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Effect of Lysine and Methionine Deficiency on Immunity in Fresh Water Fish

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ABSTRACT

The effects of methionine and lysine amino acids deficiency on freshwater fish through study the effects on: different immunological parameters . The clinical signs of fish were severe emaciation, lethargy and its immunity against different fish diseases become very low. The results revealed that, the lymphocytes, monocytes, basophils and eosinophils percentages decreased severly in deficiency of methionine and lysine followed by methionine only but the deficiency of lysine not affected severly on lymphocyte . 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

Keywords:

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 addition and improving the lysine and methionine in the fish diet improve feed intake, feed conversion ratio, body weight, 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 through study the effect of the deficiency of them on differential leucocytic counts, phagocytic activity and index and relative level of protection.

MATERIALS AND METHODS

Materials

1) Fish :

A total number of 120 healthy fish *Oreochromis niloticus*, were obtained from private fish farm. The fish were in good healthy conditions without any clinical manifestation . Fish were transported a live to the laboratory Departement of poultry and fish disease of Faculty of veterinary medicine, Alexandria Unveristy in plastic bags containing water enriched by air (2/3).Average body weight of fish about (50 ± 5 gm).

2) Fish diets :

Fish were fed on a manually prepared fish diet containing 34.15 crude protein. The diet was daily provided at 3% of body weight as described by Eurell *et al.* (1978). The daily amount of food was offered on two concessions over the day. (at 9 AM and 12 PM). The structure of the diet used is presented in Table (1).

Table (1) : ***Show the ingredients of diet Deficient in Lysine:-***

INGREDIENTS	%
Ground yellow corn	30
Soya bean meal (44 % c.p.)	30
Corn gluten meal	15
Wheat bran	12.124
Fish meal (72 % c.p.)	5
Oil (corn)	5
Binders*	2
Vitamin mix & mineral mix**	0.3
Di calcium phosphate	0.216
Limestone	0.08
L-lysine (less than the normal value)	0.18
Vitamin C	0.1

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

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

Methods

One hundred and twenty fish of *(Oreochromis niloticus)* were divided into four groups for experimental work as in the following table:-

3) Haematological examination :

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

a. Serum samples separated to determine the different biochemical parameters (Total protein, Globulin and Albumin).

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

Table (2) : *Show the ingeredients of diet deficient in Lysine and Methionine:-*

INGREDIENTS	G/KG	%	G/KG	4 K
GROUND YELLOW CORN	**30**	**0.3**	**300**	**1200**
SOYA BEAN MEAL (44 %)	**30**	**0.3**	**300**	**1200**
CORN GLUTEN MEAL	**15**	**0.15**	**150**	**600**
WHEAT BRAN	**12.124**	**0.12124**	**121.24**	**484.96**
FISH MEAL (72 %)	**5**	**0.05**	**50**	**200**
OIL (CORN)	**5**	**0.05**	**50**	**200**
BINDERS*	**2**	**0.02**	**20**	**80**
VITAMIN MIX & MINERAL MIX**	**0.3**	**0.003**	**3**	**12**
DI CALCIUM PHOSPHATE	**0.216**	**0.00216**	**2.16**	**8.64**
LIMESTONE	**0.08**	**0.0008**	**0.8**	**3.20**
L-LYSINE	**0.18**	**0.0018**	**1.8**	**7.2**
VITAMIN C	**0.1**	**0.001**	**1**	**4**

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 . Blood hemoglobin (Hb gm %) was assessed by cyanomtahemoglobin method (Drubkin, 1964).

Differential leucocytic count

Blood film was taken and prepared according the method described by Schalm (1986).

Table (3): *Show the design of experiment*:

Groups	No. of fish	Total no. of fish
Methionine deficint feed group	10 10 10	120
Lysine 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	

Determination of phagocytic activity and phagocytic index

Phagocytic activity was determined according to Kawahara *et al.* (1991).

Clinico-biochemical analysis

Determination of serum total protein, serum albumin and serum globulin

Serum total protein was determination according to Doumas *et al.* (1981), Serum albumin was determined according to Reinhold (1953), Serum globulin was determined by subtract the total serum albumin from total serum protein according to (Coles, 1974).

RESULTS

I. Clinical signs and postmortem findings of experimentally infected fish

The clinical signs of fish which proved to be suffered from lysine and methionine deficiency, suffered from severe emaciation, lethargy and its immunity against different fish diseases become very low, appearance of some nervous signs, lower feed utilization with reduction in weight gain and growth in fish, haemorrhages on the body surfaces with exophthalmia.

Post-mortem examinations revealed the presence of enlargement of different body organs with congestion of all internal organs plus presence of the bloody fluid in the abdomen.

II. Results of haematological studies

Haematological examinations of the blood samples collected from ***O. niloticus*** are summarized in Table (4) .

The results revealed that, the **lymphocytes, monocytes, basophils and eosinophils** percentages decreased severly in deficiency of methionine and lysine followed by methionine only but the deficiency of luysine not affected severly on lymphocyte , but its effect on monocyte and basophils is severly than the methionine deficiciency. But the main limiting AA that affecting eosinophils is the methionine deficiency followed by methionine and lysine deficiency. And the deficiency of Lysine and methionine only or with each other causes severe deficiency of lymphocyte, monocyte, basophils 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 are summarized in Table (5) . The results indicated that the total WBCs count decreased severly in deficiency od lysine and methionine, followed by lysine

Table (4) : Effect of dietary methionine and lysine deficiency on differential leucocytic counts at different weeks.

Weeks	Groups	N	Lymphocyte	Monocyte	Basophils	Eosinophils	Neutrophils
			Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1st week	Lysine deficient group	3	B 41.33±0.33	A 1.33±0.33	A 5.00±0.58	A 8.00±0.58	B 44.33±0.67
	Methionine deficint group	3	B 41.00±0.58	B 0.67±0.33	C 2.33±0.33	A 8.33±0.88	A 47.67±2.03
	Methionine + Lysine deficient group	3	B 39.33±0.33	A 1.33±0.33	C 2.67±0.33	A 8.33±0.33	A 48.33±1.20
	Control group	3	A 45.00±0.58	A 1.33±0.33	B 3.00±0.58	A 8.33±0.88	B 42.33±1.20
2nd Week	Lysine deficient group	3	B 41.00±0.58	D 0.67±0.33	A 3.00±0.58	C 7.00±0.58	B 48.33±0.33
	Methionine deficint group	3	B 40.00±1.53	C 1.00±0.58	C 2.33±0.33	B 8.00±0.58	B 48.67±1.20
	Methionine + Lysine deficient group	3	C 36.33±0.33	B 1.33±0.33	D 1.67±0.33	A 9.33±0.33	A 51.33±0.88
	Control group	3	A 44.67±0.33	A 1.67±0.33	B 2.67±0.33	D 6.33±0.88	C 44.67±1.45
3rd Week	Lysine deficient group	3	B 40.00±0.58	C 0.67±0.33	B 2.67±0.33	B 6.67±0.33	A 50.00±0.58
	Methionine deficint group	3	B 41.00±0.58	D 0.33±0.33	B 2.67±0.33	B 7.33±0.33	B 48.67±1.20
	Methionine + Lysine deficient group	3	C 38.67±0.33	B 1.33±0.33	A 3.00±0.58	A 8.33±0.33	B 48.67±0.88
	Control group	3	A 42.67±1.20	A 1.67±0.33	B 2.67±0.33	A 8.33±0.88	C 44.67±0.67
4th Week	Lysine deficient group	3	C 38.00±0.58	C 0.67±0.33	A 3.00±0.58	B 8.33±0.33	A 50.00±0.58
	Methionine deficint group	3	B 40.33±0.33	B 1.33±0.33	B 2.33±0.33	C 7.33±0.33	A 48.67±0.67
	Methionine + Lysine deficient group	3	D 35.67±0.33	C 0.67±0.33	B 3.33±0.33	B 8.33±0.33	B 52.00±1.15
	Control group	3	A 44.00±0.58	A 2.33±0.33	B 2.33±0.33	A 9.33±0.33	C 42.00±0.58
	Total	48	40.56±0.40	1.15±0.10	2.79±0.13	7.98±0.17	47.52±0.48

The results of lysine and methionine deficiency was tabulated.

deficiency and all of them lower than the control group which have a higher WBCs count.

The **Hb %** showed in Table (5) showed severe decrease in its concentration in methionine followed by lysine followed by deficiency of both lysine and methionine and all of them of lower concentration than the control group.

The level **PA and PI** showed severe decrease in deficiency of methionine and lysine, followed by lysine deficient groups then the methionine deficient group and all of them lower than that of the control group. (Table, 6) .

Table (5): ***Effect of dietary methionine and lysine deficiency on.WBCs, RBCs, Hb and PCV% at different weeks.***

Weeks	Groups	N	WBCs	RBCs	Hb	PCV%
			Mean Std. Error	Mean Std. Error	Mean Std. Error	Mean Std. Error
1st Week	Lysine deficient group	3	B 20.33±0.33	B 1.53±0.03	B 9.33±0.33	B 26.00±0.58
	Methionine deficint group	3	C 19.00±0.58	B 1.80±0.06	C 7.67±0.33	BC 25.00±0.58
	Methionine + Lysine deficient group	3	D 17.33±0.33	B 1.47±0.03	D 7.00±0.58	C 24.67±0.88
	Control group	3	A 21.33±0.33	A 2.00±0.06	A 9.67±0.33	A 30.33±0.33
2nd Week	Lysine deficient group	3	A 21.33±0.33	B 1.67±0.03	B 8.00±0.58	B 25.00±0.58
	Methionine deficint group	3	B 20.33±0.33	B 1.77±0.09	C 7.00±0.58	B 24.33±1.76
	Methionine + Lysine deficient group	3	C 18.00±0.58	C 1.47±0.03	D 6.33±0.33	C 21.33±0.33
	Control group	3	A 21.00±0.58	A 2.03±0.03	A 9.00±0.58	A 28.33±1.45
3rd Week	Lysine deficient group	3	B 19.33±0.88	B 1.80±0.06	B 8.67±0.33	B 25.00±0.58
	Methionine deficint group	3	B 20.33±0.33	BC 1.77±0.03	D 8.00±0.58	C 23.33±0.88
	Methionine + Lysine deficient group	3	C 18.33±1.45	C 1.60±0.17	C 8.33±0.33	D 20.67±0.33
	Control group	3	A 23.00±0.58	A 2.10±0.06	A 9.67±0.33	A 31.33±0.33
4th Week	Lysine deficient group	3	C 18.00±0.58	B 1.73±0.03	C 7.67±0.33	B 20.00±0.58
	Methionine deficint group	3	B 18.33±0.33	B 1.77±0.09	D 7.00±0.58	C 18.33±1.45
	Methionine + Lysine deficient group	3	D 16.00±0.58	C 1.50±0.06	B 8.00±0.58	D 15.67±0.33
	Control group	3	A 22.67±0.33	A 1.97±0.09	A 10.33±0.33	A 23.00±0.58
	Total	48	19.67±0.30	1.75±0.03	8.23±0.19	23.90±0.61

Table (7) explain that the**, Total protein, Albumin, Globulin and Albumin/Globulin ratio .**The level of Total protein, albumin and albumin / globulin ratio showed a higher level in control group. Meanwhile the lower level observed in the groups fed on methionine and lysine deficiency, followed by lysine deficiency and the most lower level observed in the groups fed on lysine and methionine deficient fed. Meanwhile the globulin level showed higher value the group defcient in methionine and lysine, followed by methionine deficient and lysine deficient groups and the lower level observed in the control group.

Table (6): *Effect of dietary methionine and lysine deficiency on Phagocytic activity and Phagocytic index at different weeks.*

Weeks	Groups	N	PA	PI
			Mean Std. Error	Mean Std. Error
1st Week	Lysine deficient group	3	B 19.33±0.88	B 1.50±0.06
	Methionine deficint group	3	C 18.33±0.33	B 1.47±0.03
	Methionine + Lysine deficient group	3	D 16.00±0.58	C 1.37±0.03
	Control group	3	A 21.00±0.58	A 1.73±0.09
2nd Week	Lysine deficient group	3	A 20.67±0.33	B 1.50±0.06
	Methionine deficint group	3	B 18.33±0.33	C 1.37±0.03
	Methionine + Lysine deficient group	3	C 16.33±0.33	D 1.30±0.06
	Control group	3	A 20.33±0.33	A 1.80±0.06
3rd Week	Lysine deficient group	3	C 21.33±0.33	B 1.57±0.03
	Methionine deficint group	3	B 20.67±0.33	C 1.37±0.03
	Methionine + Lysine deficient group	3	D 16.67±0.33	D 1.33±0.03
	Control group	3	A 23.33±2.03	A 2.00±0.06
4th Week	Lysine deficient group	3	B 20.00±0.58	B 1.50±0.06
	Methionine deficint group	3	C 18.33±1.45	C 1.37±0.03
	Methionine + Lysine deficient group	3	D 15.67±0.33	D 1.27±0.03
	Control group	3	A 23.00±0.58	A 1.87±0.09
	Total	48	19.33±0.37	1.52±0.03

Table (7): ***Effect of dietary methionine and lysine deficiency on total protein, albumin, globulin and albumin/globulin ratio at different weeks.***

Weeks	Groups	N	T.protein Mean Std. Error	Albumin Mean Std. Error	Globulin Mean Std. Error	A/G ratio Mean Std. Error
1st Week	**Lysine deficient group**	**3**	**B** **4.53±0.03**	**B** **2.77±0.09**	**A** **1.77±0.12**	**B** **1.59±0.15**
	Methionine deficint group	**3**	**C** **4.40±0.06**	**C** **2.63±0.03**	**A** **1.77±0.03**	**C** **1.49±0.02**
	Methionine + Lysine deficient group	**3**	**D** **4.30±0.12**	**D** **2.53±0.03**	**A** **1.77±0.15**	**C** **1.46±0.14**
	Control group	**3**	**A** **5.00±0.06**	**A** **3.67±0.03**	**B** **1.33±0.09**	**A** **2.78±0.20**
2nd Week	**Lysine deficient group**	**3**	**B** **4.53±0.03**	**C** **2.53±0.09**	**A** **2.00±0.12**	**C** **1.28±0.12**
	Methionine deficint group	**3**	**C** **4.47±0.03**	**C** **2.53±0.03**	**B** **1.93±0.03**	**C** **1.31±0.03**
	Methionine + Lysine deficient group	**3**	**BC** **4.50±0.06**	**B** **2.57±0.20**	**B** **1.93±0.15**	**B** **1.36±0.20**
	Control group	**3**	**A** **5.17±0.09**	**A** **3.57±0.03**	**C** **1.60±0.06**	**A** **2.23±0.06**
3rd Week	**Lysine deficient group**	**3**	**C** **4.23±0.03**	**B** **2.50±0.12**	**C** **1.73±0.15**	**B** **1.47±0.19**
	Methionine deficint group	**3**	**B** **4.33±0.15**	**C** **2.33±0.09**	**B** **2.00±0.20**	**C** **1.19±0.14**
	Methionine + Lysine deficient group	**3**	**D** **4.10±0.06**	**D** **1.80±0.06**	**A** **2.30±0.10**	**D** **0.79±0.06**
	Control group	**3**	**A** **5.03±0.03**	**A** **3.63±0.03**	**D** **1.40±0.06**	**A** **2.61±0.13**
4th Week	**Lysine deficient group**	**3**	**C** **4.20±0.06**	**B** **2.63±0.15**	**C** **1.57±0.13**	**B** **1.72±0.26**
	Methionine deficint group	**3**	**B** **4.40±0.06**	**C** **2.50±0.21**	**B** **1.90±0.20**	**C** **1.36±0.23**
	Methionine + Lysine deficient group	**3**	**D** **4.07±0.03**	**D** **2.07±0.03**	**A** **2.00±0.06**	**D** **1.04±0.04**
	Control group	**3**	**A** **5.00±0.06**	**A** **4.03±0.09**	**D** **0.97±0.07**	**A** **4.22±0.34**
	Total	**48**	**4.52±0.05**	**2.77±0.09**	**1.75±0.05**	**1.74±0.12**

For each week : means within the same column of different litters indicated that there is significant difference at (P < 0.01) between different treatment group.

DISCUSSION

The clinical signs of fish which proved to be suffered from lysine and methionine deficiency, suffered from severe emaciation, lethargy and its immunity against different fish diseases become very low, appearance of some nervous signs, lower feed utilization with reduction in weight gain and growth in fish, haemorrhages on the body surfaces with exophthalmia. These results agreed with those of (*Lee et al.,2007)* where they reported that, the clinical signs due to methionine and/or lysine deficiency attributed to the poor feed utilization and efficiency appear in the form of emaciation, nervous manifestation and haemorrhages on the body surface.

Meanwhile, the results of Post-mortem examinations revealed the presence of enlargement of different body organs with congestion of all internal organs plus presence of the bloody fluid in the abdomen this results agreed with those of (*Wergedahl et al., 2004)* where they reported that the appearance of Post-mortem lesions attributed to the disturbances in feed metabolism and all vital metabolic process in the body due to methionine and lysine deficiency or anly one of them.

The most important Histopathologic results indicated that, generally, the nature of the encountered lesions due lysine and methionine deficiency depend up on concentration of the methionine and lysine and if the deficiency lysine only or methionine only or both with each other. This results agreed with those of *(Rollin et al., 2003)* where they reported that, the effect of amino acids in fish differ according to the concentration of amino acids in the diet, fish species and environmental conditions around the fish.

The results of haematological studies indicated that, the the lymphocytes, monocytes, basophils and eosinophils percentages decreased severly in deficiency of methionine and lysine followed by methionine only but the deficiency of luysine not affected severly on lymphocyte , but its effect on monocyte and basophils is severly than the methionine deficiciency. And the deficiency of Lysine and methionine only or with each other causes severe deficiency of lymphocyte, monocyte, basophils 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 severly in deficiency od lysine and methionine, followed by lysine deficiency and all of them lower than the control group which have a higher WBCs count. The Hb % indicated that, there is a severe decrease in its concentration in methionine followed by lysine followed by deficiency of both lysine and methionine and all of them of lower concentration than the control group.

This results may be attributed due to the deficiency of methionine and/or lysine or with each other causes degredation of the lymphjoid 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, the deficiency of lysine and/or methionine the lymphoid organs and tissues weights might be degenerated that decreased cellular humeral synthsis from lymphoid tissues and organs. Also, *Ali (2006)* observed that, deficiency of methionine and lysine causes of all the blood parameters examined, the red blood cell count and mean cell volume showed significant decrease than the control. Other haematological values were lower than that of the normal values. The RBCs and WBCs counts showed lower than the the control, also, *Chaiyapoom et al. (2006)* noticed that, hematocrit value, Hb % were lower than the control with deficiency of methionine and/or lysine or with each other.

Meanwhile, the level PA and PI showed sever decreased 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 of phagocytic activity and phagocytic index of methionine and/or lysine deficiency or both 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 serm cortisol. The increase of cortisol level may lead in turn to suppression of phagocytosis process. This suppression may may be mediated directly via the corticosteriod receptors on macrophages or ndirectly through the enhanced production of certain factros by the macrophages themselves, which suppress the screation of other macrophage products the suppressive effect of corticoides 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).*

The, Total protein, Albumin, Globulin and Albumin/Globulin ratio (Serum proteins). 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, followed by lysine deficiency and the most lower level observed in the group fed on lysine and methionine deficient fed group. Meanwhile the globulin level showed higher value the group defcient 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. noticed that, hematocrit value, Hb % were lower than the control with deficiency of methionine and/or lysine or with each other.

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

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تأثير نقص الليسيين والميثايونين على المناعة في أسماك المياه العذبة

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تأثير نقص الأحماض الأمينية مثــل الليســين الميثيــونين علــى الأســماك المستزرعة من خلال العناصر المناعية المختلفة

من حيث الأعراض فكانت فى صورة الضعف العام والكسل , قلة المقاومــة المناعية أما من ناحية المقاييس الدموية والسيرم .

فإن الليمفوسيت والبروتين الكلى والزلال والجلوبيولين فكانت ناقصة بشــكل واضح فى حالة نقص الليسين والميثيونين معاً عن المجموعات الأخرى .