SOME STUDIES ON THE IMMUNE STATUS AND GROWTH PERFORMANCE OF CULTURED *OREOCHROMIS NILOTICUS*

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ABSTRACT

The current study investigated the effects of methionine and lysine amino acids deficiency on freshwater fish on growth performances and different immunological parameters. A total number of 120 healthy fishes were divided into 4 groups each group subdivided into 3 sub group that distributed in 3 aquaria 10 fishes per each aquarium . The first group was fed on lysine deficient feed group, the second group was fed on methionine deficient feed group, the third group was fed on methionine and lysine deficient feed group, the fourth group fed on a basal diet as control formulated according nrc (1984) for fish recommendation.

The results concerning growth performance demonstrated that the deficiency of methionine and lysine causes decreasing of body weight, body weight gain and feed conversion ratio than the control group .

The results of blood revealed that, the lymphocytes, monocytes, basophiles and eosinophiles percentages decreased severely in deficiency of methionine and lysine followed by methionine only but the deficiency of lysine not affected severely on lymphocyte .

The results of serum glutamic oxalacetic transaminase (sgot) and serum glutamic pyruvic transaminase (sgpt) showed increase in their levels than control group .

The level of total protein, albumin and albumin / globulin ration showed a higher level in control group. Meanwhile, the lower level observed in the groups fed on methionine and lysine deficiency .

INTRODUCTION

In recent years, the need for an increase in the world's food supply is generally acknowledged. The serious shortage of animal proteins, manifested by the poor health condition of people in many regions of the world, together with the relative high price of animal proteins created a great demand towards fish, which provides protein of high digestibility and nutritive value. ***(Haggag and Saad, 2006).***

The surplus addition of lysine and methionine in the fish diet improve feed intake, feed conversion ratio, body weight growth, body weight gain, yield and immunity of the fish against different fish diseases ***(Eduardo et al., 2009).***

Therefore, the present work was designed to investigate the effects of methionine and lysine amino acids deficiency on freshwater fish production and immunity through study the effect of methionine and lysine on immunity and growth performances.

MATERIALS AND METHODS

2. 1. Experimental Fish:

A total number of 120 (42 ± 1 g) healthy fishes *Oreochromis niloticus*, were obtained from private fish farm. The fish were in good healthy conditions without any clinical manifestation . Fish were transported a live in plastic bags containing water enriched by air (2/3) to the Department of Poultry and Fish Disease laboratory, Faculty of Veterinary Medicine, Alexandria University, Egypt.

2. 2. Experimental feeding program and design :

Fish were fed on 4 manually prepared experimental fish diets (table, 1) as follow, (1) basal diet (NRC, 1984) , (2) metionine deficient diet, (3)lysine deficient diet, and (4) methionine with lysine deficient diet. The diets was daily provided at 3% of body weight (**Eurell *et al.*,1978).** The daily amount of food was offered on two concessions over the day (9 AM and 12 PM).

Table (1): Ingredient composition (%) of the basal diet

Ingredients	Diets physical composition %				Nutrients	Diets calculated chemical composition			
	(1)	(2)	(3)	(4)		(1)	(2)	(3)	(4)
Yellow corn	29.93	30.00	29.00	29.40	ME Ca l/Kg	3037	3020	2946	2940
Wheat bran	12.00	12.00	20.00	20.00	Crude protein	30.44	30.21	2946	29.03
SBOM (44%)	30.00	30.00	20.00	20.00	Lysine	1.43	1.41	1.07	1.07
Vegetable oil	5.00	5.00	4.00	4.00	Methionine	0.90	0.56	0.93	0.55
Fish meal (72%)	5.00	5.00	3.50	3.50	Methionine + Cystine	1.37	1.01	1.39	0.99
Glotin	15.00	15.00	20.50	20.50	Calcium	0.34	0.34	0.28	0.28
Di Ca PHO4	0.22	0.22	0.22	0.22	Available phosphorus	0.20	0.20	0.20	0.20
Limestone	0.08	0.08	0.08	0.08	Sodium	0.83	0.83	0.82	0.82
Lysine	0.11	0.08	0		Chlorine	1.29	1.29	1.28	1.28
Methionine	0.36	0.00	0.40	0.00					
Mineral Mix	0.15	0.15	0.15	0.15					
Vitamin Mix	0.15	0.15	0.15	0.15					
Binder	2.00	2.32	2.00	2.00					

*Chemical composition was calculated according to *NRC, 1984* tables of nutrient contents of feedstuffs.

**Vitamin mix and mineral mix were prepared according to *Jaouncy and Ross (1982)*

***Binders: Sodium carboxyl methyl cellulose (high viscosity) according to *Shiau et al. (1988).*

Table (2): Experimental Design

Groups	No. of fish	Total no. of fish
Lysine deficient feed group	10 10 10	120
Methionine deficient feed group	10 10 10	
Methionine + Lysine deficient feed group	10 10 10	
Control fed on ration complete in methionine and lysine	10 10 10	

2.3. Experimental parameters :

2.3.1. Effect of lysine and methionine deficiency on growth performances of *O. niloticus* :

During the experiment the fish were weighed weekly and the body weight gain, feed intake, feed conversion ratio was calculated (table, 3):

$$\textbf{Feed conversion ratio} = \frac{\text{Amount of feed intake (g) /Fish / period}}{\text{Body weight gain (g) /Fish / period}}$$

2.3.2. Hematological examination :

1) Fresh blood samples were collected weekly from caudal blood vessels from both treated and control fish):

Serum samples separated to determine:

a. Biochemical parameters: serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) according to ***Reitman and Frankle (1957)***, total protein ***(Doumas et al.,1981)***, Albumin ***(Reinhold (1953)*** and globulin was determined by subtract the total serum albumin from total serum protein according to ***(Coles, 1974).***

b. Citrated blood for measuring (white blood cells counts, differential leucocytic count, Phagocytic activity and Phagocytic index) according to ***(Lied et al., 1975).***

White blood cells, Red blood cells count, Blood hemoglobin and Packed cell volume (PCV %) PCV were determined according to ***(Stoskopf, 1993)*** where Red blood cell (RBCs) and white blood cell (WBCs) were counted by haemocytometer . Differential leucocytic count Blood film was taken and prepared according the method described by ***Schalm (1986).*** Phagocytic activity was determined according to (***kawahara et al, 1991***).

2.4. Statistical analysis:

The data of hematological and biochemical examinations of exposed fish were statistically analyzed using t-test, Duncan-test after ANOVA and simple correlation according to ***SAS (1987)*** to examine the significant effect of the main variables on the studied parameters. After that the results presented in the form of figures according to Harvard graphics 4 computer program.

RESULTS AND DISCUSSION

The results of growth performance: body weight, body weight gain, feed intake and feed conversion ratio (table 3) showed a significant differences among different weeks and different treatments of methionine and lysine supplement . The higher body weight showed in control group compared to deficient methionine and lysine fed group. Also, the methionine deficiency affected negatively on body weight, body weight gain and feed conversion than the lysine deficiency. This

results may be attributed to the methionine and lysine improvement of the feed utilization, body weight, body weight gain and feed conversion. This results agree with those of ***(Ren et al. (2000); Valentincic et al. (2000)*** ; ***Hrbek and Meyer (2003); Rolen et al. (2003); Rollin et al. (2003) ; Eduardo et al.(2009) and Liebert (2009)*** who indicated that the addition of lysine and methionine in the fish diet improve the body weight, body weight gain, and feed conversion. The addition of the lysine and methionine in the fish diet improved feed intake, feed conversion ratio, body weight, body weight gain, yield and immunity of the fish against different fish diseases ***(Rollin et al., 2003; Velez et al., 2005; Eduardo et al., 2009 and Liebert, 2009).*** And also addition of methionine and lysine in the fish diet improved the immunity of fish against the different diseases so it will improve the body weight and body weight gain with improvement of feed conversion and utilization ***(Caprio and Byrd,1984; Nordrum et al., 2000; Velez et al., 2005; Lee et al.,2007 and Eduardo et al., 2009).***

The amount of feed intake results showed that, the amount of feed intake not differ among different treated groups as it calculated on the base of 3 % from the body weight of fish this results agreed with those of ***(Saad, 2002)*** who reported that the amount of feed intake fed to fish differ according to the fish weight, species and diseases incidence and prevalence but commonly reached to about 3 % of the fish body weight.

The results of hematological studies (table 4) indicated that the lymphocytes, monocytes, basophiles and eosinophils percentages decreased severely in deficiency of methionine and lysine followed by

methionine only but the deficiency of lysine not affected severely on lymphocyte , but its effect on monocyte and basophiles is severely than the methionine deficiency. And the deficiency of Lysine and methionine only or with each other causes severe deficiency of lymphocyte, monocyte, basophiles and eposinophils but the control group of higher percentage than the deficiency of methionine and lysine alone or mixed with each other, and these results become very clear when we reach toward the end of the experiment. The results of WBCs, RBCs and PCV % count indicated that the total WBCs count decreased severely in deficiency of lysine and methionine, followed by lysine deficiency and all of them lower than the control group which have a higher WBCs count.

This results may be attributed to the deficiency of methionine and/or lysine causes degradation of the lymphoid tissue in the fish that causes decrease of the blood cells. This results agreed with those of ***(Fasuyl and Aletor, 2005)*** where they concluded that in the deficiency of lysine and/or methionine the lymphoid organs and tissues weights might be degenerated that decreased cellular humeral synthesis from lymphoid tissues and organs. Also, ***Ali (2006)*** observed that, deficiency of methionine and lysine causes decrease of all the blood parameters examined, the red blood cell count and mean cell volume showed significant decrease than the control. Other hematological values were lower than that of the normal values. The RBCs and WBCs counts showed lower than the control, also, ***Chaiyapoom et al. (2006)*** noticed that, hematocrit value, Hb % were lower than the control with deficiency

of methionine and/or lysine. Meanwhile, the level of phagocytic activity (PA) and phagocytic index (PI) showed sever decrease in deficiency of methionine and lysine, followed by lysine deficient group then the methionine deficient group and all of them lower than that of the control group.

The significant ($P< 0.01$) decrease PA and PI of methionine and/or lysine deficiency may be attributed to the destructive effect of their deficiency on liver, kidney, spleen and other haemopiotic organs , so it causes leucocytpenia and decrease the phagocytic activity and phagocytic index. This results may suggest a stress effect of methionine and lysine deficiency on fish which leads to increased level of serum cortisol. The increase of cortisol level may lead in turn to suppression of phagocytosis process. This suppression may be mediated directly via the corticosteroid receptors on macrophages or indirectly through the enhanced production of certain factors by the macrophages themselves which suppress the secretion of other macrophage products. The suppressive effect of corticoids is due to enhanced production of certain factors by the macrophages themselves (e.g. α-2 macroglobulin) which suppress other macrophage products. ***(Brunt and Austin, 2005).***

Biochemical analysis (table 5) showed that the serum enzymatic examinations GOT and GPT concentration indicated higher level in the groups fed methionine and lysine deficient diet followed by methionine deficient group, then by lysine deficient group and the lower level of GOT and GPT observed in control group. The significant increase of GOT and GPT enzymes in lysine and /or methionine deficiency may

reflect myocardial and hepatic degeneration that leading to extensive liberation of the enzymes into blood circulation ***(Saad, 2002 and Liebert, 2009)*** and this due to the lysine and methionine responsible for the healthy condition of liver and heart (***Ali, 2006 and Zhan et al., 2006***) .

The level of total protein, albumin and albumin / globulin ration showed a higher level in control group. Meanwhile the lower level observed in the groups fed on methionine and lysine deficient diet, followed by lysine deficient diet and the most lower level observed in the group fed on lysine and methionine deficient diet. Meanwhile the globulin level showed higher value in the group deficient in methionine and lysine, followed by methionine deficient group and lysine deficient group and the lower level observed in the control group. ***Ali (2006)*** observed that, deficiency of methionine and lysine causes the values of serum and liver total protein, albumin and globulin were lower, than, the control and noticed that hematocrit value.

These results proved by ***Nadia et al. (2004)*** who stated that the liver disorder is usually accompanied by hypoalbuminaemia. Both hypogamma globulinaemia and hypoalbuminaemia confirmed the recorded hypoproteinaemia, which was associated with liver damage, also indicated that the fish under stresses usually showing hypoalbuminemia ***(Maning and Wyatt, 1984)***.

It could be concluded that the dietary deficiency of methionine and /or lysine may alter the immune status and growth performance of cultured *Oreochromis niloticus*.

Table (3): Effect of dietary methionine and lysine deficiency on growth performance of *O. niloticus*.

Groups / Performance	1	2	3	4
Initial weight	A 42.80 ±0.86^{a}	A 42.20±0.97^{a}	A 42.20±1.11^{a}	A 42.60±0.51^{a}
Final weight	B 49.00±0.71^{b}	B 47.60±0.75^{b}	B 47.20±0.80^{b}	A 54.60±0.60^{a}
Total gain	B 6.2±0.85^{b}	C 5.40±0.85^{c}	C 5.00±0.90^{c}	A 12.0±0.55^{a}
Daily weight gain	B 0.22±0.02^{b}	C 0.19±0.01^{c}	D 0.17±0.01^{d}	A 0.42±0.02^{a}
Feed conversion	C 5.16±1.50^{c}	B 5.92±1.92^{c}	A 6.40±1.14^{a}	D 2.66±1.16^{d}

Means within the same row of different superscripts are significantly different at ($P < 0.01$).

Table (4): Effect of dietary methionine and lysine deficiency on hematological parameters , phagocytic activity and phagocytic index at the end of experiment.

Groups / hematological parameters	Lysine deficient group	Methionine deficient group	Methionine + Lysine eficient group	Control group
Lymphocyte	38.00±0.58^{c}	40.33±0.33^{b}	35.67±0.33^{d}	44.00±0.58^{a}
Monocyte	0.67±0.33^{c}	1.33±0.33^{b}	0.67±0.33^{c}	2.33±0.33^{a}
Basophiles	3.00±0.58^{a}	2.33±0.33^{b}	3.33±0.33^{b}	2.33±0.33^{b}
Eosinophils	8.33±0.33^{b}	7.33±0.33^{c}	8.33±0.33^{b}	9.33±0.33^{a}
Neutrophils	50.00±0.58^{a}	48.67±0.67^{a}	52.00±1.15^{b}	42.00±0.58^{c}
WBCs	18.00±0.58^{c}	18.33±0.33^{b}	16.00±0.58^{d}	22.67±0.33^{a}
RBCs	1.73±0.03^{b}	1.77±0.09^{b}	1.50±0.06^{c}	1.97±0.09^{a}
PCV	20.00±0.58^{b}	18.33±1.45^{c}	15.67±0.33^{d}	23.00±0.58^{a}
PA	20.00±0.58^{b}	18.33±1.45^{c}	15.67±0.33^{d}	23.00±0.58^{a}
PI	1.50±0.06^{b}	1.37±0.03^{c}	1.27±0.03^{d}	1.87±0.09^{a}

Means within the same column with different superscripts are significantly different at ($P < 0.01$).

Table (5): Effect of dietary methionine and lysine deficiency on biochemical parameters at the end of experiment.

Groups / Biochemical parameters	Lysine deficient group	Methionine deficient group	Methionine + Lysine deficient group	Control group
SGOT	67.00±1.15^{c}	69.00±0.58^{b}	72.33±0.33^{a}	64.33±0.88^{d}
SGPT	78.33±0.33^{b}	79.00±1.15^{b}	81.67±0.33^{a}	71.67±0.33^{c}
T. protein	4.20±0.06^{c}	4.40±0.06^{b}	4.07±0.03^{d}	5.00±0.06^{a}
Albumin	2.63±0.15^{b}	2.50±0.21^{c}	2.07±0.03^{d}	4.03±0.09^{a}
Globulin	1.57±0.13^{c}	1.90±0.20^{b}	2.00±0.06^{a}	0.97±0.07^{d}
A/G ratio	1.72±0.26^{b}	1.36±0.23^{c}	1.04±0.04^{d}	4.22±0.34^{a}

Means within the same column with different superscripts are significantly different at ($P < 0.01$).

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بعض الدراسات على الحالة المناعية و الإنتاجية لأسماك البلطى النيلى المستزرع

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يعتبر الليسين و الميثيونين من أهم العناصر الغذائية اللازمة لتغذية الأسماك والمحافظة على نموه وكفاءته الإنتاجية فهما من أهم العوامل المحددة للاستزراع السمكى وأريحية مزارع الأسماك ونجاحها ونقص الليسين و الميثيونين يسببا خسائر إنتاجية عالية و تعرض الأسماك لكثير من الأمراض.

لذا تهدف هذه الدراسة إلى تقييم مدىتأثير نقص الليسين والميثونين فى علائق أسماك البلطى النيلى المستزرععلى المناعة والكفاءة الإنتاجية.

لقد أجريت هذه التجربة على عدد 120 سمكة بلطى تم تقسيمها إلى أربع مجموعات حيث تتكون كل مجموعة من ثلاث مكررات وكل مكرر يحتوى على 10 أسماك حيث غذيت المجموعة الأولى على عليقة بها نقص فى الليسين وغذيت المجموعة الثانية على عليقة بها نقص فى الميثونين وغذيت المجموعة الثالثة على عليقة بها نقص فى كل من الليسين والميثونين بينما غذيت المجموعة الرابعة على عليقة تحتوى على النسب المقررة من الليسين والميثونين واستخدمت كمجموعة ضابطة.

وكان من أهم النتائج التى تم الحصول عليها من هذه التجربة أن نقص اليسين والميثونين يؤدى إلى نقص حاد فى أوزان الأسماك ومعدلات الزيادة فى أوزان الأسماك ، كما يقلل من معدل التحويل الغذائى للأسماك.

وأوضحت دراسة عينات الدم وجود تناقص شديد فى إجمالى عدد كرات الدم البيضاء والحمراء ونقص فى نسبة الهيموجلوبين بالدم فى الأسماك التى تعانى من نقص فى الليسين والميثونين، كما أوضحت الدراسة أن نقص الليسين والميثونين أدى إلى تناقص شديد فى الخلايا الليمفاوية والخلايا أحادية الخلية والخلايا القاعدية، والخلايا الحامضية الصبغة.

وأظهرت النتائج أيضاً وجود نقص فى مستوى البروتينات وأنزيمات الدم نظراً لنقص الليسين والميثونين.

ويستخلص من هذه الدراسة يتضح أن المحافظة على الليسين والميثونين والنسبة بينهما فى علائق الأسماك تزيد من الحالة المناعية للأسماك وتحسن من الكفاءة الإنتاجية للأسماك.