EFFECT OF DIETARY PROTEIN LEVELS ON DISSOLVED OXYGEN DYNAMICS AND GROWTH PERFORMANCE OF NILE TILAPIA

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ABSTRACT

Nile tilapia juveniles with initial weights range of 129.6-140.3 grams /fish were distributed into 18 concrete tanks with a constant water depth of 75 cm. Two feeding rates (inputs) were employed at 8 and 9 grams diet/m^2/day, six days a week. The commercial feed contained three levels of dietary protein (25, 30 and 35%). The rearing experiment lasted 86 days and took place during April –June 2014. Increasing dietary protein content did not have any effect on oxygen concentrations at sunset among treatments. Dissolved oxygen (DO) concentrations at sunset decreased sharply from 8.9 - 10.8 g O_2/m^2 during the second month to 3.4 - 6.9 g O_2/m^2 during the third month, with significant differences between the two periods (p<0.05). There was a sharp decline in secchi disk readings caused by the excessive increase in algal abundance during the third month. Dissolved oxygen concentrations at sunrise and sunset were drastically reduced during the third month due to the excessive algal bloom observed in the latter period. Better environment during the second month improved treatment performance in terms of nighttime oxygen availability. However, adverse environments were observed during the third month with fish suffering oxygen deficiency. The 25% crude protein diet in both the 8 gram and 9 gram feeding rates had better oxygen budget and better environmental conditions for fish production. The better environment of the 25% crude protein treatments were caused by the lower algal blooms (secchi disc readings 10.0 - 13.3 cm) observed in those treatments during the third month compared to most of the other treatments. The 25% crude protein treatments also produced better feed conversion ratios (2.42-3.44:1) and protein efficiency ratios (1.16 - 1.66) compared to those of the 30 and 35% crude protein treatments.

Key words: Nile tilapia, protein levels, oxygen dynamics, growth performance.

1.INTRODUCTION

Within semi-intensive pond-based farming systems, the dietary nutrient requirements of the cultured species is largely met through the consumption of natural food organisms produced within the pond and/or through the direct consumption of supplied "supplementary" feed inputs (Tacon and De Silva, 1997). Protein is a major component in fish feeds, because it provides the essential and nonessential amino acids to synthesize body protein and in part provides energy for maintenance (Gan *et al*., 2012). By giving a high level of protein in artificial diets, production costs are considerably increased: for this reason, it is important to use diets rationally to attain optimal yield (Gan *et al*., 2012). Knowledge of the optimum levels of protein and other nutrients, such as lipids and carbohydr in artificial diets can reduce feeding costs (Taboada *et al*., 1998). The reason for quantifying protein requirements is to develop the lowest-cost feed that provides adequate or maximum growth (Brunty *et al.,* 1997).

On the other hand, total pond respiration consists of three components: fish, plankton, and sediment. Evaluation of the relative contribution of each component allows assessment of options for manipulation of fish crops, water levels, and pond design to improve the availability of dissolved oxygen (DO) and minimize costs associated with aeration (Steeby *et al*., 2004).

There is also an urgent need to comprehend the dynamics of the pond ecosystem and "integrated" farming system as a whole so as to further improve the nutritional efficiency and benefit of the farming system with the minimum of external feed inputs (Tacon and De Silva, 1997).

The presebt experiment was conducted to evaluate growth and feed performance as well as water quality dynamics in rearing tanks under different dietary protein levels. The effect of feed inputs and crude protein levels were also investigated in terms of their effect on oxygen budgets among treatments.

2. MATERIALS AND METHODS
2.1. Experimental design

The current study was conducted at the Fish Research Unit, Faculty of Agriculture, Cairo University, Egypt, during spring 2014. The experiment was performed in outdoor concrete tanks. Each tank had a surface water area of 2.5 m^2 and water volume of 2 m^3.

Nile tilapia juveniles with a range initial weights of 129.6-140.3 gr./fish were distributed into 18 concrete tanks which had a constant water depth of 75 cm. A total of 180 fish were randomly distributed into tanks at the rate of 10 juveniles per tank. Treatments were equally replicated and arranged in completely randomized design. Two feeding rates (inputs) were employed in the experiment at 8 and 9 g diet/m^2/day, six days a week. This was equivalent to 20 and 23 gr. diet per tank per day, respectively, six days a week, with constant feed input per tank per day during the whole experiment. The commercial diet contained three levels of dietary protein (25, 30 and 35%). Therefore, the experiment consisted of 6 treatments, with three replicate tanks per treatment. The rearing experiment lasted 86 days and took place during April –June 2014.

Fish in each tank were individually weighed and counted at the start of the experiment. At the end of each experiment, concrete tanks were completely drained and the fingerlings were harvested, counted and individually weighed to the nearest 0.1 g The proximate composition of the commercial diets is illustrated in Table (1).

Water temperature and DO were measured four times daily (early morning at 07:00 a.m., dusk at 06:00 p.m., nighttime at 00:00 h and next early morning at 07:00 a.m.). The duration of nighttime hours (from dusk to dawn) was approximately (11:00 to 12:00 hours). Oxygen dynamics data and water quality parameters were only measured during the second month and third month of the experiment (from 17th May to July12[th]) since most water quality problems were expected to occur during that period. Consequently, oxygen dynamics and

Table (1): Chemical composition of the experimental diets (%).

Proximate Composition (%)	25% C.P.	30% C.P.	35% C.P.
Moisture	9.61	9.64	9.65
Crude protein	25.8	29.2	32.5
Crude lipid	2.46	2.7	2.00
Ash	9.37	8.30	8.58
Crude fiber	2.89	2.60	2.25
NFE	49.87	47.56	45.02
Total	100	100	100

water quality parameters were compared during the second and third months. Oxygen dynamic parameters were calculated as indicated below.

2.2. Oxygen dynamics
1. Nighttime community respiration per hour ($nCRh^{-1}$) = (dusk oxygen concentration – nighttime oxygen concentration) /nighttime period (hours).
2. Nighttime community respiration (nCR) = hourly nighttime community respiration * dark period (hours).
3. Daytime net primary production (dNPP) = dusk oxygen concentration – dawn oxygen concentration.
4. Dawn oxygen surplus or deficit = dNPP – nCR.

Optimal nighttime community respiration (nCR) = hourly early night community respiration * nighttime period (hours).

2.3. Water quality parameters
All determinations of water quality parameters were carried out according to Boyd and Tucker (1992). Water temperature and dissolved oxygen were measured using dissolved oxygen meter (Hanna model 55 USA). Secchi disc visibility was measured using a wooden apparatus. Total Ammonia concentration was measured by phenate method (Boyd and Tucker (1992). Filterable orthophosphate was measured by ascorbic acid method (Boyd and Tucker (1992).

2.4. Growth and Feed Performance
Growth performance of cultured fish was measured in terms of final individual fish weight (g), weight gain (g/fish), daily weight gain (g/fish/day), daily weight gain /m^2, specific growth rate (SGR-%/day). Feed performance

was measured in terms of feed conversion ratio (FCR) and protein efficiency ratio (PER). The growth and feed performance parameters were calculated as follows:

2.4.1. Body weight

Individual weights of fish were measured at the start and end of the experimental period using digital balance for weight to the nearest 0.1 g.

2.4.2. Weight gain (WG)

Weight gain was calculated as:

WG =WF-Wi

Where:

WG=weight gain (gram/fish)

WF=final weight per fish at the end of the experiment

Wi = initial weight per fish at the start of the experiment

2.4.3. Daily weight gain (DWG)

DWG = (final body weight -initial body weight) /experimental period (days).

2.4.4. Specific growth rate (SGR)

Specific growth rate based on weight (SGRW) was determined:

SGR (W) = (ln Wt – ln W0)*100 / t.

Where Wt: is weight at time t, W0 weight at time 0, and t is the duration of time in days.

2.4.5. Feed conversion ratio (FCR)

Feed conversion was determined as the grams dry diet consumed per gram wet weight gain of fish.

FCR= dry weight of feed fed (g) / fish weight gain (g).

2.4.6. Protein efficiency ratio (PER)

Protein efficiency ratio was calculated as the grams of protein consumed per gram wet weight gain of fish.

PER =fish weight gain (g) / protein fed (g).

2.5. Statistical analysis

Statistical analysis was performed using two way analysis of variance (ANOVA) for treatment effect. Duncan's multiple range test was used to separate treatment means at significance level of 0.05. Statistical tests on parameter values were conducted using SPSS computer software (SPSS Inc., 1997).

3. RESULTS AND DISCUSSION

3.1. Oxygen concentrations at sunset

Oxygen dynamics and water quality parameters are shown in Table (2). During the second month of the experiment, oxygen concentration at sunset ranged 9.2-10.8 g O_2/m^2 among the 8 gram treatments and 8.9 to 10.4 g O_2/m^2 among the 9 gram treatments, with no significant differences between means ($p>0.05$).

Increasing dietary protein content did not have any effect on oxygen concentrations at sunset among treatments.

During the third month of the experiment, oxygen concentrations at sunset ranged 3.4 to 4.6 g O_2/m^2 among the 8 g treatments, with no significant differences between treatments ($p>0.05$). Similarly, DO concentrations at sunset ranged 4.4 to 6.9 g O_2/m^2 between the 9 g treatments. Dietary protein content did not affect DO concentrations at sunset among treatments during the third month of the experiment ($p>0.05$).

Dissolved oxygen concentrations at sunset decreased sharply from 8.9 to 10.8 g O_2/m^2 during the second month to 3.4 to 6.9 g O_2/m^2 during the third month, with significant differences between the two periods ($p<0.05$). This could be due to the gradual accumulation of algal detritus and feces overtime and the subsequent bacterial respiration needed to degrade the organic matter.

Oxygen deficiency can be induced by decaying algal blooms, long term nutrients and associated organic matter enrichment (Best *et al.*, 2007). Assuming that the decomposition of sediment organic matter is desirable, mixing ponds during daylight hours when DO concentrations $>8mgl-1$ will allow maximum mineralization of organic matter in the ferrous and sulfur sediment layer (Steeby *et al.*, 2004).

The only factor that created differences during the experiment is the time of measurements, where the second month had optimal environmental conditions in terms of oxygen concentrations needed to support optimal growth rates in contrast to those of the third month. The increase in dietary protein contents from 25% to 35%, did not affect dusk oxygen concentrations between treatments during the third month ($p>0.05$).

The overall oxygen concentrations at sunset gradually decreased overtime from 8.9 – 10.8 g O_2/m^2 during the second month to 3.4 – 6.9 g O_2/m^2 during the third month, with significant differences among the two periods. This may be due to the shallower secchi disc readings (*i.e.* excessive algal blooms) observed during the third month of the experiment. Heaps of algal detritus and feces were gradually accumulated during the third month of the experiment, which consumed a large amount of oxygen needed for bacterial activities during daytime period. This helped decrease oxygen accumulation in water

Table (2) : Oxygen dynamics in concrete tanks under different feed and protein levels   (g feed/m²/day).

Parameter	Treatment	Feed load					
		8 g/m²			9 g/m²		
		25%cp	30%cp	35%cp	25%cp	30%cp	35%cp
Oxygen concentrations at sunset (g O₂/m²)	Second month	9.56[a]	9.22[a]	10.84[a]	10.40[a]	8.97[a]	9.88[a]
	Third month	3.58[b]	4.68[ab]	3.44[b]	5.36[ab]	6.94[a]	4.40[ab]
Oxygen concentrations at sunrise (g O₂/m²)	Second month	1.82[a]	1.61[a]	2.68[a]	2.19[a]	1.61[a]	1.89[a]
	Third month	0.30[a]	0.29[a]	0.27[a]	0.31[a]	0.26[a]	0.26[a]
Oxygen concentration at midnight (g O₂/m²)	Second month	4.85[a]	4.34[a]	5.71[a]	5.34[a]	4.20[a]	4.63[a]
	Third month	0.93[a]	0.82[a]	0.41[a]	0.77[a]	1.08[a]	0.44[a]
Optimal oxygen respiration per hour (g O₂/m²/hour)	Second month	0.93[a]	0.97[a]	1.02[a]	1.00[a]	0.95[a]	1.04[a]
	Third month	1.05[ab]	0.76[b]	0.72[b]	0.91[ab]	1.16[a]	0.78[b]
Oxygen gain (g O₂/m²/daytime)	Second month	8.36[a]	7.90[a]	9.17[a]	8.81[a]	7.68[a]	8.58[a]
	Third month	6.46[ab]	4.45[b]	3.86[c]	5.13[bc]	6.54[ab]	4.17[c]
Optimal oxygen loss (g O₂/m²/nighttime)	Second month	9.40[a]	9.76[a]	10.26[a]	10.12[a]	9.54[a]	10.51[a]
	Third month	10.53[ab]	7.73[b]	7.31[b]	9.18[ab]	11.72[a]	7.90[b]
Oxygen budget (g O₂/m²)	Second month	-1.04[a]	-1.85[a]	-1.09[a]	-1.30[a]	-1.86[a]	-1.93[a]
	Third month	-4.07[a]	-3.28[a]	-3.44[a]	-4.05[a]	-5.18[a]	-3.73[a]
Temperature at 7 a.m. (°C)	Second month	27.72[a]	27.50[a]	27.83[a]	27.73[a]	27.22[a]	27.25[a]
	Third month	29.55[a]	29.92[a]	29.93[a]	30.25[a]	29.94[a]	29.93[a]
Temperature at 6 p.m. (°C)	Second month	23.94[a]	29.89[a]	30.41[a]	30.58[a]	29.84[a]	30.02[a]
	Third month	31.25[a]	32.20[a]	32.09[a]	32.44[a]	32.24[a]	32.14[a]
Heat gain (°C)	Second month	2.27[c]	2.38[c]	2.57[b]	2.84[a]	2.61[b]	2.77[ab]
	Third month	1.70[b]	2.27[a]	2.15[a]	2.18[a]	2.37[a]	2.36[a]
Secchi disc (cm)	Second month	13.5[a]	16.2[a]	13.1[a]	16.7[a]	17.7[a]	15.7[a]
	Third month	13.3[a]	6.0[c]	5.9[c]	10.0[abc]	11.8[ab]	7.6[bc]
Ammonia NH₃-N (mg/l)	Overall	ND	0.40[a]	0.50[a]	0.42[a]	0.43[a]	0.31[a]
Phosphate PO₄-P (mg/l)	Overall	ND	0.92[ab]	0.90[ab]	0.74[ab]	0.58[b]	0.78[ab]
Means in the same row or column with different letters are significantly different (p<0.05).							

during daytime period, resulting in reduced oxygen concentration at sunset during the third month. Phytoplankton blooms involving excessive algal growth are common occurrences in aquaculture ponds (Casé *et al*., 2008).

3.2. Oxygen concentrations at sunrise

During the second month of the experiment, oxygen concentrations at sunrise ranged 1.6 to 2.6 g O₂/m² in the 8 g treatments. Similarly, oxygen concentrations ranged 1.6 to 2.1 g O₂/m² at sunrise in the 9 g treatments. Dietary protein content did not adversely affect DO concentrations at sunrise in all treatments during the second month (p>0.05).

During the third month of the experiment, sharp decline in DO concentrations at sunrise took place in all the treatments, with significant differences over time between the second and third months (p<0.05). Oxygen concentrations at sunrise ranged 0.2 to 0.3 g O₂/m² in the 8 g

treatments, and 0.2 to 0.3 g O₂/m² in the 9 g treatments, with no significant differences between means (p>0.05).

In shallow water, depending on the balance between production and respiration, a natural diel cycling of DO from super saturation during the day to hypoxic or near anoxic during the night can occur (Diaz and Breitburg , 2009).

Higher nutrient loading will lead to more eutrophic conditions with associated water quality deterioration. For example, excessive algal growth can lead to elevated pH levels (*via* photosynthesis), fluctuations of dissolved oxygen concentrations, production of cyanobacterial toxins in nutrient-rich waters causing stress to the fish (Jacob and Culver, 2010).

The sharp decrease in sunrise oxygen concentrations from the second month to the third month over time may be due to two

reasons, namely the excessive algal blooms during the third month accompanied by a decrease in oxygen concentration at sunset. Moreover, oxygen cycling due to the increase of bacterial respiration needed to degrade algal detritus (sediments) accumulated above the tank bottom. It is well known that excessive algal bloom is always accompanied by a decrease in oxygen production (rate of photosynthesis) and oxygen problems in aquaculture ponds. Moreover, the algal bloom is always accompanied by an increase in algal sedimentation (algal detritus) which induce oxygen cycling.

Consequently, DO concentration at sunrise and sunset were drastically reduced during the third month due to the excessive algal bloom observed in the latter period. Increasing dietary protein content within the 8 g and the 9 g treatments during the second month did not affect oxygen concentrations among treatments either at sunset or at sunrise. Moreover, increasing diet inputs from 8 g to 9 g had no effect on oxygen concentrations among the treatments.

3.3. Oxygen concentration at midnight

Oxygen concentration at midnight ranged from 4.2 to 5.7 g O_2/m^2 during the second month of the experiment, with no significant differences among treatments (p>0.05). Oxygen concentrations at midnight were optimal for fish growth during the second month since optimal oxygen respiration per hour during nighttime within that period ranged from 0.9 to 1.0 g O_2/m^2/hour, indicating surplus of oxygen concentration at daybreak with no oxygen stress on fish. Comparing midnight and sunrise oxygen concentrations among treatments, it was obvious that dawn oxygen had surplus concentration during the second month of the experiment. Water was well aerated in the pre-dawn period with no respiratory stress on fish.

Manufactured feeds were the main source of nitrogen and phosphorus nutrients in the ponds and these nutrients should be available in adequate concentration to provide optimum phytoplankton growth (Cremen *et al.*, 2007).

Feed load during the second month just equaled the assimilative capacity of water in terms of oxygen availability needed for the metabolic oxidation of all feed load. However, feed load plus algal detritus and feces gradually accumulated during the third month. Consequently, the sum of feed load and algal detritus were more than the assimilative capacity

of water in terms of oxygen availability. Therefore, nighttime oxygen concentrations sharply deteriorated during the third month, with oxygen concentrations at midnight ranging from 0.4 to 1.0 g O_2/m^2, which were not sufficient to sustain nighttime respiration in the pre-dawn hours.

Oxygen concentration at midnight sharply deteriorated to sublethal limits during the third month since optimal oxygen respiration rates per hour during nighttime ranged 0.7 to 1.1 g O_2/m^2/hour, indicating severe shortage in oxygen concentration during most of the nighttime period. Fish were observed to surface water and respire through surface respiration at the air-water interface all nighttime period from midnight to dawn, which induced respiratory stress in all treatments during the third month of the experiment.

The form of periodic hypoxia with the most frequent occurrence is diel cycling hypoxia, which appears to be common in shallow systems, and is driven by the balance between oxygen production during daylight and respiration at night (Diaz and Breitburg, 2009).

The duration of depleted oxygen during the third month ranged from 4 to 5 hours in the pre-dawn hours, with fish respiring oxygen at the air-water surface interface which induced respiratory stress on fish and visible signs of oxygen stress were observed in all treatments.

Torrans (2004) reported that although visible signs of oxygen stress were never observed in the low oxygen treatment, feed consumption was reduced by 45% and the average fish weight in the low oxygen treatment was 30% less than the control, moreover net production was also cut in half. Depending on how low the dissolved oxygen concentration is and how long it remains low, fish may consume less feed, grow more slowly, convert feed less efficiently, be more susceptible to infectious diseases (Tucker, 2005).

Better environment during the second month improved treatment performance in terms of nighttime oxygen availability, however, adverse environments were observed during the third month with fish suffering oxygen deficiency after midnight in most of the pre-dawn hours. This was due to the excessive algal bloom and oxygen cycling in all the treatments during the third month with dissolved oxygen deterioration. The presence of excess organic load in terms of algal detritus and accumulated feces during the third month was above the waste assimilative

capacity of water and sediment, which caused lower daytime net primary production.

3.4. Oxygen gain, loss and budget

Increasing dietary protein level within the 8 gram treatments or the 9 g. treatments during the second month did not affect daytime oxygen gains which ranged 7.9-9.17 g O_2/m^2 and 7.68-8.81 g O_2/m^2, respectively, with insignificant differences among treatments.

Similarly, optimal nighttime oxygen loss did not differ significantly among the treatments with increasing dietary protein level from 25% to 35% within the 8 g treatments (9.4-10.2 g O_2/m^2) or within the 9 g treatments (9.5-10.5 g O_2/m^2) during the second month of the experiment. Oxygen budgets during 24 hours had slightly negative values ranging from -1.04 to -1.85 within the 8 g treatments and from -1.3 to -1.93 within the 9 g treatments, with no significant differences among the treatments.

Consequently, Nile tilapia did not suffer respiratory oxygen stress during the second month of the experiment. Adequate oxygen concentrations in water were available in most of the nighttime period during the second month as indicated by acceptable sunrise concentrations.

Daytime oxygen gains during the third month (3.86 to 6.54g O_2/m^2) were greatly reduced compared with those of the second month (7.68 to 9.17 g O_2/m^2), with significant differences over time ($p<0.05$). This was caused by a combined effect of excess accumulation of algal detritus and feces over time during the third month. The increase in feed metabolism and algal detritus degradation overtime was above the normal capacity of water to produce oxygen.

Blooms of phytoplankton in surface waters can potentially deplete the water column of oxygen during the night (Jones *et al.,* 1982). Oxygen depletion is more commonly observed as a consequence of the sinking and decay of blooms (Best *et al*., 2007). Microbial respiration can deplete the water column and sediments of dissolved oxygen in the presence of organic matter (Best *et al*., 2007).

Respiration resulting from waste feed and algal detritus decomposition exceeded daytime net primary production, resulting in a severe negative oxygen budget during the third month of the experiment, with oxygen shortage lasting few hours after midnight.

Daytime oxygen gains and nighttime optimal oxygen losses ranged 4.17 – 6.54 g O_2/m^2 and 7.9 – 11.72 g O_2/m^2 among all treatments,

respectively, with significant differences among the treatments ($p<0.05$). Severe negative oxygen budgets were observed during the third month among treatments with large negative values ranging from -3.28 to -5.18 g O_2/m^2, signifying severe oxygen stress on fish during most of the nighttime period after midnight.

Increasing dietary protein content within the 8 g and 9 g treatments did not have significant effects on oxygen budgets among the treatments which ranged from -3.28 to -4.07 and -3.73 to – 5.18 g O_2/m^2, respectively. Both the 8 gram and 9 gram treatments were above the assimilative capacity of water. Consequently, increasing dietary protein content within the tested diets had no effect on oxygen budgets within each feed load.

Optimal nighttime community respiration per hour ranged from 0.93 to 1.04 g O_2/m^2/hour during the second month and 0.72 to 1.16 g O_2/m^2/hour during the third month of the experiment, with no significant difference between the two periods overtime ($p>0.05$). Consequently, oxygen problems during the third month were due to the reduced rates of oxygen production through photosynthesis as well as the high daytime oxygen respiration rates under excessive algal blooms and accumulation of algal detritus. This was evident through the reduced oxygen concentrations at sunset in the 8 g (3.44 – 4.68 g O_2/m^2) and the 9 grams (4.4 – 6.94 g O_2/m^2) treatments during the third month of the experiment compared to those of the second month of the experiment which ranged 9.22 to 10.84 g O_2/m^2 and 8.97 to 10.4 g O_2/m^2, respectively.

3.5. Secchi disc readings

Moderate algal blooms were observed during the second month of the experiment where secchi disc readings ranged 13.1-16.2 cm in the 8 g treatments and 15.7-17.7 cm in the 9 g treatments, with no significant differences among means ($p>0.05$). Increasing protein level within the 8 g treatments or the 9 g treatments did not have significant effects on secchi disc visibility due to moderate algal blooms observed among treatments.

Fish can retain 20–50% of feed N and 15–56% of feed P (Schneider *et al*., 2004). The remaining N and P are released into the water and can be converted to valuable products by phototrophic and heterotrophic organisms (Schneider, 2006). The nutrients not retained in fish act as a fertilizer, stimulating the production of natural food organisms, including algae,

periphyton, zooplankton and benthos, which may be consumed by the fish (Verdegem, 2013).

However, excessive algal blooms took place in most treatments during the third month where secchi disc averaged 5.9-13.3 cm in the 8 g treatments and 7.6-11.8 cm in the 9 g treatments, respectively. There was a sharp decline in secchi disc readings caused by the excessive increase in algal abundance during the third month. Excessive algal blooms may be responsible for oxygen cycling and the deterioration in oxygen concentrations in cultured ponds which may lead to reduced oxygen concentrations at both sunrise and sunset.

Phytoplankton blooms involving excessive algal growth are common occurrences in aquaculture ponds (Case *et al*., 2008). Monthly decline in Secchi disc transparency with a corresponding increase in phytoplankton and chlorophyll-a also suggest that the ponds might have experienced an increase in the amount of mineral turbidity in the water column (Islam *et al.,* 2004).

Increasing protein level within the 8 gram treatments during the third month negatively affected secchi disc readings where readings decreased from 13.3 to 5.9 cm with the increase of protein level, with significant differences among treatments ($p<0.05$). This was due to the positive effect of protein content on the excretion rate of metabolic by-products (ammonia and phosphate) leading to algal blooms overtime during the course of the experiment. Similarly, increasing protein level in the 9 gr. treatments negatively affected secchi disc readings where readings decreased from 10.0 to 7.6 cm with increasing protein level, with significant differences among means ($p<0.05$). Major protein metabolic products are in the forms of ammonia and phosphate which are considered as vital nourishment for algal bloom in aquatic system. Consequently, increasing the protein level would nourish algae with ammonia and phosphate, resulting in an excessive algal bloom overtime during the third month of the experiment.

With increasing feed input, the concentration of suspended solids (including phytoplankton) and inorganic dissolved nutrients (ammonium, nitrite and nitrate) in the water column increased (Jiménez-Montealege *et al*., 2002). According to Wang *et al*. (1998), the N and P excreted by tilapia function to yield slow, even fertilization,

the effect of which is to maintain a constant, but effective biomass of phytoplankton.

3.6. Algal sedimentation and sediment respiration

Excessive algal bloom always results in higher dead algal sedimentation to the bottom sediment and negatively affects oxygen budget through oxygen cycling. Excessive algal bloom and higher dead algae load at the bottom sediment affect the waste assimilative capacity of static water since most of the oxygen content in static water is consumed by phytoplankton respiration (Hargreaves and Tucker, 2003). In the longer term, the release of nutrients by fish excretion and organic matter mineralization stimulates the development of dense phytoplankton population that constitute the major component of pond respiration (Hargreaves and Steeby, 1999).

Oxygen budget in catfish ponds described in the literature as whole pond respiration (WPR) included phytoplankton respiration (57.5%), fish respiration (22.5%) and sediment respiration (19.4%) (Santa and Vinetea, 2007). Algal respiration consumes 49% of the total oxygen budget available during 24 hours as indicated by Guo-Cai *et al*. (2000). Consequently, excessive algal blooms and the consequent higher dead algal sedimentation rates cause negative oxygen budget and oxygen problems after midnight hour. In the current study, the total ammonia-nitrogen and orthophosphate concentrations ranged 0.31-0.5 and 0.58-0.92 mg/l, respectively among the treatments, which indicated eutrophic conditions in the experimental tanks.

Nutrient enrichment (metabolic products of protein metabolism) in the high protein level treatments increased algal blooms and sedimentation rates of dying algae, consequently, decomposition rate (oxygen demand) by bacterial activities increased, resulting in oxygen recycling. The combined effect of algal respiration and bacterial activities on sedimented algae resulted in a negative oxygen budget after midnight in the third month of the experiment. Pond sediment oxygen demand (SOD) is a major contributor to total pond respiration, accounting for as much as 38-80% of the total respiration in shallow aquaculture ponds (Steeby *et al*., 2004).

The oxygen budgets observed during the third month of the experiment indicated unfavorable oxygen concentrations in water after midnight till dawn time. The decomposition

of sediment (dead) algae by bacterial activities resulted in oxygen cycling and unfavorable oxygen budget.

Hargreaves and Tucker (2003) indicated that excessive algal bloom (due to nutrient enrichment with high nitrogen inputs) and its sedimentation rate resulted in a higher dead algae load at the bottom sediment which affect the waste assimilative capacity of static water. The same authors indicated that excessive algal bloom reduces the photosynthetic activities and the rates of oxygen production in water on areal basis.

In catfish ponds, sediment concentration of organic matter increased from the beginning to the end of culture period (from 3.4 to 6.3%), which resulted in a higher oxygen demand by decomposing bacteria (Steeby et al., 2004). This may explain why oxygen budget deteriorated during the third month of the experiment.

Dense algal bloom in water column during the third month negatively affected oxygen budgets due to an increased algal sedimentation and shortening the photic zone depth according to Boyd (1990). Shallower photic zone observed during the third month was explained by nutrient enrichment due to high dietary protein inputs. Boyd (1985) indicated that one kilogram of pelleted feed applied in earthen ponds, produced approximately 1.25 kilogram of algae on a dry matter basis.

Dietary protein metabolism by fish results in the excretion of ammonia and phosphate into water column which are transformed into algal protein (biomass), through the process of algal photosynthesis. Liquidized heaps of algal sediments with green color were observed at the tank bottom at the time of harvest when water was completely drained. Shallower secchi disc readings were observed during the third month of the experiment as protein level increased within treatments (p<0.05), due to high dietary protein inputs. Intensive nutrient inputs lead to abundant community of phytoplankton (Ray et al., 2010). Moreover, excessive plankton abundance may be detrimental to fish production by causing problems with water quality (Boyd, 1979). Most of oxygen demand in pond aquaculture is accounted for by plankton and sediment respiration rather than by fish (Tucker, 2005).

Previous studies indicated that algae consume at least 50% of its total daytime oxygen production on a daily basis (Madenjian et al., 1987; Robson, 2005). Problems with low

dissolved oxygen become more frequent and severe (Boyd, 1990) due to the increase in the settled algal sediments (organic detritus) which exert high oxygen demands.

High algal sedimentation rates to tank bottom resulted in the accumulation of huge algal matter in the dark zone, with high sediment oxygen demand resulting from bacterial activities. Excessive algal blooms are always accompanied by high algal sedimentation rates (Boyd, 1990). When insufficient oxygen existed for the decomposition of algal sediments, algal matter gradually accumulated overtime, resulting in negative oxygen budgets (Hargreaves and Tucker, 2003).

High sediment respiration rates lead to oxygen problems in the pre-dawn hours. Moreover, sediment respiration rates will be also high during daylight hours, resulting in lower oxygen concentrations at dusk time during the third month of the experiment.

3.7. Growth and feed performance

Growth and feed performance parameters of Nile tilapia are shown in Table (3). The 25% crude protein diet in both the 8 gram and 9 gram treatments had better oxygen budget and better environmental conditions for fish production. Weight gains (47.4 - 48.9 g/fish) and daily weight gains (0.57 - 0.59 g/fish/day) were improved with the 25% crude protein diets compared to those of the 30% and 35% crude protein diets which ranged 33.3 - 41.6 g/fish and 0.4 - 0.5 g/fish/day, respectively. The better environment of the 25% crude protein treatments were caused by the lower algal blooms (secchi disc readings of 10.0 - 13.3 cm) observed in those treatments during the third month compared to most of the other treatments (5.9 - 7.6 cm). Lower algal blooms are always positively correlated with lower oxygen cycling and better oxygen budgets in farm pond aquaculture (Boyd, 1990).

Tilapia showed a better performance in pond cultures, when they were fed on a low protein diet in combination with bacterial produced in the pond than tilapia, which were fed with a high protein diet (Schneider et al., 2005).

Akiyama (1993), however, demonstrated that the use of sub-optimal quality feeds (feeds which were not adequate on their own to provide complete nutrition of the species) resulted in good, acceptable yields, comparable to those obtained with use of high-quality feeds. Indirectly, these trials demonstrate the usability of the "economically optimal protein level"

Table (3) : Growth and feed performance of Nile tilapia under different protein and feed levels (g feed/m² /day).

Treatment / Parameter	Feed load					
	8g/m²			9g/m²		
	25%cp	30%cp	35%cp	25%cp	30%cp	35%cp
Initial weight (grams/fish)	140.31[a]	139.49[a]	139.18[a]	129.69[a]	132.85[a]	131.19[a]
Final weight (grams/fish)	189.30[a]	181.14[ab]	172.50[b]	177.18[ab]	166.71[b]	168.74[b]
Weight gain (g/fish)	48.98[a]	41.65[a]	33.32[a]	47.48[a]	33.86[a]	37.55[a]
Daily weight gain (g/fish/day)	0.59[a]	0.50[a]	0.40[a]	0.57[a]	0.40[a]	0.45[a]
SGR (%)	0.35[a]	0.31[a]	0.25[a]	0.37[a]	0.27[a]	0.29[a]
Survival (%)	90%	90%	95%	100%	95%	100%
Daily weight gain/m² DWG/m²(g/m²/day)	2.65[ab]	2.25[ab]	1.88[b]	2.86[a]	1.92[b]	2.26[ab]
Feed conversion ratio (FCR)	2.42[b]	3.79[ab]	4.68[a]	3.44[ab]	5.15[a]	4.53[a]
Protein efficiency ratio (PER)	1.66[a]	0.87[bc]	0.62[c]	1.16[b]	0.65[c]	0.65[c]
Means in the same row with different letters are significantly different ($p<0.05$).						

concept developed for tilapia (De Silva *et al.,* 1989).

Similarly, feed performance in the 25% crude protein treatments was better than those of the 30% and 35% treatments. Better feed utilization was due to the better oxygen budget and lower algal blooms observed in the 25% crude protein treatments. Higher protein diets always produce high amounts of metabolic ammonia and phosphate excretions in water, followed by excessive algal blooms and adverse oxygen budgets as observed during the current experiment. The better environment of the 25% crude protein treatments improved treatment performance in terms of daily weight gain, feed conversion ratio and protein efficiency ratio.

Increasing the dietary level of non-protein digestible energy increases nitrogen retention by decreasing nitrogen losses (Kaushik and Oliva-Teles, 1985; Medale *et al.,* 1995; Engin and Carter, 2001). Higher nitrogen retention efficiency would be predicted at lower dietary protein intake (Engin and Carter, 2001). Protein content and availability in feeds affect water quality via nitrogen excretion, therefore, feeds that contain the least amount of protein necessary for optimum growth should be formulated (Gomez *et al.,* 2005).

Feed conversion ratios (3.7-5.15:1) and protein efficiency ratios (0.62-0.87) were significantly deteriorated in the high protein (30% and 35% crude protein diets) treatments. The 25% crude protein treatments produced better feed conversion ratios (2.42-3.44:1) and protein efficiency ratios (1.16 - 1.66) compared to those of the 30 and 35% crude protein treatments ($p<0.05$).

Both protein efficiency ratio and productive protein value decreased with increasing dietary protein levels (Yang *et al.,* 2002). Similar findings have also been reported for other fish species (Brown *et al.,* 1992; Arzel *et al.*, 1995; Gunasekera *et al*., 2000). This is probably because more dietary protein is used as energy when high protein diets are fed to fish (Kim *et al.*, 1991).

The deterioration in feed performance in the 30 and 35% crude protein treatments may be due to the adverse effect of high metabolic excretions of ammonia and phosphate on oxygen cycling. Negative oxygen budgets have been associated with reduced feed consumption and growth deterioration of fish (Boyd, 1990; Bhujel, 2000 and Tucker, 2005). This indicates why the high crude protein treatments did not have good growth and feed performances under excessive algal blooms.

Net protein utilization (NPU) in C. macropomum fish decreased with increasing protein ration (Gflnther and Boza 1993; Van der Meer *et al.,* 1995). Knowledge of protein utilization rates will allow selection of the most economical feed mix to minimize protein wastes and reduce both costs and ammonia production (Brunty *et al.*, 1997).

Visible signs of oxygen stress among fish were observed in all treatments after midnight time. Fish were experiencing surface water breathing after midnight until dawn time. The deterioration in feed conversion may be due to the adverse effect of excessive metabolic excretions of ammonia and phosphate on oxygen budget and midnight oxygen concentrations.

Conclusion

Feed efficiency and feed intake by fish were always low with negative oxygen budget. Depending on how negative the oxygen budget, Nile tilapia may lose appetite and decrease growth rate.

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تأثير مستويات بروتين العليقة على ديناميكية الأكسجين الذائب و أداء النمو فى البلطى النيلى

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ملخص

تم توزيع إصبعيات البلطى النيلى ذات متوسط أوزان 129.6 – 140.3 جرام/سمكة فى 18 حوض أسمنتى بعمق ثابت للمياه 75 سم. كما تم إستخدام اثنين من معدلات التغذية فى التجربة بمعدل 8 ، 9جرام عليقة/م²/يوم ، 6 أيام فى الإسبوع. احتوت العليقة الصناعية داخل كل معدل من معدلات التغذية على ثلاثة مستويات من البروتين (25% ، 30% ، 35%). إستمرت تجربة النمو 86 يوم خلال الفترة من أبريل وحتى يونيو 2014. عند زيادة محتوى بروتين العليقة لم يؤثر ذلك على تركيزات الأكسجين فى المياة عند غروب الشمس فى المعاملات المختلفة. انخفضت تركيزات الأكسجين الذائب عند غروب الشمس بمعدل سريع من 8.9 – 10.8 جرام أكسجين/ م² خلال الشهر الثانى من التجربة مع وجود فروق معنوية بين الفترتين (عند معنوية 5%). وقد لوحظ انخفاضا حادا فى قراءات قرص سيكى خلال الشهر الثانى للتجربة وهذا يرجع الى الزيادة المفرطة فى الوفرة النسبية للطحالب وقد انخفض تركيز الأكسجين انخفاض حاد عند شروق الشمس وغروب الشمس خلال الشهر الثانى وهذا يرجع الى الإزدهار الحاد للطحالب خلال الفترة الأخيرة . وقد تميز الشهر الثانى بوجود بيئة أسماك جيدة بالنسبة لتركيز الأكسجين فى وقت الليل مما أدى الى الآداء الجيد عبر المعاملات. بالرغم من ذلك لوحظ تدهور البيئات خلال الشهر الثالث حيث كانت الأسماك تعانى من نقص الأكسجين. وقد تميزت معاملة البروتين الخام (25%) بميزانية أكسجين جيدة وظروف بيئية جيدة تناسب إنتاج الأسماك فى معاملات 8 ، 9 جرام. وقد أدى انخفاض إزدهار الطحالب (قراءات قرص سيكى: 10 – 13.3) بصورة ملحوظة فى هذه المعاملات خلال الشهر الثالث الى تحسن البيئة المائية فى معاملات 25% بروتين خام بالمقارنة بالمعاملات الأخرى. بالإضافة الى ذلك فإن معاملات 25% بروتين خام انتجت معاملات تحويل غذائى جيدة (2.42 – 3.44 : 1) ومعاملات كفاءة بروتين جيدة (1.16 – 1.66) بالمقارنة بالمعاملات التى احتوت على 30% ، 35% بروتين خام.