EGYPTIAN JOURNAL OF AQUATIC RESEARCH **ISSN 1110-0354**
VOL. 30(A), 2004:25-42

EFFECTS OF PULP AND PAPER INDUSTRIAL EFFLUENT ON SOME BLOOD PARAMETERS, GONADS AND FLESH PROTEINS IN EXPERIMENTALLY EXPOSED STRIPED SEABREAM *LITHOGNATHUS MORMYRUS*

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Key words: *Industrial effluent – Blood cells – protein – amino acids - flesh – gonads Orthognathus mormyrus.*

ABSTRACT

Study of the effect of Pulp and Paper industrial effluent was performed on the striped seabream (Lithognathus mormyrus). Fish were exposed to sublethal concentrations of effluent for 4- week . Erythrocytes count RBC decreased significantly ($0.88x\ 10^6$ /m m^3 p <0.0 5) at concentration of 20 ml /L of effluent. A significant reduction in both haemoglobin content Hb (4.3 gm/100mL $p<0.01$) and packed cell volume PCV (34.9 % $p<0.01$) were detected at concentration of 20 ml/L of effluent compared to control 6.50gm/100mL and 43.0 %respectively. The corpuscular indices, as mean corpuscular volume MCVand mean corpuscular heamoglobin MCH increased with effluent concentrations compared to control (318 um^3 and 47.1pg respectively). The MCV was 386.5 um^3 at concentration of 20 ml/L of effluent ,while MCH was 49.5 pg at concentration of 10ml/L of effluent. Also the results indicated that the mean corpuscular heamoglobin concentration MCHC decreased significantly (12.9%$p<0.05$) at 20ml/L of effluent. These changes in corpuscular indices, MCVandMCH compensate the decrease in RBC,Hb,and PCV values resulting in macrocytic hypochromic anemia.The total white blood cells count WBC increased significantly with increasing effluent concentration (leucocytes count at control was $10.99x10^3mm^3$). Blood smears and kidney prints revealed that increasing waste concentration caused increase in small lymphocytes S.L and neutrophiles NT, that emphasize the compensatory and defensive reaction of fish to effluent. Protein content of gonads and flesh showed directly proportional reduction with effluent concentration compared to control. Seabream protein is characterized by high values of Aspartic and Glutamic amino acids. Flesh of seabream has high values of Alanine and Lycine, while ovaries and testis have higher values of Leucine, Isoleucine, and Arginine. Total amino acid content decreased by increasing effluent concentrations. Overall the changes in the TEAA and TNEAA reflected that of the amino acid pool. In contrast the FAA increased with effluent exposure. The change being reflected in both FEAA and FNEAA. It is concluded that the rate of breakdown of protein was higher than the anabolic processes during effluent exposure. Data also suggest that FAA is an unlikely primary energy substrate during stresses.

INTRODUCTION

The rapid development of industry and especially chemical industry has created serious problems of water pollution .Many of the toxic substances are lipophilic and weren't adversely affected by water. These substances

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accumulate in fish fatty tissues or become protein bound, so it is of importance to know the critical concentration above which humanbeing are affected and the commercial fish species become unsuitable food (EL-Ezaby, 1994).

Pollutants generally produce relatively rapid changes in blood characteristics of fish (Johansen *et al*.,1994; Moussa *et al*.,1994; Ezzat *et al*., 1998 and Rizkalla *et al*., 1999). Therefore haematological data can provide valuable information in assessing the health of fish and in monitoring stress responses. Larsson *et al*., (1980) noticed increased (PCV) and (Hb) values with a slightly decrease in number of lymphocytes on exposing flounders, *Platichthys flexus* to two concentrations of industrial effluents. Fish living in water polluted with bleached kraft mill effluents display increased (Hb) and (PCV) values and decreased white blood cells count (Oikari *et al*., 1985 and Andersson *et al* .,1988) . *Sparus auratus* and *Solea vulgaris* exposed to industrial effluents showed haemolytic anemia accompanied by leucocytosis (Wahbi,1992 and 1998) . Mourad (1995) recorded an increase in (Hb) content of *Tilapia zilli* exposed to copper works effluent for 14 days.

The toxic waste impact on protein of fish may lead to alteration in its structure, and subsequently in its building units of amino acids. In addition to the protein bound amino acids, the cellular tissue and fluid of the living organisms contain a permanent reservoir of free amino acids, which take part in metabolic reactions. Certain amino acids may be mobilized preferentially from the muscle due to exposure to various environmental influences. Oikari *et al.*,(1985); Kan (1987); Ghoneim (1989); EL –Sayed (1990); Khadre and Shabana (1991) and Wahbi (1992,1998) found a decrease in protein content on contamination with industrial effluents . An alteration in amino acids content was detected by Koehn (1978) due to salinity change. Jana *et al*.,(1986) and Abd-EL-Moneim *et al*., (1990) also found variation in amino acid values as a result of exposure to industrial waste containing heavy metals . Investigations during the last decade, have demonstrated free amino acids as a fuel in the energy metabolism (Fyhn, 1989; Ronnested and Fyhn, 1992). Siddiqui *et al.,* (1973) stated that free amino acids has no anatomical reality but represents the amino acids derived from food and as a result of tissues breakdown.

The objectives of the present study are: first to give a through and deep practical view about the biochemical changes in seabream exposed to different levels of paper and pulp industrial waste altering its flesh constituents as a major food source for mankind, and reserved protein of gonads that promote gonadal maturation. Second, the change in the amount and nature of the amino acids required by an organism to overcome physiological and ecological changes .Third this study also trying to assess the haematological changes which may help in displaying the toxic effect of the effluent.

MATERIALS AND METHODS

Eighty healthy seabream of an average total length of 150 mm (range 128 - 166 mm) and average weight of 99 g (range 85 - 105 g) were brought to laboratory from eastern harbor. They were maintained for acclimatization for two weeks in aerated aquarium of 80-liter capacity. They were fed on small shrimp through the holding and experimental period. The median lethal concentration (LC50-96 hr.) was carried out according to Franson (1980).

Effluent samples were collected weekly in plastic containers. Stored in refrigerator at $4C^0$ till analyzed according to Franson (1980).

Fish were randomly distributed among 8 glasses tanks with density of 10 fish per tank and assigned to the concentrations of 5,10,20 ml/L (80 L) of effluent and blank for 4- weeks (2 replicate aquarium for each concentration). Blood samples were collected by caudal peduncle excision. Erythrocytes count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular

haemoglobin concentration (MCHC) were determined according to Dacie and Lewis (1975), haemoglobin content (Vankampen, 1961) and haematocrit value (Britton, 1963). White blood cells count (WBC) was calculated using Shaw, 1930) method. Giemsa stain was found to be quite suitable for leucocyte differential count.Kidney prints were stained by Wright's stain.

Determination of total protein content was done according to Tsuyosh and James (1978).Total amino acids using Davis and Thomas (1973) method .Free amino acids were determined as described by Huggins and Colley (1971).The analysis and composition of total and free amino acids have done by Bechman 118/119 CI amino acid analyzer (faculty of agriculture).All amino acids values were expressed as gram percent of protein on dry bases, while the free amino acids are expressed as gram percent of dry tissue . Results were subjected to T test and Duncan's multiple range test for comparison of the means among different concentrations studied at the 5% probability level.

RESULTS AND DISCUSSION

Common features of the effluent

The physico-chemical characteristics of the effluent are summarized in Table (1). From this table it is cleared that the total solids, suspended solids, volatile solids, heavy metals and sulfate were higher than standards limits.

The high lethality of the effluent can be attributed to combine effect created by presence of sulfate, heavy metals resulting from pigments additives and inks used in printing that had toxic effect on fish. Also organic matter originated during paper converting processes causing oxygen depletion stress on fish. This oxygen depletion increase lethality of the waste. The LC_{50} (96h r) was found to be 35 ml/L of effluent.

Table 1: Characteristics of Varta paper processing waste water.

Parameters	Standerd Limits	Measured values
PH	6-9	7.4 ± 0.50
Turbidity (N.T.U.)	≤ 50	79.9 ± 5.50
Colour (P.C.S.)	Free	260 ± 23.5
Total solid (mg/L)	1200	1365 ± 95
Suspended solid(mg/L)	30	580 ± 35.5
Volatile solid(mg/L)	-	980 ± 47.0
Phosphate (mg/L)	1	14.5 ± 1.02
Sulphate(mg/L)	1	42.0 ± 2.5
Chemical **oxygen** demande (mg/L)	40	1870 ± 98.0
Trace metals		
Copper(ug/L)		180 ± 10
Ferrous(ug/L)		3550 ± 210
Zinc(ug/L)		12.5 ± 1.2
Manganese(ug/L)		270 ± 25
Lead(ug/L)		320± 30
Cadmium(ug/L)		2.3 ± 0.2
Total trace metals(mg/L)	1	4. 3348

Variation in cellular elements of blood

The physiological stress resulting from waste poisoning is clearly reflected by blood patterns of the experimented fish as shown in Table (2). It was found that the red blood cells count (RBC) decreased by increasing effluent concentrations from 1.27 at concentration of 5ml/L to 0.88 at concentration of 20ml/L of effluent . R ed blood cell mass as measured by packed cell volume (PCV) and haemoglobin content (Hb) of effluent exposed fish groups showed a progressive fall parallel to pollutant concentrations reaching a minimum values of 34.9,4.3 at concentration 20ml/L of effluent respectively. Khangarot and Tripathi (1991) attributed the decrease in the RBC to haemolytic crisis that results in severe anaemia in fish exposed to copper pollutant or due to reduction of haem synthesis affected by pollutants (Wintrobe, 1978).The decrease in both Hb and PCV in this work indicates that effluent exposed fish are anaemic .This results are in affirmative agreement with that investigated by Wahbi (1992 & 1998) and El-Ezaby (1994).Also Sastry and Sachdeva (1994) nd Nounou *et al*. (1997) found that RBC,Hb,and PCV decrease in *Channa punctatus* and *Clarias lazera* exposed to heavy metals .Nounou *et al*.,(1997) stated that this decrease resulted in macrocytic hypochromic anaemia from effluent stress.

Concerning the calculated blood indices mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH), Table (2) showed an increase in their values by increasing effluent concentration . MCV has a maximum value of 386.5 at concentration of 20ml/L of effluent, while MCH value was 49.5 at 10ml/L of effluent. The increase in (MCV) together with decrease in (RBC) and in (Hb) content confirms the occurrence of haemolytic anaemia in fish and exaggerates disturbances that occurred in both metabolic and haemopoietic activities of fish due to waste exposure.

The increase in (MCV) and (MCH) with decrease in (RBC), (Hb) and (PCV) values was previously recorded by Mukherjee and Sinha (1993) for major carp esposed to cadmium chloride for 2 weeks. It is clear also from Table (2) that the percentage of mean corpuscular heamoglobin concentration (MCHC) is significantly decreased (12.9 ± 1.15 $p \leq 0.05$) at 20ml/L of effluent. This result is also found by Woo and Chiu (1995) working on the effect of nitrite on sea bass, *Lates calcarifer***.** Abd-Alla *et al.,* (1991) assumed that variation in values of blood indices (MCV, MCH, and MCHC) may be a defensive mechanism against effluent toxicity through stimulation of erythropoesis.

In present work, the total leucocytes count (WBC) of control and polluted fish can be seen in Table (3). It is obvious that the total number of (WBC) increase from $10.99x10^3 \pm 0.68$ in control ones to $15.46x10^3 \pm 0.94$ at concentration of 20ml/L of effluent. Also, the percentage of both small lymphocytes (S.L) and neutrophiles(Nt) increased reaching maximum percentage of(69.5 & 21%) respectively at 20ml/L of effluent. This increase in number of leucocytes is a defensive reaction against pollutant stress. These investigations are in agreement with that of Abidi and Srivastava (1988) following exposure of *Channa Punctatus* to endosulfan , *Channel Catfish* to aflatoxin B, (Lovell and Jantrarotai,1990), *Mugil cephalus* to crude oil (Khadre and Shabana,1991) and *Sparus auratus* and *Solea vulgaris* to industrial effluents (Wahbi , 1992 & 1998).

Examination of Giemsa stained blood smears of control fish showed well developed erythrocytes and neutrophil (Nt) with bilobed nucleus, (plate A fig.1) Smears also showed developmental stages of erythrocytes and small number of senile erythrocyte, (plate A fig .2). Kidney prints of control fish showed developmental stages of white blood cells (plate A fig. 3). Examination of Giemsa stained blood smears

of polluted fish revealed that the number of both neutrophil (Nt) and small lymphocytes (S.L) cells were large at concentration of 10,20ml/L of effluent respectively (plate B Fig. 1&2). Also (plate B fig. 3&4) demonstrated that the number of senile erythrocytes(S.E) and developing erythrocytes(D.E) increased at higher concentrations of 10, 20 ml/Lof effluent. Kidney prints show increase in number of pronormoblast (PrN) at concentration of 20ml/L of effluent (plate B Fig.5) .Also an increase in number of myelocytes and lymphoblasts is detected at concentrations of 10,20 ml/L of effluent (plate B fig. 6,7). Neutrophilia and lymphocytosis processes occurring in peripheral blood smears, and an increase in number of developing erythrocytes myelocytes, and lymphocytes in kidney prints emphasize the compensatory and defensive reaction of fish to waste concentrations.

Table (2): Haematological values of control and waste treated striped seabream after exposure to different effluent concentrations For 4- weeks.

Items	**Concentration ml/L**			
	0%(Blank)	**5ml/L**	**10ml/L**	**20ml/L**
RBCx(10^6/mm ^{3})	1.35 ±0.22	1.27 ±0.20	1.11 ±0.14	0.88 ±0.13*
Hb(g/100ml)	6.50 ±1.43	6.22 ±1.01	5.54 ±0.90*	4.3 ±0.09**
PCV(%)	43.0 ±3.37	40.8 ±3.10	38.4 ±2.84*	34.9 ±2.20**
MCV(um^3)	318 ±55.9	322 ±40.6	346 ±35.1	386.5 ±26.2*
MCH(pg)	47.1 ±3.6	48.7 ±3.4	49.5 ±4.05*	48.9 ±5.50
MCHC(%)	15.1 ±1.50	15.1 ±1.42	14.6 ±1.02	12.9 ±1.15*

*Significant at p< 0.05

**Significant at p<0.01

Table (3): Total and differential white blood cells count of control and treated striped seabream after exposure to different concentrations of effluent for4- weeks.

Conc.	**TotalWBCx (10^3/mm^3)**	**Differential WBC**						
		Mean Agran%	**Mean S.L%**	**Mean L.L%**	**Mean Gran%**	**Mean Nt%**	**Mean Es%**	**Mean Bs%**
0% (Blank)	10.99± 0.68	67. 50	66. 50	10. 00	23. 50	18. 00	5. 00	0. 50
5ml/L	11. 75 ±0. 80	75. 50	67. 50	8. 00	24. 50	19. 00	5. 00	0. 50
10ml/L	14. 20 ±0. 88*	75. 00	68. 00	7. 00	25. 00	20. 50	4. 50	0. 00
20ml/L	15. 46 ±0. 94*	74. 50	69. 50	5. 00	25. 50	21. 00	4. 00	0. 50

*Significant at 0.05 level

Variation in flesh and gonads protein content

The effluent lead to alteration in flesh and gonads protein content of fish. Table (4) illustrated the total protein content in flesh and gonads of seabream during long term exposure to paper and pulp effluent. It is obvious that effluent exposure leads to reduction in total protein content of flesh and gonads of exposed fish compared to control . Maximum fail was at ovaries of fish exposed to 20 ml/L of effluent 42.33 g/100g dry protein (control 63.05 g/100g dry protein). Since the amino acids are the building units of fish protein , therefore the toxicity of waste will has its impact on amino acids . This is apparent in the results achieved in Table (5). Total amino acid content of fish flesh decreased by increasing waste level . The percentage of total amino acids content in the muscular protein of seabream fish in the control group is 88.00 %, thus, t is higher than those exposed to both5, 10 and 20ml/L concentrations waste groups (81.21%, 77.23%, and 75.50% respectively). The changes in the total essential amino acid (TEAA) and total non-essential amino acid (TNEAA) reflected that of the amino acid pool. At concentration of 20ml/ L of effluent, however the exceptions to the general decline were four NEAA(Aspartic acid, Glycine, Serine and cysteine). Qualitatively the predominant in TAA pool were Lysine, Aspartic acid and Glutamic amino acids. The sum of these amino acids percentages in fish exposed to 10ml/L of waste were (28.68%) decreased from that of the control group (31.46 %) . The changes in the free essential amino acids (FEAA), and free non - essential amino acids (FNEAA) in the free amino acids (FAA) pool with effluent concentrations in flesh of seabream is given in Table (6).The FAA content increased with effluent to a value of 17.69±0.42 at concentration of 10ml/L of effluent compared to 13.76±0.5 at control . It accounted for 22.91% of the TAA (in control 15.64%). It is evident from the table that in all instances there was a gradual increase in amount of FAA in the TAA. All the nine FEAA identified in this study increased significantly with effluent. They were dominated by Argenine and Threonine. The eight FNEAA identified showed increased values with effluent. They were dominated by Alanine and Glutamic acid.

Table 4: The mean values of muscular and gonads protein content (g/ 100 g dry tissue) of seabream exposed to paper and pulp industrial effluent .

	Effluent concentrations			
	0 ml/L	**5ml/L**	**10ml/L**	**20ml/L**
Flesh	70.04±2.7	64.78±2. 0	58.60± 1.8*	52.36± 1.6*
Ovary	63.05±1. 5	55.31± 1.3	49.75± 1.1*	42.33± 0.9**
Testis	58.89±1. 2	54.47± 1.2	49.27±1.0	44.3 4±0.8*

* Significant at 5%

** Significant at 1%

Table 5: Total amino acid content (±S E) in g/ 100 g protein on dry basis in flesh of seabream at different concentrations of waste as well as control. Values with the same superscript in each row are not significantly different (p>0.05)*

AMINO ACIDS	CONCENTRATIONS			
	CONTROL	5ml/L	10 ml/ L	20ml/L
Arginine	6.83±0.3[a]	5.46±0.2[b]	5.21±0.2[b]	4.70±0.1[c]
Histidine	2.89±0.1[a]	2.81±0.09[a]	1.91±0.07[b]	1.82±0.05[b]
Isoleucine	4.70±0.2 [a]	4.30±0.2[b]	4.39±0.2[b]	4.10±0.1[c]
Leucine	6.80±0.3[a]	6.17±0.2[b]	6.11±0.2[b]	5.60±0.1[a]
Lysine	8.73±0.2[a]	8.71±0.2[a]	8.70±0.2[a]	8.90±0.1[a]
Methionine	2.60±0.1[a]	2.30±0.1[b]	2.11±0.1[b]	1.75±0.09[c]
Phenil-alanine	4.35±0.1[a]	4.12±0.1[a]	3.79±0.1[b]	2.77±0.08[c]
Threonine	3.93±0.2[a]	3.80±0.2[a]	3.31±0.1[b]	2.41±0.09[c]
Valine	4.80±0.6[d]	4.51±0.5[c]	4.11±0.3[b]	3.91±0.3[a]
TEAA	44.80±0.6[a]	42.11±0.91[a]	39.11±0.82[b]	36.2±0.70[b]
Alanine	6.10±0.4[a]	5.50±0.3[b]	5.11±0.2[c]	4.31±0.2[d]
Aspartic acid	8.83±0.2[b]	7.88±0.2[a]	7.88±0.2[a]	9.60±0.3[c]
Cysteine	1.20±0.3[a]	1.25±0.2[a]	1.26±0.2[a]	2.16±0.3[b]
Glutamic	13.90±0.9[a]	12.40±0.8[b]	12.10±0.7[b]	11.70±0.5[c]
Glycine	4.39±0.2[a]	4.39±0.2[a]	4.38±0.2[a]	4.44±0.3[a]
Serine	3.25±0.1[a]	3.28±0.1[a]	3.25±0.1[a]	3.45±0.1[a]
Tyrosine	3.10±0.1[a]	2.77±0.2[b]	2.30±0.3[c]	1.90±0.3[d]
Proline	2.40±0.2[a]	2.10±0.2[b]	2.15±0.1[b]	1.80±0.1[c]
TNEAA	43.20±1.3[a]	39.10±1.20[b]	38.12±1.20[b]	39.30±1.0[b]
TAA	88.00±3.0[a]	81.21±2.11[b]	77.23±2.02[c]	75.50±1.70[c]

Table 6: Free amino acid content (±S E) in g /100g dry tissue on dry basis in flesh of seabream at different waste concentrations as well as control . Values with the same superscript in each row are not significantly different (p >0.05)* .

AMINO ACIDS	CONCENTRATIONS			
	CONTROL	5ml/L	10ml/ L	20ml/L
Arginine	0.96±0.05^{a}	1.18±0.04^{b}	1.17±0.04^{b}	1.23±0.05^{bc}
Histidine	0.78±0.06^{a}	0.76±0.05^{a}	0.94±0.03^{b}	1.02±0.04^{bc}
Isoleusine	0.65±0.04^{a}	0.70±0.03^{b}	0.74±0.02^{b}	0.92±0.04^{c}
Leusine	0.89±0.02^{a}	1.19±0.02^{b}	1.24±0.02^{bc}	1.21±0.01^{b}
Lysine	0.78±0.03^{a}	0.90±0.02^{b}	0.88±0.02^{b}	0.99±0.03^{bc}
Methionine	0.62±0.06^{a}	0.72±0.05^{b}	0.93±0.05^{c}	0.97±0.06^{c}
Phenylalanine	0.68±0.05^{a}	0.80±0.04^{b}	0.79±0.03^{b}	0.88±0.04^{bc}
Threonine	1.05±0.08^{a}	1.20±0.07^{b}	1.29±0.07^{b}	1.32±0.08^{c}
Valine	0.76±0.04^{a}	0.80±0.03^{a}	0.91±0.01^{b}	0.96±0.01^{b}
TFEAA	7.15±0.31^{a}	8.21±0.29^{b}	8.88±0.21^{c}	9.3±0.33^{d}
Alanine	1.11±0.10^{a}	1.23±0.09^{b}	1.31±0.09^{c}	1.39±0.10^{cd}
Aspartic acid	0.83±0.03^{a}	0.89±0.02^{a}	0.98±0.02^{b}	0.95±0.01^{b}
Cysteine	0.29±0.01^{a}	0.45±0.01^{b}	0.43±0.01^{b}	0.51±0.02^{bc}
Glutamic acid	1.23±0.04^{a}	1.34±0.03^{b}	1.42±0.03^{bc}	1.47±0.02^{cd}
Glycine	0.88±0.04^{a}	1.03±0.02^{b}	1.12±0.02^{bc}	1.09±0.02^{b}
Serine	0.83±0.04^{a}	0.98±0.03^{bc}	0.91±0.02^{b}	0.95±0.02^{b}
Tyrosine	0.69±0.02^{a}	0.75±0.01^{b}	0.79±0.01^{b}	0.91±0.02^{c}
Proline	0.76±0.02^{a}	0.94±0.01^{b}	1.09±0.02^{cd}	0.99±0.01^{bc}
TFNEAA	6.61±0.28^{a}	7.59±0.21^{b}	8.81±0.21^{d}	8.26±0.20^{c}
FAA	13.76±0.59^{a}	15.80±0.50^{b}	17.69±0.42^{c}	17.56±0.53^{c}

The mean amount of total essential amino acids (TEAA) and total non - essential amino acids (TNEAA) in the total amino acid (TAA) pool (protein bound + free) for each concentration in ovary of seabream are given in Table (7). It is clear that total amino acid content of fish ovary decreased by increasing effluent level. The percentage of total amino acids content in ovary protein of seabream fish in the control group is 82.52% , while in exposed fish to both 5,10 and 20ml/L concentrations of waste were (77.09%, 75.24% and 68.79% respectively) i.e. it decreased by a value of 16.64% at concentration of 20ml/L of effluent than control . The changes in the TEAA and

TNEAA reflected that of the amino acid pool. At concentration 20ml/L of effluent, however the exceptions to the general decline was Lysine. Qualitatively the predominant in TAA pool were Arginine, Leucine, Lysine, Aspartic acid and Glutamic acid. The sum of these amino acids percentages in fish exposed to 20ml/L of waste was (39.44 %), while at control group it was (43.07 %).

The FAA content of ovary protein of control and treated seabream fish were illustrated at Table (8). The FAA content increased with effluent to a value of 17.35±0.66 at concentration of 20 ml/L of effluent compared to 13.08±0.78 at control. It accounted for 25.22% of the TAA (in control 15.85%). All the nine free essential amino acids identified in this study increased significantly with effluent. They were dominated by Arginine, Leucine, Isoleucine and Threonine. The eight free non-essential amino acids identified showed increased values with effluent. They were dominated by Alanine, Aspartic acid, Glutamic acid Glycine and Serine .

Table (9) show total amino acids of seabream testis on exposing to different concentrations of waste . It is obvious that there is a gradual decline in TAA content of testis as effluent concentration increase, with maximum fail at concentration of 20ml/Lof effluent being 67.43% compared to 80.19% at control. This decline is reflected in both EAA and NEAA. The EAA and NEAA percentages at concentration of 20ml/L of waste were (35.62% and 31.81%) decreased from that of control (42.02% and 38.17%) by(15.24% and 16.66%) respectively. The predominant in TAA pool were Arginine, Leucine, Lysine, Aspartic acid and Glutamic acid.

The changes in free amino acids with effluent concentrations in testis of seabream were given in Table (10). The FAA content increased with concentration to a value of 16.79±0.44 at concentration of 20ml/L of effluent compared to 12.31±0.58 at control. It accounted for 24.90% of the TAA (in control 15.35%). From the table it is clear that there was a gradual increase in amount of FEAA and FNEAA with waste concentration. The nine FEAA identified were dominated by Arginine , Leucine , and Isoleucine . The eight FNEAA identified were dominated by Alanine, Glycine and Glutamic acid . From the above result it can be concluded that the waste decreases the protein content in seabream fish during long term exposure . This finding coincided with that reported by Oikari et al., (1985); Kan (1987); Ghoneim (1989); El-Sayed (1990); Khadre and Shabana (1991) and Wahbi (1992&1998) on contamination with industrial effluents. Also, the decrease in amino acids content recorded here was approved by Abd- El Moneim *et al*., (1990) who reported decrease in amino acids content of *Tilapia nilotica* exposed to subtoxic levels of plastic and electric company effluent. The increase in tissues and gonads content of free amino acid may account for the enhancement of protein catabolism and / or heavy metals constituting the paper and pulp manufacturing liquid waste to be extracted away in order to maintain cell volume as a result of hypersomatic stress effect . Koehn (1978) has postulated that reduced salinity leads to an increase in free amino acids content, this increase in FAA is due to protein catabolism. Jana *et al*., (1986) noted that an increase in free amino acid contents was recorded in all organs of *Clarias batrachus L*. by heavy metals in the order lead >aresenic >mercury. The decrease in essential amino acids as Arginine ,Lysine ,Isoleucine and Methionine with effluent concentrations indicate a decrease in the nutritional value of exposed fish .This result agree with the findings of Harper and Rodwell (1975) who found failure to support maximum growth in fish feed Lysine deficient diet.

Finally it appeared that the waste in its present form entails a special risk to fish and probably to the consumer .Laws and regulations should be updated for treatment of waste before discharging into sea.

Table7: Total amino acid content (± SE) in g/100g protein on dry basis in ovary of seabream at different concentrations of waste as well as control . Values with the same superscrip in each row are not significantly different (p>0.05)* .

AMINO ACIDS	CONCENTRATIONS			
	CONTROL	5ml/ L	10ml/L	20ml/L
Arginine	7.03±0.4^{a}	6.04±0.3^{b}	5.22±0.2^{b}	5.21±0.2^{b}
Histidine	2.25±0.2^{a}	1.83±0.1^{b}	1.80±0.1^{b}	1.79±0.1^{b}
Isoleucine	5.20±0.2^{a}	3.98±0.2^{b}	3.52±0.2^{b}	1.29±0.1^{c}
Leucine	7.13±0.3^{a}	6.39±0.3^{a}	6.00±0.2^{b}	7.06±0.3^{a}
Lysine	7.50±0.3^{b}	6.68±0.2^{a}	7.33±0.3^{b}	7.74±0.3^{c}
Methionine	2.31±0.1^{b}	1.83±0.2^{a}	1.80±0.2^{a}	1.79±0.2^{a}
Phenyl-alanine	4.13±0.2^{b}	3.83±0.2^{a}	3.79±0.2^{a}	3.72±0.1^{a}
Threonine	3.51±0.2^{a}	3.80±0.2^{a}	3.76±0.2^{a}	2.12±0.1^{b}
Valine	4.25±0.1^{a}	4.31±0.2^{a}	3.98±0.1^{b}	3.81±0.1^{b}
TEAA	43.02±1.8^{a}	38.50±1.5^{b}	37.01±1.2^{bc}	34.32±1.1^{d}
Alanine	4.95±0.2^{a}	4.45±0.3^{b}	4.44±0.3^{b}	4.09±0.2^{c}
Aspartic acid	8.61±0.3^{a}	8.70±0.3^{a}	8.68±0.3^{a}	7.58±0.2^{b}
Cysteine	1.10±0.1^{a}	1.15±0.1^{a}	1.12±0.1^{a}	1.05±0.09^{a}
Glutamic acid	12.80±0.8^{a}	12.50±0.6^{b}	12.37±0.7^{bc}	11.85±0.5^{a}
Glycine	4.20±0.2^{a}	4.22±0.2^{a}	4.20±0.2^{a}	4.07±0.1^{b}
Serine	3.12±0.1^{a}	3.24±0.2^{a}	3.17±0.1^{a}	1.66±0.2^{b}
Tyrosine	2.99±0.2^{a}	2.94±0.2^{a}	2.73±0.1^{b}	2.71±0.1^{b}
Proline	2.22±0.2^{a}	1.83±0.1^{b}	1.80±0.1^{b}	1.71±0.09^{bc}
TNEAA	39.50±1.7^{a}	38.59±1.8^{a}	38.23±1.5^{a}	34.47±1.9^{b}
TAA	82.52±3.5^{a}	77.09±3.3^{b}	75.24±2.7^{b}	68.79±3.0^{c}

Table 8: Free amino acid content (±SE) in g/100g dry tissue on dry basis in ovary of seabream at different waste concentrations as well as control . Values with the same superscript in each row are not significantly different (p>0.05)* .

AMINO ACIDS	CONCENTRATIONS			
	CONTROL	5ml/L	10ml/L	20ml/L
Arginine	0.99±0.05[a]	1.10±0.04[b]	1.19±0.04[c]	1.28±0.05[d]
Histidine	0.61±0.04[a]	0.79±0.03[b]	0.91±0.04[c]	1.05±0.04[d]
Isoleucine	0.89±0.04[a]	0.91±0.06[b]	0.95±0.05[b]	1.10±0.06[c]
Leucine	0.93±0.06[a]	0.93±0.05[a]	0.99±0.05[b]	1.08±0.06[c]
Lysine	0.67±0.03[a]	0.81±0.03[b]	0.98±0.01[c]	0.97±0.03[c]
Methionine	0.55±0.03[a]	0.69±0.02[b]	0.84±0.02[c]	0.93±0.02[d]
Phenyl-alanine	0.65±0.04[a]	0.71±0.04[b]	0.78±0.03[c]	0.83±0.03[d]
Threonine	1.02±0.06[a]	1.11±0.06[b]	1.17±0.05[c]	1.32±0.06[d]
Valine	0.72±0.05[a]	0.76±0.04[a]	0.82±0.04[b]	0.85±0.05[b]
FEAA	7.00±0.38[a]	7.77±0.51[b]	8.51±0.30[c]	9.35±0.35[d]
Alanine	0.92±0.06[a]	0.91±0.05[a]	0.98±0.04[b]	1.21±0.06[c]
Aspartic acid	0.81±0.04[a]	0.82±0.03[a]	0.91±0.04[b]	0.97±0.03b[c]
Cysteine	0.27±0.02[a]	0.31±0.02[a]	0.42±0.02[b]	0.51±0.02[c]
Glutamic acid	1.13±0.09[a]	1.17±0.07[a]	1.22±0.07[b]	1.31±0.06[c]
Glycine	0.84±0.07[a]	0.80±0.06[a]	0.93±0.05[b]	0.97±0.05[b]
Serine	0.80±0.07[a]	0.91±0.04[b]	0.98±0.04[b]	1.12±0.03[c]
Tyrosine	0.67±0.04[a]	0.76±0.04[b]	0.83±0.04[c]	0.94±0.03[d]
Proline	0.70±0.05[a]	0.82±0.03[b]	0.88±0.05[b]	0.99±0.04[c]
FNEAA	6.08±0.40[a]	6.10±0.29[a]	7.09±0.25[b]	8.00±0.31[c]
FAA	13.08±0.78[a]	13.87±0.80[a]	15.60±0.55[b]	17.35±0.66[c]

Table 9: Total amino acid content (± SE) in g /100 g protein on dry basis in testis of seabream at different concentrations of waste as well as control . Values with with the same superscript in each row are not significantly different (p>0.05)*

AMINO ACIDS	CONCENTRATIONS			
	CONTROL	5ml/L	10ml/L	20ml/L
Arginine	6.95±0.3^{a}	6.35±0.3^{b}	6.20±0.2^{b}	5.11±0.1^{c}
Histidine	2.21±0.1^{a}	2.01±0.2^{a}	1.78±0.1^{b}	1.90±0.1^{b}
Isoleucine	4.90±0.1^{a}	4.65±0.2^{b}	4.41±0.1^{c}	4.01±0.1^{d}
Leucine	7.08±0.2^{a}	6.85±0.2^{b}	6.40±0.2^{c}	6.10±0.1^{d}
Lysine	7.25±0.2^{a}	7.01±0.2^{b}	6.90±0.1^{b}	6.64±0.1^{c}
Methionine	2.12±0.1^{a}	1.90±0.1^{b}	2.01±0.1^{ab}	1.81±0.1^{b}
Phenyl-alanine	4.08±0.2^{a}	3.93±0.2^{a}	3.71±0.2^{b}	3.50±0.1^{c}
Threonine	3.45±0.2^{a}	3.13±0.2^{b}	3.25±0.1^{b}	2.88±0.2^{c}
Valine	4.10±0.1^{a}	4.05±0.1^{a}	4.09±0.1^{a}	3.91±0.2^{b}
TEAA	42.02±1.2^{a}	39.50±1.3^{b}	38.57±0.9^{b}	35.62±1.0^{c}
Alanine	4.80±0.2^{a}	4.20±0.2^{b}	4.00±0.1^{bc}	3.89±0.1^{cd}
Aspartic acid	8.35±0.2^{a}	8.31±0.2^{a}	8.11±0.2^{b}	7.84±0.1^{c}
Cysteine	1.02±0.1^{a}	0.91±0.1^{b}	0.84±0.1^{b}	0.77±0.1^{b}
Glutamic acid	12.31±0.7^{a}	11.69±0.6^{c}	11.90±0.7^{b}	10.13±0.5^{d}
Glycine	4.05±0.2^{a}	3.76±0.1^{b}	3.80±0.1^{b}	3.49±0.1^{c}
Serine	3.10±0.1^{a}	3.0±0.1^{b}	2.79±0.1^{c}	2.61±0.1^{d}
Tyrosine	2.79±0.1^{a}	2.13±0.2^{bc}	2.24±0.2^{b}	2.00±0.3^{cd}
Proline	2.05±0.2^{a}	1.88±0.1^{b}	1.78±0.1^{b}	1.17±0.09^{c}
TNEAA	38.17±1.2^{a}	35.58±1.3^{b}	35.10±1.1^{b}	31.81±0.9^{c}
TAA	80.19±2.4^{a}	75.08±2.6^{b}	73.67±2.0^{b}	67.43±1.9^{c}

Table 10: Free amino acid content (±SE) in g /100 g dry tissue on dry basis in testis of seabream at different waste concentrations as well as control . Values with the same superscript in each row are not significantly different (p>0.05)*

AMINO ACIDS	CONCENTRATIONS			
	CONTROL	5ml/L	10ml/L	20ml/L
Arginine	0.98±0.05^{a}	1.16±0.04^{b}	1.21±0.03^{b}	1.26±0.03^{b}
Histidine	0.60±0.04^{a}	0.87±0.03^{b}	0.90±0.02^{b}	1.11±0.02^{c}
Isoleucine	0.84±0.04^{a}	1.06±0.04^{b}	1.15±0.05^{b}	1.39±0.04^{c}
Leucine	0.92±0.06^{a}	0.91±0.04^{a}	1.06±0.04^{b}	1.09±0.03^{b}
Lysine	0.65±0.04^{a}	0.85±0.02^{b}	0.92±0.02^{c}	0.98±0.02^{cd}
Methionine	0.50±0.03^{a}	0.72±0.02^{b}	0.94±0.01^{c}	0.94±0.01^{c}
Phenyl-alanine	0.64±0.04^{a}	0.73±0.04^{b}	0.76±0.03^{b}	0.78±0.03^{b}
Threonine	1.00±0.07^{b}	0.91±0.05^{a}	1.01±0.06^{b}	1.27±0.06^{c}
Valine	0.69±0.02^{a}	0.71±0.02^{a}	0.84±0.02^{b}	0.89±0.02^{b}
FEAA	6.50±0.30^{a}	7.85±0.25^{b}	8.71±0.25^{c}	9.65±0.21^{d}
Alanine	0.89±0.04^{a}	0.86±0.03^{a}	0.88±0.03^{a}	1.01±0.04^{b}
Aspartic acid	0.79±0.03^{a}	0.78±0.03^{a}	0.85±0.02^{b}	1.00±0.01^{c}
Cysteine	0.25±0.02^{a}	0.25±0.02^{a}	0.33±0.02^{b}	0.39±0.01^{b}
Glutamic acid	1.09±0.06^{a}	1.09±0.05^{a}	1.17±0.06^{b}	1.22±0.05^{b}
Glycine	0.81±0.07^{b}	0.71±0.04^{a}	0.84±0.05^{b}	0.88±0.05^{b}
Serine	0.79±0.04^{a}	0.84±0.04^{b}	0.86±0.04^{b}	1.07±0.05^{c}
Tyrosine	0.62±0.03^{b}	0.55±0.03^{a}	0.68±0.03^{b}	0.69±0.03^{b}
Proline	0.65±0.03^{a}	0.84±0.02^{b}	0.87±0.02^{b}	0.98±0.01^{c}
FNEAA	5.81±0.28^{a}	5.90±0.21^{a}	6.40±0.23^{b}	7.14±0.23^{c}
FAA	12.31±0.58^{a}	13.75±0.46^{b}	15.11±0.48^{c}	16.79±0.44^{d}

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EXPLANATION OF PLATE

PLATE (A)

Fig:(1) .Blood smear- Giemsa stain of control fish group showing neutrophil (Nt) .X 1250

Fig :(2) .Blood smear- Giemsa stain of control fish group showing Basophilic normoblast (BN),Polychromatophil normoblast(PN), Orthochromatophil normoblast (ON) , Mature erythrocyte (ME), Senile erythrocyte (SE) and Eosinophil(ES) . X1250.

Fig: (3) . Kidney imprints - Wright's stain of control fish group Showing early myelocyte (ElM) , meta myelocyte (MM) and late myelocyte (LtM) . X1250.

PLATE (B)

Fig.(1): Blood smear-Giemsa stain of fish at concentration 10ml/L of effluent showing neutrophil (N) . X125

Fig.(2):Blood smear-Giemsa stain of fish at concentration 20ml/Lof effluent showing lymphocytes (L) . X125

Fig.(3): Blood smear-Giemsa stain of fish at concentration 10ml/L of effluent showing senile erythrocytes (SE). X1250

Fig.(4): Blood smear-Giemsa stain of fish at concentration 20ml/L Of effluent showing developing erythrocytes(DE). X1250

Fig.(5): Kidney imprints-Wright's stain of fish at concentration 20ml/L of effluent showing haemocytoblast (arrow), pronormoblast (PrN) and basophilic normoblast (BN).x1250

Fig.(6): Kidney imprints - Wright's stain at concentration 10ml/L showing haemocytoblast (Hcb) , early myelocyte (ElM) and late myelocytes (LtM) .X1250.

Fig.(7): Kidney imprints-Wright's stain at concentration 20ml/L showing developing erythrocytes (DE) and developing lymphocytes (DL). x 125.

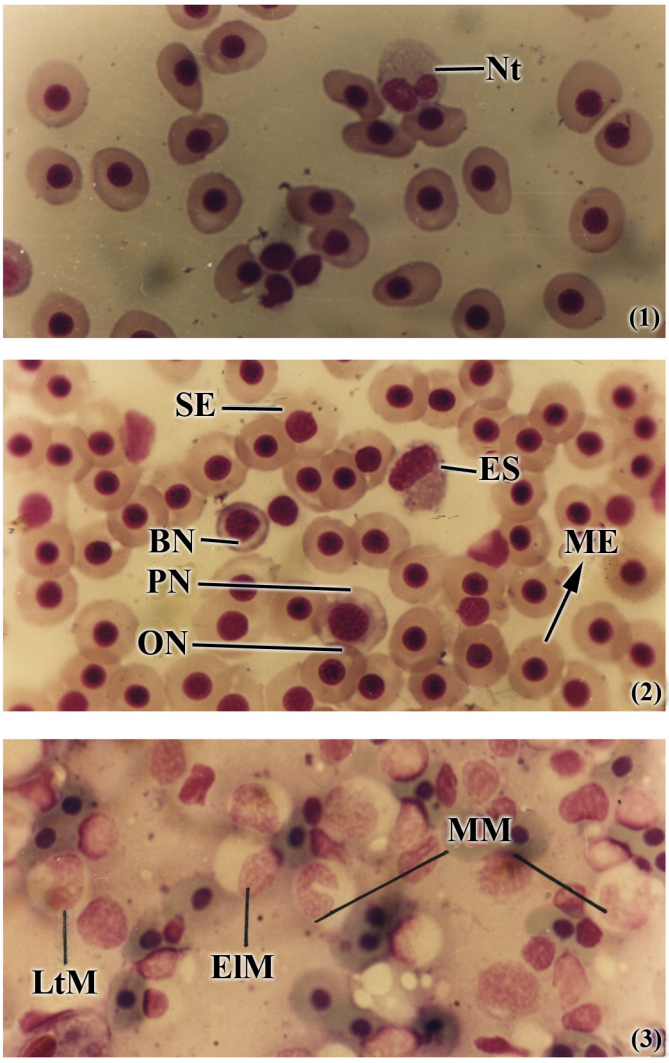

Plate (A)

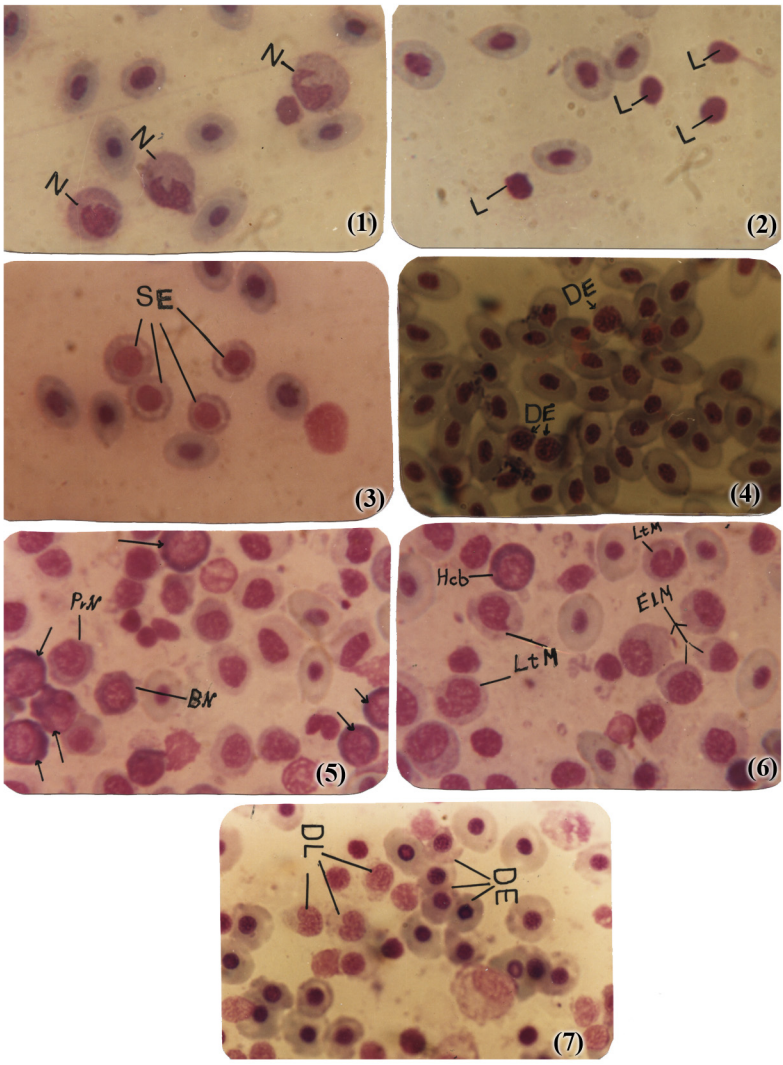

Plate (B)