VITAMIN A IN DIETS FOR NILE TILAPIA

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ABSTRACT: Dietary vitamin supplementation decrease stress caused by high stocking density, and boosts immunological system of farmed fish. A studied was carried out to determine vitamin A requirements of Nile tilapia (*Oreochromis niloticus*) in an all male group (13.8 ± 1.2 g) and a mixed sex population (9.8 ± 2.3 g). Fish stocked in 100-L plastic aquaria (26.0 ± 1.0°C) were fed to near satiety, twice a day, seven days a week, during 75 days with vitamin A-free, semi-purified diets supplemented with 0; 600; 1,200; 1,800; 2,400; 3,000; 3,600; 4,200; 4,800 and 5,400 International Units (IU) of retinyl palmitate (30% vitamin A) per kg of diet in a completely randomized experimental design, factorial arrangement 2χ10 (n = 4). Deficiency signs of vitamin A were observed in fish fed 0 to 1.200 IU vitamin A kg^{-1} diet; moderate signs were observed in fish fed diets with 1.800 to 3.600 IU vitamin A kg^{-1} diet; no interactions group*level ($p < 0.05$) were detected. Dietary levels of vitamin A up to 5.400 IU kg^{-1} influenced final weight and weight gain of fish ($p < 0.05$), but did not influence feed consumption ($p > 0.05$). A group effect was observed regarding all performance variables ($p < 0.0001$). Quantification of hepatic retinol (HPLC) detected vitamin A only in fish fed 5.400 IU retinol kg^{-1} of diet, therefore characterizing that dietary retinol was used and stored. The quantity of 5.400 IU of retinol kg^{-1} of diet is recommended for adequate nutrition of Nile tilapia.
Key words: *Oreochromis niloticus*, retinol, nutrition

VITAMINA A EM DIETAS PARA TILÁPIA DO NILO

RESUMO: A suplementação de vitaminas na dieta diminui o estresse e estimula o sistema imunológico causado por altas densidades de estocagem dos peixes. Este trabalho determinou a exigência em vitamina A para a tilápia do Nilo em uma população monosexo masculina (13.8 ± 1.2 g) e em uma população original (9.8 ± 2.3 g). Os peixes foram estocados em aquários plásticos de 100 L (26.0 ± 1.0°C) e alimentados "ad libitum", duas vezes ao dia, sete dias da semana, durante 75 dias com dieta semi-purificada suplementada com 0; 600; 1.200; 1.800; 2.400; 3.000; 3.600; 4.200; 4.800 e 5.400 UI de retinyl palmitato (30% de vitamina A) por kg de dieta, em um delineamento experimental totalmente ao acaso e arranjo fatorial 2χ10 (n = 4). Deficiência nutricional severa foi observada em peixes alimentados com 0 a 1.200 UI vitamina A kg^{-1} de dieta; sinais moderados foram encontrados em peixes alimentados com 1.800 a 3.600 UI vitamina A kg^{-1} de dieta; interações grupo*nível ($p < 0,05$) não foram detectadas. O aumento de nível de vitamina A influenciou no peso final e no ganho de peso dos peixes ($p < 0,05$), mas não influenciou o consumo de ração ($p > 0,05$). Foi observado efeito de grupo no desempenho dos peixes ($p < 0,0001$). Foi detectado o retinol hepático através de HPLC somente no grupo alimentado com 5.400 UI de retinol kg^{-1} de dieta, caracterizando assim que o mesmo foi utilizado e armazenado. A quantidade de 5.400 IU de retinol kg^{-1} de dieta é a mínima recomendada para tilápia do Nilo.
Palavras-chave: *Oreochromis niloticus*, retinol, nutrição

INTRODUCTION

Commercial production of tilapia requires the use of high quality, complete feed. Increasing vitamin supplementation of complete diets decreases stress caused by high stocking density, and boosts immunological system of fish (Davis et al., 1998; Halver, 1985; Toguyeni et al., 1997). On the other hand, inadequate dietary vitamin supplementation can result in diseases outbreaks or reduced growth in a confined fish population (Hepher, 1988; NRC, 1993; De Silva & Anderson, 1995; Harikumar et al., 1996; Goswami & Dutta, 1991; Taveekijaran, 1994; Thompson et al., 1995).

Excess dietary fat-soluble vitamins are stored in hepatic lipid deposits (NRC, 1993; Ornsrud et al.,

2002); 90% of the stored vitamin A is found in the liver (Katuyama & Matsuno, 1988). Therefore, quantifying vitamin A depots in hepatic tissue elicits establishing metabolic and nutritional requirements (Hole & Taylor, 1996).

Vitamin A requirements were determined for channel catfish (1,000 to 2,000 International Units (IU) kg^{-1} of diet), salmonids (2,500 IU kg^{-1} of diet), carp (4,000 to 20.000 IU kg^{-1} of diet), Japanese flounder (10.000 IU kg^{-1} of diet) and greasy grouper *Epinephelus tauvina* (3,101 IU kg^{-1} of diet) (NRC, 1993; Hepher, 1998; Hernandez et al., 2007; Mohamed et al., 2003). Saleh et al. (1995) determined that vitamin A requirement of Nile tilapia is 5,000 IU kg^{-1} diet. Hu et al. (2006) determined that vitamin A requirement of hybrid tilapia *O. niloticus* × *O. aureus* ranges on 5,850 to 6,970 IU kg^{-1}. This same author also registered that tilapia can utilize β-carotene to fulfill the dietary vitamin A requirements. However, Kubitza et al. (1998) and Kubitza & Cyrino (1999) reported that Brazilian commercial feeds for omnivorous, tropical fish may contain from 3,000 to 22,000 IU kg^{-1} vitamin A. The aim of this study was to verify the use of vitamin A in diets for Nile tilapia, through the determination of hepatic vitamin A storage capacity and double check discrepant, reported dietary vitamin A requirement of juvenile Nile tilapia fed semi-purified diets, through the evaluation of growth rate and deficiency signs.

MATERIAL AND METHODS

Fish (19 per aquarium) were kept in 40 100-L plastic aquaria, supplied by a closed recirculation system. Aeration was provided continuously throughout the experiment. Water pH, dissolved oxygen (OD) and temperature (26 ± 1.0°C) were monitored on a daily basis. Fish were fed for 11 weeks with vitamin A-free, semi-purified diets supplemented with 0, 600, 1.200, 1.800, 2.400, 3.000, 3.600, 4.200, 4.800 and 5.400 IU of vitamin A kg^{-1} diet (Table 1), in a completely randomized experimental design, factorial arrangement 2χ10 (n = 4). Retinyl palmitate (Rovimix TO 500 Roche®; 30% vitamin A) was used as dietary vitamin A source. The diet was formulated based on albumin and gelatin protein (Table 1). The mixture was extruded through a mincer (ML-4.0 WEG-μline); pellets were collected, dried overnight in a forced air oven (55°C); grinded to 1 mm pellets, sized, hermetically packed and stored under refrigeration until use. Fish were fed to near satiety twice a day (6:00 am and 6:00 pm).

The trial was duplicated with (i) an all-male, sex-reversed Nile tilapia juvenile population (SR), (13.76 ± 1.21 g), and (ii) a mixed-sex population (NSR) (9.83 ± 2.30 g). Fish were acclimated to the aquaria and fed for fifteen days prior to the beginning of the feeding trials with the non-supplemented diet to simulate or induce deficiency (NRC, 1993).

Table 1 - Formulation and proximate composition of the experimental diets.

Ingredients	Contents (%)
Albumin	32.10
Gelatin	7.70
Corn starch	44.13
Soybean oil	6.00
a - Cellulose	6.00
Bicalcium Phosphate	3.00
Vitamin A-free and mineral mix	0.50
Vitamin C[2]	0.05
Sodium choride	0.50
BHT[3]	0.02
Proximate composition	
Dry matter (%)	92.09
Gross energy (kcal kg^{-1})	4791
Crude protein (%)	34.70
Crude lipid (%)	2.65
Crude fiber (%)	3.83
Ash (%)	4.79

[1]Units kg^{-1} of diet: vit D_3 200,000 UI; vit E 12,000 mg; vit. K_3 2,400 mg; vit B_1 4,800 mg; vit B_2 4,800 mg; vit B_6 4,000 mg; vit B_{12} 4,800 mg; folic acid 1,200 mg; pantothenic acid 1,200 mg; vit C 48,000 mg; biotin 48 mg; niacin 24,000 mg; Fe 10.000 mg; Cu 600 mg; Mn 4,000 mg; Zn 6,000 mg; I 20 mg; Co 2 mg; Se 20 mg. [2]Vit C (Lutavit C- Aquastab BASF®). [3]BHT= Butil hidroxi tolueno.

Apparent signs of vitamin A deficiency – e.g. exophthalmia, depigmentation, clouding of corneal epithelium, anorexia, warped gill operculum, reduced growth, poor feed efficiency, and high mortality (Tacon, 1992) – were recorded through visual observations along the experimental period and at the end of the trial in all fish. The following growth data were recorded at the beginning and ending the experimental period: initial and final weigh; weight gain [(final weigh) - (initial weigh)]; feed consumption; feed conversion ratio [(feed consumption) ÷ (weight gain)]; and survival rate [100 × (final number of animals) ÷ (initial number of animals)]. At the end of the trials, hepatic tissue was sampled from fish and stored in liquid nitrogen. High pressure liquid chromatography (HPLC) was utilized to quantify vitamin A in the hepatic tissue lipid depots (Landen-Júnior & Eitenmiller, 1979).

Data were submitted to ANOVA and regression analysis by the PROC GLM, SAS software (SAS In-

stitute, 2001), for linear and quadratic effects of the treatments on final weight (FW), weight gain (WG), feed conversion rate (FCR) and survival (S).

RESULTS AND DISCUSSION

Deficiency signs

The same clinical deficiency signs were observed for animals of all groups fed vitamin-A deficient diet. Normally colored livers with dark-colored gal bladders, a characteristic sign of clinical stress (Halver, 1989; Roberts, 1981; Post, 1987; Steffens, 1989; Tacon, 1992; Plumb, 1999) was also recorded (Table 2).

Vitamin A deficiency signs in *O. niloticus* include: abnormal swimming behavior; internal hemorrhages; protruded, blind eyes; anemia; hemorrhage in the base of fins and in the skin (Saleh et al., 1995). In advanced deficiency condition Saleh et al. (1991) also observed widespread depigmentation and edemas in the abdomen, sometimes associated with ascites; reduction of mucus secretion and dry, hard mucous tissue. Lesions observed post-mortem appeared as ascites, clubbed gills and hemorrhagic kidneys. Hemorrhagic, amorphous, granulomatous spleen; necrotic, granulomatous, amorphous liver were conspicuously found in the present work in fish receiving less than 1,200 IU vitamin A kg^{-1} diet. Spleen severe conditions were also registered to a lesser extent in fish fed diets containing 1,800~2,400 IU vitamin A kg^{-1} diet.

Cherry salmon *Oncorhynchus massou* fed vitamin A-deficient diets for 15 weeks presented clinical signs similar to those described above (Taveekijaran et al., 1994). Similar observations were reported for catfish *Heteropneustes fossilis*, greasy grouper *Epinephelus tauvina*, Atlantic halibut *Hippoglossus hippoglossus L.* and sunshine bass juvenile *Morone chrysops* × *M. saxatilis* (Harikumar et al., 1996, Mohamed et al., 2003; Moren et al., 2004; Hemre et al., 2004). The use of advanced juveniles, which may have adequate body reserves of vitamin A, may explain the low incidence of ocular problems, opposing to observations of Poston et al. (1977) with rainbow trout.

Growth parameters

Weight gain (WG), feed conversion ratio (FCR), survival (S) and feed consumption rate (FCR) data are presented in Table 3. A linear effect ($p <= 0.01$) was detected for FW and FCR, but no effect was detected regarding WG ($p >= 0.05$) (Figure 1 and 2). This may have resulted from differences in fish initial weight. Several research reports are in accord to these results. Hu et al. (2006) reported that hybrid tilapia fed diets supplemented with 50,000 IU vitamin A kg^{-1} present better weight gain (601%) and better feed conversion ratio (1.00). Saleh et al. (1995) also observed that Nile tilapia juveniles fed diets supplemented with 5,000 IU vitamin A kg^{-1} presented better weight gain (23.9 g), better feed consumption rate (60.2 g), and better feed

Table 2 - Percent incidence of clinical signs of vitamin A deficiency recorded in all Nile tilapia juveniles fed diets varying vitamin A levels.

Diet	Deficiency signs of vitamin A recorded (%)																					
	0		600		1,200		1,800		2,400		3,000		3,600		4,200		4,800		5,400			
	mg vit A kg^{-1}																					
Clinical signs during the experiment period	SR	NSR	SR	NSR	SR	NSR	SR	NSR	SR	NSR	SR	NSR	SR	NSR	SR	NSR	SR	NSR	SR	NSR	SR	NSR
Pale liver		0.13		0.13						0.52		0.39										0.13
Skin without scale		0.13				0.13												0.13				
Cataract			0.26					0.13			0.13		0.13									
Hemorrage lateral fin														0.13								
Exophtalm															0.13							
Clinical signs after the experiment period																						
Ascites						0.13						0.13										
Granulomatous, necrotic spleen	0.65	0.65	2.63	0.65	2.10	27	1.71	1.44	1.44	0.39	0.65	0.13		0.13		0.13						
Granulamatous, amorphous liver	3.94	5.00	3.94	3.94	2.10	0.65								0.13		0.13		0.13				
Amorphous kidney	2.63		2.63	3.94	3.94																	

SR = sex-reverted; NSR = non sex-reverted

Table 3 - Growth performance of the juveniles fed diets varying vitamin A levels.

Group	Growth performance				
	IW	FW	WG	FC	FCR
	g				
SR	13.76	43.70	29.94	33.82	1.12
NSR	9.83	27.03	17.22	25.45	1.49
Vitamin A level (IU kg^{-1})					
0	11.14	31.61	20.46	32.46	1.58
600	10.64	30.09	19.45	29.62	1.53
1200	11.94	33.29	21.35	28.83	1.38
1800	11.70	32.84	21.14	26.04	1.28
2400	12.93	36.72	23.79	28.16	1.20
3000	12.73	37.24	24.51	29.10	1.20
3600	12.56	38.58	26.02	30.85	1.23
4200	10.97	37.02	26.05	29.90	1.17
4800	11.74	37.49	25.75	29.34	1.16
5400	11.55	38.81	27.26	32.04	1.20

SR = sex-reversed; NSR = non sex-reversed, IW= initial weight; FW = final weight; WG = weight gain; FC = food consumption; FCR = food conversion rate.

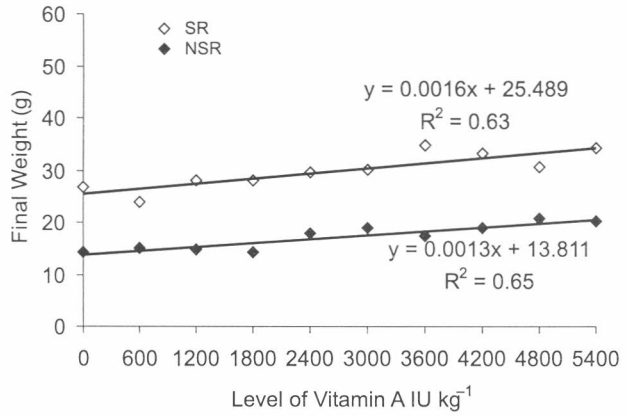

Figure 1 - Final weight (FW) of the animals (SR: sex-reverted; NSR: non sex-reversed) in response to the levels of vitamin A inclusion.

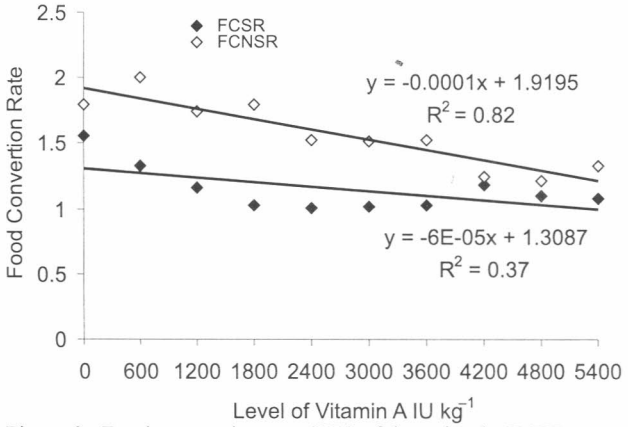

Figure 2 - Food conversion rate (CR) of the animals (FCSR: sex-reverted; FCNSR: non sex-reversed in response to the levels of vitamin A inclusion.

conversion ratio (2.5), than fish fed diets containing 0, 10,000 or 40,000 IU vitamin A kg^{-1}. Mohamed et al. (2003) observed that diets supplemented with 3,764 mg vitamin A kg^{-1} for greasy grouper led to a better weight gain (420.94%), better feed conversion ratio (1.42) and better protein efficiency ratio (2.08). Sunshine bass fed diets supplemented with 509 – 40,516 μg vit A kg^{-1} had no difference in weight gain (269-285%) or feed efficiency (0.88-0.89) (Hemre et al., 2004). On the other hand, Hernandez et al. (2007) observed that Japanese flounder *Paralicthys olivaceous* fed fish meal-based diets supplemented with 0.00 IU vitamin A kg^{-1} presented better specific growth rate (4.9%). Also, Atlantic halibut fed diets supplemented with 0-250 mg of retinal kg^{-1} had no differences in final weight or mortality (Moren et al., 2004).

Survival rate obeyed to a quadratic effect ($p <= 0.001$) (Figure 3). Saleh et al. (1995) reported that groups of Nile tilapia receiving 5,000 and 10,000 IU vitamin A kg^{-1} diet had 93% survival ratio. Thus increasing dietary vitamin A levels up to 10,000 IU kg^{-1} do not significantly reduce survival rate of Nile tilapia. Mortality rate of rainbow trout juveniles was not influenced when feeding on either vitamin A-free diet or diets supplemented with 10,000 IU of retinyl palmitate kg^{-1} for a maximum 20 weeks (Poston et al., 1977). This data for rainbow trout corroborates results for Japanese flounder, greasy grouper, hybrid tilapia, and sunshine bass (Hernandez et al., 2007; Mohamed et al., 2003; Hemre et al., 2004; Hu et al., 2006).

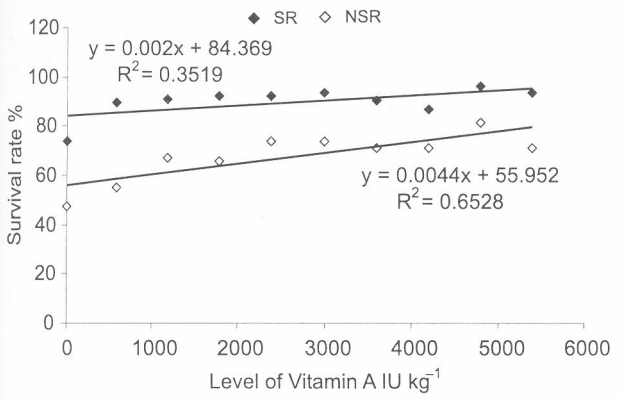

Figure 3 - Survival rate (S) in response to the different levels of vitamin A inclusion (SR: sex-reversed; NSR: non sex-reverted).

Regardless of dietary vitamin A level, sex-reversed fish had better growth rates in comparison to the mixed sex groups. Actually, monosex tilapia populations usually present better growth rates as a result of altered endocrine status induced by populational skewed sex ratio (Toguyeni et al., 1997).

Hepatic retinol

High performance liquid chromatography (HPLC) analyses did not detect vitamin A in hepatic tissue sampled from fish receiving 0 to 4,800 IU of retinyl palmitate kg^{-1} diet. The detectable level was of 45 ± 10 mg of vitamin A 100 g^{-1} hepatic tissue. Only fish receiving the 5,400 IU kg^{-1} presented detectable amounts – 136 ± 10 mg of vitamin A 100 g^{-1} hepatic tissue. Therefore only this level of dietary vitamin A supplementation exceeded fish metabolic requirements and so, only after meeting the metabolic needs of the animals, it could be stored in the liver. Fontagné et al. (2006) fed Siberian sturgeon *Acipenser baeri* larvae with vitamin A and also found retinal palmitate as the main storage form of vitamin A with 6.7 μg g^{-1} in larvae fed diets with the highest vitamin A level, that was 772,500 IU kg^{-1} .

Camargo et al. (1975) determined concentrations of retinol (mg g^{-1}) and other compounds derived from vitamin A in hepatic lipid depots of six neotropical, fresh water fish captured in the Moji-Guaçu river, State of Sao Paulo, Brazil: curimbatá *Prochilodus scrofa* 3.32; dourado *Salminus maxillosus* 7.62; piapara *Leporinus piapara* 4.30; mandiuva *Pimelodus clarias* 2.85; piava *Leporinus copelandi* 3.40. Hole & Taylor (1996) reported that the dogfish *Squalus acanthias* concentrate as little as 0.047 mg g^{-1} retinol in the liver. Hernandez et al. (2007) also did not detect retinol in livers of Japanese flounder fed fish meal-based diet not supplemented with vitamin A, and the level of retinol in the fish liver increased respectively, in the diets supplemented with 10,000 IU vitamin A kg^{-1} and 25,000 IU vitamin A kg^{-1} respectively. Hemre et al. (2004) reported for sunshine bass fed diets supplemented with 0-509 μg of retinyl acetate kg^{-1}, the level detected by HPLC where over 924 μg of retinyl acetate kg^{-1} was supplemented in the diet. Vitamin A retention was not significant in hybrid tilapia fed diets supplemented with levels below 6,000 IU vitamin A kg^{-1} when detected by HPLC (Hu et al., 2006).

In exception to the dourado, all other fish studied by Camargo et al. (1975) had plant material as major food items; this same feeding behavior is true for Nile tilapia (Lowe-McConnel, 1975; Beveridge & McAndrew, 2000). Rodriguez-Amaya (1999) reported that the concentration of β-carotene, a conspicuous pro-vitamin A in plant tissue tops 360 IU g^{-1}; actually, any plant tissue which contains 46~74 IU g^{-1} β-carotene is considered a concentrated source of vitamin A. In exception to the control (no supplementation), any other tested level within the scope of this work (600~5,400 IU of vitamin A kg^{-1} of the diet) lies within the high to the mega-dose of dietary vitamin A supplementation. Therefore, the amount of hepatic retinol detected in this study meets any assumed expectation, and the fact that the highest dietary vitamin A contents – 5,400 IU kg^{-1} – elicited best growth performance do not surprise either.

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