

EXAMINATION OF THE EFFECTS OF DIETARY PROTEIN AND LIPID ON
GROWTH AND STRESS RESPONSE OF NILE TILAPIA
CULTURED IN HIGH INTENSITY SYSTEMS

by

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ABSTRACT

Tilapia is the second most consumed farmed fish, after carp, and the most widely grown of any farmed fish. Significant feed price increases in recent years threaten sustainability of the industry. In the US tilapia are often subjected to extended hauling to reach live-fish markets. Therefore, the objectives of the current study were to optimize dietary protein and lipid levels for juvenile tilapia cultured in high-intensity recirculating-water system and assess how dietary changes alter hauling stress-tolerance. To achieve these objectives, a 3 X 3 factorial design was used with practical-type diets formulated to contain three levels of dietary protein (28, 32 and 36%) and three levels of dietary lipid (3, 6, and 9%). Juvenile tilapia (34.5 ± 0.4 g initial weight) were fed one of the nine diets, three feedings/d to apparent satiation, six d/wk for 12wk. Fish were weighed and counted every three weeks and feed consumed recorded weekly. At the conclusion of the feeding trial, three fish per tank were sampled for proximate composition analyses. One week post-conclusion of the feeding trial, tilapia remaining in each tank were subjected to a simulated live haul in which fish were transferred to insulated container (2lbs/gallon) with supplemental oxygen for 24h, and then returned to their source tank and allowed to recover for an additional 48h. Hematocrit, glucose, lactate and cortisol measurements were collected at time 0, 24h, and 72h. Increasing dietary protein significantly improved tilapia weight gain ($P=0.01$), feed conversion (FCR, $P=0.03$), feed intake ($P=0.02$), protein retention ($P=0.01$) and filet ratio ($P=0.01$). Increasing dietary lipid also significantly improved weight gain ($P=0.05$) and FCR ($P=0.01$) but at 9% decreased feed intake ($P=0.02$). Blood chemistry values were also altered by dietary protein and lipid levels (Figure 1). No significant interactions between dietary protein and lipid levels on growth performance or blood chemistry values were measured. Results of this study suggest that while increasing protein and lipid levels in tilapia diet formulations improved production of tilapia cultured in high intensity systems and that, stress tolerance during live hauls appeared to be reduced.

INTRODUCTION

Tilapia are the second most consumed fish after carp and are the most widely grown of any farmed fish (El-Sayed 2004). Tilapia production grew at an average annual rate of 13.4% during 1970–2002 and continues to be one of the fastest growing farming activities (El-Sayed 2004). Tilapia is increasing in popularity due to its year round availability, mild flavor and its ability to be reared in a variety of culture conditions (Lim and Webster 2006). Although the majority of tilapia production has historically been conducted in low density pond systems, an increasing shift toward culture intensification, including fish rearing in high intensity recirculating systems, is occurring. In pond culture, natural feed organisms such as microorganisms account for 30-50% of overall growth of tilapia (Pompa and Masser 1999). However, many current diet formulations do not take into account the fact that high intensity systems lack these natural feed stuffs and thus may not maximize growth and health of intensively-reared tilapia. Additionally, significant price increases for ingredients (40-60% for some ingredients; David Brock personal communication) in recent years threatens the continued profitability of the industry because feed is estimated to contribute 40- 60% of total production costs (Fodetor 2004).

Protein is a major dietary nutrient that affects tilapia growth (Lovell and Limsuwan, 1986) by providing essential amino acids and energy for maintenance. The ability of tilapia to utilize dietary protein is related to both dietary protein level and the availability of non-protein energy sources (Sans et al. 2000); if insufficient non-protein energy is available or if the protein is of poor quality, protein will be de-aminated in the

body to supply energy for metabolism. To minimize protein catabolism for energy, increasing dietary lipid levels has been used as an effective strategy in numerous aquaculture species. However, upper limits for dietary lipid inclusion are species-specific with some warm-water fish species, including tilapia, displaying decreased growth and performance when levels are excessive. Decreased performance of tilapia is of concern because it affects the ability of farmers to deliver product to market.

Additionally, the majority of United States-cultured tilapia are sold at a premium to live fish markets located on the coasts and can be up to 48h away from tilapia farms (Colt et al. 2011). For these markets, the quality and performance of the live fish is dependent on their ability to withstand the stress associated with hauling. Stress increases incidences of disease and reduces pathogen resistance (Pickering and Chistie 1982; Sumpter et al 1985; Wedemeyer 1997). To date, there have been few studies that have examined how diet quality altered stress responses of tilapia subjected to live-hauling.

LITERATURE REVIEW

Tilapia

Tilapias are a warm-water, omnivorous, fish in the cichlid family. In Africa, the earliest discovered tilapia fossils were estimated to be 18 million years old (Fryer and Iles 1972). Tilapia are native to Africa, Jordan and Israel and in these three locations there are 70 known species of tilapia (Philippart and Ruwet 1982, McAndrew 2002.) Although there are a plethora of known tilapia species, only a few are used in commercial aquaculture. The most extensively cultured species of tilapia is the Nile tilapia (Oreochromis niloticus), which accounts for about 60% of total tilapia culture (Lim and Webster 2006), because of fast growth, acceptability to different culture conditions and consumer preference (Macintosh and Little 1995, Shelton 2002a).

Growth of Tilapia Aquaculture

Tilapia are cultured in more than 100 countries due to their ability to withstand a wide variation of different environments. Tilapia aquaculture represents 5% of the total quantity of fish cultured and is second only to carp as the world's most commonly cultured fish (Lim and Webster 2006). The popularity of tilapia culture has increased due to its year round supply of seed stock, consumer preference for a mild flavor as a food fish and its ability to be reared in a variety of culture conditions (Lim and Webster 2006). Tilapia culture had an average annual growth rate of 13.4% during 1970–2002 and was one of the fastest growing farming activities (El-Sayed 2004). In 1974, tilapia was

introduced into the United States and by 1996, sales surpassed rainbow trout sales (Pompa and Masser 1999).

Tilapia Diets

Tilapia in adapted environments feed on micro-organisms, detritus and zooplankton (Bowen 1982). In pond-cultured tilapia, these natural feed organisms account for 30-50% of overall growth (Pompa and Masser 1999). In high-intensity systems, natural feed organisms are lacking and tilapia require nutritionally balanced prepared diets. It has been estimated that aquaculture feed costs are between 40- 60% of total production costs (Fodetor 2004).

Tilapias are commonly cultured in flow-through, clear-water systems in the Western US (Colt et al 2011). Tilapia have the ability to survive in this area in part due to geothermal water supplies. The dietary requirements for tilapia raised in high intensity systems are likely to be substantially different than pond-cultured tilapia because the former rely on a commercially prepared diets due to the lack of natural organisms that are commonly found in tilapia pond culture.

Protein Requirements of Tilapia

Protein is a major dietary nutrient that affects fish growth (Lovell and Limsuwan 1986) by providing essential amino acids. Amino acids are essential in that the fish cannot synthesize them in adequate amounts and therefore they must be provided by the diet for producing muscle, assist with enzymatic functions and supply energy to the fish

which in turn help them grow. Fish require ten essential amino acids: (Methionine, Threonine, Tyrosine, Lysine, Arginine, Isoleucine, Histidine, Valine, Leucine, Proline). Protein (amino acid) requirements for fish change as the maturity level of the fish changes as well (Craig 2009), where mature fish require less protein to support growth than do juvenile fishes. Almost all commercially available feedstuffs lack some of the ten essential amino acids that are necessary for promoting adequate growth for fish (NRC 1993). For this reason, fish generally can utilize high protein prepared feeds for growth and other important physiological factors but can lose up to 65% of dietary protein to the environment when consuming prepared diets (Craig 2009).

Dietary protein requirements of tilapia have been reported to be between 20-56% depending on fish size and environmental factors (El-Sayed and Teshima 1991). Protein requirements also depend heavily on the type of culture system in which the fish is grown (NRC 1993). As an example, in Egypt, Khattab et al. (2000) reported that the optimum dietary protein levels for growth was between 27 and 37% CP depending upon the culture location.

Dietary protein and amino acid requirements are generally higher in young fry and juvenile tilapia than older tilapia (NRC 1993). Bahnsanaway (2009) found that in fertilized tanks, monosex juvenile tilapia grew faster when fed a 35% crude protein (CP) diet than when fed a 17% CP diet. However, there were no differences in fish weights when juveniles were fed 25, 30 and 35% CP diets. In earlier research, De Silva et al. (1989) found that juvenile tilapia grew best with a dietary crude protein level of 34-36% and these results agree with those of Balarin and Halfer (1982) who found that tilapia

ranging from 5-25 g/BW had protein requirement between 25-35% CP. More recently, Wilkinson (2003) found that optimum growth was obtained at 30-34% CP and interestingly higher levels of CP depressed growth rates in juveniles cultured in cages. The latter observation contrasts with the results of Jauncey (1982) who found that increasing dietary protein to 38-40% CP further increased growth rate.

Dietary protein of mature (reproductively capable) tilapia has been more modestly studied with available literature focusing on providing an adequate amount of energy to produce mature gonads. Researchers have reported that dietary protein requirements for adult tilapia are between 30-40% CP for optimum spawning (Gunasekera, Shim & Lam 1996; El-Sayed, Mansour & Ezzat 2003). In contrast, Cisse (1982) reported that 20% CP was sufficient to maintain growth of mature fish fed fishmeal plus cottonseed-based diets. More recent research by El Sayed and Kawanna (2008) found significant differences between protein levels in tilapia eggs, when mature were fed varying levels of protein and energy. Additionally, when diets containing levels of 30, 35 and 40% CP were fed to sexually mature fish it was found that both male and female fish receiving 40% CP displayed improved growth compared to fish fed 30% CP even when the sexes were raised in different tanks. Although, fewer studies have examined dietary effects on the onset of maturity in tilapia, El Sayed et al. (2002) found a significant linear increase in growth of mature tilapia fed 25, 30, 35, and 40% CP.

Lipid Requirements of Tilapia

Lipids supply essential fatty acids that are necessary for maintaining biological structures and cellular membranes (Sargent et al. 1989). Numerous studies have indicated that tilapia require both n-3 and n-6 fatty acids for growth (Stickney and Wurts 1986; Stickney and Hardy 1989; Chou and Shiau 1999). Tilapias have been reported have the ability to elongate and desaturate 18:3n-3 fatty acids to 20:5n-3 and 22:6n-3 as well as desaturating 18:2n-6 to 20:4n-6 (Lim et al. 2009.)

Lipids are high in energy that supplies approximately about twice the amount of energy as carbohydrates or proteins and contain triglycerides that aid in the transportation of fat soluble vitamins (Craig 2009). Although dietary lipids supply a flux of energy to many fish species, in omnivorous fish such as tilapia lipids have less of a role when compared to carbohydrates (Lim et al. 2011). However, tilapia digest lipids from protein sources better than lipids that are present in carbohydrate sources (Lim et al. 2009).

Numerous studies have attempted to identify optimum dietary lipid levels that provide adequate energy for tilapia with conflicting results. Chou and Shiau (1996) reported that the dietary lipid requirement for Nile x Blue tilapia hybrids was between 5-12%. In that study, tilapia had better growth rates when fed 10 and 15% dietary lipid compared to tilapia fed 5% dietary lipid in isocaloric and isonitrogenous diets. Results suggest that a 5% dietary lipid was the lowest amount of dietary lipid needed to meet the minimum requirement of hybrid tilapia for acceptable growth rate. Earlier research by Stickney and Wurts (1986) found growth differences in fish fed dietary lipid less than 3% when compared with fish fed 7.5-10% dietary lipid. However, Hanley (1991) found no

differences in growth rates of tilapia in semi-intensive fish culture systems when fish were fed diets containing 5, 7 and 12% dietary lipid. Similarly, Fitzsimmons et al (1997) found that isocaloric and isonitrogenous diets fed to tilapia containing 3, 6, and 8% dietary lipid were not significantly different in rate and efficiency of growth. Based on these studies, Fitzsimmons (2009) recommended that the dietary lipid requirement of tilapia under 2g BW should represent 10% of the total diet. The variability in species and size of the tilapia used for these studies may explain some of the discrepancies in the reported lipid requirements of tilapia.

Because some researchers have observed depressed growth at higher lipid levels (Jauncey and Ross 1982; Jauncey 2002; Han et al. 2010), Fitzsimmons (2009) recommended that tilapia from 2g BW to harvest should require no more than 6-8% of lipid in the total diet. Jauncey and Ross (1982) and Jauncey (2002) found that at 12% dietary lipid, growth and performance began to decrease. In a more recent study, Han et al (2010) found that tilapia fed 5.5 and 8.5 % performed better than fish fed either 2.2% or 15.0% of dietary lipid. Other research, however, disagrees with these conclusions. El-Sayed and Kuwana (2008) found that broodstock tilapia grew better when fed increased dietary lipid levels. In that study, brood tilapia were fed varying levels of dietary lipid (14.6, 16.7, 18.8 %) and there was a positive growth effect for increasing dietary lipid on growth with brood tilapia fed 18.8 % growing faster than brood tilapia fed 14.6% dietary lipid.

Protein Sparing by Lipid in Tilapia

Protein is the primary nutrient behind rising feed costs and dietary protein alone can be up to 50% of the overall diet cost in intensive tilapia culture systems (El-Sayed 2004). Excessive levels of dietary protein that are metabolized for energy should, where appropriate, be replaced by cheaper alternative sources of energy. A protein sparing effect has been observed in numerous other fish species including catfish (*Pseudobagrus fulvidraco*; Lee and Lee 2005) and grass carp (*Ctenopharyngodon idella*; Du et al. 2005). Tilapias are effective at utilizing dietary lipid as an energy source and research has indicated that dietary lipid can have a sparing effect on dietary protein (Jauncey and Ross, 1982; Li et al. 1991). Jauncey and Ross (1982) were able to increase performance of tilapia fed lower protein levels by increasing lipid from 6 to 10% of the diet. Li et al. (1991) found that by increasing dietary lipid from 5.7% to 9.4% and carbohydrates from 31.9% to 36.7%, it was possible to simultaneously reduce protein in the diet from 33.2% to 25.7% while maintaining acceptable growth rates. De silva et al. (1991) investigated these interactions further and his results indicate that by increasing lipid to 18% of the diet this would decrease dietary protein requirement while still maintaining similar growth. Jauncey (2000) found similar results in Nile tilapia x blue tilapia hybrids fed dietary lipid up to 12%. Dietary protein levels could be reduced from 40 to 30% without decreasing growth. However, a notable contrast between the De silva et al. (1989) study and the Jauncey (2000) study was that in the latter when dietary lipid levels exceeded 12%, suppressed growth was observed. Research in tilapia by Han et al. (2010) found no positive effects of lipid on protein sparing and suggested that hybrid tilapia may not be as

effective at lipid utilization when compared to natural strains. A caveat of using increasing lipid levels to spare protein however is that by increasing dietary lipid levels were fed to tilapia, excessive fat may be deposited in the visceral cavity thus creating fattier fish that may alter consumer preference (Goa et al. 2009) and potentially reducing stress tolerance (Barton et al. 1988).

Stress in Fish

Stress is a general response that fish have an adaptive reaction to cope with stressors that are present (Barton 2002). One type of stressors are classified as physical stressors that act directly on fish. Physical stressors can also have no focal contact with fish, but rather result due to stress from specific cues and memory retrieval of stressful events (Moreirro and Valpato 2004). In numerous species in fish, stressors cause an increase of plasma catecholamines as a primary response to stress (Mazeaud et al. 1977). This flux of catecholamines into the blood stream happens within minutes of the stressor (Mazeaud 1973a) and can last for many hours after termination of the stressor (Nakano and Tomlinson 1967).

Cortisol is the principal glucocorticoid secreted by the interrenal tissue (steroidogenic cells) located in the head-kidney of fish (Iwama et al. 1999). This hormone is released by the activation of the hypothalamus-pituitary-interrenal axis (HPI axis; Mommsen et al. 1999). In stressful conditions chomaffrin cells release catecholamines, adrenaline and nonadrenaline hormones into blood circulation (Reid et

al. 1998). With elevated levels of cortisol and the stress hormones glucose levels begin to be elevated through gluconeogenesis and glycogenolysis (Iwawa et al.1999).

Interactions Between Diet and Stress

The stress response by Nile tilapia has been documented in a number of experiments (Barcellos et al. 1999; Delaney et al. 2004; Barreto and Velpato 2006). However, few have examined the interaction between stress and diet in mixed-tank tilapia culture although Cheng et al. (2006) has examined this interaction in orange spotted grouper (*Epinephelus coioides*). Cheng et al. (2006) examined glucose levels in orange grouper fed differing dietary protein and lipid levels and then subjected fish to an environmental stressor. Grouper were fed one of four diets; 1) low protein-low fat, 2) high protein-low fat, 3) high protein-low fat, 4) high protein-high fat. Cheng et al. (2006) found significant differences in plasma glucose; fish fed nutrient dense, high protein and high fat diets, had plasma glucose levels that suggested these fish were less stressed than fish fed low protein low fat diets.

Lemly et al. (1996) suggested that lipid reserves are important in energy mobilization for maintaining acceptable body condition. Lipids because of their rapid metabolic transformation are considered transient body material, but they represent the major source of stored chemical energy and their presence or absence reflects the physiological capacity of fish (Scheck and Moyle 1990).

The influence of stress on lipid metabolism in fish has been studied by several groups (Abo- Hegab et al. 1993 and El- Nagar et al. 2000). Barton (1997) found that

with higher levels of free fatty acids in diets, fish responded better to stressors due to the ability of fish to utilize stored lipids. This theory was supported by El- Sayed et al. (1996) and Barton (1988). El-Sayed et al. (1996) hypothesized that the decrease in body protein and lipid observed in fish maintained in an inappropriate habitat was a direct result of utilization of body protein and / or fat as an energy source to meet the increase in physiology demands. Barton (1988) using chinook salmon (*Oncorhynchus tshawytscha*) found that fish supplemented with high energy diets had higher plasma glucose levels suggesting a better ability to cope with stress. A more recent study by Falcon et al. (2007) suggested that in tilapia there was no effect of dietary lipid level on blood glucose and cortisol levels following a cold stress test. These authors did, however, see differences in blood chemistry when dietary lipid was supplemented with varying levels of vitamin C. Kumar et al. (2011) found feeding Labeo rohito fingerlings varying levels of dietary protein altered the fish's stress response. Specifically, fish fed 20% CP diets had higher levels of serum cortisol after a temperature challenge when compared to fish fed 30, 40 and 45% CP.

MATERIALS AND METHODS

Experiment 1Objectives

The objective of experiment one were to determine optimum dietary protein and lipid requirements for diets fed to juvenile tilapia grown in high intensity systems and secondly to examine the potential of these diets to alter stress in tilapia stress response during transportation.

Experimental Design

A 12-week feeding trial with juvenile tilapia was conducted as a three by three factorial design with three CP levels 28%, 32%, 36% and three crude lipid (CL) levels 3%, 6%, and 9% (Table 1,2). At the conclusion of the feeding trial, tilapia were exposed to a simulated hauling experiment and the effects of dietary protein and lipid level on survival, blood chemistry and stress tolerance were assessed.

Table 1. Ingredients¹ of diets for juvenile tilapia used in the feeding trials.

Protein	Diets								
	28% CP			32% CP			36%CP		
Lipid	3%	6%	9%	3%	6%	9%	3%	6%	9%
BM ²	3	3	3	4	4	4	5	5	5
PBM ³	2	2	2	2.7	2.7	2.7	3.3	3.3	3.3
MF ⁴	2	2	2	2.7	2.7	2.7	3.3	3.3	3.3

Table 1 Continued

SM ⁵	21	21	21	28	28	28	35	35	35
WM ⁶	44	40	36	34.8	30.8	26.8	25.7	21.7	17.7
WGL ⁷	0	1	2	0	1	2	0	1	2
WF ⁸	20	20	20	20	20	20	20	20	20
PF ⁹	0.9	2.9	4.9	0.7	2.7	4.7	0.5	2.5	4.5
MFO ¹⁰	1	2	3	1	2	3	1	2	3
L ¹¹	1	1	1	1	1	1	1	1	1
Stay-C 35	1	1	1	1	1	1	1	1	1
VP ARS ¹²	1	1	1	1	1	1	1	1	1
TM ARS									
640 ¹³	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DP ¹⁴	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
CCL 50% ¹⁵	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6

¹Origin of ingredients: Nelson & Sons, Murray, UT, USA.; ² Blood Meal ³Poultry by product meal. ⁴Menhaden fish meal. ⁵ Soybean Meal ⁶Wheat Midds ⁷Wheat Gluten Meal, ⁸ Wheat Flour ⁹Poultry Fat ¹⁰Menhaden fish oil, ¹¹ Lecithin, ¹² Contributed per kilogram of diet: vitamin A (as retinol palmitate), 10,000 IU; vitamin D₃, 720 IU; vitamin E (as DL- α -tocopherylacetate), 530 IU; niacin, 330 mg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; menadione sodium bisulfate, 25 mg; folacin, 13 mg; biotin, 1 mg; vitamin B₁₂, 30 ug. ¹³Contributed in mg/kg of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3.

¹⁴Dicalcium Phosphate. ¹⁵Choline CL 50%.

Table 2. Analyzed composition(SD)¹ of diets fed to juvenile fish for 12 weeks²

Diet	Gross Energy	Crude Protein	Crude Lipid	Moisture	
		Kcal/g	%	%	%
28% CP	3%CL	4392(.10)	28.8(0.00)	3.2(0.08)	4.4(0.08)
	6%CL	4603(33)	29.0(0.17)	5.7(0.10)	3.1(0.10)
	9% CL	4860(70)	29.7(0.15)	8.6(0.01)	3.4(0.01)
32% CP	3%CL	4340(19)	31.5(0.13)	3.1(0.01)	1.8(0.01)
	6%CL	4607(0.47)	32.3(0.27)	5.4(0.22)	3.3(0.22)
	9% CL	4862(9)	32.7(0.13)	8.5(0.10)	2.2(0.10)
36% CP	3%CL	4472(88)	36.1(0.04)	2.6(0.17)	3.1(0.17)
	6%CL	4578(21)	36.7(0.06)	4.6(0.10)	3.3(0.08)
	9% CL	4842(31)	37.1(0.14)	8.3(0.12)	1.6(0.17)

¹Standard deviation of ²Means of two analysis/diet.

All treatments had triplicate tank replication except for the 3% CL levels which were run in duplicate due to tank space limitations. Duplicates were applied to the 3% CL because the existing literature suggested that this level was below optimum (Chou and Shiau 1996) but had not previously been examined simultaneously with increasing protein levels.

Fish and Culture System

Juvenile tilapia, Oreochromis niloticus, were obtained as fry from the University of Arizona and grown to an average initial weight of 34.5g (± 0.4 g) and were fed a commercial tilapia diet (Arkat Tilapia Grower; 32%CP, 6% CL) at the Fish Technology Center, Bozeman, Montana. At the beginning of the feeding trial, fish were randomly selected and stocked at a density of 30/tank. Fish were cultured in twenty-four, 75L fiberglass tanks with an initial water depth of 0.25m. Lighting was maintained on a 13:11 diurnal fluctuation and water quality characteristics (dissolved oxygen, temperature, pH, ammonia, and nitrates), were maintained within acceptable ranges for tilapia (Lim and Webster 2006). All rearing and sampling protocols were approved by the USFWS, Bozeman Fish Technology Center Animal Care and Use Committee.

Experimental Diets and Feeding

All diets (Table 1) were formulated to meet or exceed nutrient requirements for tilapia (NRC 1993). Diets were manufactured at the Fish Technology Center using cooking extrusion (DN DL-44, Buhler AG, Uzwil, Switzerland) and dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland). Fish were acclimated to their respective

experimental diets for one week before the study began. Throughout the feeding trial, each diet was offered to fish 3x/d a day by hand feeding to apparent satiation for 6d/wk.

Fish Sampling

At the beginning of the feeding trial, three fish from the original population were sacrificed for determination of initial whole body proximate composition. Throughout the study, fish were weighed every 3wk and feed conversion ratio (FCR), feed intake and weight gain were calculated. At the conclusion of the study, 3 fish from each tank were murdered for whole body composition and three additional fish were sampled for determination of hepatosomatic index (HIS), fillet ratio (FR) and visceral somatic index (VSI).

Proximate Analyses

Dry matter and ash analysis was performed according to AOAC (1995). Crude protein (N x 6.25) was determined by Dumas method (AOAC, 1995) on a Leco TruSpec N nitrogen determinator (Leco Corporation, St. Joseph, MI). Total energy was determined by isoperibol bomb calorimetry (Parr6300, Parr Instrument Co. Inc., Moline, IL). Lipid was determined using an Ankom XT10 (Ankom Technologies, Macedon, NY).

Growth and Nutrient Retention Efficiency Calculations

The following formulae were used:

$$\text{Weight gain (WG)} = \text{final average fish weight (g)} - \text{initial average fish weight (g)}$$

$$\text{FCR} = \text{dry diet fed (g)} / \text{wet weight gained (g)}$$

Feed intake = wet feed fed (g) * 100 / (((initial tank weight (g) - final tank weight (g)) / 2) / # of d)

Hepatosomatic index (HSI) = Liver weight (g) / whole body weight (g) * 100

Visceral somatic index (VSI) = Gut weight (g) / whole body weight (g) * 100

Fillet ratio (FR) = Fillet weight * 2 (g) / whole body weight (g) * 100

Energy retention efficiency (ERE) = energy gain in fish (g) / energy intake (g) x 100

Protein retention efficiency (PRE) = protein gain in fish (g) / protein intake (g) x 100

Simulated Fish Hauling Trial

At the conclusion of the feeding trial, the remaining fish continued to be fed their respective diets and were cultured as described above for an additional two weeks.

Twenty-four hours prior to hauling, feeding was discontinued. Simulated hauling trials were performed by approximating conditions used by live-haulers in the Western United States (Cole et al. 2011). Briefly, tilapia were dry-netted from their respective culture tanks and placed in an 8 L insulated container at constant ratio of 0.24kg /L into 22°C spring water supplemented with 3 ppt salt. Compressed oxygen was supplemented to each container via an airstone and was kept above saturation for the entirety of the 24 h period. Following the 24 h haul, fish were returned to their respective culture tanks and survival was monitored for an additional 48 h. Water quality parameters (pH, O₂, un-ionized ammonia, nitrite, and temperature) were determined at the beginning and end of

each simulated haul using an YSI 550A (Yellow Springs, OH) and a Hach NI-HDT (Loveland, CO) water quality system

Blood Chemistry Sampling

At time 0 (prior to the haul), two fish were quickly netted from each culture tank and bled via caudal puncture with a 26-gauge needle and 1 cc syringe. At 24 h post-haul, two fish from each hauling container and 48 h post-haul (time 72 h), from each recovery tank, were also bled. All fish were bled within 2 min of netting. Hematocrit was determined in duplicate on whole blood using a microhematocrit centrifuge and reader (Critospin, Norwood, MA). Remaining blood was placed in heparinized tubes on ice until centrifugation at 4000 g for 8 min. Resultant sera were stored frozen at -80C until analysis.

Glucose and lactate levels were determined using a Vitros DT60 II (Johnson and Johnson, Roshestor, New York). Cortisol was analyzed by radio-immuno assay protocol as previously described by Forster (1974) and modified by Redding et al. (1984). Briefly, 10 ul of plasma was combined with 200 ul of phosphate-buffered saline, and immersed in a 100C water bath for 15 min to denature the proteins. The samples were then cooled to room temperature in a cold water (4C) bath for 5 min prior to assay. All samples were analyzed in duplicate. The lower limit of detection was 0.98 ng/ml. The intra and interassay coefficients of variation for all assays were less than 5% and 10%, respectively. Steroid levels (determined by RIA) were validated by verifying that serial dilutions were parallel to standard curves.

Statistical Analyses

Data was subjected to factorial analysis of variance using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) to determine the effect of dietary protein and lipid and their potential interactions on fish growth, performance and body composition and blood chemistry following a simulated hauling trial. Tukey's (1953) mean separation was used to determine any differences within main effects. Differences were considered significant at $P < 0.05$.

Experiment 2

Objective

The objective of experiment two were to determine optimum dietary protein and to lipid requirements for practical diet formulations in mature tilapia grown in high intensity systems and examine the potential of these diets to reduce stress responses during transportation.

Experimental Design

An 18-week feeding trial with mature tilapia was conducted as a three by three factorial with three crude protein (CP) levels 28%, 32%, 36% and three crude lipid (CL) levels 3%, 6%, and 9% (Table 1) as previously described for experiment 1. At the conclusion of the feeding trial, mature tilapia were similarly exposed to a simulated hauling experiment and the effects of dietary protein and lipid level on survival, blood chemistry and stress tolerance were assessed.

Replication mirrored that of the juvenile study in that all mature fish treatments had triplicate tank replication except for 3% CL levels which were run in duplicate due to tank space limitations. Duplicates were applied to the 3% CL because the existing literature suggested that this level was below optimum (Chou and Shiau 1996) but had not previously been examined in mature fish simultaneously with increasing protein levels.

Fish and Culture System

Mature tilapia, Oreochromis niloticus, were obtained as fry from the University of Arizona and grown to an average initial weight of 129.6g (± 3.8 g) at Bozeman Fish Technology Center. At the beginning of the feeding trial, fish from the previously described population were randomly selected and stocked at a density of 15 fish/tank. Fish were cultured in twenty-four, 75 L fiberglass tanks with an initial water depth of 0.25 m. Lighting was maintained on a 13:11 diurnal fluctuation and water quality characteristics (dissolved oxygen, temperature, pH, ammonia, and nitrates), were maintained within acceptable ranges for tilapia (Lim and Webster 2006). All rearing and sampling protocols were approved by the USFWS, Bozeman Fish Technology Center Animal Care and Use Committee.

Experimental Diets and Feeding

Diets were manufactured at the Bozeman Fish Technology Center using cooking extrusion (DNDL-44, Buhler AG, Uzwil, Switzerland) with a 2 mm die head and dried in

a pulse bed drier (Buhler AG, Uzwil, Switzerland). Pellet durability was determined as previously described with a Holmen NHP 100 Portable pellet tester (Norfolk, England).

Fish were acclimated to their respective experimental diets for one week before the study began. Throughout the feeding trial, each diet was offered to fish three times a day by hand feeding to apparent satiation for six days a week.

Fish Sampling

At the beginning of the feeding trial, three fish from the original population were sacrificed for determination of initial whole body proximate composition. Throughout the study, fish were weighed every three weeks and feed conversion ratio (FCR), feed intake and weight gain were calculated. At the conclusion of the study, three fish were sampled for determination of HSI, FR and VSI.

Proximate Analyses

Dry matter and ash analysis was performed according to AOAC (1995). Crude protein (N x 6.25) was determined by Dumas method (AOAC, 1995) on a Leco TruSpec N nitrogen determinator (Leco Corporation, St. Joseph, MI). Total energy was determined by isoperibol bomb calorimetry (Parr6300, Parr Instrument Co. Inc., Moline, IL). Lipid was determined using an Ankom XT10 (Ankom Technologies, Macedon, NY).

Growth and Nutrient Retention Efficiency Calculations

The following formulae were used:

Weight gain (WG) = final weight (g) - initial weight (g)

FCR= dry diet fed (g)/ wet weight gained (g)

Feed intake= wet feed fed (g) * 100 (((initial tank weight (g)-final tank weight (g)/2))/ # of d

Hepatosomatic index (HSI) = Liver weight (g)/ whole body weight (g) * 100

Visceral somatic index (VSI) = Gut weight (g)/ whole body weight (g) * 100

Fillet ratio (FR) = Fillet weight *2 (g) / whole body weight (g) * 100

Energy retention efficiency (ERE) = energy gain in fish (g)/energy intake (g) x 100

Protein retention efficiency (PRE) = protein gain in fish (g)/protein intake (g) x 100

Simulated Fish Hauling Trial

At the conclusion of the feeding trial, fish remaining post-sampling continued to be fed their respective diets and were cultured as described above for an additional two weeks. Twenty-four hours prior to hauling feeding was discontinued. Simulated hauling trials were performed by approximating conditions used by live-haulers in the Western United States (Cole et al. 2011). Briefly, tilapia were dry-netted from their respective culture tanks and placed in a 8L insulated container at constant ratio of 0.24kg /l into 22C spring water supplemented with 3 ppt salt. Compressed oxygen was supplemented to each container via an airstone and was kept above saturation for the entirety of the 24 h period. Following the 24 h haul, fish were returned to their respective culture tanks and survival was monitored for an additional 48 h. Water quality parameters (pH, O₂, un-

ionized ammonia, nitrite, and temperature) were determined at the beginning and end of each simulated haul using a YSI 550A (Yellow Springs, OH) and a Hach NI-HDT (Loveland, CO) water quality system.

Blood Chemistry Sampling

At time 0 (prior to the haul), two fish were quickly netted from each culture tank and bled via caudal puncture with a 26 gauge needle and 1cc syringe. At 24 h post-haul, three fish from each hauling container. At 48 h post haul, all remaining fish from each recovery tank were also bled. All fish were bled within 2 min of netting. Remaining blood was placed in heparanized tubes on ice until centrifugation at 4000 rpms for 8 min. Resultant sera were stored frozen at -80C until analysis.

Glucose and lactate levels were determined using a Vitros DT60 II (Johnson and Johnson, Roshestor, NY). Cortisol was analyzed by radio-immuno assay protocol as previously described by Forster (1974) and modified by Redding et al. (1984). Briefly, 10 ul of plasma was combined with 200 ul of phosphate-buffered saline, and immersed in a 100C water bath for 15 min to denature the proteins. The samples were then cooled to room temperature in a cold water (4C) bath for 5 min prior to assay. All samples were analyzed in duplicate. The lower limit of detection was 0.98 ng/ml. The intra and interassay coefficients of variation for all assays were less than 5% and 10%, respectively. Steroid levels (determined by RIA) were validated by verifying that serial dilutions were parallel to standard curves.

Statistical Analyses

Data was subjected to factorial analysis of variance using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) to determine the effect of dietary protein and lipid and their potential interactions on fish growth, performance and body composition and blood chemistry following a simulated hauling trial. Tukeys (1953) mean separation was used to determine any differences within main effects. Differences were considered significant at $P < 0.05$.

RESULTS

Experiment 1: Juvenile TilapiaGrowth and Body Composition

Analyzed composition of the experimental diets in the juvenile feeding trial reflected formulation targets (Table 2). Juvenile tilapia showed no significant interactions between CP and CL were observed for weight gain, FCR or feed intake. Weight gain, feed conversion and feed intake were significantly ($P < 0.05$) affected by diet (Table 3). Fish fed 36% CP had faster gains than fish fed 28 or 32% CP and lower FCRs than fish fed 28% CP. Fish fed 3% CL had significantly slower gains and higher FCRs than fish fed 6 or 9% CL which were similar. Feed intake was significantly decreased ($P < 0.02$) in fish fed 6 and 9% CL; however, dietary protein had no ($P < 0.10$) effect on feed intake

Table 3. Growth Performance of Juvenile tilapia fed 28 32 or 36% crude protein and 3 6 or 9% crude lipid for 12 weeks

Diet	Growth Performance ¹			Body indices ²			
	Weight gain ³	FCR ⁴	Feed Intake ⁵	VSI ⁶	FR ⁷	HSI ⁸	
	0-12wk	(feed/gain)	(%)				
28% CP	3%CL	1627 ^{b,x}	1.71 ^{a,x}	3.13 ^x	9.8 ^a	35.6 ^b	1.5 ^a
	6%CL	2100 ^{b,y}	1.46 ^{a,y}	2.91 ^y	9.9 ^a	34.2 ^b	1.8 ^a
	9% CL	2177 ^{b,y}	1.43 ^{a,y}	2.91 ^y	9.8 ^a	35.0 ^b	1.7 ^a
32% CP	3%CL	2033 ^{b,x}	1.48 ^{ab,x}	2.95 ^x	9.7 ^a	36.1 ^{ab}	1.3 ^b
	6%CL	2151 ^{b,y}	1.41 ^{ab,y}	2.86 ^y	9.4 ^a	36.8 ^{ab}	1.4 ^b
	9% CL	1997 ^{b,y}	1.40 ^{ab,y}	2.74 ^y	9.7 ^a	35.4 ^{ab}	1.5 ^b
6% CP	3%CL	2176 ^{a,x}	1.48 ^{b,x}	2.99 ^x	7.5 ^b	38.4 ^a	1.4 ^b
	6%CL	2261 ^{a,y}	1.35 ^{b,y}	2.76 ^y	8.6 ^b	37.0 ^a	1.4 ^b

Table 3 Continued

	9% CL	2479 ^{a,y}	1.37 ^{b,y}	2.89 ^y	8.4 ^b	37.4 ^a	1.3 ^b
Pooled SE		1.10	0.66	0.44	59.35	1.56	1.44
ANOVA, Pr > ⁶		0.01	0.05	0.13	0.03	0.12	0.19
Protein		0.02	0.03	0.10	0.01	0.01	0.02
Lipid		0.04	0.03	0.02	0.81	0.63	0.71
Protein*Lipid		0.52	0.62	0.46	0.89	0.73	0.75

¹ Means of three replicate tanks (30fish/tank);two replicate tanks for 3% lipid treatments ; six fish per treatment 3% lipid treatment. ² Means of nine fish per treatment; three replicate tanks per diet. ³(Final average fish weight –initial average fish weight/initial average fish weight. ⁴FCR = feed conversion ratio; (g feed fed (dry)/g gain (wet)). ⁵ Feed intake= (total wet feed fed(g)*100)/((Individual fish weight(g) + Initial individual fish weight(g))/2)/days on feed). ⁶ Gut weight (g)/ whole body weight (g) * 100. ⁷ Fillet weight *2 (g) / whole body weight (g) * 100 ⁸ Liver weight (g)/ whole body weight (g) * 100. ⁹Significance probability associated with the *F*-statistic Values within columns with a common superscript letter do not differ significantly at $P < 0.05$; lower case superscripts a,b,c when present refer to significant of protein while lower case subscripts refer to significant effects of lipid when present.

Visceral Somatic Index, HSI and FR of tilapia were altered ($P < 0.02$) by diet (Table 3). VSI was significantly higher in fish fed 6 or 9% CL compared to fish fed 3% CL. Hepatosomatic index was higher for in fish fed 28% CP compared to fish fed 32 or 36% CP which were similar. No significant interactions between CP and CL were observed for VSI, HSI or FR in juvenile tilapia.

Whole body proximate composition and nutrient retention efficiency were altered by diet (Table 4). Fish fed 28% CP had whole body moisture levels that were lower ($P < 0.03$) than fish fed 32 and 36% CP. Whole body lipid levels were higher ($P < 0.03$) in fish fed 28% CP than fish fed 36% CP. Fish fed 3% and 6% CL had whole body moisture levels that were higher than fish fed 9% CL. Fish fed 3% CL had lower whole body lipid

levels that were higher than fish fed 6% and 9% CL. Fish fed 32 and 36% CP had whole body protein levels higher ($P < .01$) than fish fed 28% CP. Whole body energy was significantly altered by dietary CL level in that fish fed 9% CL had significantly higher whole body energy levels as compared to fish fed 3% CL. No significant interactions between CP and CL were observed for whole body proximate composition or nutrient retention efficiencies.

Table 4. Proximate composition and nutrient retention efficiency of juvenile tilapia fed 28, 32 or 36% crude protein and 3, 6 or 9% crude lipid for 12 weeks.¹

Diet		Moisture (%)	Lipid (%)	Crude protein (%)	Gross energy kcal/g	PRE ² (%)	ERE ³ (%)
28% CP	3%CL	70.24 ^{b,x}	10.16 ^{a,x}	16.48 ^b	1767 ^y	34.03 ^a	25.35
	6% CL	69.40 ^{b,x}	8.65 ^{a,y}	16.85 ^b	1860 ^{x,y}	37.87 ^a	27.18
	9%CL	66.08 ^{b,y}	9.41 ^{a,y}	16.74 ^b	2067 ^x	36.21 ^a	30.68
32% CP	3%CL	71.13 ^{a,x}	12.10 ^{a,x}	17.19 ^b	1701 ^y	37.51 ^b	24.71
	6% CL	70.04 ^{a,x}	8.82 ^{a,y}	16.60 ^b	1740 ^{x,y}	36.31 ^b	24.63
	9%CL	68.54 ^{a,y}	7.81 ^{a,y}	16.85 ^b	1909 ^x	38.52 ^b	25.16
36% CP	3%CL	69.61 ^{a,x}	9.67 ^{b,x}	17.64 ^a	1809 ^y	40.68 ^b	27.48
	6% CL	71.45 ^{a,x}	9.98 ^{b,y}	18.11 ^a	1828 ^{x,y}	39.01 ^b	26.62
	9%CL	69.46 ^{a,y}	10.13 ^{b,y}	18.16 ^a	1805 ^x	41.02 ^b	25.72
Pooled SE		1.10	0.66	0.44	59	1.56	1.44
ANOVA, $Pr > F^4$		0.01	0.05	0.13	0.03	0.12	0.19
Protein		0.02	0.03	0.01	0.07	0.01	0.07
Lipid		0.04	0.03	0.92	0.02	0.87	0.54
Protein*Lipid		0.52	0.62	0.80	0.20	0.56	0.29

¹ Means of duplicate fish pools per tank; three replicate tanks per diet on an as-fed basis. ² Apparent protein retention efficiency (PRE) = protein gain in fish (g)/protein intake (g) x 100. ³ Apparent energy retention efficiency (ERE) = energy gain in fish (g)/energy intake (g) x 100. ⁴ Significance probabilities associated with the *F*-statistic. Values within columns with a common superscript letter do not differ significantly at $P < 0.05$; lower case superscripts a,b,c when present refer to significant of protein while lower case subscripts refer to significant effects of lipid when present.; No significant interactions were observed.

Protein retention efficiency (PRE) was significantly reduced in juvenile tilapia fed 28% CP as compared to fish fed the 32 or 36% CP (Table 4). Energy retention efficiency (ERE) tended to be higher ($P=0.07$) in fish fed 28 and 36% CP as compared to fish fed 32% CP. No significant interactions between CP and CL were observed for proximate composition or retention efficiencies.

Juvenile Tilapia Hauling Trial

Water quality in the hauling container at 24 h post-haul was not significantly altered by diet (Table 5). Temperature, DO, nitrite and unionized ammonia ranged from 20.9C - 22.5C, 26.0-11.1 mg/L, 0.01-0.04 ppm and 0.1-1.0 ppm, respectively at 24 h post-haul.

Table 5. Effects of feeding 28, 32, 36%CP and 3,6 and 9%CL to juvenile tilapia for 12weeks on water quality 24h after start of simulated live haul.

Diet		Nitrite mg/l	pH	Temperature (°C)	DO mg/l	TAN mg/l	Un-ionized Ammonia mg/l
28% CP	3%CL	0.0	7.9 ^a	20.9	23.5	17.4	0.5
	6%CL	0.0	7.4 ^b	22.4	16.0	14.8	0.2
	9% CL	0.0	7.3 ^b	22.5	16.9	13.4	0.1
32% CP	3%CL	0.1	7.3 ^a	22.3	11.1	13.5	0.1
	6%CL	0.0	7.4 ^b	21.9	17.6	9.2	0.1
	9%CL	0.0	7.5 ^b	22.2	17.6	15.2	0.1
36% CP	3%CL	0.0	8.2 ^a	21.7	26.0	10.2	1.0
	6%CL	0.0	7.5 ^b	22.2	13.9	17.7	0.3

Table 5 Continued

	9%CL	0.0	7.3 ^b	22.4	17.3	11.9	0.1
Pooled SE		0.01	0.2	0.5	3.0	3.8	0.2
ANOVA, $Pr > F^5$		0.24	0.12	0.72	0.21	0.91	0.20
Protein		0.08	0.47	0.98	0.62	0.71	0.40
Lipid		0.59	0.05	0.34	0.31	0.97	0.11
Protein*Lipid		0.34	0.18	0.60	0.11	0.68	0.26

¹ Means of duplicate assays per tank; three replicate tanks per diet except 3% lipid where n= 2 tanks/diet. ² Significance probabilities associated with the F -statistic.

Hematocrit levels of the juvenile tilapia in the hauling containers were affected by diet at 72 h; fish fed 32% CP had higher levels of red blood cells than fish fed 28% and 36% CP levels (Figure 1). Lactate levels were altered by diet at time 0 and time 24 h specifically, by an effect of protein source (Figure 1; Table 6). At time 0, fish fed 32% CP had higher ($P<.02$) lactate levels than fish fed 36% CP. At time 24 h, fish fed 36% CP had s higher ($P<.05$) lactate levels than fish fed 28% CP. Glucose levels of fish in the simulated haul were significantly altered ($P<. 01$) by diet at time 0; fish fed 32% CP had lower glucose levels than fish fed 36% CP and 28% CP (Figure 2; Table 6).

Table 6. Effects of feeding 28, 32 or 36% CP and 3, 6 or 9% CP to juvenile tilapia for 12¹ weeks on blood lactate², glucose³, and cortisol⁴ during a simulated hauling trial.

Diet	Lactate ¹			Glucose ¹			Cortisol ¹		
	Time 0	24h	72h	Time 0	24h	72h	Time 0	24h	72h
28% CP 3%CL	0.9 ^{ab}	2.6 ^b	0.7	39.0 ^a	59.0	43.0	62.5	130.5	109.0
6%CL	0.9 ^{ab}	2.6 ^b	0.6	39.0 ^a	83.0	41.5	72.8	228.3 ¹	88.5
9%CL	0.8 ^{ab}	4.6 ^b	0.7	44.8 ^a	67.8	45.8	84.8	125.8	61.3
32% CP 3%CL	1.0 ^a	4.9 ^{ab}	2.0	39.5 ^b	78.5	51.0	69.5	69.5	82.3

Table 6 Continued

6%CL	1.2 ^a	5.4 ^{ab}	1.1	30.2 ^b	80.0	39.0	40.7	134.0 ²	72.3
9%CL	0.8 ^a	5.5 ^{ab}	1.3	37.2 ^b	87.7	40.7	49.3	188.1	41.5
36% CP 3%CL	0.8 ^b	4.9 ^a	0.9	40.0 ^a	81.7	39.0	72.8	170.8	105.8
6%CL	0.6 ^b	1.3 ^a	1.1	45.0 ^a	101.8	39.0	55.8	129.8 ²	63.7
9%CL	0.9 ^b	8.5 ^a	7.3	45.3 ^a	88.5	50.8	66.0	160.3	83.5
Pooled SE	0.1	1.3	0.4	4.0	23.1	6.3	18.4	38.6	27.1
ANOVA, $Pr > F^5$	0.07	0.09	0.39	0.02	0.91	0.44	0.4	0.03	0.40
Protien	0.02	0.05	0.07	0.01	0.40	0.96	0.13	0.52	0.41
Lipid	0.77	0.14	0.68	0.20	0.64	0.24	0.52	0.26	0.12
Protien*Lipid	0.18	0.32	0.72	0.25	0.99	0.31	0.63	0.01	0.69

¹ Means of duplicate fish per tank; three replicate tanks per diet except 3% lipid where n= 2 tanks/diet. ²average of two fish/tank at each time period measured as mmol/L. ³average of two fish/tank at each time period measured as mg/dL. ⁴average of two fish/tank measured in ng/mL. ⁵Significance probability associated with the *F*-statistic. Values within columns with a common superscript letter do not differ significantly at $P < 0.05$; lower case alphabetic superscripts refer to significant main effects; numeric subscripts within table refer to significant interactions.

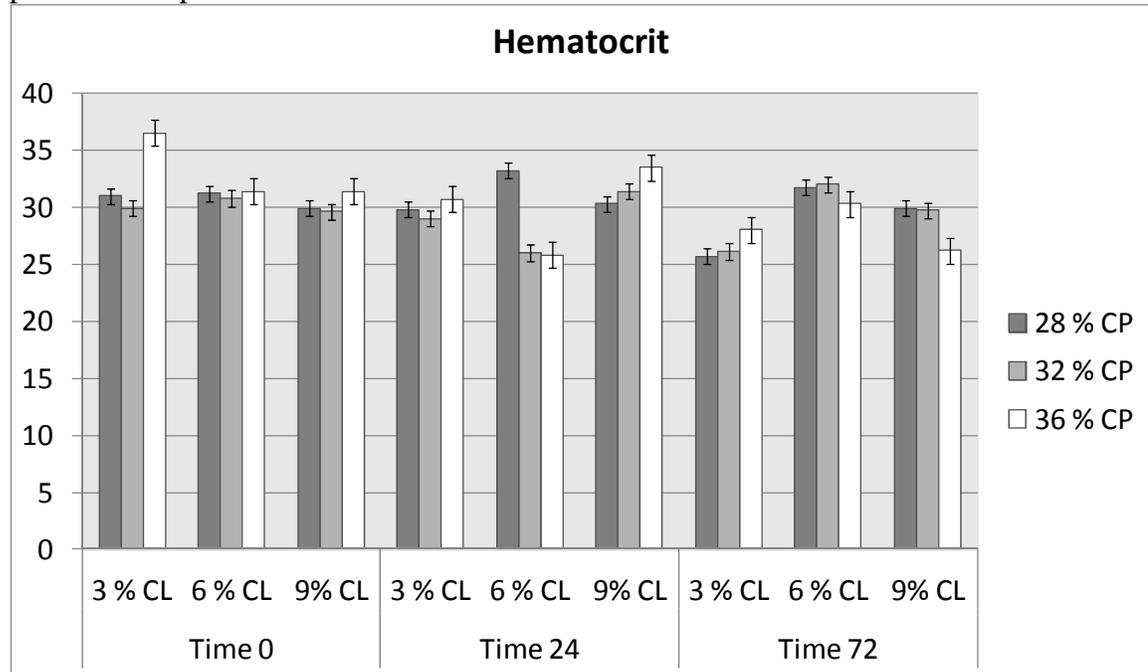
Figure 1. Lactate levels (\pm standard error) of juvenile tilapia fed varying levels of dietary protein and lipid for 12 weeks

Figure 2. Lactate levels (\pm standard error) of juvenile tilapia fed varying levels of dietary protein and lipid for 12 weeks

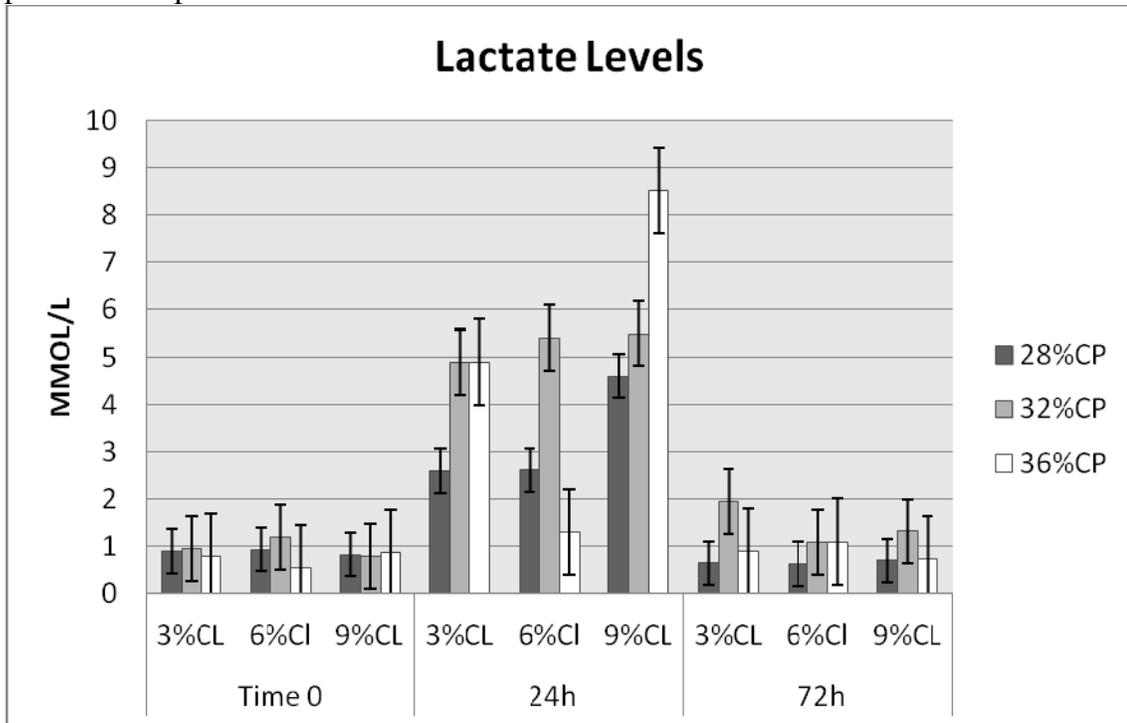
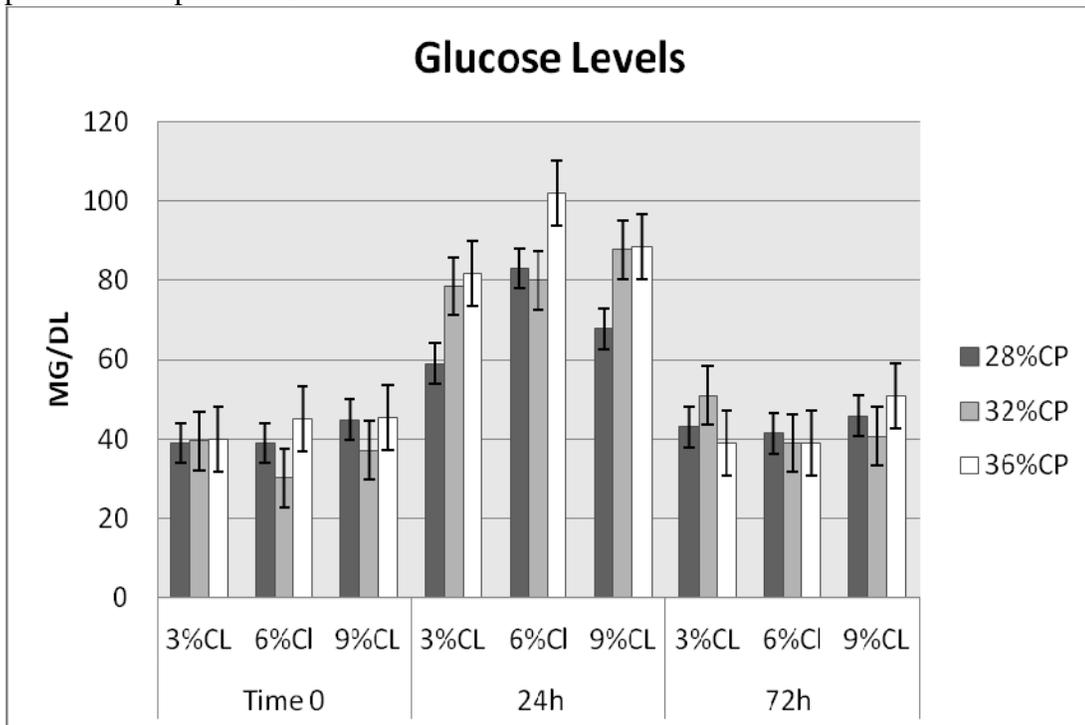
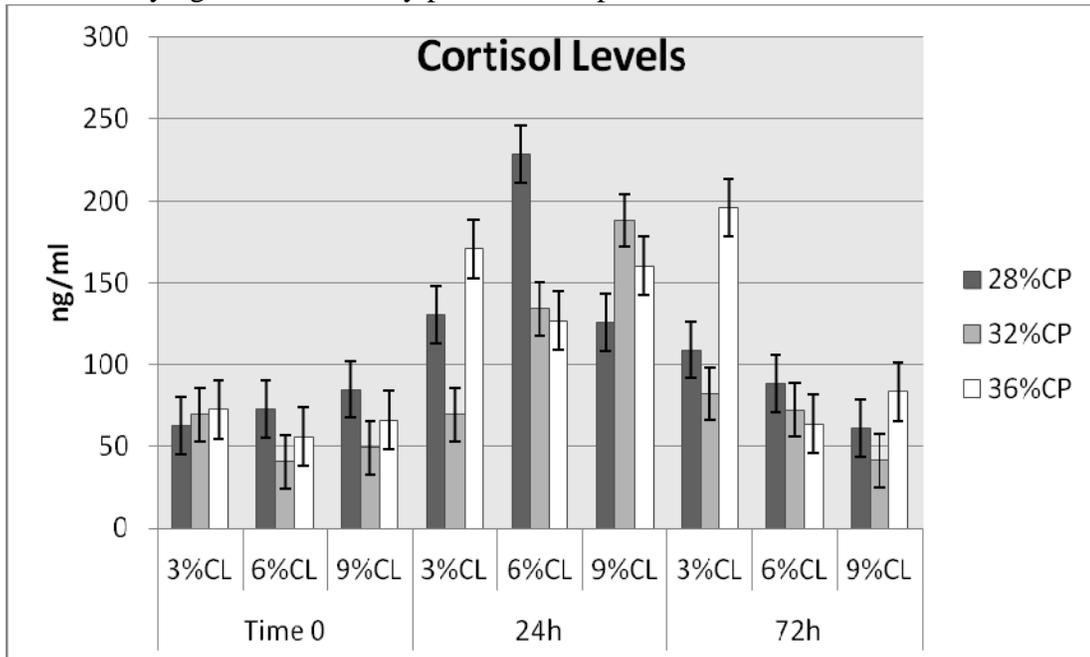


Figure 3. Glucose levels (\pm standard error) of juvenile tilapia fed varying levels of dietary protein and lipid for 12 weeks.



Juvenile tilapia displayed higher in cortisol levels at time 24 h as than at time 0 and time 72 (Figure 3). There was a protein by lipid interaction at time 24 h where fish fed 28% CP and 6% CL had higher cortisol levels (Table 6) than fish fed 36%CP and 6%CL as well as fish fed 32%CP and 6%CL dietary combinations at time 24 h. There were no significant main effects of dietary protein and or lipid on juvenile tilapia cortisol levels.

Figure 4. Cortisol levels (\pm standard error) of juvenile tilapia measured at time 0, 24 and 72h fed varying levels of dietary protein and lipid for 12 weeks



Cost Effectiveness

Cost effectiveness on a \$/lb diet basis was calculated via a commercial feed company and \$/lb of gain was measured at the conclusion of the study. The diet containing 36% CP and 6% CL was found to be the most cost effective diet in growing juvenile tilapia (Table 7).

Table 7. Cost evaluation of protein and lipid fraction of diets fed to juvenile tilapia for 12wks

Diet	Gain (0-12wk)	Feed (g)	Cost/lb of feed ¹	Cost/lb of gain ²
28% CP 3%CL	1627	2780	0.35\$	0.60\$
6%CL	2100	3056	0.37\$	0.54\$
9% CL	2177	3110	0.40\$	0.57\$
32% CP 3%CL	2033	3015	0.36\$	0.53\$
6%CL	2151	3037	0.39\$	0.55\$
9%CL	1997	2772	0.41\$	0.58\$
36% CP 3%CL	2176	2506	0.38\$	0.56\$
6%CL	2261	3044	0.40\$	0.54\$
9%CL	2479	3389	0.43\$	0.59\$

¹Cost provided by a commercial feed manufacturing company. ²Calculated as \$/lb of feed fed/0-18wk gain in lbs.

Experiment 2: Mature Tilapia

Analyzed dietary composition reflected formulation targets (Table 8). Pellet durability index (PDI) was different between diets (Table 9). Diets containing 36% CP had ($P < 0.001$) more breakage during testing than diets containing 32 and 28% CP. There was also a significant effect of lipid on PDI. Diets containing 9% CL had ($P < 0.01$) less breakage during testing than diets containing 6% CL. A protein by lipid interaction ($P < 0.01$) also was observed for PDI in diets containing 32% CP. PDI values in the 32% CP diet with 3, 6, 9 % CL had similar PDI values regardless of lipid level when compared to 36 and 28%CP which were similar.

Table 8. Analyzed composition(standard deviation)¹ of diets fed to juvenile fish for 18 weeks²

Diet		Gross Energy Kcal/g	Crude Protein %	Crude Lipid %	Moisture %	PDI ³ %
28% CP	3%CL	4392(5)	27(.11)	3.4(0.06)	4.8(0.01)	74.6(0.49) ^{axy}
	6%CL	4461(5)	27(0.01)	5.3(0.09)	5.7(0.27)	65.6(0.77) ^{ay}
	9%CL	4647(10)	28(0.00)	8.8(0.08)	5.7(0.73)	83.9(.0.64) ^{bx}
32% CP	3%CL	4417(15)	30(3.3)	4.0(0.28)	4.3(0.03)	76.9(0.28) ^{axy}
	6%CL	4542(5)	32(0.81)	5.7(0.09)	4.7(0.16)	63.2(12.3) ^{ay}
	9%CL	4718(5)	32(0.17)	8.6(0.01)	4.1(0.24)	71.9(0.99) ^{bx}
36% CP	3%CL	4354(40)	35(0.10)	3.1(0.18)	5.8(0.13)	58.5(1.20) ^{axy}
	6%CL	4561(20)	36(0.12)	5.8(0.00)	3.7(0.13)	66.5(0.57) ^{ay}
	9%CL	4731(0)	36(0.21)	8.6(0.01)	3.3(0.59)	66.4(0.78) ^{bx}

¹ Standard deviation. ²Means of two analyses/diet. ³Pellet Durability Index ability of pellets to hold together when placed through a Holmen pellet quality tester.

Growth and Body Composition

Tilapia weight gain, feed conversion and feed intake were not significantly ($P < 0.05$) affected by diet (Table 9). There were no significant interactions between CP and CL observed for weight gain, FCR or feed intake.

Visceral somatic index and hepatosomatic index of mature tilapia were not significantly altered by diet (Table 9). In contrast, muscle ratio was significantly affected by diet. Muscle ratio was significantly lower in fish fed 3% CL than fish fed 6 or 9% CL. No significant interactions between CP and CL were observed for VSI, HSI or FR.

Table 9. Growth and performance of mature tilapia fed 28, 32 or 36% crude protein and 3, 6 or 9% crude lipid for 12 weeks.

Diet		Growth Performance ¹			Body indices ²			Pellet Quality ³
		Weight gain ⁴	FCR ⁵	Feed Intake ⁶	VSI ⁷	FR ⁸	HSI ⁹	PDI
28% CP	3%CL	1644	2.10	3.07	8.0	29.8 ^y	1.9	74.6(0.5) ^{axy}
	6%CL	1815	1.88	3.03	8.1	38.6 ^x	2.1	65.6(0.8) ^{ay}
	9%CL	1528	2.16	2.94	9.2	35.6 ^y	2.5	83.9(0.6) ^{bx}
32% CP	3%CL	1314	2.39	2.83	7.5	29.3 ^y	2.1	76.9(0.3) ^{axy}
	6%CL	1924	1.96	3.23	7.1	37.1 ^x	2.2	63.2(12) ^{ay}
	9% CL	1828	1.98	3.17	8.5	35.7 ^y	2.0	71.9(1.0) ^{bx}
36% CP	3%CL	1993	1.93	3.31	7.2	38.3 ^y	1.8	58.5(1.2) ^{axy}
	6%CL	2003	1.87	3.24	7.3	37.2 ^x	2.0	66.5(0.6) ^{ay}
	9% CL	1835	2.01	3.20	6.8	37.6 ^y	1.9	66.4(0.8) ^{bx}
Pooled SE		0.005	8.30	0.74	1.1	0.50	4.59	0.54
ANOVA, $Pr > F^{10}$		0.30	0.25	0.42	0.70	0.08	0.54	<.0001
Protein		0.21	0.41	0.16	0.24	0.22	0.35	<.0001
Lipid		0.25	0.11	0.69	0.65	0.03	0.47	<.0001
Protein*Lipid		0.42	0.38	0.44	0.83	0.23	0.52	<.0001

¹ Means of three replicate tanks (30fish/tank) except for 3% lipid where n=2 tanks. ²

Means of nine fish per treatment except 3% lipid where n=6 fish. Means of two analyses/diet ³Pellet Durability Index ability of pellets to hold together when placed through a Holmen pellet quality tester. ⁴Weight gain(Final average fish weight –initial average fish weight). ⁵FCR = feed conversion ratio; (g feed fed (dry)/g gain (wet)). ⁶Feed intake= (total wet feed fed(g)*100)/((Individual fish weight(g) + Initial individual fish weight(g))/2)/days on feed). ⁷Visceral somatic index¹⁰Significance probability associated with the *F*-statistic Values within columns with a common superscript letter do not differ significantly at $P < 0.05$; lower case a,b,c when present superscripts refer to significant protein effects while lowercase x,y,z subscripts when present refer to significant lipid effects.

Whole body proximate composition and nutrient retention efficiency were not significantly altered by diet in mature tilapia (Table 10). Moisture levels ranged from 68.5-71.8 %, lipid ranged from a 7.3 to 9.3%, protein ranged from 16.5 to 17.4 % and

gross energy ranged from 1645-1854 kcal/g. PRE ranged from 21.0-25.5 and ERE ranged from 15.5- 20.6 in mature tilapia fed the various protein and lipid levels.

Table 10. Proximate composition and nutrient retention efficiency of juvenile tilapia fed 28, 32 or 36% crude protein and 3, 6 or 9% crude lipid for 18 weeks.¹

Diet		Moisture (%)	Crude Lipid (%)	Crude protein (%)	Gross energy kcal/g	PRE ² (%)	ERE ³ (%)
28% CP	3%CL	71.4	7.95	16.8	1645	23.3	15.6
	6% CL	69.1	9.33	16.5	1756	22.9	19.8
	9%CL	69.8	8.70	16.8	1792	25.5	19.5
32% CP	3%CL	68.5	8.85	17.3	1778	22.9	17.0
	6% CL	69.5	8.63	17.4	1677	21.0	17.0
	9%CL	70.0	8.73	17.0	1659	21.5	15.5
36% CP	3%CL	70.4	8.25	16.7	1730	25.5	20.6
	6% CL	71.8	7.33	17.0	1854	21.2	19.0
	9%CL	70.8	7.27	17.1	1636	21.8	17.1
Pooled SE		1.51	1.35	3.46	0.01	0.63	0.43
ANOVA, $Pr > F^4$		0.54	0.56	0.54	0.76	0.47	0.79
Protein		0.16	0.13	0.16	0.81	0.27	0.46
Lipid		0.96	0.95	0.97	0.69	0.33	0.81
Protein*Lipid		0.53	0.71	0.58	0.48	0.60	0.67

¹ Means of duplicate fish pools per tank; three replicate tanks per diet on an as-fed basis except for 3% lipid treatment where n=two tanks per diet. ² Apparent protein retention efficiency (PRE) = protein gain in fish (g)/protein intake (g) x 100. ³ Apparent energy retention efficiency (ERE) = energy gain in fish (g)/energy intake (g) x 100. ⁴ Significance probability associated with the *F*-statistic.

Mature Fish Hauling Trial

Water quality in the hauling container at 24 h post-haul was not significantly altered by diet. Temperature, DO, nitrite and unionized ammonia ranged from 20.9 C - 22.5 C, 26.0-11.1 mg/L, 0.01-0.04 ppm and 0.1-1.0 ppm, respectively at 24 h post-haul (Data not shown).

Lactate levels of mature tilapia were significantly altered by diet at time 24 h and time 72 h, by an effect of lipid source (Figure 4; Table 11). At time 24 h, fish fed 3% CL had significantly lower lactate levels than fish fed 6 and 9% CL. At time 72 h, fish fed 9% CL had significantly higher lactate levels than fish fed 6 and 3% CL. There were also significant interactions at time 24 and 72 h. At time 24 h a significant interaction was observed where fish fed diets containing 28% CP and 6% CL had significantly higher lactate levels than fish fed the 32% CP and 6% CL and the 36% CP and 6% lipid diet. At time 72 h a significant interaction between dietary protein and lipid was observed in fish fed 32% CP and 6% CL had significantly lower lactate levels than fish fed 28% CP and 6% CL and fish fed 36% CP and 6% CL. In contrast, glucose levels of mature fish in the simulated haul were not significantly altered by diet (Figure 5; Table 12).

Table 11. Effects of feeding 28, 32 or 36% CP and 3, 6 or 9% CP to juvenile tilapia for 18 weeks¹ on blood lactate², glucose³, and cortisol⁴ during a simulated hauling trial.

Diet	Lactate			Glucose			Cortisol		
	Time 0	24h	72h	Time 0	24h	72h	Time 0	24h	72h
28%CP 3%CL	0.9	3.9 ^y	1.4 ^x	49.3	130	63.0	70.1	356.8 ^a	185
6%CL	0.4	8.0 ^x	1.7 ^x	53.8	44.7	49.0	90.6	293.6 ^a	92.3
9%CL	0.0	8.9 ^x	1.9 ^y	50.0	49.0	57.4	69.1	346.9 ^a	74.3
32% CP 3%CL	0.8	7.9 ^y	1.5 ^x	43.7	62.2	63.4	81.4	248.2 ^b	67.3
6%CL	0.4	7.8 ^x	1.0 ^x	45.2	65.3	52.6	126.0	178.0 ^b	93.6
9%CL	0.4	5.7 ^x	1.3 ^y	69.0	33.5	50.9	67.8	252.2 ^b	70.3
36% CP 3%CL	0.1	4.3 ^y	2.3 ^x	48.8	28.0	56.2	67.1	141.3 ^b	45.3
6%CL	0.6	6.8 ^x	1.3 ^x	48.7	102	60.6	89.6	200.6 ^b	104
9%CL	0.4	6.8 ^x	1.2 ^y	40.8	49.2	62.9	106.5	226.4 ^b	191
Pooled SE	0.28	1.24	0.42	10.4	24.9	16.8	30.1	86.7	80.9
ANOVA, Pr>F ⁵	0.21	0.002	0.001	0.55	0.31	0.96	0.56	0.11	0.45

Table 11 Continued

Protien	0.60	0.16	0.13	0.62	0.66	0.85	0.67	0.01	0.68
Lipid	0.20	0.01	0.02	0.82	0.28	0.71	0.28	0.47	0.96
Protien*Lipid	0.15	0.001	0.002	0.26	0.21	0.81	0.49	0.78	0.15

¹Means of duplicate fish per tank; three replicate tanks per diet except 3% lipid where n= 2 tanks/diet. ²Significance probability associated with the F-statistic. Values within columns with a common superscript letter do not differ significantly at P< 0.05; lower case a,b,c superscripts refer to significant protein effects while lower case x,y,z superscripts when present refer to significant lipid effects numeric subscripts refer to significant interactions

Figure 5. Lactate levels (\pm standard error) of adult tilapia measured at time 0, 24 and 72h fed varying levels of dietary protein and lipid.

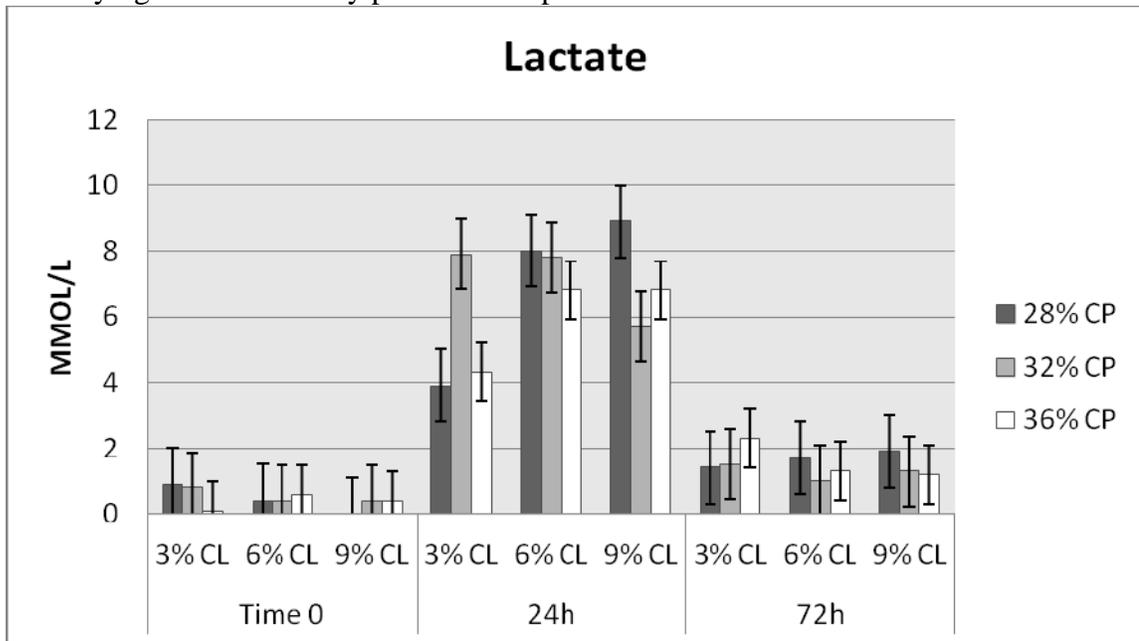
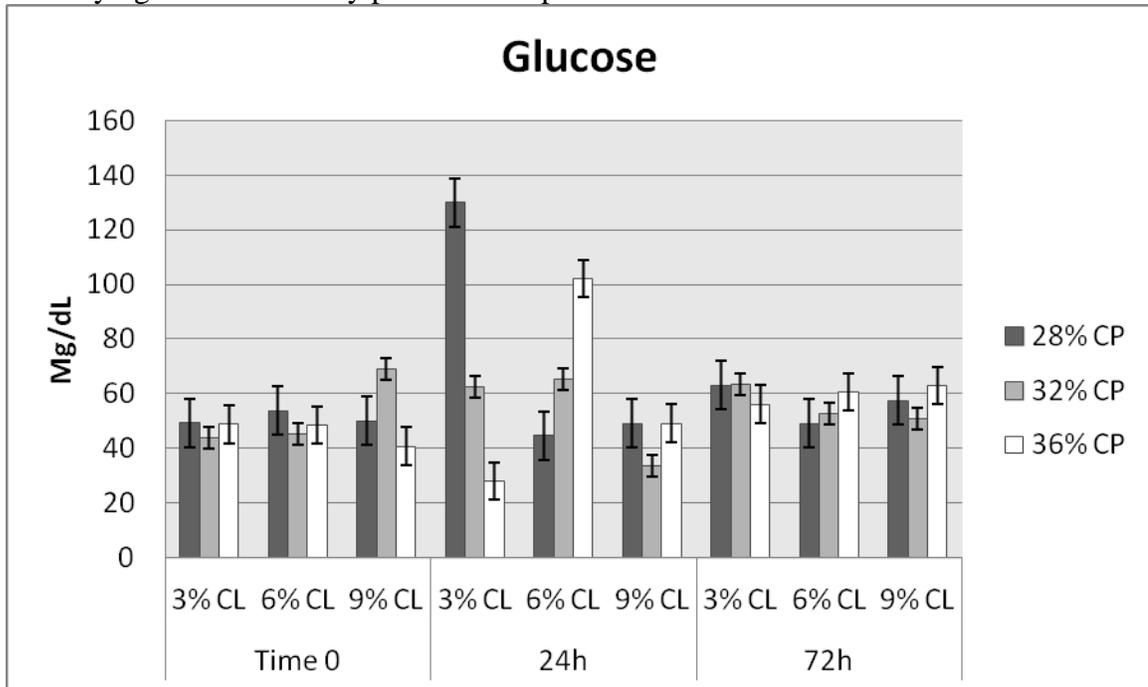
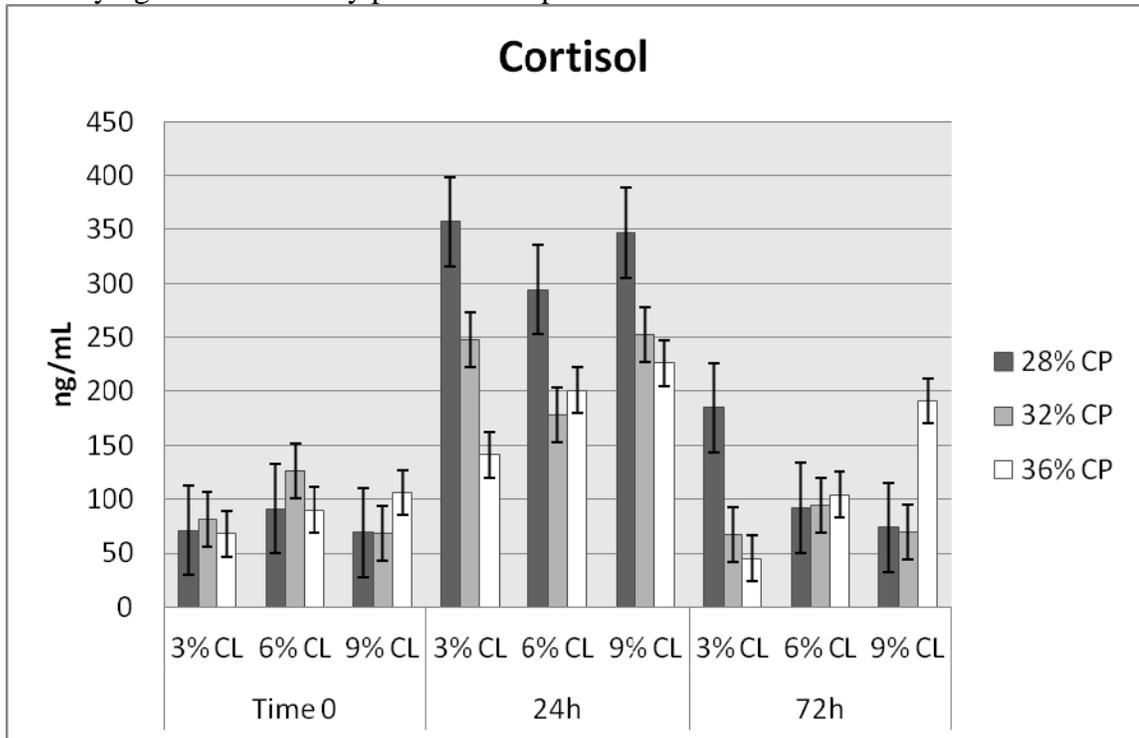


Figure 5. Glucose levels (\pm standard error) of adult tilapia measured at time 0, 24 and 72h fed varying levels of dietary protein and lipid.



Mature tilapia displayed 100 fold increases in cortisol levels at time 24h as compared to time 0h and time 72h (Figure 6). A significant effect of CP was observed at time 24 h where fish fed 28% CP had significantly higher cortisol levels than fish fed 32 and 36% CP, (Figure 6, Table 11). There were no significant interactions between dietary protein and lipid on mature tilapia cortisol levels.

Figure 7. Cortisol levels (\pm standard error) of adult tilapia measured at time 0, 24 and 72h fed varying levels of dietary protein and lipid.



Cost Effectiveness

Cost effectiveness on a \$/lb diet was calculated via a commercial feed company and \$/lb of gain was measured at the conclusion of the study. The diet containing 28% CP and 3% CL identified as the most cost-effective diet in growing mature tilapia (Table 12).

Table 12. Cost evaluation of protein and lipid fraction of diets fed to juvenile tilapia for 18wks

Diet	Gain (0-18wk)	Feed (g)	Cost/lb of feed ¹	Cost/lb of gain ²
28% CP 3%CL	1644	3460	0.35\$	0.73\$
6%CL	1815	3335	0.37\$	0.69\$
9% CL	1528	3265	0.40\$	0.85\$
32% CP 3%CL	1314	3119	0.36\$	0.86\$
6%CL	1924	3694	0.39\$	0.75\$
9%CL	1828	3607	0.41\$	0.82\$
36% CP 3%CL	1993	3484	0.38\$	0.73\$
6%CL	2003	3704	0.40\$	0.74\$
9%CL	1835	3669	0.43\$	0.86\$

¹Cost/ lb of feed provided by a commercial feed manufacturing company. ²Calculated as \$/lb of feed fed/0-18wk gain in lbs.

Comparing Juvenile Tilapia vs. Adult Tilapia

A 3x3x2 factorial analysis was performed to compare the results from the juvenile and mature fish studies to test for significant effects of fish. Significant interaction between size of the fish and the optimum dietary protein level to maximize intake was observed (Table 3:10). Mature fish fed 36 and 32% CP had higher feed intakes than juvenile fish fed 36 and 32% CP however, at the 28%CP level there was no difference (Table 3;10) between mature and juvenile fish feed intake.

Significant effects (Table 3;10) of maturity on gain ($P<0.0001$), FCR ($P<0.0002$) and feed intake ($P<0.0002$) were observed. These results suggest that juvenile fish grew better than adult fish. There were also effects of dietary protein on gain ($P<0.01$) observed; fish fed 36% CP gained more than fish fed 32 and 28%CP when both studies were combined. FCR was also affected by dietary components and the combined data showed a statistically significant effect due to dietary lipid level ($P<0.002$). This lipid

effect manifested in and was likely explain by the fact that fish fed 3% CL had elevated feed intakes when compared to fish fed 6 and 9 % CL.

Significant effects of fish size were also observed in term of post-feeding trial compositional data (Table 4;11). Juvenile fish had higher lipid levels than mature fish ($P < 0.02$) and mature fish stored less whole body energy than juvenile fish ($P < 0.02$). Effects of protein on fat storage were also observed, as previously described for the juvenile fish, in that fish fed 36%CP fed had less whole body lipid stores when compared to 32 and 28%CP fed fish. Whole body protein storage also were affected by protein level ($P < 0.03$) with fish fed 28% CP storing less lipid than fish fed 32 and 36% CP.

A significant interaction was measured ($P < 0.004$) between lipid and size of tilapia, with tilapia being fed 3% CL increasing MR in fish fed 36%CP when compared to fish fed 32 and 28%CP. Significant differences in all indices were observed between mature and juvenile fish studies (Table 3;10). The VSI was affected by size ($P < 0.002$), with adult fish having lower VSI weights when compared to juvenile fish. Protein level was also affected ($P < 0.003$) with fish being fed 36% CP having lower VSI than 32 and 28% CP. HSI was also significantly affected by size ($P < 0.0001$) with juvenile fish having lower HSI than that of mature fish. An effect was also measured for protein level ($P < 0.001$). Fish fed 28% CP had higher HSI when compared to fish fed 32 and 36% CP levels. There was an effect of protein ($P < 0.002$) on MR, with fish fed 36% CP having higher MR than fish fed 32 and 28% CP.

When comparing hauling stress between mature and juvenile fish significant effects on lactate were observed. However, there were no significant effects of lactate

levels at time 0 (Table 6; 12, Figures 1; 4). At 24 h there was a significant effect of size ($P<0.0001$) on lactate levels with mature fish being significantly more stressed than juvenile fish. At time 24 h there was an interaction ($P<0.004$) of size by dietary protein level with mature fish having significantly higher lactate levels when fed 32% CP than those of juvenile fish fed 32% CP. There was also a protein by lipid interaction ($P<0.005$) at time 24 h with fish being fed 32% CP diets having significantly higher lactate levels than fish fed 36 or 28% CP diets at the 3 and 6 %CL lipid levels. The same effect was observed at time 72 h. There was a significant effect of size ($P<0.0001$) and lipid ($P<0.03$) on lactate levels at 72 h, with adult fish having significantly higher lactate levels than juvenile fish. The lipid effect was seen at the 6% CL lipid level. A significant interaction was also observed when looking at both studies combined at time 72 h in regards to a lipid by protein interaction ($P<0.03$). Tilapia fed 6% CL and 32% CP had significantly higher lactate levels than fish being fed 6% CL.

Glucose levels were significantly different at time 0 due to size ($P<0.0001$) of fish, with mature fish having higher glucose levels than juvenile fish (Table 6; 12, Figures 2; 5). A significant size by dietary protein interaction ($P<0.02$) was also observed at time 0. This was observed in fish fed 36% CP having higher glucose levels in the juvenile fish study when compared to the mature fish.

Cortisol was significantly affected by size of fish at time 0 ($P<0.008$) and 24 h ($P<0.0001$) with mature fish having significantly higher cortisol levels as compared to juvenile fish (Table 6; 12, Figures 3; 6). In contrast, there was no effect of size on 72 h cortisol levels. There was also a size independent significant effect of protein on cortisol

levels of fish at 24 h. Fish that were fed 28% CP had significantly higher cortisol levels ($P < 0.004$) than fish fed 32 and 36% CP.

DISCUSSION

Experiment 1: Juvenile Tilapia

Results demonstrate that the greatest weight gain for juvenile tilapia were observed for juvenile tilapia fed 36% CP dietary protein and agree with results of El Sayed and Teshima (1991). El Sayed and Teshima (1991), found that optimal dietary protein levels for Oreochromis niloticus fed practical diets ranged from 40-45% for fish weighing less than a gram and decreased to 30% CP for fish weighing 46-260 g. This review indicated that no studies have previously investigated protein requirements for fish in the 17-45 g range which would encompass the 34 g (initial weight) fish used in the present study. Although the lowest dietary protein level examined in the current study (28%) was below the minimum requirement described by El Sayed and Teshima (1991) but adequate according to NRC (1993) recommendations, the 32% should have exceeded the recommended requirements for this size class; however, both displayed inferior growth compared to fish fed the diet containing 36% protein. These results suggest a growth benefit for feeding juvenile tilapia of this size range dietary protein levels higher than those previously reported. Body condition indices further support this theory as increased FR and decreased VSI were both indicative of improved nutrient utilization and retention were observed in juvenile tilapia fed 36% CP. In contrast, Bahnasawy (2009) observed no difference in growth when tilapia (2.5g initial weight) were fed either 30 or 35% dietary crude protein levels. However, a notable difference between our study and that of Bahnasawy (2009) was that Bahnasawy (2009) was fertilizing tanks to increase

natural feedstuffs within the system. Thus, in the absence of natural feedstuffs, a higher level of dietary protein may be required as was shown by the fact that 32% CP was not sufficient to optimize growth and performance of juvenile tilapia in our clear-water recirculating system.

Data from the juvenile study also demonstrate that a minimum lipid level of >3% is required to maintain normal growth agree with results of Chou and Shiau (1996). In that study, juvenile tilapia were fed dietary lipid levels ranging from 5-12% and optimum growth was measured at 12% lipid. In the current study, lipid levels lower and higher levels of 3 and 9% lipid respectively were investigated because they bracket the currently utilized commercial tilapia diet lipid level of 6% (David Brock, Rangen Aquafeeds, personal communication). Because dietary lipid levels greater than 9% were not investigated it is problematic to speculate whether higher lipid level would have increased growth as was observed by Chou and Shiau (1996). However, in the current study, feed intake was significantly decreased in fish fed 6 and 9% lipid and agrees with results of Jauncey (2000) with 10-40 g tilapia when dietary lipid was greater than 12%. Of note, the fish in our study were approximately 20 times the size of the tilapia used in the study by Chou and Shiau (1996). Previously, Fitzsimons (1997) stated that optimal dietary lipid levels of juvenile tilapia >2g range from 6-8%. Additional research is needed to clarify these inconsistencies in lipid need of juvenile tilapia and determine optimal dietary lipid levels for juvenile tilapia for the size class examined in the current study.

Results from the juvenile study growth data also does not indicate a sparring effect of dietary protein by lipid which was suggested by Jauncey (2000) and (Li et al. 1991). Li et al. (1991) demonstrated that by increasing dietary lipid from 5.7 to 9.4%, and decreasing in dietary protein (dropping the level from 36.9% to 31.9%) did not result in growth depression. Jauncey (2000) found similar results when assessing the effects of dietary lipid on hybrid tilapia, where dietary protein in juvenile tilapia could be decreased from 40% to 30% and growth maintained when dietary lipid was increased from 5 to 12% lipid. The dietary protein and lipid levels examined in the current study may have limited our ability to detect protein sparring effects and requires additional study.

Whole body proximate composition data from the current study does not indicate dietary protein sparring effect by lipid in juvenile tilapia. Whole body composition of juvenile tilapia differed substantially among fish fed the various dietary protein and lipid levels and trends and levels observed are in general agreement with results reported by other researchers (Bahnsanawy 2009, Gao et al. 2011). Bahnsanawy (2009) reported that tilapia fed 35%CP had significantly higher whole body protein than tilapia fed 17, 25, and 30% CP diets. Similarly, Gao et al. (2011) found that increasing dietary lipid from 4 to 10% increased whole body lipid levels of juvenile tilapia. Results from the current and the previous studies in regards to increased tissue nutrient stores with nutrient-dense diets are particularly relevant to tilapia culture in the western US because live-hauling of tilapia can cause stress and in severe cases, mortality (Colt et al. 2011).

Numerous studies have examined the blood chemistry responses of warm-water fish species during hauling (Carmicheal et al. 1984; Dobsikova et al., 2006; Raune et al.,

2001). Carmicheal et al. (1984) found that hybrid striped bass (Morone chrysops X Morone saxatilis) transported for hrs in 1.0% NaCl had lower blood cortisol and glucose levels than fish transported in 0.1% CaCl and freshwater treatments. In contrast, Dobsikova et al., (2006) studied the stress response of common carp during a twelve hour haul and found no significant differences in blood cortisol and glucose prior to, during or at the end of the haul. However, they did detect significant differences in blood lactate with the highest levels being detected at the time 0. Previously, Raune et al. (2001) had demonstrated that physically altering (netting, transportation, increasing densities) of carp in any way led to elevated plasma cortisol, glucose and lactate. Data from these studies indicate that the blood chemistry parameters examined in the current study were probably adequate in detecting stress in tilapia when handling protocols were standardized. However a limitation of the previous studies that examined blood chemistry responses during a hauling trial was that none of them controlled for the potential effects of previous dietary history.

High-energy, nutrient-dense diets have also been hypothesized to reduce stress tolerance in some fish species (Cheng et al. 2006). Cheng found that intakes of diets with varying protein and lipid levels resulted in varying levels of blood glucose in orange spotted Grouper (Epinephelus coioides). During our simulated live-haul, glucose and lactate levels increased during the live haul and returned to normal levels at 48h post-haul indicating that the study was successful in inducing an acute stress event in tilapia when conditions that simulate live hauling in the Western US were followed (Colt et al. 2011). However, even though substantial differences in fish size and whole body composition

were observed in the current study, no effects of diet on survival (data not shown).

Although we did see dietary effects on blood cortisol, lactate and glucose levels there was no clear dietary combination of protein and lipid observed that reduced stress. Prior to the simulated live haul in the current study, tilapias were fasted for 24 h. Wright (1992) found fasting tilapia for 48 h lowered plasma glucose levels in tilapia 75.4 ± 3.0 mg/dl ($n = 140$). These values compare favorably with mean non-fasting and fasting plasma glucose values in man (i.e., 90 mg/dl and 63 mg/dl, respectively) (Aoki, 1985). Similarly in the current study the use of the 3 ppt salt treatment may have altered our ability to detect differences between blood chemistry parameters. However, both fasting for 24-48h and salt supplementation to the transport container (3 ppt) are common tilapia hauling practices (Colt et al. 2011).

The lack of effects of dietary protein or lipid on blood chemistry responses may also simply reflect a limited ability to detect differences due to the limited number of fish examined at each time point and the high variability in responses between fish within a treatment. Of note, significant interactive effects were observed for lactate at 36%CP and; however it is unclear whether these statistically significant differences have physiologically relevant implications. Alternatively and although not heavily studied, it has been previously reported by Falcon et al. (2007) that dietary lipid level does not alter stress parameters, specifically blood cortisol and glucose in mono-sex populations of juvenile tilapia. Thus our data may also simply indicate a lack of dietary effect on hauling stress of tilapia.

Experiment 2: Mature Tilapia

In contrast to the substantial dietary effects observed in the juvenile study, results from the mature tilapia trial suggest that there is no significant effect of feeding mature tilapia dietary levels of CP varying from 28-36%. These results are in agreement with Cisse (1988) who found adequate growth in mature tilapia fed 20% CP when fish meal and cottonseed meal were the primary protein sources. The results from the mature fish trial also are supported by the work of El-Sayed (2004) who found that 20-30% CP was the optimal dietary level for mature tilapia. In contrast, El-sayed and Kuwana (2008) found that increasing dietary protein from 30-40% CP produced significantly better growth for both female and male tilapia reared in mono-sex culture tanks. In the same study, El-Sayed and Kuwana (2008) also found that broodstock tilapia grew better with increasing dietary lipid level which contrasts with the results from the current study. In their study, fish were fed varying levels of dietary lipid 14.6, 16.7, 18.8 % and there was a significant effect of lipid percentage on growth in that tilapia fed 18.8% lipid grew better than fish fed 14.6% lipid.

A primary difference between our studies and those of El- Sayed and Kuwana (2008) is that in the current mature fish study mixed-sex populations within each tank were used. Even though in the current study, stocking densities were maintained at levels previously reported to reduce breeding incidences and no spawning vessels were provided, viable eggs were observed in some tanks as early as six weeks post-feeding and periodically throughout the rest of the feeding trial. El Sayed (2004) stated that 35-45% CP was necessary to produce maximum fecundity. However they stated that for mature

tilapia to maintain growth only 20-30% CP was needed. This is supported by the current study where significant difference in growth was observed due to dietary protein levels ranging from 28-36% CP.

Faster growth has been observed for numerous tilapia species cultured in a mono-sex populations and it has been hypothesized that this is due, in part, to reallocation of energy into the production of gametes, as well as, the courting process (Dan and Little 2000; Tran-Duy et al. 2008). In a more recent study, Chakrobaty et al. (2007) found that tilapia reared for six months in mixed-sex populations performed worse than mono-sex populations fed the same diets and cultured in the same manner. Chakrobaty (2007) hypothesized that this was due to the fact that in mono-sex culture fish rely more on nutrition for growth and less on nutrition to produce gametes. In the current mature tilapia study, mixed-sex tilapia were cultured for 18 wks and fed diets ranging from 36-down to 28% CP with no significant effects of dietary protein level on growth observed which agrees with the optimal level of 20-30% CP that El-Sayed stated was necessary to maintain growth and performance in mature tilapia. However, the unanticipated gamete production and an inability to account or control for variation in this parameter and the subsequent reallocation of dietary nutrients for that purpose also could explain why we were unable to detect dietary effects on growth in the mature fish study. Lending support to this theory in the mature fish study, a decrease in PRE was observed as compared to the juvenile tilapia trial. This is in agreement with what was found by Chakrobaty et al (2007) when comparing PRE in monosex vs mixed sex tilapia in mature tilapia.

Another relevant observation of the mature tilapia trial was that the mature tilapia cortisol levels were higher (85 ± 20.4 ng/ml) at initial sampling when compared to previous research. Moreira and Volpato (2004) found tilapia cortisol levels prior to environmental stressors were between 27-55ng/ml while Auperin et al. (1997) found similar but even lower cortisol levels ranging from 5-50 ng/ml. This suggests that tilapia in the mature study may have been more stressed due to culture conditions (stocking rate or courting stress) thus obscuring our ability to detect dietary effects on hauling stress tolerance. Lending support for this theory is that when comparing our juvenile tilapia plasma cortisol levels to mature tilapia plasma cortisol levels in the current studies, higher levels were observed at time 0 and thus an elevated may have limited our ability to detect differences in the mature tilapia study. However, in the mature fish study we saw a significant difference in cortisol levels at the 28% CP level at time 24 h when water quality and temperature were at their highest reported levels though out the hauling period. This data is in agreement with Kumar et al. (2011) where they fed Labeo rohito fingerlings varying levels of dietary protein. Kumar et al. (2011) found that fish fed 20% CP diets had higher levels of serum cortisol when compared to 30, 40 and 45% CP after a temperature challenge. This suggests that environmental stressors such as high temperatures and poor water quality can cause disruptions in protein metabolism thus creating an elevated cortisol response in lower protein feeds. Additionally, in the mature fish study, a lipid effect on blood lactate was observed that was not observed in the juvenile fish study. This effect may be explained by the increased energy demand of the sexually mature tilapia wherein dietary lipids were being used more for gamete

production than storage creating a deficit that prevented them from mounting an appropriate response post-hauling.

CONCLUSIONS

The plasticity of tilapia culture and markets coupled with large variations in dietary protein and lipid levels (and subsequently costs) observed in commercially available feeds for tilapia complicates the ability of producers to identify appropriate diets for various size classes of tilapia grown in high-intensity systems. Therefore the purpose of the current research was to determine optimum dietary protein and lipid requirements of juvenile and mature tilapia grown in high density clear water conditions and examine the potential of these diets to affect tilapia during transportation.

In the juvenile tilapia study, substantial dietary effects on growth of juvenile tilapia were observed and hauling stress was only minimally dietary responsive. These results suggest that feeding juvenile tilapia higher protein and lipid levels will produce better growth and performance. However, when comparing the cost effectiveness of the observed by growth rate in juvenile tilapia, the 36% CP and 6% CL diet is recommended.

In the mature fish study, no significant effects of diet on growth were observed. For the mature fish study we found the most cost effective diet to be at 28% CP and 6% CL. For this reason we suggest that there is no need to increase protein above 28% CP and 6% CL in mature tilapia mixed-sex culture.

From these studies we suggest that juvenile and mature tilapia need to be managed in different ways when reared in mixed-sex culture in a high intensity system. Juvenile fish studies that could be undertaken include pushing the upper limits of dietary lipid inclusion and to further test the potential of protein sparing as well as producing optimum growth and stress response for juvenile tilapia. Protein should also be looked at

in order to effectively evaluate the ability of tilapia to utilize other energy sources to produce maximum growth and development. In order for protein to be utilized effectively and efficiently the fish should exhibit growth for the least amount of money.

Further studies that could lend evidence on producing optimal growth for mature tilapia could include mono-sexing the tilapia and then conducting a grow-out style study with the dietary ingredients used in the current study. By doing this, it would be possible to test the potential benefits using sex change hormones on growth and performance of fish fed diets at various protein and lipid levels. Another interesting study would be looking at the ability of juvenile tilapia to utilize higher levels of protein for maximum growth. At 36% CP, fish showed maximum growth. However, by further increasing the amount of dietary protein it might be possible to further improve growth in juvenile fish. This study could potentially lead into a complete life stage dietary analysis where fish are fed the same diet throughout their entire culture cycle. This would allow you to see more precisely when the lower protein diets can catch up to the higher protein diets so that you would have better recommendations for farmers regarding when to switch to lower protein diets.

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