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PRODUCTIVE PERFORMANCE OF TILAPIA (*Oreochromis niloticus*) FINGERLINGS FED ON DIETS CONTAINING FISH MEAL STORED UNDER DIFFERENT STORAGE METHODS AND SUPPLEMENTED WITH BHT ANTIOXIDANT.

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SUMMARY

Fish meal was stored for 26 weeks under three different storage methods (in store, under shade and in sun) with or without supplemented antioxidant (Butylated Hydroxy Toluene BHT). Two levels of BHT were used (125 and 250 ppm). Nine formulated diets were fed for 12 weeks to 460 tilapia fingerlings (*O. neloticus*, L) of about 2.8g/ fish. All treated diets contained about 37% crude protein and 4800 kcal GE/Kg diet. The results of storing experiment showed that the best storage method is in store, and no need to add BHT, because the origin added ethoxyquin protected fish meal against oxidation and any changes in its chemical composition. In the feeding experiment, stored fish meal in store and without adding BHT offered the best results of growth parameters, feed efficiency and chemical composition of whole fish body.

Keywords: *Fish meal, Storage, antioxidant, Tilapia, Productive performance.*

INTRODUCTION

Aksnes *et al.* (1983), reported that an extensive oxidation was found after ten months storage in the unprotected fish meals, whereas only minor changes were found in fish meals containing ethoxyquin. The fat oxidation had taken place mainly during the first 4 to 6 months. EL-Lakany and March (1974), found that after 5 months storage, the growth response and the efficiency of feed conversion to the untreated fish meal stored at 21 C° was lower at all levels of dietary inclusion (4,7 and 11% of protein supplement) than the response to the antioxidant-stabilized fish meals stored at the same temperature. After 10 months storage, a similar pattern of relative response to the different fish meals was prevailed. The present study was conducted to study the effects of storing imported fish meal under different storage methods supplemented with BHT antioxidant on the productive performance of tilapia fish (*Oreochromis niloticus*,L).

MATERIALS AND METHODS

Two separate experiments were designed to evaluate imported fish meal {originally treated with 125 ppm. ethoxyquin (EMQ)}. 1- Storing experiment: to study the effect of three storing methods (in store, under shade and in sun) on the chemical composition and peroxide number of fish meal for six months storing period. Within each method of storing three levels of Butylated Hydroxy Toluene (BHT) antioxidant were used; 0, 125 and 250 ppm. So, this first experiment included nine treatments. 2- Feeding experiment:

was to study the effect of incorporation the stored fish meal (nine treatments) in diets on the productive performance of tilapia fingerlings (*Oreochromis niloticus*). Four hundred and sixty tilapia fingerlings with an average initial weight of 2.8g/fish were used in this experiment. Nine fish groups (20 fish/each) were randomly distributed among the nine treatments. Each treatment had two replicates (aquarium). One hundred tilapia fingerlings were killed at the beginning of the experiment as a zero group and were frozen at -20 °C until chemical analysis. The average daily water temperature ranged between 23 and 29°C with an average of 26°C. A period of two weeks before the feeding experiment, fish fingerlings were acclimated to the aquarium condition and feeding regime.

From the nine fish meal samples, nine isonitrogenous (37% crude protein and isocaloric 4800 kcal GE/kg according to NRC, 1983) formulated feed mixtures were used and fed through 12 weeks experimental period. All the experimental diet component, were the same, except that fish meal was stored with three methods and treated with three levels of BHT antioxidant. The experimental diets consisted of 35% fish meal , 38% yellow corn, 20% soybean meal, 5% corn oil, 1.5% vitamin & mineral premix and 0.5% Ca Co_3. Chemical compositions of the experimental diets are shown in Table (1).

Feeding level of all experimental diets was 3% of the total fish biomass per day. Fish were fed three times daily (at 9.00, 12.00 and 15.00 h) and were weighed weekly.

Moisture, crude protein (CP), ether extract (EE), crude fiber (CF) and ash content of storing fish meal, diets and fish carcass were determined according to methods of A.O.A.C. (1984), while nitrogen free extract (NFE) was calculated by difference. Gross energy in feeds and fish carcass were calculated from their chemical composition, using the factors 5.65, 9.40, 4.00 and 4.00 (kcal/g) for protein fat, fiber and carbohydrate, respectively (Jobiling, 1983). The peroxide value was measured weekly for the experimental fish meal samples according to the method of Wheeler (1932). Data were statistically analyzed by SAS program (1988) SAS/STAT users guide, Release 6.03 Edition SAS Inst. Inc. Cary NC. USA.

RESULTS AND DISCUSSION

1-Storage experiment:

Results in Table (2) clearly indicated that fish meal stored in sun recorded the lowest ($P<0.05$) values of DM, EE and CP. Whereas the highest ($P<0.05$) values were noticed for that stored in store. However, approximately similar values were shown regarding Ash for fish meal stored with the three different methods. The present results are in full agreement with earlier observation of Lea *et al.*, (1960). They suggested that the losses of fat extractable by ether or light petroleum which occur when fish meal is stored in air are due to combination with the protein. However, their losses of extractable lipid are much smaller when chloroform methanol is the extracting solvent. With respect to effect of storage period on chemical composition of fish meal, data of Table (2) showed decreasing values of DM ($P> 0.05$), ether extract ($P< 0.05$) and CP ($P> 0.05$) as storage period increased. No differences ($P> 0.05$) were observed between ash content of the different storage periods. The significant EE reduction of fish meal after 26 weeks storage period is in line with that reported by Opstvedt *et al.*, (1970a) who found that ether extract of marine origin decreased with the length of storage of both stabilized and unstabilized fish meal. Inspection of the effect of BHT antioxidant on chemical composition of fish meal (Table 2) indicated similar values of DM and ash for fish meal

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

Item	Moisture %	Crude Protein %	Ether Extract %	Crude Fiber %	Ash %	N.F.E %	Energy kcal/g DM
Base diet	10.70	37.00	11.40	3.20	9.30	39.10	4831

Table 2. Effects of storage conditions, storage period and BHT antioxidant on chemical composition of fish meal (%on DM basis).

Treatment	D.M %	E.E %	C.P %	Ash %
Storage condition:				
S_1 (in store)	92.88[a]	10.70[a]	71.83[a]	16.67
S_2 (under shade)	92.70[b]	10.60[a]	71.79[a]	16.64
S_3 (in sun)	92.49[c]	10.30[b]	71.50[b]	16.68
L.S.M std. Err.	0.042	0.029	0.047	0.028
Storage period (weeks)				
0	93.00	11.00[a]	72.00	16.50
13	92.66	10.60[ab]	72.00	16.80
26	92.42	9.96[b]	71.20	16.70
L.S.M std. Err.	0.042	0.029	0.047	0.028
Antioxidant:				
L_1(without BHT)	92.68	10.40[c]	71.65[b]	16.67
L_2(with 125 ppm.)	92.71	10.50[b]	71.69[ab]	16.66
L_3(with 250 ppm.)	92.69	10.60[a]	71.82[a]	16.65
L.S.M std. Err.	0.042	0.029	0.047	0.028

a, b: Means with different superscripts in the same column are significantly different (P< 0.05).

treated with the different levels of the antioxidant (0, 125 and 250 p.pm). However, as the level of BHT was increased, the percentages of ether extract and CP were gradually increased (P< 0.05). The present results are in accordance with that of March *et al.*, (1962) who postulated that BHT treatment of herring meals retarded the decrease in ether extract. On the contrary, Moodie and Wessels (1974) treated fish meal with 0, 100 and 400 mg ethoxyquin (EQ) /kg, they found that moisture content varied while fat percentage declined with time in the fish meal that were not stabilized or that stabilized through the addition of only 100 mg EQ/Kg.

Lowest significant (P<0.05) peroxide value (149.2) was observed in fish meal stored in store (table 3). However, values of those stored under shade and in sun were higher (P< 0.05) than that in store by about 32.7 and 26.6%, respectively. Peroxide number of fish meal stored under shade was higher (P< 0.05) than that in sun by about 4.8%. The lowest value of peroxide number of fish meal stored in store may probably be attributed to the best condition in the store in which a relatively lower storage temperature was found. The relative higher storage temperature of the other tow storage

Table 3. Effects of storage condition and antioxidant on peroxide number of fish meal.

Item	Storage condition			Antioxidant		
	S_1 (in store)	S_2 (under shade)	S_3 (in sun)	L_1 (without BHT)	L_2 (with125 ppm)	L_3 (with250ppm)
Peroxide number	149.2^c	198.0^b	188.9^b	172.1^b	182.1^a	181.9^a

a, b: Means with different superscript in the same column are significantly different (P< 0.05).

conditions may accelerate oxidation. Such results are in agree with that of Hardy (1980) who concluded that the most effective prevention of oxidation is storage at low temperature. Also, kulikov (1978) found in his experiment on production of fish meal, oil and protein-vitamin preparation, that the increase in peroxide value could be attributed to oxidation of nutrients such as lipids and fatty acids, fat-soluble vitamins and vitamin C. He added that in order to decrease the rate of oxidation, the storage temperature should be reduced and storage condition must be properly controlled. Opstvedt (1973) reported that some oxidation took place during the drying fish meal in hot air.

It was surprised to observe that both levels of BHT (125 and 250 ppm) caused a significant (P<0.05) increase in peroxide number than control by 5.8 and 5.7%, respectively. These results clearly showed no beneficial effect of adding BHT on peroxide number of fish meal. This may indicate that treated fishmeal with 125 ppm ethoxyquin alone (from the origin) is sufficient for stabilization against oxidation. However, a way of interaction between the two antioxidants (Ethoxyquin and BHT) was happened and negatively affected the peroxide number. These results are in accordance with the finding of Murai and Andrews (1974). They concluded that 125mg/kg ethoxyquin alone was sufficient to curb oxidation when added to diet with 100 g menhaden oil/kg diet during 16 weeks of storage.

2-Feeding experiment:

It is clearly shown (table 4) that although averages of initial weight of fish for the different experimental groups were the same, however, all the tested growth parameters (gain, ADG, SGR) showed highest values (P> 0.05) for groups fed fish meal stored in store while lowest values were obtained for those fed fish meal stored in sun. The relative or apparent obvious negative effects of fish meal stored either under shade or in sun on the tested growth parameters of fish may probably be attributed to their higher storage temperature than that in store. Hence, storage of fish meal at high temperature may cause fat oxidation and also reduce the available lysine with different percentages (Lea *et al.*, 1960). Also, fish meal stored at high temperature may cause sever damage to the hepatopancreatic cells (De La Cruz *et al.*, 1989), and according to Travis (1955) and Johnson (1980), the hepatopancreas is responsible for active metabolic reactions and also for storage of lipids used in cellular metabolism, moulting and reproduction. With regard to FCR, the lowest (P>0.05) value was noticed for group fed fish meal stored in store, while highest (P>0.05) value was obtained for group fed fish meal stored in sun. These results are in accordance with those of De La Cruz *et al.*, (1989), who found that feed conversion rate value was higher for prawns fed diet stored at the high temperature (40°C), than those fed diet stored at the low temperature (0 and 10 °C). Also, feed stored at high temperature

Table 4. Effect of storage condition and antioxidant supplementation of fish meal on growth parameters of *O. niloticus* fingerlings.

Treatment	Initial weight	Final weight	Gain	A.D.G.	S.G.R..	feed intake	F.C.R.	P.E.R.	P.P.V.	Energy
	g/fish	g/fish	g/fish	Mg/d/fish	%/d.	g/fish	g DM/g gain		%	Utilization
Storage condition										
S1 in store	2.86	9.97	7.11	84.58	1.48	13.58	1.91	1.42	33.00	26.88
S2 under shade	2.85	9.66	6.81	81.22	1.43	13.23	1.94	1.36	31.02	25.10
S3 in sun	2.87	8.71	5.84	69.63	1.33	12.77	2.19	1.23	28.35	22.33
L.S.M. Std Error ±	0.02	0.66	0.64	0.07	0.07	0.497	0.12	0.08	2.34	1.92
Energy										
Antioxidant										
L1without BHT	2.87	10.32	7.45	88.70	1.49	13.22	1.87	1.49	34.58	27.08
L2with125 ppm BHT	2.87	8.78	5.91	70.23	1.32	13.00	2.20	1.22	28.55	23.87
L3with250 ppm BHT	2.82	9.24	6.43	76.50	1.41	13.37	2.08	1.30	29.22	23.37
L.S.M. Std Error ±	0.022	0.66	0.65	7.72	0.07	0.497	0.12	0.08	2.34	1.91

develops oxidative rancidity, which affects growth and consumption in amphipods (Stuart *et al.*, 1985). The same trend was obtained with regard to PER and PPV% (table 4). Fish group fed fish meal stored in store showed the highest value of PER and PPV% ($P>0.05$), while the lowest was for that fed fish meal stored in sun. Also, energy utilization had the same trend, the best ($P>0.05$) was shown for group fed fish meal stored in store while the worst was that of group fed fish meal stored in sun. The relative or apparent obvious negative effects of fish meal stored either in sun or under shade on feed efficiency may be possibly attributed to their higher temperature than that of in store. Storage of fish meal at high temperature may cause protein damage due to peroxidizing lipids (Narayan and kumerow, 1958 and Tappel, 1955). Numerous studies have been conducted to investigate the mechanisms involved in the reaction between protein and oxidizing lipids. The findings have been discussed by Eichner (1969) and Varma (1967). The most pronounced results of the protein-lipid reaction are the formations of polymers by cross-linking of proteins and/or by cross-linking of protein through oxidation products of fatty acids. The polymers formed are resistant to enzymatic hydrolysis (Cuq *et al.*, 1973; Tannenbaum *et al.*, 1969). In addition, individual amino acids may be rendered unavailable to animal by the reaction of carbonyl compounds from oxidizing fatty acids with the ϵ-amino group of lysine and the terminal groups of proteins (Bjarnarson & Carpenter, 1970) and by oxidative changes of labile amino acids (Njaa, 1962).

With respect to the effects of antioxidant, data of Table (4) clearly showed that both antioxidant (BHT) supplemented groups (L_2 and L_3) had insignificant effects compared to control (L_1) on weight gain, ADG, SGR, PER, PPV% and poorest values of FCR. Such results confirm no beneficial effects of adding the BHT antioxidant on fish performances. These may indicate also that treated imported fish meal with 125 ppm ethoxyquin (EQ) alone (from the origin) is sufficient to protect fish meal against oxidation and probably a way of interaction was happened between the two antioxidants (ethoxyquin and BHT) that decrease the studied growth parameters. Murai and Andrews (1974), observed significant growth reduction ($P<0.05$) during the first 8 weeks in fish fed diet without supplemental α- tocopherol and ethoxyquin compared with fish fed a diet containing either antioxidant. Also, Murai and Andrews (1974), found that feed conversion data were highly correlated to growth results. The poorest conversion was obtained from fish fed diets containing 100 mg/Kg oxidized menhaden oil, low levels of α- tocopherol and no ethoxyquin. In the remainder of dietary groups, which contain ethoxyquin or no oxidized menhaden oil, conversion ratios were 1.1-1.3 g feed/g gain, respectively.

Data of Table (5) indicated approximately similar values of moisture content, CP and Ash of the whole body for fish fed fish meal stored by the different experimental methods. Results of ether extract content showed very close values for whole body of fish fed fish meal stored either in store or under shade (26.6 and 26.5%, respectively), while significant ($P<0.05$) lower EE value was recorded for fish fed fish meal stored in sun. (25.8%). A positive relationship was clearly observed between energy (kcal/g) and ether extract contents. The highest value of energy content was noticed for fish fed fish meal stored in store while, the lowest ($P<0.05$) one was shown for that fed diet containing fish meal stored in

Table 5. Effect of storage condition and antioxidant supplementation of fish meal on body composition and energy content of *O. niloticus* and their retained.

Treatment	Moisture	C.P	E.E	Ash	Energy*	Nutrient retained g/fish			
	%	%	%	%	kcal/g	Protein	Fat	Ash	Energy kcal/fish
Storage condition									
S1	65.80	55.80	26.60[a]	16.20	5.30[a]	1.67	0.85	0.48	17240.30
S2	65.90	55.50	26.50[a]	16.20	5.28[a]	1.55	0.82	0.45	16287.30
S3	66.10	55.90	25.80[b]	16.60	5.23[b]	1.35	0.66	0.42	13803.40
Antioxidant									
L1	66.10	56.30[a]	26.30	16.30	5.30[a]	1.73	0.85	0.49	17662.10
L2	65.70	55.50[b]	26.30	16.40	5.26[b]	1.38	0.72	0.41	14452.20
L3	66.00	55.40[b]	26.30	16.40	5.25[b]	1.46	0.76	0.44	15216.30
S.E.	0.42	0.25	0.17	0.15	13.757	0.162	0.074	0.04	1579.00

a,b: Means with the same superscript in each column are not significantly different (P>0.05).

*Calculated by the factors of Jobiling (1983) as 4.0, 5.6 and 9.44 kcal/g DM for carbohydrate, protein and fat, resp.

sun. This may probably be attributed to the relative high fat oxidation that occurred in fish meal stored in sun. The present results of chemical composition are in accordance with those obtained by Hung *et al.*, (1981) who found that no significant differences in carcass composition from control, when more highly oxidized oil was used in the practical diet.

It is of interest to observe similar trend of storage conditions on protein, fat, ash and energy retention in fish body (Table 5). The highest overall mean values ($P>0.05$) of these parameters were recorded for fish fed fish meal stored in store, while the lowest mean values were obtained for fish fed fish meal stored in sun. A positive relationship between fat and energy retention was noticed.

The relative or apparent obvious negative effects of fish meal stored either under shade or in sun on the retention of protein, fat, ash and energy of fish may by attributed to their higher storage temperature than that in store. Hence, storage of fish meal at high temperature may cause fat oxidation. The most likely mechanisms whereby the nutritive value of the protein of fish meal could be reduced as a result of oxidation of the lipid would be by reaction of aldehydes, peroxides, free radicals or other products of fat oxidation with amino acid and others reactive groups (Bjarnarson & Carpenter, 1970).

The effect of BHT antioxidant on body composition and energy content of *O.niloticus* (table 5) indicated similar values of moisture, E.E and ash for fish fed diets contained fish meal treated with the different levels of the antioxidant (0, 125 and 250 ppm). However, crude protein decreased significantly ($P<0.05$) when fish fed fish meal with both levels of BHT antioxidant. These results clearly indicate no beneficial effects of BHT antioxidant supplementation on chemical composition of the imported fish meal. Such results are in contrast with those of Opstvedt *et al.*, (1970b), who postulated that the feeding of EQ stabilized fish meal, reduced fat and increased water content of the carcass, compared to the feeding of unstabilized fish meal, but had apparently negligible effects on protein content.

Data of Table (5) clearly indicated higher values ($P>0.05$) of protein, fat, ash and energy retention of control group than those treated by the both levels of BHT (125 and250 ppm). However, fish group treated with the high BHT level (250 ppm) recorded higher values ($P>0.05$) than the low BHT level treated group. Here again, fat retention related positively with energy retention. The present results clearly indicate no beneficial effects of BHT antioxidant supplementation on the retention of protein, fat ash and energy.

Moodie and Wessels (1974) suggested that the stabilized pilchard meals did not give any greater nitrogen retention than the comparable unstabilized pilchard meal. But the difference between the nitrogen retention of chickens receiving the well-stabilized anchovy meal and that of the chickens receiving the comparable unstabilized anchovy meal was statistically significant.

It could be concluded from this study that the best way for storing the imported herring meal is in store and putted on wood floor in a vertical position, and there was a way of each row to allow a good ventilation around them, and a periodic check of peroxide levels should be done in order to detect the early stage of deterioration.

There isn't need for adding antioxidant for imported herring meal when it already has antioxidant (from the origin) which is sufficient for stabilization against oxidation.

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الأداء الإنتاجي لإصباعيات البلطى النيلى المغذاة على عليقة تحتوى على مسحوق سمك مخزن بطرق تخزين مختلفة و مزود بمضاد التأكسد (BHT).

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تم تخزين مسحوق السمك لمدة ٢٦ أسبوع تحت ثلاث طرق تخزين مختلفة (فى المخزن، تحت مظلة، فى الشمس) بدون إضافة أو إضافة مضاد التأكسد (BHT) وذلك بمستويين (١٢٥ و ٢٥٠ جزء فى المليون). تم تكوين تسع علائق و غذيت لمدة ٢٦ أسبوع ل٤٦٠ إصباعية بلطى نيلى بمتوسط وزن أولى ٢,٨ جم/سمكة. كل العلائق كانت تحتوى على حوالى ٣٧% بروتين و ٤٨٠٠ ك كالورى طاقة كلية/كجم عليقة. أظهرت نتائج تجربة التخزين أن أحسن طريقة لتخزين مسحوق السمك هى فى المخزن، و ان ليس هناك حاجة لإضافة مضاد التأكسد(BHT) و أن مضاد التأكسد المضاف فى الأصل مع مسحوق السمك المستورد (Ethoxyquin) وفر الحماية لمسحوق السمك ضد التأكسد و حدوث تغيرات فى التركيب الكيميائي. كما أظهرت نتائج تجربة التغذية أن تخزين مسحوق السمك فى المخزن و بدون اضافة مضاد التأكسد(BHT) أعطى أحسن النتائج فى قياسات النمو و الكفاءة الغذائية و التركيب الكيميائى لجسم السمكة.