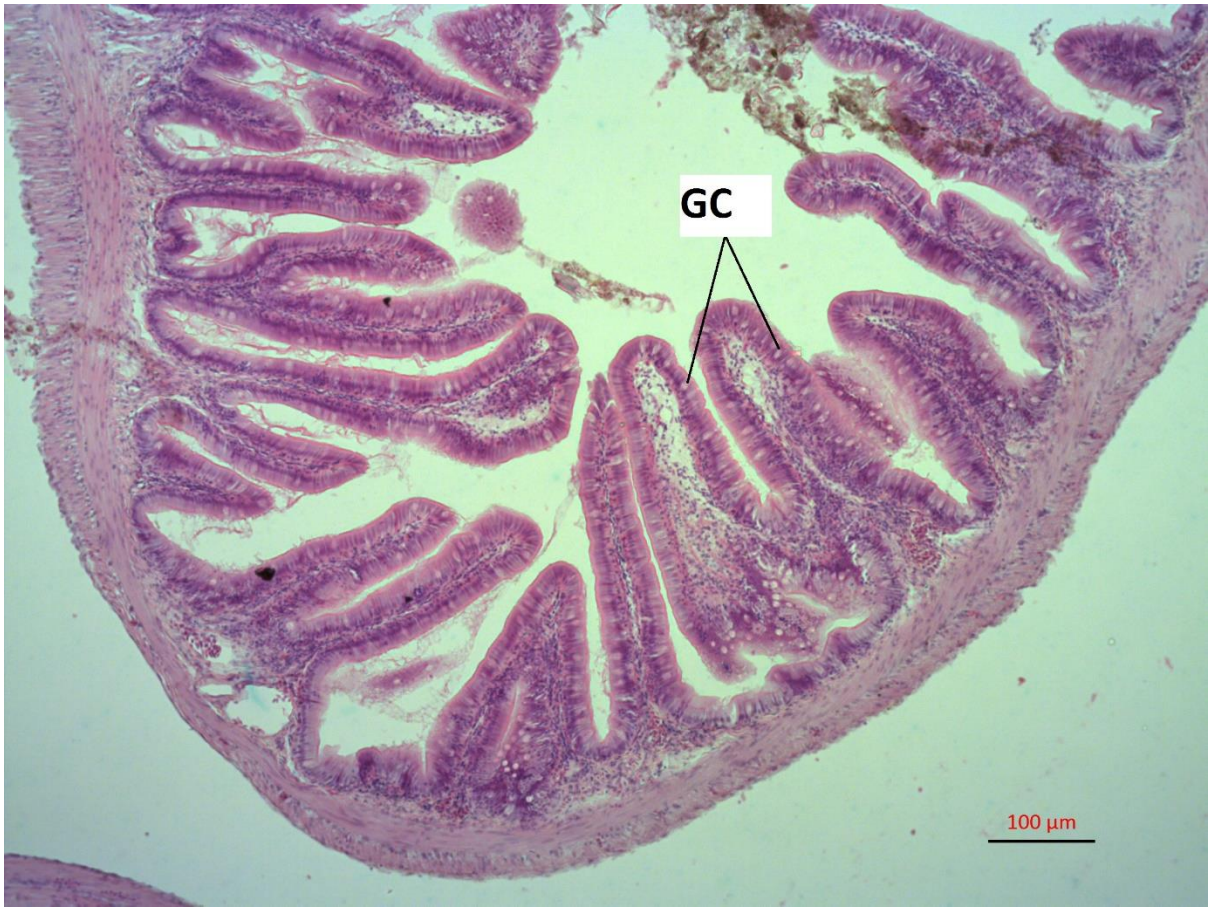

Figure 18: Morphological alteration of distal intestinal mucosa of *P. commersonnii* fed on *Ulva*: GC, goblet cells (hematoxylin & eosin, x10).

Discussion

Hepatosomatic index and visceral fat index

Visceral fat is regarded as a good indicator of poor nutrient utilization and health status related to fish nutrition (Wang *et al.*, 2005). The visceral fat becomes easily rancid at post-harvest as a result of its unpleasant odour making fish products to be undesirable for consumer's acceptance (Love, 1992; Kabahenda *et al.*, 2009), thus reducing the commercial value of final products by affecting fish yield, product quality (Wang *et al.*, 2005; Kabahenda *et al.*, 2009) and storage stability (Webster *et al.*, 2004). The VFI of fish fed on *C. utilis* and *U. lactuca* containing diets was relatively low when compared to other treatments after a feeding period of 12 weeks. In contrast, a drastic increase in VFI on fish fed *S. cerevisiae*, *S. platensis*, LIV-UP® as well as UNP PB-20® supplemented diets was observed. The seaweed, *U. lactuca* is highly rich in ascorbic acid which plays a significant role in emulsification of fats (Ortiz *et al.*, 2006). Therefore, the reduced VFI observed in fish fed on *Ulva* might be attributed to the high content of vitamin C that is present in seaweed. Fats are the primary form of energy storage in fish and fish feed to meet their energy requirements. However, excess fat accumulation in fish can reduce carcass yield and can negatively influence the health status of fish (Otwell & Rickards, 1981). In this study, all the diets contained the same lipid levels and differed only in tested ingredients. Therefore, the observed increased VFI could suggest that in future studies, dietary lipid inclusion may be reduced in supplemented fish diets; and this, then could also reduce the production cost of commercial diets.

The HSI is sensitive to nutritional conditions and highly associated with energy storage and fish condition (Lambert & Dutil, 1997; Yong *et al.*, 2015). Liver is the primary site of fat and glycogen deposition (Peres & Oliva-Teles, 1999; Chatzifotis *et al.*, 2010). Peres and Oliva-Teles (1999) reported that HSI corresponds with dietary levels of carbohydrates and hepatic glycogen content. The results of the present study show that, fish fed on plant-based feed additive (i.e. *Ulva*) had significantly increased HSI value when compared to the fish that fed on other diets. The *Ulva* has high content of carbohydrates (Ortiz *et al.*, 2006, Khairy & El-Shafay, 2013; Anuradha *et al.*, 2015). Excess carbohydrates get deposited in the liver as fat and consequently increase energy content. Therefore, a significant increase observed in

HSI of fish fed on *Ulva* could be attributed to excess energy from the feed additive (*Ulva*) that accumulated into the liver. These results compare favourably with the findings of Ighwela *et al.* (2014) and Li *et al.* (2016) who found that VFI and HSI increased with increase in carbohydrate levels.

Blood glucose and glycogen

Blood glucose concentration is a biochemical indicator of environmental stress, health condition and dietary deficiency in fish (Silbergeld, 1974; Hemre *et al.*, 1996; Barton, 2002). Blood glucose levels are also affected by external and internal environmental factors which includes seasons (Coban & Sen, 2001; Patriche, 2009), reproductive activities, fish nutritional state (Bianca, 2008) and presence of diseases (Wenderlaar-Bonga, 1997). The addition of torula yeast significantly increased the blood glucose concentration compared to the control. However, there was no significant difference observed in blood glucose levels of *P. commersonnii* fed on *Ulva*, brewer's yeast, LIV-UP®, UNP PB-20® and the control. Coban and Sen (2011) reported that blood glucose level increases when fish are under stress and make use of the glycogen reserved in the muscles and liver. In the current study, the fish were stocked at a density of 15 fish per tank in all the replicates to avoid overstocking, fish were fed regularly (twice a day at 3.6% of their body weight) and water quality parameters (temperature, pH and dissolve oxygen) were the same in all the tanks. Therefore, high glucose observed in fish fed with torula yeast diets cannot be attributed to common stressors. It is more likely that torula yeast containing diet contained more glucose than other diets in the form glucan. This possibility conforms to the results previously obtained by Salnur *et al.* (2009). The yeast cell walls contain glucan which is a polysaccharide that consists of glucose. These results suggest that the observed glucose increase in spotted grunter fed on torula yeast diet might be attributed to presence of glucose units from the yeast diet.

Glycogen deposition in fish livers was also affected ($p < 0.00001$) by different diets in the current study. The results of the study demonstrated that fish fed on *Spirulina*, LIV-UP® and UNP PB-20® containing diets were significantly lower than the control while fish fed on the *Ulva*, torula and brewer's yeast did not differ significantly from the control. The glycogen content of the fish fed on torula and *Ulva* was higher than that of the control group. Fish fed on UNP PB-20® has a significantly low glycogen

content than all the diets. The glycogen content is affected by physical, chemical and biological factors (Coban & Sen, 2011). The findings of the study suggest that during the experiment most fish stored adequate glycogen in the liver which suggests that they were not negatively affected by different diets. The UNP PB-20® containing diet did not provide adequate carbohydrate energy and consequently fish were unable to reserve enough glycogen in their liver. However, lipid reserves can serve as alternative energy resource as a relative high VFI was measured for the fish fed this dietary supplement.

Intestinal histological examination

Regarding the morphological aspect of the intestine, it was observed that fish fed diets supplemented with plant-based protein (*U. lactuca*) and growth promoters (LIV-UP® UNP PB-20®) showed that fish had intestinal enteritis to some extent in the distal intestine, characterised by shortening of mucosal folds, increased number of goblet cells, widening of lamina propria and alteration in the amount of vacuolization. Similar to the results of the present study, previous investigations with Nile tilapia (*O. niloticus*; Silva *et al.*, 2015) and gilthead seabream (*S. aurata*; Cerezuela *et al.*, 2012) shows that seaweed (*Gracilaria*, *Porphyra* and *Ulva*) and *Bacillus amyloliquefaciens* supplementation led to evident changes in fish intestinal morphology with reduction in the length of mucosal folds. On the contrary, previous studies with rainbow trout (*Oncorhynchus mykiss*; Heidarieh *et al.*, 2013) have shown that algal bioactive compounds and dietary *Aloe vera* can modulate rainbow trout digestive system morphology by increasing the microvilli length in order to improve nutrient absorption from the feed. Moreover, Emire *et al.* (2013) and De Oliveira *et al.* (2009) reported that seaweeds and plants contain anti-nutritional factors (which include lectins, protease inhibitors, allergens, tannins and toxins) which can hamper fish growth due to reduced nutritional quality. Our findings suggest that the morphological alterations of spotted grunter might be attributed to the presence of saponins in plant-based proteins that a carnivorous fish species is not able to digest.

The intestinal histology of fish fed on brewer's yeast supplemented diet had high density and length of intestinal villi. These results are consistent with the findings of

Gatesoupe (2007) who reported that brewer's yeast enhanced the length and density of the intestinal folds resulting in improved feed digestion and absorption. The improved intestinal villi observed in the gut of fish fed on *S. cerevisiae* could also be attributed to the beneficial effects of nucleic acid and mannan oligosaccharide (MOS) present in yeast. According to Li *et al.* (2007), yeasts have high content of nucleic acids which plays a significant role in improving mucosal surface and gastrointestinal microbiota. Additionally, Dimitrouglou *et al.* (2009) also argues that the MOS present in yeast cell walls helps to modulate intestinal microflora and also increases of density of intestinal villi. Torrecillas *et al.* (2013), later confirmed that the addition of MOS in European sea bass (*Dicentrarchus labrax*) diets had a positive influence on the gut epithelial integrity and functionality after 8 weeks feeding. Similar results were also reported by Denji *et al.*, (2015) after feeding rainbow trout with dietary MOS for 60 days. Therefore, the results of the current study confirm that brewer's yeast improves the intestinal villi and density in fish.

CHAPTER 4

THE EFFECT OF REPLACING FISHMEAL WITH GRADED LEVELS OF TORULA YEAST (*C. UTILIS*) ON GROWTH PERFORMANCE AND HEALTH OF SPOTTED GRUNTER, *POMADASYS COMMERSONNII*

Introduction

Fishmeal is a limited feed resource and concerns about its future availability could facilitate the incorporation of feed in fish diets (Naylor *et al.*, 2009). In aquaculture, it is important to supplement feed with feed additives in order to address nutritional requirements of the culture species for enhanced growth and production. In order to ensure normal growth and physiological functions, feed supplements in fish diets must provide adequate proteins, lipids, energy, vitamins, and minerals (Nakagawa, 2010). Therefore, the evaluation of cost-effective alternative protein sources to fishmeal is the research priority for aquaculture nutritionists.

Yeast is one such feed supplement that has been widely used in fish diets. The yeasts are also considered as a cost-effective dietary supplement as they can be easily produced in industries (Schulz & Oslage, 1976). In aquaculture, yeasts have been shown to promote growth performance in tilapia, *O. mossambicus*, Nile tilapia, *O. niloticu*s, rainbow trout, *O. mykiss*, African catfish, *C. gariepinus* and common carp, *C. carpio* (Oliva-Teles & Gonçalves, 2001; Pooramini *et al.*, 2009; Bob-Manuel, 2014; Manoppo & Kolopita, 2016), counteract intestinal inflammation induced by experimental diets (soybean meal) (Grammes *et al.*, 2003), promote diseases resistance when fish are challenged with a pathogen (Abu-Elala *et al.*, 2013) and improves intestinal integrity (Dimitroglou *et al.*, 2009; Abu-Elala *et al.*, 2013). The mannan oligosaccharide (MOS) present in yeast can prevent bacterial communities in the gut by agglutination and to regulate intestinal microbiota which eventually improves intestinal absorptive area. Due to these reasons, when yeasts are provided at graded levels they can positively affect host health and growth rate (Myers, 2007).

Few studies have focused on the potential of supplementing yeast as a feed additive in fish diets. Consequently, there is no literature on the use of yeasts as a replacement for fish meal in spotted grunter diets. Torula yeast has been used in few

studies with carnivorous and herbivorous fish species (Atlantic salmon and tilapia) as a protein source (Olvera-Novoa *et al.*, 2002; Øverland *et al.*, 2013) with promising results. The authors reported that 30% of *C. utilis* increased the growth performance of *O. mossambicus* after 63 days of feeding. Similarly, Øverland *et al.*, 2013 reported that among other dietary yeasts, torula yeast is a promising protein source for Atlantic salmon after a feeding trial of 89 days. Siwicki *et al.*, (1994) also showed that *C. utilis* supplemented diets can result in immunostimulation and protection against pathogens such as *Aeromonas salmonicida* in rainbow trout. Hence, the aim of the present experiment was to evaluate the effects of replacing fish meal with increasing levels of torula yeast (*C. utilis*) in the diets of *P. commersonnii* on growth performance, whole body composition and health status of fish.

Materials and methods

Experimental system

The experimental system described in Chapter 2 was used during this study: 465 L per tank with a sea water flow rate of 540 L/h, aeration and water temperature of 24 - 25ºC.

Experimental fish

Captive bred spotted grunter juveniles of approximately 0.99 g were acclimated in the same experimental tanks described in Chapter 2, at the DAFF Marine Research Aquarium in Sea Point (Cape Town) for three weeks. During acclimation the fish were fed an imported feed: 200-500 µm pellets (59% protein; 14% lipid; Skretting GEMMA Micro 300, Italy), followed by the commercial trout meal (45% fish meal protein; 14% lipid; Aquanutro, Nutroscience Pty Ltd, South Africa) to apparent satiation, four times a day.

Stocking density and acclimation

Two weeks before commencement of the experiment, a group of 270 fingerlings of spotted grunter weighing 1.23 ± 0.3 g (mean ± standard error) were randomly stocked in 18 tanks (i.e. three replicates per treatment) at a density of 15 fish per

tank. The juveniles were fed trout meal twice a day at 09h00 and 15h00 at a maximum of 3.6% of their body weight per day, to condition them for the experimental feeding program.

After the acclimation period, all the experimental fish were purged for 24 hours prior to the feeding trial for gastric evacuation, anesthetized with 2-phenoxyethanol (Merck laboratories, Johannesburg) at 0.2 mL/L, weighed to the nearest gram and standard length was measured to the nearest millimeter. The experimental period was set for 8 weeks and the morphometric data was recorded after every seven days.

Experimental diets

The torula yeast was obtained from a local market (Nature's Choice, Johannesburg) Experimental diets were formulated to contain 0.0 (control), 4, 8, 12, 16 and 20% of torula yeast. The formulation and proximate composition of the diets are presented in Table 6. The predetermined yeast quota was mixed with the basal composition (fish meal and vitamin/mineral premix) prior to mixing thoroughly with enough water to produce a homogeneous dough. The paste was oven-dried at 38ºC for 16 hours in the laboratory and the feed was ground using an adjustable corn kernel hand grinder into desirable particle sizes. The feed was packed in sealed plastic bags and kept at -20°C for further use during the experiment. Prior to feeding, the pellets were coated with cod liver oil at 10% of the ration (*m*:*v*).

Each of the formulated diets (Table 6) were fed to three replicates per treatment (thus, n = 18 tanks stocked with 15 fish per tank) for the duration of eight weeks.

Table 6: Formulation and chemical composition of experimental diets.

Ingredients (%)	Diets					
	Yeast 0%	Yeast 4%	Yeast 8%	Yeast 12%	Yeast 16%	Yeast 20%
Fish meal[1]	81.95	77.95	73.95	69.95	65.95	61.95
Torula yeast[2]	-	4	8	12	16	20
Binder	7	7	7	7	7	7
Premix[3] (Min/Vit mix)	1.05	1.05	1.05	1.05	1.05	1.05
Cod liver oil[4]	10	10	10	10	10	10
Diet proximate composition						
Crude protein	49.7	50.1	49.7	49.8	49.8	49.5
Crude Fat	15.3	15.1	14.9	14.8	15.6	15.3
Ash	0.81	0.81	0.76	0.82	0.8	0.89
Moisture	10.2	11.4	11.1	10.4	10.6	9.7

[1]Aquanutro (Pty) Ltd, 5 Aqua Crescent, Malmesbury, South Africa
[2]Nature's Choice, Meyerton, Cape Town, South Africa
[3]Kyron Laboratories (Pty) Ltd, Benrose, Johannesburg, South Africa
[4]Alpha Pharmaceuticals, Mayville, Durban, South Africa

Data collection for growth performance, proximate analysis and health status were measured as described in Chapter 2 and 3 respectively.

Water quality parameters

Water quality parameters were measured as per methods described in Chapter 2. The dissolved oxygen in the tanks was maintained at 6.5 ± 0.2 mg/L. Total ammonia-nitrogen (ammonia and ammonium) ranged between 0.00 to 0.25 mg/L and the water pH ranged between 7.7 and 7.9. The water salinity remained at approximately 35 mg/L throughout the feeding trial.

Statistical analysis was determined as described in Chapter 2.

Results

The results obtained for growth performance and diet utilization during the spotted grunter feeding trial are summarized in Table 7. All the yeast supplemented diets were readily accepted by fish and no mortalities were observed during the feeding period. The data presented in Table 7 indicates that there was no significant difference on the initial weight of fish with overall average means of 1.53 ± 0.34 g ($F_{(5, 12)} = 1.65$, $p = 0.22$), length 3.79 ± 0.25 mm ($F_{(5, 12)} = 1.59$, $p = 0.24$) and condition factor 2.74 ± 0.54 ($F_{(5, 12)} = 1.25$, $p = 0.35$).

Dietary yeast inclusions did not significantly improve weight gain ($F_{(5, 42)} = 1.08$, $p = 0.38$), SGR ($F_{(5, 42)} = 2.13$, $p = 0.08$), final length ($F_{(5, 42)} = 2.00$, $p = 0.15$) and condition factor ($F_{(5, 42)} = 0.89$, $p = 0.52$), after the feeding period of 8 weeks (Figures 19 to 23). The weight gain of fish did not follow any trend among all treatments. However, all the fish fed with the successively increasing levels of yeast increased their average body weight gain when compared to the control (0% yeast diet) which had the lowest mean weight gain (3.75 ± 1.74 g). Fish fed with 16% yeast diet supported the highest average body weight gain of 5.07 ± 0.20 g compared to all other diets.

Similarly, the condition factor of spotted grunter was not affected by increasing levels of torula yeast after the feeding trial ($F_{(5, 12)} = 0.89$, $p = 0.52$; Table 7 and Figure 21). There was a decreasing trend in condition factor of fish fed with different yeast levels, decreasing from 3.11 ± 0.06 for the non-supplemented diet to 2.88 ± 0.11 for the 12% yeast containing diet. An apparent trend of increase in the average SGR was observed ranging from 2.03 ± 0.42 for the control to 3.07 ± 0.44%/day for 12% yeast diet with increasing yeast levels (Table 7), although there was no significant difference observed among the diets.

Table 7: The weight, length, condition factor (CF), weight gain and specific growth rate (SGR) of *P. commersonnii* juveniles fed different diets over 8 weeks. Data given as mean ± standard error, n = 3 per treatment

	Yeast 0%	Yeast 4%	Yeast 8%	Yeast 12%	Yeast 16%	Yeast 20%	F value	p- value
Initial weight (g)	1.19±0.06	1.52±0.47	1.83±0.02	1.34±0.26	1.65±0.34	1.65±0.34	$F_{(5, 12)} = 0.22$	1.65
Initial length (mm)	3.64±0.10	3.89±0.18	3.63±0.09	3.98±0.25	3.96±0.07	3.65±0.44	$F_{(5, 12)} = 1.59$	0.24
Initial CF	2.47±0.09	2.40±0.51	3.16±0.75	3.16±0.76	2.60±0.17	2.64±0.44	$F_{(5, 12)} = 1.25$	0.35
Weight gain (g)	3.75±0.23	4.85±1.24	4.38±0.54	4.38±0.89	5.07±0.20	4.72±0.13	$F_{(5, 42)} = 1.08$	0.38
Final length (mm)	5.75±0.39	6.21± 0.22	6.21±0.16	6.44±0.22	6.74±0.45	6.32±0.69	$F_{(5, 12)} = 2.00$	0.15
Final CF	3.11±0.06	3.00±0.15	3.01±0.21	2.88±0.11	3.00±0.08	2.93±0.19	$F_{(5, 12)} = 0.89$	0.52
Final weight	5.95±0.33	7.16±0.16	7.23±0.78	7.74±0.88	9.15±0.15	7.69±0.50	$F_{(5, 12)} = 1.18$	0.37
SGR (% body weight per day)	2.03±0.42	2.44±0.48	2.77±0.17	3.07±0.44	3.03±0.64	2.85±0.24	$F_{(5, 42)} = 2.13$	0.08

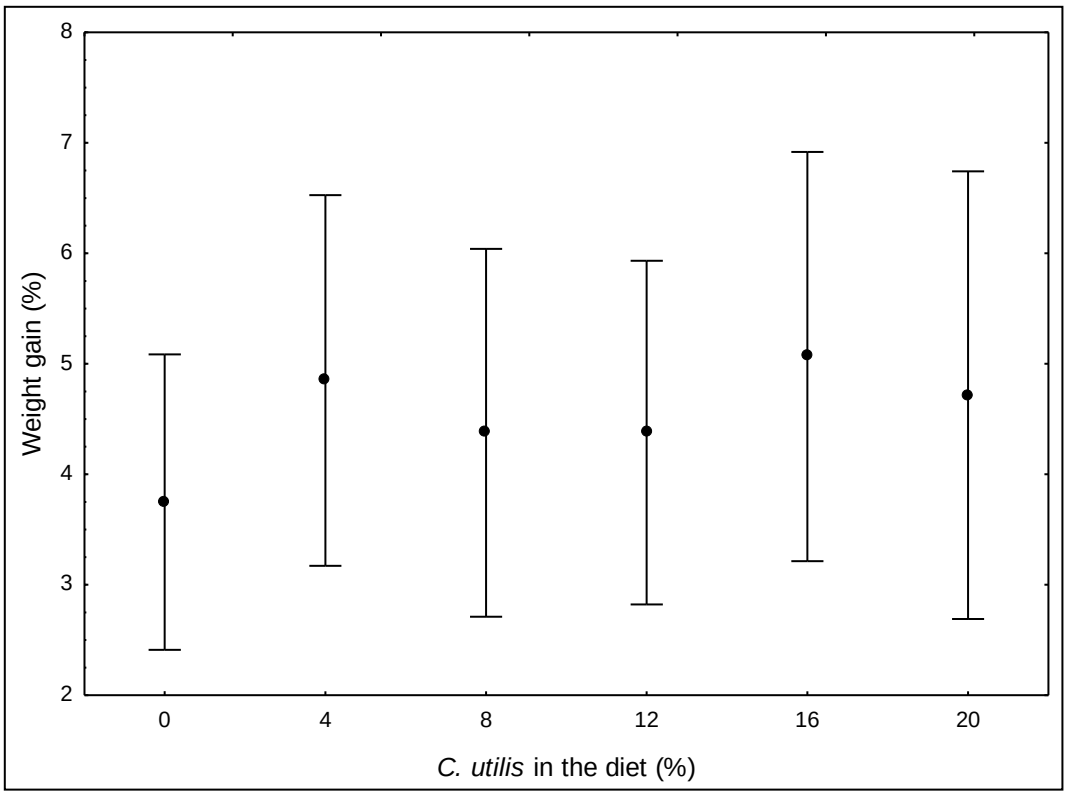

Figure 19: Weight gain (g) of *P. commersonnii* (n = 3) fed diets with increasing levels of torula yeast for 56 days. Data represented in mean ± S.E.

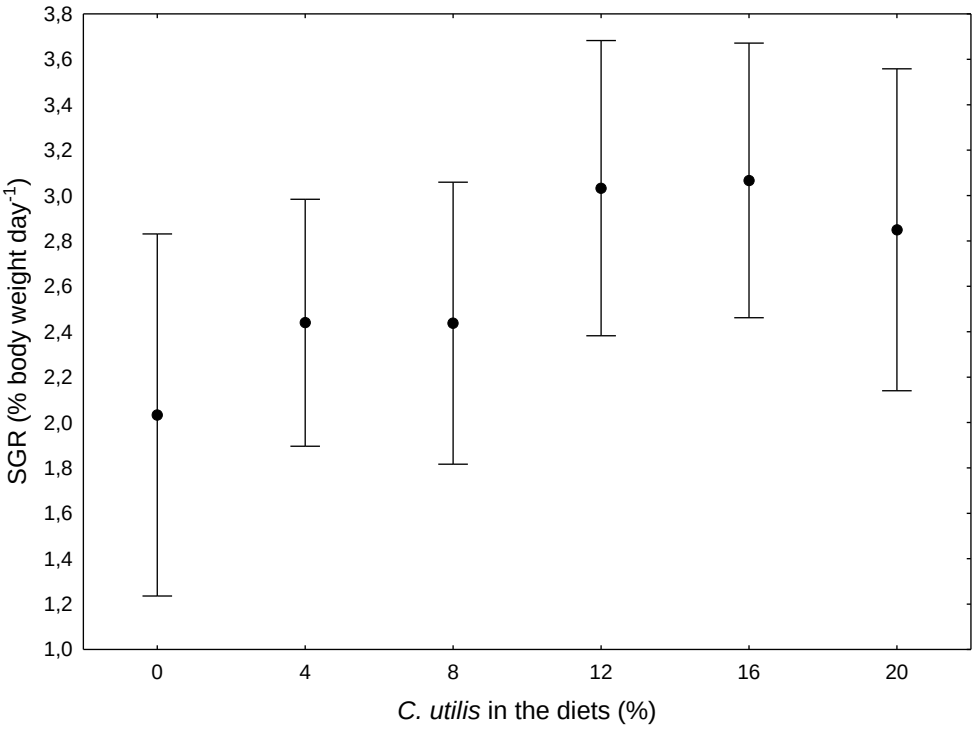

Figure 20: Specific growth rate (%/day) of *P. commersonnii* (n = 3) fed diets with increasing levels of torula yeast 56 days. Data represented in mean ± S.E.

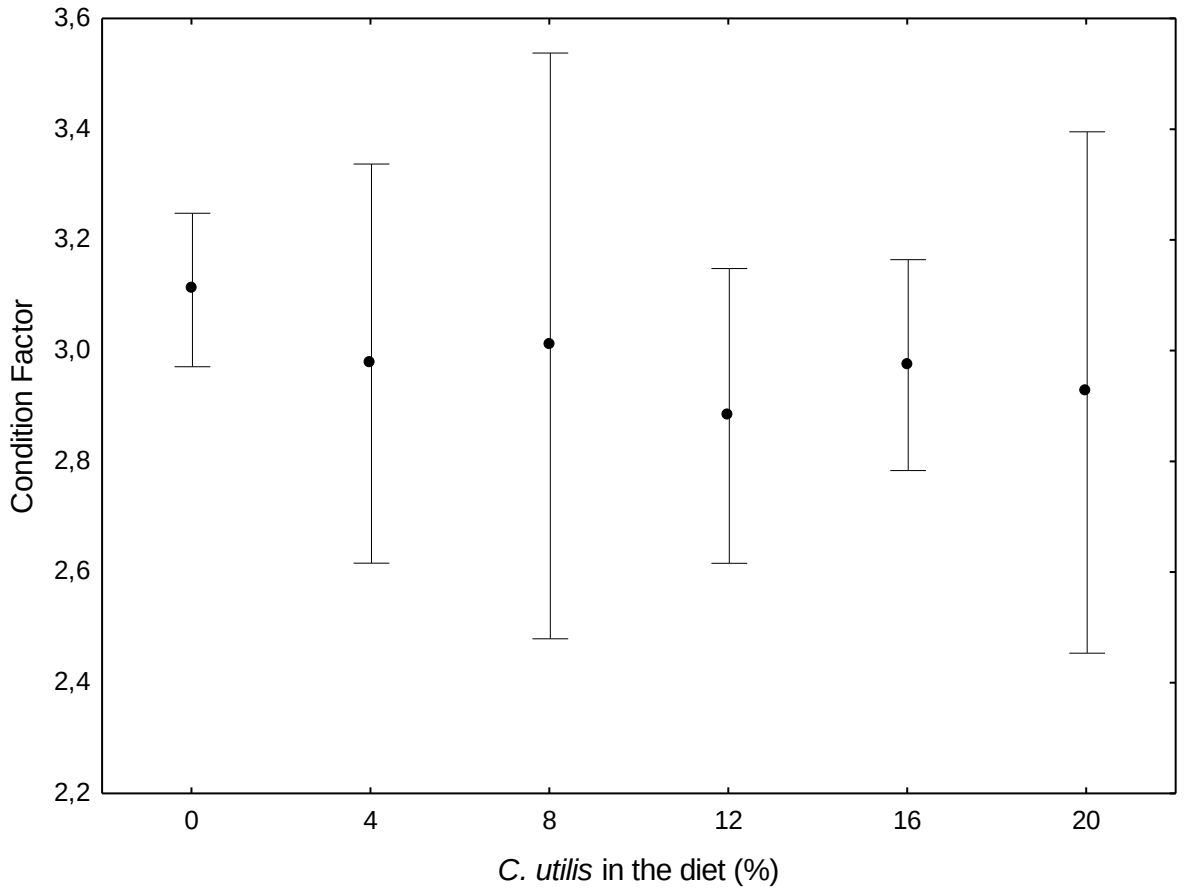

Figure 21: Condition factor (%) of *P. commersonnii* (n = 3) fed diets with increasing levels of torula yeast for 56 days. Data represented in mean ± S.E.

After 56 days of feeding graded levels of yeast, there was no significant difference observed in final weight ($F_{(5, 12)} = 1.18$, $p = 0.37$). However, there was an apparent trend of increase in the final weight ranging from 5.95 ± 0.33 for fish fed non-supplemented diet to 9.15 ± 0.15 g for fish fed 16% yeast containing diet (Table 7 and Figure 22). A comparative trend of average value fluctuations was observed in final length measurements, although there was no significant difference observed among the treatments ($F_{(5, 12)} = 2.00$, $p = 0.15$; Table 7 and Figure 23).

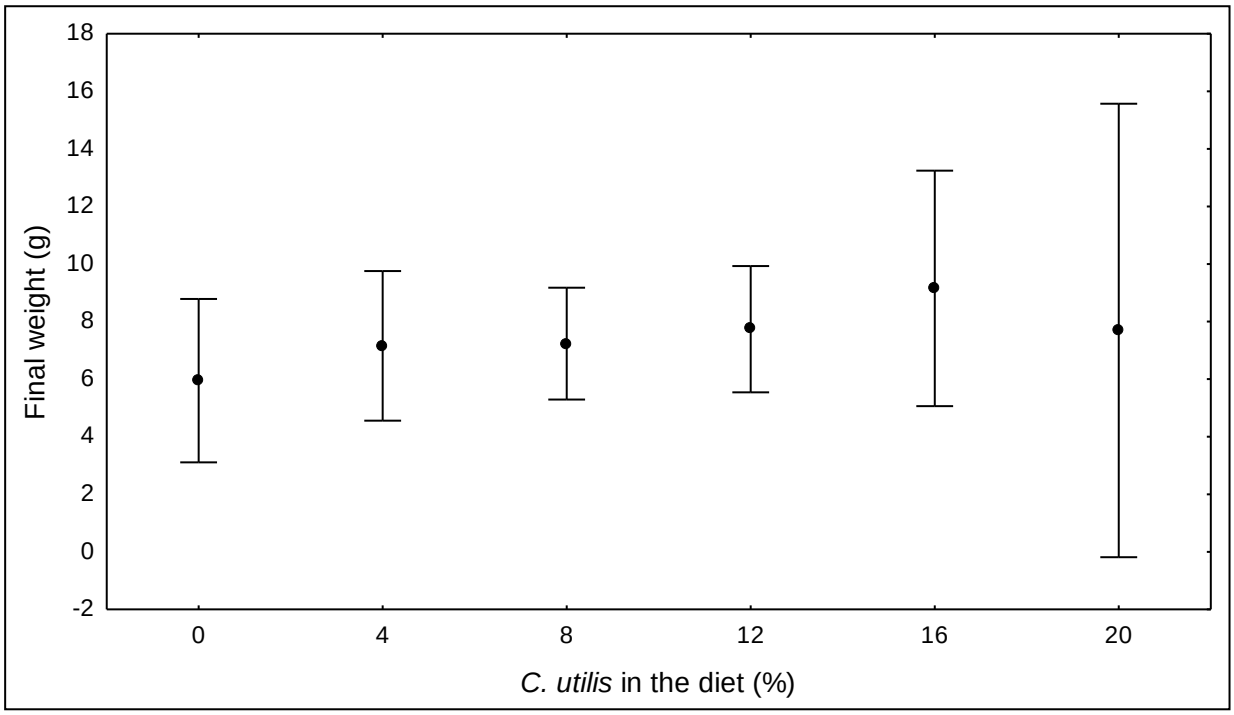

Figure 22: Final weight (g) of *P. commersonnii* (n = 3) fed diets with increasing levels of torula yeast for 56 days. Data represented in mean ± S.E.

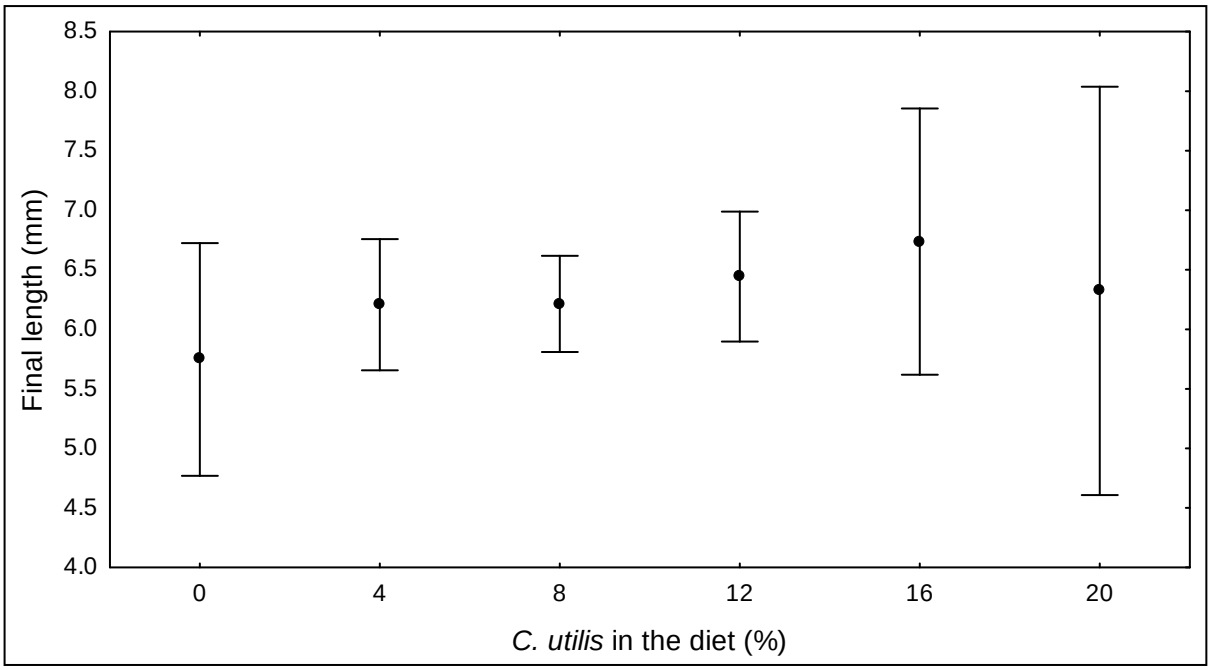

Figure 23: Final length (mm) of *P. commersonnii* (n = 3) fed diets with increasing levels of torula yeast for 56 days. Data represented in mean ± S.E.

In general, FCR tended to improve with weight gain. Fish fed on 20% yeast supplemented diet supported the better feed conversion ratio, and the other yeast-

containing diets had lower feed conversion ratios than the control. The best FCR was recorded for the diet containing 20% of yeast compared to the control (Table 8). Other yeast containing diets (i.e. 16, 4, 12 and 8%) also mediated lower FCR (i.e. 1.14, 1.15, 1.18 and 1.19, respectively) when compared to the control (Table 8). The PER followed the same sequence as FCR where 16% yeast supplemented diet attained the highest PER when compared to control (Table 8).

Table 8: Feed conversion ratio (FCR) and protein efficiency ratio (PER), of *P. commersonnii* fed with diets containing increasing levels of yeast for 56 days.

	Yeast 0%	Yeast 4%	Yeast 8%	Yeast 12%	Yeast 16%	Yeast 20%
FCR	1.32	1.15	1.19	1.18	1.14	1.1
PER	0.73	0.94	0.93	0.95	1.02	0.95

Chemical composition of the fish

At the end of the 56 days feeding trial, nine fish per treatment (i.e. three fish per replicate) were sacrificed for chemical analyses. The results of the chemical analysis of fish body at the end of the feeding period are shown in Table 9. The obtained results for the carcass composition showed that crude protein and fat were generally affected by the experimental diets compared to the control with an overall mean (± standard error) of 19.31 ± 0.33 ($F_{(5, 12)} = 201.15$, $p < 0.0001$) and 7.21± 0.94% and ($F_{(5, 12)} = 428.43$, $p < 0.0001$, Table 9), respectively. It was observed that carcass lipid content tended to decrease as the dietary protein level increases. Fish fed with 4% yeast supplemented diet supported a significantly higher lipid content (8.21 ± 0.19%) when compared to all the diets and those that were fed 20% of yeast containing diet were significantly lower than other treatments (5.46 ± 0.02%).

Table 9: Proximate carcass composition of *P. commersonnii* fed with experimental diets (n = 3). Data represented in mean ± S.E.

	Yeast 0%	Yeast 4%	Yeast 8%	Yeast 12%	Yeast 16%	Yeast 20%
Crude Protein	18.83±0.01	18.16±0.13	19.5±0.01	19.5±0.05	19.8±0.01	19.03±0.03
Crude Fat	6.75±0.02	8.21±0.19	8.01 ±0.03	7.54 ±0.02	7.31 ±0.06	5.46 ± 0.02

HSI, VFI and blood glucose

After 56 days of feeding spotted grunter with graded levels of torula yeast there was no significant difference observed in HSI 1.63 ± 0.40% ($F_{(5, 48)} = 0.91$, $p = 0.49$), VFI 4.60 ± 1.20% ($F_{(5, 48)} = 0.86$, $p = 0.51$) and blood glucose values 5.77 ± 1.06 mmol/L ($F_{(5, 48)} = 1.68$, $p = 0.16$), (Figures 24 to 26). There was no specific trend observed after the feeding trial in HSI and blood glucose levels of spotted grunter, although the diets containing 8 and 16% of *C. utilis* exhibited the highest HSI (1.93 ± 0.82%) and blood glucose (6.71 ± 0.64 mmol/L), respectively (Table 10). The average VFI increased with increasing levels of torula yeast in the diets but the values were statistically non-significant.

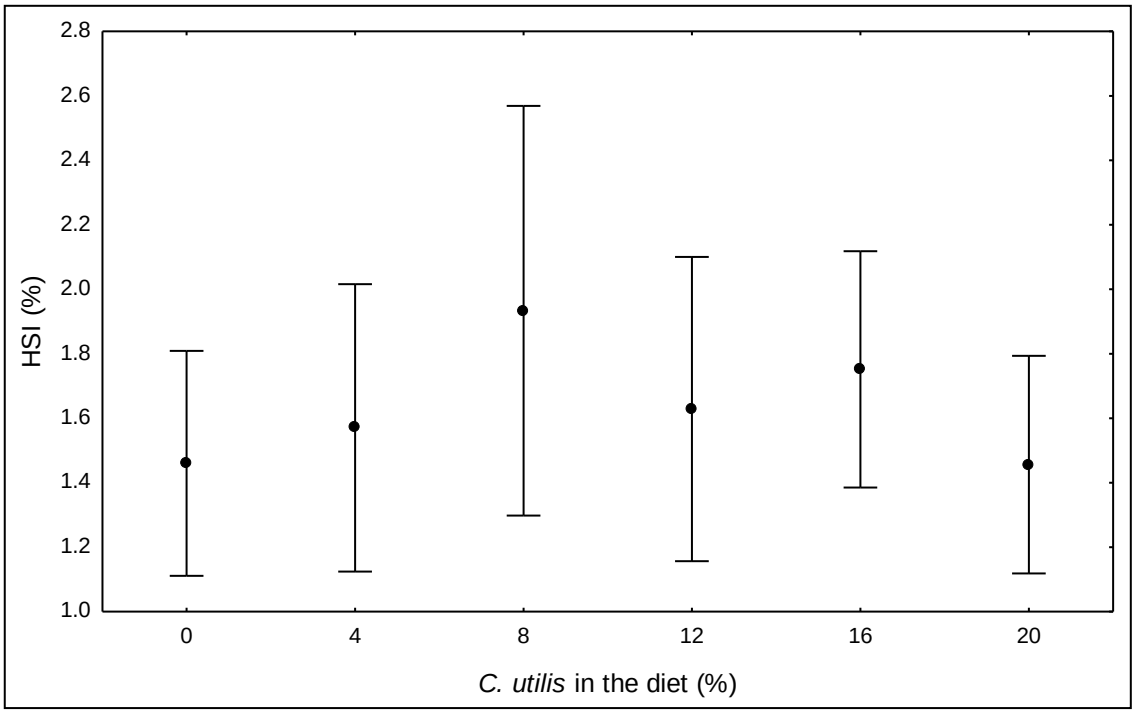

Figure 24: Hepatosomatic index (%) of *P. commersonnii* (n = 3) fed diets with increasing levels of torula yeast for 56 days. Data represented in mean ± S.E.

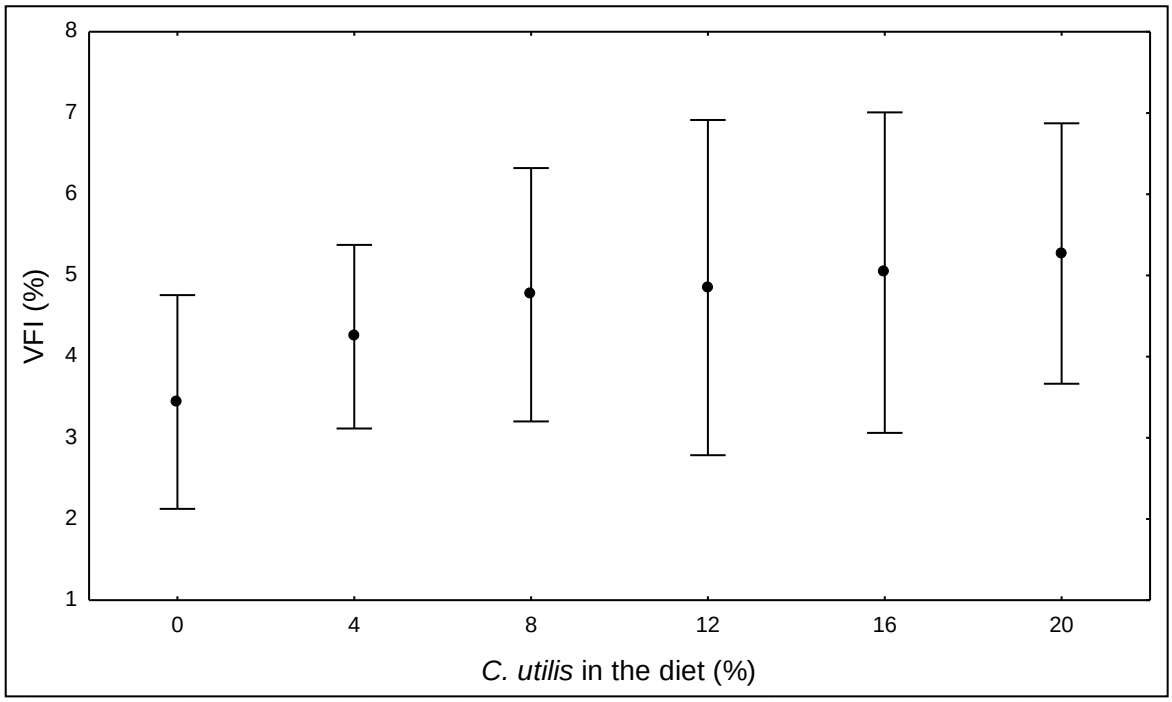

Figure 25: Visceral fat index (%) of *P. commersonnii* (n = 3) fed diets with increasing levels of torula yeast for 56 days. Data represented in mean ± S.E.

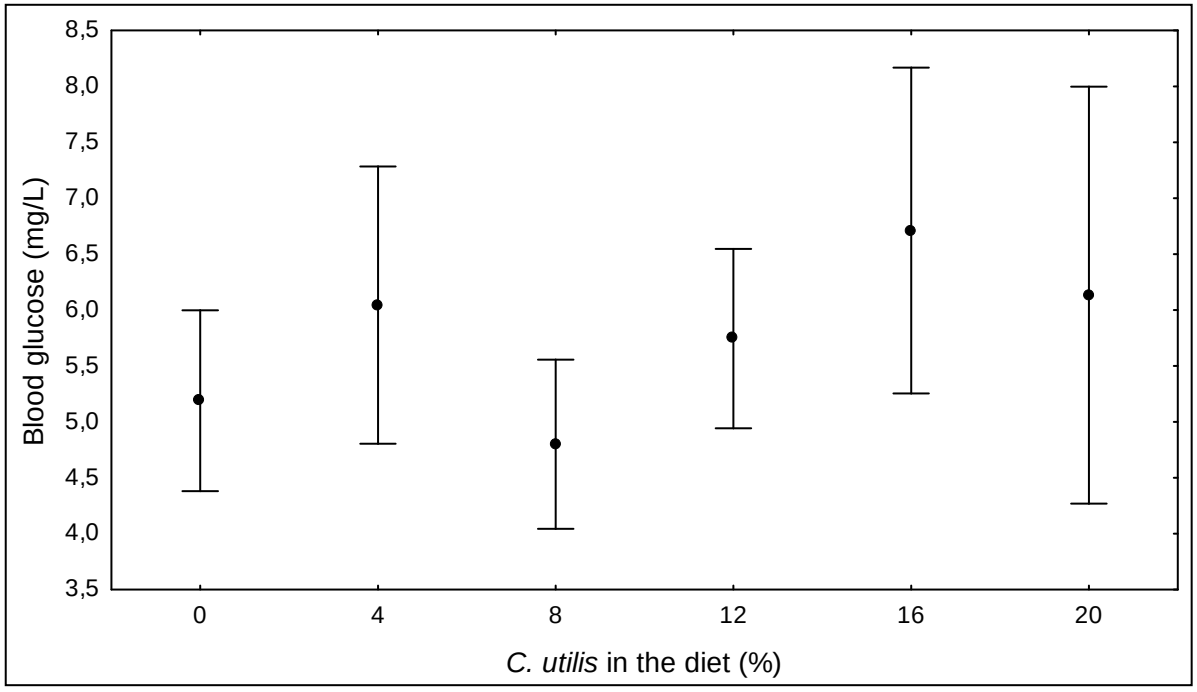

Figure 26: Blood glucose (mmol/l) of *P. commersonnii* (n = 3) fed diets with increasing levels of torula yeast for 56 days. Data represented in mean ± S.E.

The data collected on liver glycogen levels indicated that the control group was significantly different ($F_{(5, 48)} = 33.96$, $p < 0.0001$) from all other treatments except for

fish fed on 20% yeast containing diet (p = 0.53). Moreover, fish fed on 4 and 8%, as well as 12 and 16% *C. utilis* containing diets did not show any significant variation, p = 0.99 and p = 1.00, respectively. Glycogen content in the liver generally decreased with increasing levels of *C. utilis*. However, the diet containing 8% of *C. utilis* stored the highest liver glycogen content (74.19 ± 5.37 mg/g) compared to the liver storage values of all the treatments Figure 27.

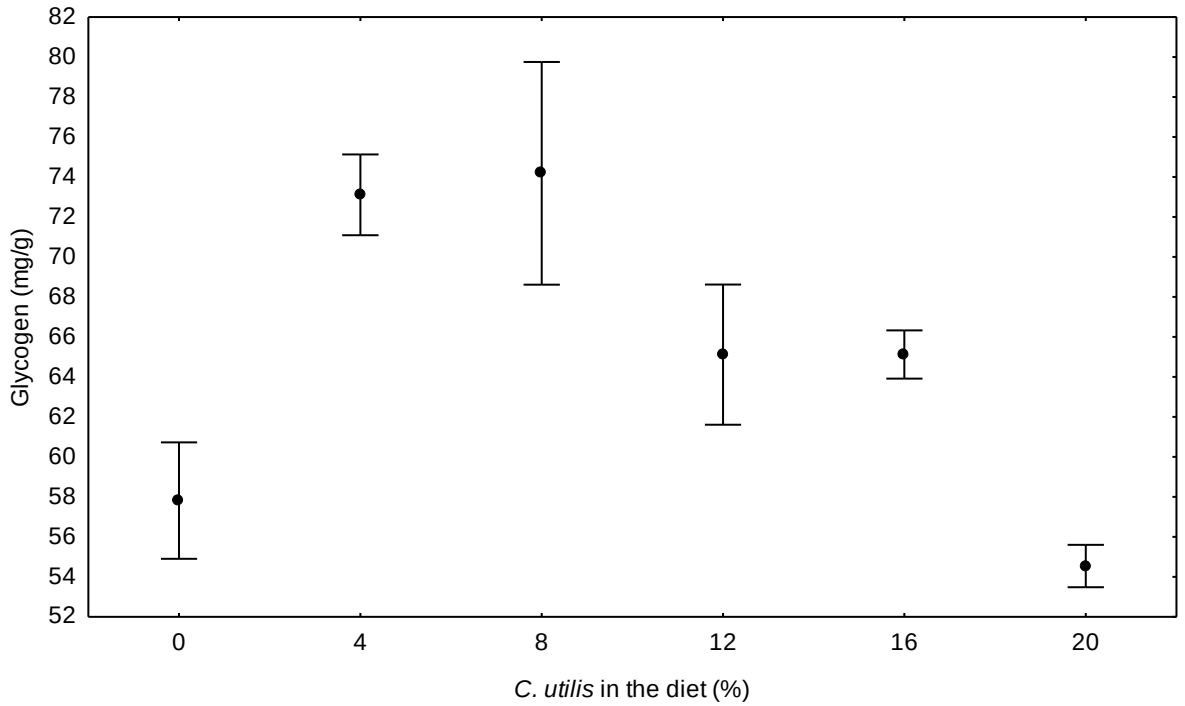

Figure 27: Glycogen (mg/g) of *P. commersonnii* (n = 3) fed diets with increasing levels of torula yeast for 56 days. Data represented in mean ± S.E.

Table 10: Hepatosomatic index (%), visceral fat index (%), blood glucose (mmol/L) and glycogen content (mg/g) of *P. commersonnii* (n = 3 per treatment) fed diets with increasing levels of torula yeast for 56 days. Data represented in mean ± S.E.

Parameters	Diets					
	Yeast 0%	Yeast 4%	Yeast 8%	Yeast 12%	Yeast 16%	Yeast 20%
HSI (%)	1.46 ± 0.38	1.57 ± 0.12	1.93 ± 0.82	1.63 ± 0.11	1.75 ± 0.35	1.46 ± 0.36
VFI (%)	3.44 ± 1.28	4.25 ± 0.96	4.76 ± 1.59	4.85 ± 0.67	5.03 ± 1.62	5.27 ± 0.92
Blood glucose (mmol/L)	5.19 ± 0.49	6.04 ± 1.47	4.8 ± 0.48	5.74 ± 0.80	6.71 ± 0.64	6.13 ± 1.55
Glycogen content (mg/g)	57.82 ± 3.68	73.11 ± 1.96***	74.19 ± 5.37***	65.12 ± 4.86**	65.12 ± 1.18**	54.54 ± 1.01***

ANOVA followed by Tukey's *D*-test was used to compare means between treatments at $p = 0.05$. Footnotes: *significantly different from each other at $p < 0.05$; ** significant at $p < 0.005$; *** significant at $p < 0.0001$.

The histological examination of the distal intestine showed that all the fish fed on 20% *C. utilis* containing diet exhibited well-established signs of intestinal enteritis characterised by reduced height of the intestinal folds, widening of the submucosa and lamina propria, increased frequency of goblet cells and diminishing absorptive vacuoles (Figure 28A). Fish fed 12 and 16% torula yeast included diets showed insignificant signs of enteritis in the distal intestine though the incidence of occurrence was not different from the control fish or the fish fed 4 and 8% yeast inclusion diets (Figure 28B). An increased mucosal fold height, decreased goblet cell frequency and high (normal) enterocyte vacuolisation was observed in fish fed the diet supplemented with 12 and 16% of torula yeast when compared to fish fed with 20% yeast containing diet.

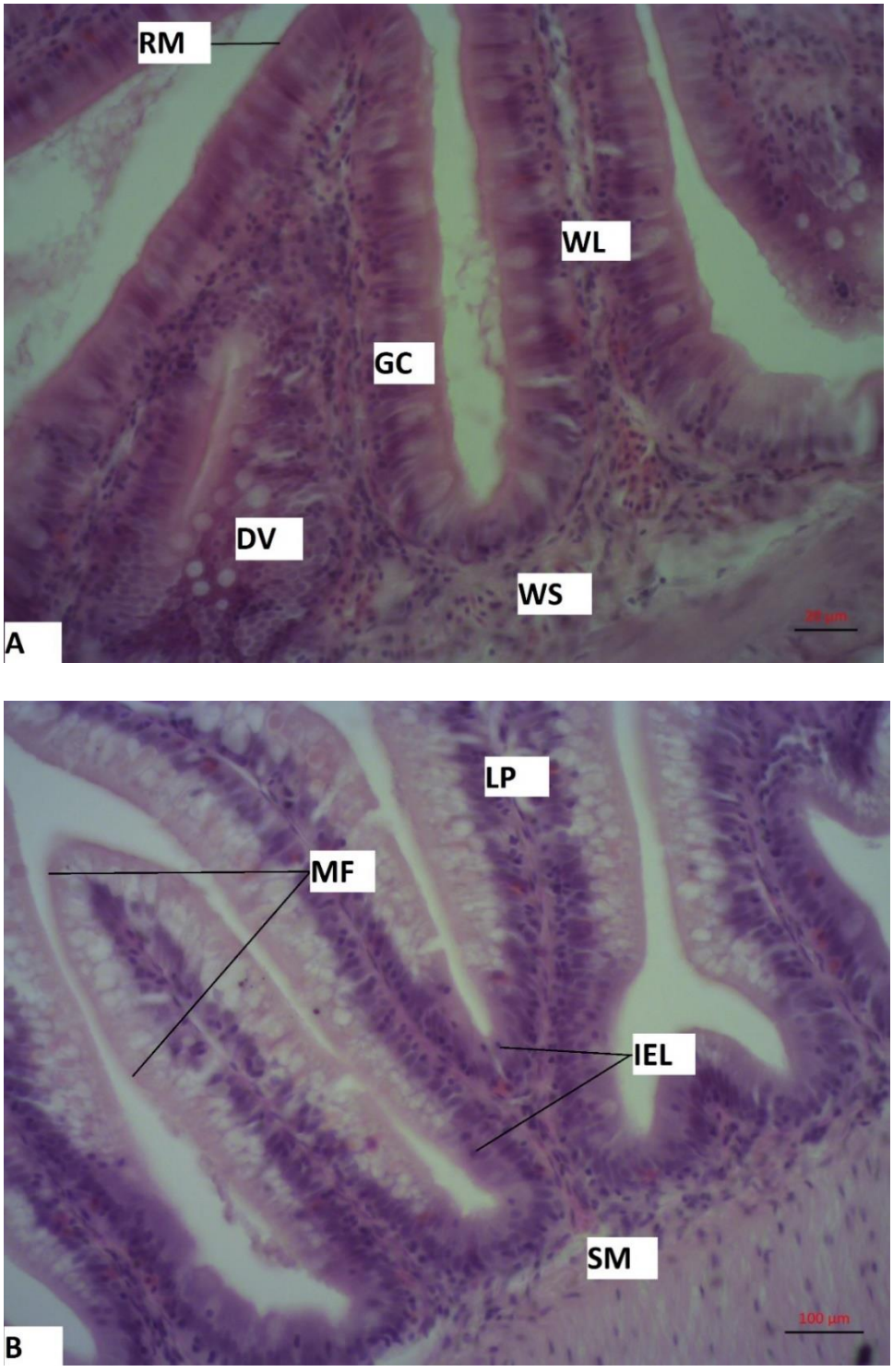

Figure 28: Distal intestinal mucosa in *P. commersonnii* showing some histopathological alterations. **(A):** RM, reduction in mucosal fold height, WL, widened lamina propria; WS, widened submucosa; DV, diminishing absorptive vacuoles; GC, goblet cells (hematoxylin and eosin, 10x). **(B)**: MF, elongated mucosal fold; LP, lamia propria; SM, submucosa and IEL, intraepithelial lymphocytes (hematoxylin and eosin, 10x).

Discussion

The results of the present study show that the diet containing 16% yeast provided the best average values in terms of body weight and length gain than fish fed with the control diet, while diets with 8 and 12% yeast inclusion achieved a better condition factor and SGR respectively. However, no significant differences between these treatments were observed. The results of this work are in agreement with the work of Ovie & Eze (2014) in which, *S. cerevisiae* diets for catfish (*C. gariepinus*) did not affect the growth performance. Lee *et al.* (2000) evaluated the effects of supplementing the diets of Korean rockfish (*Sebastes schlegeli*) with *Kluyveromyces fragilis*, *C. utilis* and brewer's yeast. There was no significant difference observed in growth performance and feed utilization of juvenile Korean rockfish when fed with yeast supplemented diets. These results also compare favourably with the work of Olvera-Novoa *et al.* (2002) where yeast based diet composition successfully replaced a portion of fishmeal in diets of *O. mossambicus*. Conversely to this, the growth performance of convict cichlid, *Amatitlania nigrofasciata*, significantly increased when fed a diet with 2% addition of yeast (Mohammadi *et al.*, 2015). The growth performance of catfish (*C. gariepinus*), Nile tilapia (*O. niloticus*), three spot cichlid (*Cichlasoma trimaculatum*) and common carp, (*Cyprinus carpio*) were also not negatively affected by the inclusion of yeast in their diets (Lara-Flores *et al.*, 2003; Faramarzi *et al.*, 2011; Bob-Manuel, 2014; Abdel-Tawwab, 2012; Mohammadi *et al.*, 2016). The present study indicates that the replacement of fish meal with 16% of torula yeast can promote growth rate and feed utilization of spotted grunter. Further investigations are required to determine whether higher inclusion levels of yeast can replace fish meal in *P. commersonnii*.

The 20% torula yeast inclusion improves FCR as indicated by the current study since the poorest FCR was determined in response to the yeast free diet (control). The supplementation of *C. utilis* yeast in fish diets enhanced the PER. Fish fed 16% yeast supplement diet had the highest PER while the lowest PER was observed in the control. The best FCR observed with yeast containing diets suggest that the supplementation of prebiotics enhanced feed utilization. The highest PER results indicate that addition of 16% yeast in fish diets can significantly improve protein utilization in spotted grunter. These findings are in agreement with Lara-Flores *et al.*

(2003) who found that supplementation of yeast to Nile tilapia diets enhanced diet and protein digestibility hence the better feed efficiency observed in yeast supplemented diets. Welker and Lim (2011) also reported that prebiotics utilization can improve nutrient absorption and utilization by fish. According to Wang and Xu (2006), feed utilization and digestion may be improved by intestinal bacteria that assist during the decomposition of nutrients providing the macro-organisms with physiological active materials.

In the present study, the dietary supplementation of yeast significantly affected the body protein and fat contents. These results followed the same trend as those obtained by Abdel-Tawwab (2012) who found a significant difference in whole fish body lipid and protein content of fish fed graded levels of yeast. Contrary to these results Olvera-Novoa *et al.,* (2002) and Mohammadi *et al.,* (2016) did not find significant differences in whole body protein and lipid of *O. mossambicus* and *C. trimaculatum* fed different yeast levels. The alterations observed in body constituents such as protein and lipid could be associated with deposition rate in muscle, different growth and constituent synthesis rates (Fauconneau, 1984; Abdel-Tawwab *et al.*, 2006).

Dietary supplementation did not have any significant effect on both the HSI and VFI of fish fed graded levels of torula yeast. The results obtained on the HSI did not follow any significant trend. However, fish fed on 8% yeast containing diet exhibited higher HSI when compared to other diets, whereas VFI values of spotted grunter increased with increasing yeast levels. These results are similar to the finding by Hauptman *et al.* (2014), who reported an increased visceral fat deposition and whole body lipid deposition in *O. mykiss* fed increasing levels of grain distillers dried yeast. Given that fat deposition can reduce seafood commercial value, fat accumulation observed in experimental fish implies that spotted grunter was unable to metabolize yeast effectively. These results also suggest that lipid inclusion in torula yeast fed spotted grunter may be reduced which will be a significant cost-effective advantage in the production of commercial feeds. Torula yeast therefore, may have lipid sparing effect when included in spotted grunter diets.

The HSI is associated with the storage of energy in the liver and is also sensitive to the nutritional status of the fish (Pyle *et al.*, 2005). Also, the CF can be used to

monitor the general fish condition (Van der Oost *et al.*, 2003). According to Goede & Barton (1990), the CF decreases when energy reserve in fish is depleted or when fish are subjected to stress associated with nutrient availability. In this study, both the HSI and CF were not affected by dietary treatment and these results are supported by Hauptman *et al.* (2014) and Mohammadi *et al.* (2016) who did not observe any effect on HSI and CF in fish fed graded levels of yeast.

On the other hand, no significant enhancement was observed in the blood glucose of fish fed different yeast levels, while liver glycogen content was significantly different. Yeast supplementation increased blood glucose to a maximum accumulation at 16% yeast containing diets compared to all other diets (Figure 26) while liver glycogen significantly decreased with increasing levels of yeast in fish diets. Our results are inconsistent with the finding of Abdel-Tawwab, (2012) and Sanlur *et al.* (2009), who found that different yeast levels significantly increased blood glucose in Nile tilapia (*O. niloticus*) and gilthead sea bream (*S. aurata*). From the findings of the present study, it is evident that 16% yeast containing diet provided enough energy to increase blood glucose although the diet containing 8% yeast stored the highest content of glycogen in the liver. However, it is not known how the glycogen storage capacity of skeletal and heart muscle was affected by the yeast supplemented diets.

The supplementation of fish diets with 20% torula yeast induced histological enteritis in the distal intestine. All the fish fed 20% containing yeast showed typical symptoms of intestinal inflammation. The reduction of intestinal folds with infiltration of leukocytes in the submucosa and lamina propria may explain inflammation when feeding 20% yeast supplemented diet because the membrane becomes more vulnerable to all possible inducers within the diet. These results are in contrast with a four-week feeding trial by Grammes *et al.*, (2003) who reported that 20% *C. utilis* supplemented diets showed healthy intestines at the end of the trial with no signs of intestinal enteritis in Atlantic salmon (*Salmor salar*). Furthermore, the histological examination of the distal intestine demonstrated that, yeast significantly improved microvilli length of spotted grunter fed diet containing 12 and 16% of *C. utilis* yeast. Li *et al.*, (2007) reported that the nucleic acids present in dietary yeasts can repair intestines, enhance mucosal gut flora, mucosal surface and increase the length of the intestinal tract. Therefore, these results suggest that torula yeast may substitute

a maximum of 16% in spotted grunter diets in order to improve the integrity of intestinal mucosa as well as to increase the density of intestinal villi.

CHAPTER 5

CONCLUDING DISCUSSION

The expansion of aquaculture production continues to put pressure on the sustainability of ocean fisheries. Consequently, fish meal production has been decreasing at an annual rate of 1.7% (FAO, 2012). This had led to the global increase in fish and fish meal prices which are negatively affecting aquaculture industries and fish farmers. Supplementation with feed additives has been an alternative source to enhance the quality of formulated fish diets in order to improve fish growth performance and health (Yilmaz & Cengiz, 2016). Thus, the aim of the current study was to investigate the effects of selected dietary algae, probiotics and commercial herbal additives in diets for juvenile *Pomadasys commersonnii*. The aim of the study was achieved by evaluating the effects of supplementing equal portions of dietary ingredients (that is, *S. platensis*, *U. lactuca*, *C. utilis*, *S. cerevisiae*, LIV-UP® and UNP PB-20®) in the diets on growth, feed utilization proximate composition and health parameters in spotted grunter.

The results of this study show that supplemented dietary ingredients in spotted grunter diets can significantly improve growth rate and feed efficiency compared to the non-supplemented diet (control). Amongst the supplemented dietary ingredients, *C. utilis* supported the best growth performance for fish. Nonetheless, in order to achieve maximum levels of yeast replacement, the health status of the fish must also be considered. For fish to remain healthy and maintain optimum growth for a long time, they require a nutritionally balanced diet (Craig & Helfrich, 2002). Therefore, it was suggested that a maximum replacement of 16% of *C. utilis* can be incorporated in spotted grunter diets without showing any apparent negative health effects.

When analysing the results of the growth performance for spotted grunter fed with selected feed additives, fish growth was significantly high in fish fed with supplemented diets compared to the control (Chapter 2, Figure 4). The growth improvement indicates that the addition of feed additives enhanced nutrient utilization which was reflected in increased weight gain, SGR, PER, FCR and alleviated the effects of stress factors. Dietary supplementation with feed additives seems to provide adequate nutrients to enhance fish growth and stimulate appetite.

Furthermore, on the diet proximate composition point of view, the protein content in fish body (Chapter 2, Table 4) tended to decrease with increasing dietary protein concentration. These results confirm the findings by Hecht *et al.*, 2003, who reported that spotted grunter has a maximum protein requirement of 50%. Protein is one of the essential nutritional constituents in fish diets to promote normal growth rate (Lovell, 1989). Fish fed on diets containing dietary protein ranging from 48 to 50% (torula, brewer's yeast and *Spirulina,*) grew significantly faster compared to those fish that fed on diets with less or more than an optimum dietary protein content for spotted grunter (Chapter 2, Table 1). Hence, the inhibition of growth observed in fish fed with diets containing high dietary protein content could be due to the fact that fish of different sizes prefer a certain limit of protein after which excess protein could not be utilized effectively. Therefore, the protein present in fish diets was utilised effectively by spotted grunter and the fish used it to improve somatic growth rate. Also, the results of the study suggest that feed additives can be added in formulated diets to enhance the growth rate of spotted grunter while reducing feed production cost.

Concerning the effect of graded levels of *C. utilis* on growth performance of spotted grunter, the results of this feeding experiment suggest that a maximum replacement of 16% of *C. utilis* can be incorporated in spotted grunter diets to improve growth and without showing any apparent negative health effects (Chapter 4, Figure 19). Improved growth rate, PER and FCR was observed in spotted grunter fed with diets containing 16% of torula yeast compared to the control (fish meal). The better feed utilization observed in fish fed with diet containing 16% of torula yeast suggests that yeast improved protein and diet digestibility, which could explain better growth and feed efficiency observed with this diet. Yeast has been found to be effective in growth, feed efficiency and blood parameters (Ramasamy *et al.*, 2011; Abu-Elala *et al.*, 2013). This growth improvement could be due to the presence of yeast in fish diets which provides monogastric organisms with digestive enzymes, essential nutrients and vitamins with consequent improvement in feed digestion.

The histological examination of fish intestine showed elongated and dense intestinal villi in fish fed yeast supplemented diets when compared to other diets. Fish fed with yeasts (both *S. cerevisiae* and *C. utilis*) showed an increase in length and density of intestinal microvilli which is beneficial for feed assimilation (Chapter 3, Figure 16 and

Figure 17, respectively). Furthermore, the nucleic acid present in yeast diets also has several benefits which help to accelerate intestinal integrity (Li *et al.*, 2007). Similar results were reported by Dimitroglou *et al.*, 2009 and Abu-Elala *et al.*, 2013. The intestinal villi length and density observed in fish fed yeast supplemented diets could explain the apparent gradual growth increase (Chapter 3, Figure 16, 17A and Chapter 4, Figure 28B). Supplementation of fish diets with selected additives is responsible for the considerable accumulation of visceral fat and whole-body lipids observed in spotted grunter. The deposition of visceral fat was relatively high in all the fish groups that were fed with selected feed additives when compared to the non-supplemented diet (Chapter 3, Figure 10). However, the VFI analysis of the diets described in Chapter 4 (Figure 25) indicates that there was a proportional accumulation of visceral fat with inclusion of torula yeast even though the dietary lipid content was the same in all the diets. This implies that the rate of lipid deposition in spotted grunter is influenced by the addition of additives. These results also suggest that lipid inclusion in supplemented diets for spotted grunter should be reduced for a lesser expensive feed cost in aquaculture industry. Further study is required to determine the effects of dietary lipid levels on supplemented diets of spotted grunter.

The addition of selected feed additives in spotted grunter diets showed a significant increase in liver glycogen compared to the UNP PB-20® supplemented diet. In addition, the blood glucose concentration of spotted grunter was not affected by graded yeast levels after a feeding period of 8 weeks (Chapter 4, Figure 26); however, when the longer feeding experiment with diets containing different selected feed additives for 12 weeks was conducted, a significant increase in blood glucose of fish fed on torula and *Spirulina* diets was observed (Chapter 3, Figure 11). The liver glycogen and blood glucose content observed in fish fed with supplemented diets remained relatively high when compared to UNP PB-20® containing diet which had a remarkable reduced glycogen and blood glucose in spotted grunter. Blood glucose in fish is affected by health conditions and dietary deficiency (Hemre *et al.*, 1996) while glycogen content decreases when fish are exposed to stressful conditions. Therefore, the results observed in this study suggests that fish groups fed with most diets i.e. *S. platensis*, *U. lactuca*, *C. utilis*, *S.cerevisiae*, LIV-UP® and the control accumulated enough glycogen in the liver and were in good biological condition.

However, UNP PB-20® supplemented diets did not provide sufficient energy to the fish as a result fish were unable to store enough glycogen in the liver, which might explain the decrease in HSI. However, the high VFI in these fish is indicative that they also used lipids as energy for metabolic requirements.

In addition to biochemical indicators of the fish fed supplemented diets, glycogen accumulated disproportionally in the liver of fish fed with graded levels of yeast (Chapter 4, Figure 27), while glucose content was observed to increase with yeast inclusion (Chapter 4, Figure 26). Fish fed 8% yeast containing diet accumulated the highest liver glycogen content and lowest blood glucose when compared to all the treatments. These findings possibly suggest that spotted grunter was not negatively affected by 8% of yeast inclusion in their diets and might have contributed to the increase in HSI.

In conclusion, selected feed additives can be supplemented in *P. commersonnii* diets. Based on the findings of this study, a maximum concentration of 16% of *C. utilis* can be added in spotted grunter diets to enhance growth rate, feed efficiency and without any adverse effects on the well-being of the fish. Replacement of fish meal with other feed additives (i.e. Brewer's yeast, LIV-UP® and UNP PB-20®) is not advisable because of the increased accumulation of visceral fat that was observed in fish. However, reducing dietary lipid levels may reduce the VFI and consequent dietary costs. Nevertheless, further investigation is required to elucidate the effect of substituting fish meal with higher concentration of yeast on growth and health condition of spotted grunter – especially since possible nutrition additives contributing to improved intestinal wall integrity, may supress the enteritis effects of increased dietary yeast inclusions.

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