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Effects of Nutritional Supplements Phosphatidylcholine and Beta-Carotene on Growth and Selected Stress and Immune Parameters in Nile Tilapia, *Oreochromis Niloticus* (L.)

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Abstract Dietary supplements phosphatidylcholine and β-carotene, a dimer of vitamin A have been shown have positive effectson a variety of physiological and immunological parameters in humans and other vertebrates. Previous studies have demonstrated that these neutraceuticals enhance growth of Nile tilapia reared under optimal temperature. However, in those studies specially formulated diets were used. Given that tilapia is a commercially important fish, unlike previous studies we wanted to examine the effects of adding these supplements directly to the commercially available basal diet. Since weight gain can be accompanied by poor health and overdose of vitamin A can be toxic we examined condition factor, which is an indicator of overall health. Effects of these neutraceuticals on stress parameters such as blood glucose levels and hematocrit as well as on immune function as measured by macrophage phagocytic activity were also examined. All experiments in our study were conducted on tilapia grown under optimal temperature (28±2℃). Our experiments showed that both phosphatidylcholine and β-carotene supplemented diets significantly enhanced fish growth compared to basal diet.However, there were no significant differences in condition factor and stress or immune parameters. Thus it was apparent that fish remain healthy and stress free. Furthermore, there was no negative effect on immune function. These data collectively suggest that addition of phosphatidylcholine and β-carotene to commercially available basal diet used in this study can be used in tilapia aquaculture to enhance fish growth. This information can also help producers of commercial feed in improving feed composition.

Keywords Tilapia, Nutrition, Stress, Immune Response

1. Introduction

Dietary supplementation with soy lecithin has been shown to have wide range of effects on biological systems including improvement in blood lipid profile in hyperlipidimic patients [1], alteration in phagocytosis and lymphocyte response to concanavalin-A in diabetic rats [2]and reduction in cholesterolemia [3,4].Phosphatidylcholine (PC) is a major component in soy lecithin and has been implicated in aforementioned effects. Other studies have demonstrated that PC has anti-inflammatory effect as well as immunomodulatory effect in the presence of arachdonic acid[5].Active incorporation of PC by macrophages and lymphocytes *in vitro* has also been demonstrated [6].Vitamin A is another dietary supplement that is a key regulator of many biological processes including adaptive immunity and immune cell development[7, 8].

Previous studies by Craig and Gatlin [9], Kanazawa et al. [10], Kanazawa [11], Poston [12, 13](1990a, 1990b) and Kasper and Brown [14] have demonstrated beneficial effect of PC supplementation on fish growth.β-carotene, a dimer of vitamin A, is another neutraceutical of interest that has been shown to increase growth rate in Nile tilapia [15].However, effects of PC or BC supplementation on tilapiamacrophage phagocytic activity and on physiological parameters such as blood glucose levels and hematocrit has only been investigated in tilapia grown in suboptimal temperatures[16].In this study we looked at the effects of PC and BC supplementation on these physiological and immunological parameters.

2. Materials and Methods

2.1. Fish Acquisition and Maintenance

Juvenile Nile tilapia (mean weight 7.5 grams and mean length 6.8 cm) *Oreochromisniloticus* were purchased from Animas (AmeriCulture, Inc., NM) and transported to the Life Sciences Resource Center at Indiana University-Purdue University Fort Wayne. The fish were placed in 40-L tanks

containing dechlorinated water filtered and oxygenated by Millennium 2000 filters (Aquarium Systems, OH).The dechlorination phase involved filling large bins with city water, which remained uncovered under light for approximately one week with aeration. This process enabled the slow evaporation of chlorine from the water supply, which was then used to fill the tanks. The fish were randomly stocked in 6 tanks (3 groups x 2 replicates) at a density of 25 fish per tank and allowed to acclimate for 2 weeks before initiation of the experiment. Three fish groups were: fish fed control diet, fish fed PC-supplemented diet, and fish fed BC-supplemented diet. Fish were kept at 28±2^{o}C and fed diets twice daily to apparent satiation during the acclimation period. For heating water, Visi-Therm Deluxe 100W heaters (Aquarium Systems, OH) were used to maintain the optimal warm water temperature at 28^{o}C.Floating glass thermometers (Aquarium Systems, OH) were used in each tank to monitor the water temperatures. Water temperature was monitored at least twice a day. Fish were fed their respective diets twice per day to apparent satiation throughout the experimental phase (8 weeks).The diurnal light: dark cycle was maintained at 16 h light: 8 h dark. Water quality parameters were monitored and optimal water quality was maintained (dissolved oxygen was at a minimum of 6 mg/L and pH between 6 and 8.5). Fish were cared for according to approved animal care protocol.

2.2. Feed Preparation

The basal diet used in this experiment was a commercial floating pelleted feed (Purina AQUAMAX GROWER 400) purchased from the Mill and Meadow, 5210 W. Washington Center Road. Fort Wayne, IN.The L-α-Phosphatidylcholine (catalog # P7443) and β-carotene (catalog # C9750) supplements were purchased from Sigma-Aldrich (St. Louis, MO). The PC product used in our experiments contains 80 % unsaturated fatty acids. To incorporate the supplements into the basal diet commercial feed, the PC and BC were first dissolved in reagent grade hexane (Curtin Matheson Scientific, Inc., Houston, TX) and each respective solution was carefully sprayed onto the feed in small aliquots. Concentrations of PC and BC supplements were 2500 mg/kg (0.25%) and 40 mg/kg (0.004%), respectively. Feed pellets were allowed to dry between applications to allow evaporation of the hexane carrier. All feed was stored under air-tight condition at -20°C until needed for feeding.

2.3. Sampling

Six fish per treatment group (3 fish per tank X 2 replicates) were sampled during the experimental phase at weeks 0, 2, 4, 6, and 8. After removal from the tank, the fish were immediately exposed to a lethal dose of the anesthetic tricainemethanesulfonate (MS-222; ~200mg/L; Sigma-Aldrich, St. Louis, MO). The health and stress levels of these fish were then determined by the following measures: length, weight, condition factor (K), blood glucose, hematocrit, and macrophage phagocytic activity.

2.4. Growth

The length and weight of all sampled fish were measured to monitor growth and determine condition factors (K).The following equation was used to calculate condition factors from length and weight [17]:Condition Factor (K) = [(Weight x 100)/ (Length^{3})].

2.5. Plasma Glucose

To determine the blood glucose levels, methods were followed as given by Gensic et al. [18] and Schreck and Moyle [19], and validated for use in fish by Wedemeyeret al. [20].The caudal peduncle was first severed and then a drop of blood from each fish was obtained, placed on a glucose strip, and inserted into a standard glucometer (Precision Xtra, Abbott Laboratories, Abbott Park, IL).

2.6. Hematocrit

The blood drawn for this measurement was collected via capillary tube from the previously severed caudal peduncle. These tubes were then capped, centrifuged, and read using Micro-Hematocrit Capillary Tube Reader (Monoject Scientific, St. Louis, MO).

2.7. Phagocytic Activity

Isolation of macrophages and assessment of their phagocytic activity was accomplished by following the technique described in Mustafa et al. [21].Briefly, the head kidney was removed from each fish and placed in Leibovitz-15 (L15) medium (Mediatech, Inc., VA) on ice. The samples were then macerated through coarse mesh to tease apart the larger tissue fibers. After centrifuging the collected cells at 1000 x g, the supernatants were discarded, the cells were resuspended in fresh L-15, and then incubated on slides for 90 min. To determine the phagocytic activity of the macrophages, a microscopic counting technique described by Mathews et al. [22] and Brown et al. [23] was followed. Formaline killed *Bacillus megaterium*, were used and following the recommendations of Enane et al. [24], at least 100 cells were examined under 100 x magnification to determine the percentage of phagocytic cells able to take up at least five bacteria.

2.8. Data Analysis

The data obtained during the course of this experiment was statistically analyzed using Minitab® 15.1.0, 2007.The means and standard errors of the means were calculated for each assay by one-way analysis of variance (ANOVA) and differences were considered significant when $P<0.05$. A post-ANOVA comparison of multiple means test (Tukey's) was performed in order to determine any differences

between treatments. The graphs and the written data within this document are presented as means ± standard errors of the means (SEM).

3. Results

3.1. Growth

Tilapia given PC-supplemented feed had significantly higher weight than those given control feed at weeks 6 and 8 and tilapia given BC-supplemented feed had significantly higher weight than those given control feed at weeks 2, 4, 6 and 8 (Fig. 1).However, no significant differences were found when comparing the fish given PC- supplemented feed with those given BC- supplanted feed. Similarly there were no significant differences in condition factors among the fish groups (data not shown).

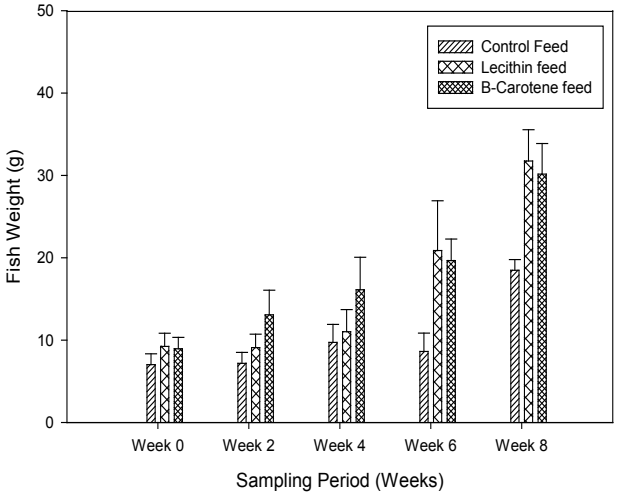

Figure 1. Mean fish weight for each sampling period with SEM. * means significantly different ($p<0.05$) than fish fed basal diet (control) within each sampling period.

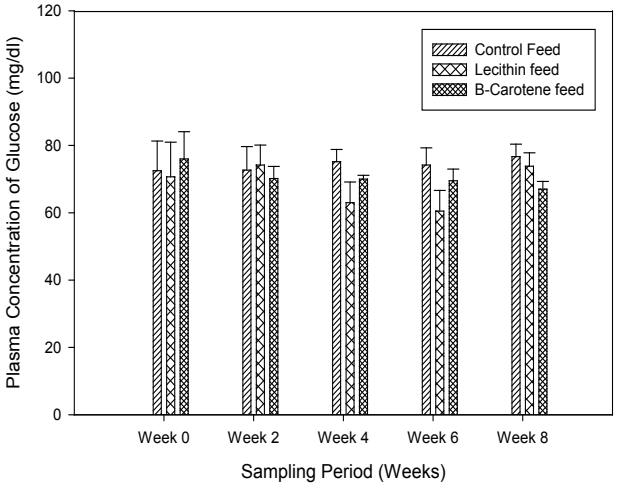

Figure 2. Mean plasma glucose levels for each sampling period with SEM.

3.2. Stress

Throughout the experimental period, there were no significant difference either in plasma levels of glucose or in hematocrit levels among the tilapia groups given either control feed or PC- or BC- supplemented feed (Figs. 2 and 3).

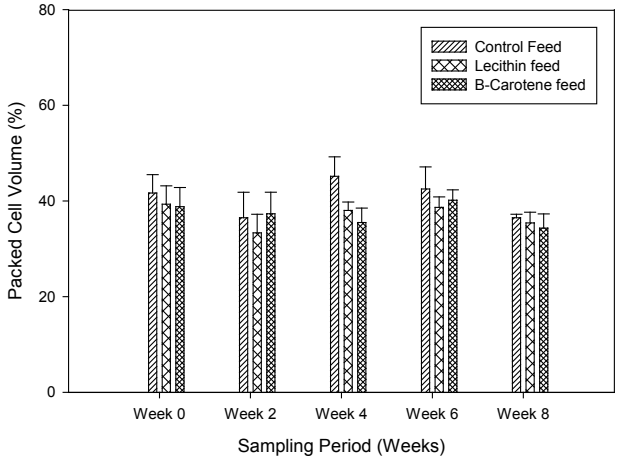

Figure 3. Mean Packed Cell Volumes for each sampling period with SEM.

3.3. Immunity

No significant differences in phagocytic capacity were seen among the treatments regardless of feed and sampling period (Fig. 4).

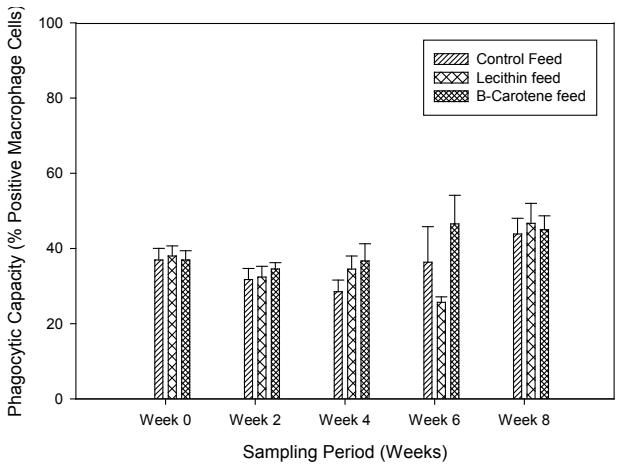

Figure 4. Mean phagocytic capacity of macrophage cells for each sampling period with SEM.

4. Discussion

Enhanced weight gain in juvenile Nile tilapia fed with laboratory-formulated diet containing PC in comparison to the diet without added PC has been previously reported[14]. Similarly vitamin Ahas been shown to increase growth rates in tilapia [15] and it is known thatNile tilapia converts BC to

vitamin A [25]. Hence, we wanted to investigate effects of supplementing commercial diet with BC and PC on tilapia grown under optimal temperature. Results presented above show that tilapia grown under optimal temperature and fed with PC or BC-supplemented commercial diet gained significantly higher weight when compared to controls fed on basal diet. In 8 weeks total weight for controls was twice that of starting weight while in fish fed with PC or BC supplemented commercial diet weight gain was five times that of starting weight. Significant differences in weight gain between controls and PC or BC fed fish were apparent in 6 weeks. Interestingly there were no significant differences between PC and BC supplementation. Given that weight gain beyond certain point can be deleterious to humans and other vertebrates including fish, we examined condition factor and found no significant differences among control or experimental samples. Thus fish do not appear to be overweight and there is no apparent toxicity associated with beta-carotine.

In a previous study Mustafa et al. [16]demonstrated that when tilapia were grown in suboptimal temperature (16 ^{0} C) PC or BC supplementation of commercial diet did not have significant effect on condition factor, blood hematocrit, plasma glucose and phagocytic activity of macrophage cells. However, as mentioned in the introduction, both PC and BC have been shown to have beneficial effects on wide range of biological systems including immune system. Therefore, we investigated effects of PC or BC supplementation on stress parameters plasma glucose levels and hematocrit levels, and found no significant differences between basal diet-fed controls and fish fed with PC or BC supplemented diet. Similarly, in tilapia grown under optimal temperature, there were no significant differences in mean phagocytic capacity of macrophage among treatments regardless of feed. These data suggest that enhanced weight gain in experimental sample is not accompanied by added perturbation of condition factor indicating normal growth and health. Furthermore, given that fish were not stressed in any way PC and BC do not have any effect on stress and immune parameters: as measured by blood glucose levels, haematocrit and phagocytic capacity of macrophages.These observations suggest that PC or BC supplementation of commercial diet can be beneficial in tilapia aquaculture with regard to fish growth.Our results also show that the enhanced growth is not deleterious to fish health or immune response.

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