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EFFECT OF REPLACING FISH MEAL AND SOYBEAN MEAL BY SWEET LUPIN SUPPLEMENTED WITH METHIONINE IN DIETS ON THE PRODUVTIVE PERFORMANCE OF BLUE TILAPIA FISH (*Oreochromis aureus*)

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ABSTRACT

In an experiment carried out in the fish wet lab of the Faculty of Agriculture, Ain Shams University, 360 fish fingerlings (*Oreochromis aureus*) with an average initial weight of 3g/ fish were used. The fish were randomly distributed in 18 twenty liter fiberglass tanks (20 fish each). The tanks were a part of closed system provided with biological and mechanical filters to keep constant water quality in all tanks. Water temperature was kept constant at 27° C throughout the experimental period (8 weeks) by an electrical thermo regulated heaters (4kW). Six experimental diets were formulated to contain 31% crude protein and 4150 cal gross energy/ kg, where diet 1 (control) contains fish meal and soy bean meal as a main sources of protein in the diet. In the second diet, soybean meal protein was replaced by sweet lupin protein to test the effect of replacing soybean meal by sweet lupin, and diets 3, 4, 5 and 6 were designed to include only plant protein sources (soybean meal and sweet lupin) supplemented with 0, 0.3, 0.6 or 0.9 % DL-methionine, respectively. Each diet was fed daily to 3 tanks at a rate of 7% of fish body weight daily. During the experimental period, fish were weighed every 7 days to adjust feed amount. A sample of fish fingerlings at the beginning of the experiment and the all fish at the end were chemically analyzed to determine the differences in body composition.

The results showed that fish groups fed diets containing both animal and plant proteins (diet 1 and 2) had the highest significant weight gain ($P<0.05$). When animal protein was replaced by plant protein without methionine supplementation (group 3) fish final weight decreased to less than 50% of the control group. With adding DL-methionine the growth performance parameter values (weight gain, SGR, feed/ gain, PER, PPV and the energy utilization) started to increase. Final body protein was not significantly ($P>0.05$) affected by the type of protein used in diets. Body fat content was decreased when sweet lupin was incorporated in diets. Ash did not indicate any changes when protein source in the diets was changed. It seems that soybean meal can be replaced by sweet lupin without adverse effects on fish productive performance and supplementation of sweet lupin with DL methionine improved growth rate and feed utilization of blue tilapia fingerlings (*Oreochromis aureus*).

Keywords: Fish meal, soybean meal, sweet lupin, *Oreochromis aureus* fingerlings, DL-Methionine.

INTRODUCTION

The need for protein has been provided in most fin fish diets through the inclusion of fish meal in the diet. However, with the continuing expansion of the aquaculture industry the need for alternative protein resources to replace fish meal is an increasing imperative (Tacon, 1996). Partial or even total replacement of dietary fish meal by soy bean meal has been successfully accomplished in number of teleost fishes (Tacon, 1994; Kaushik *et al*., 1995; Mambrini *et al*., 1999).

The quality of the protein source depends on its digestibility and amino acid profile (Kaushik and Cowey, 1991). However, soybean meal is limited in the essential amino acid methionine (Dabrowski *et al*., 1989), it is the most extensively evaluated and most commonly used plant protein source in commercial aquaculture feeds. The increased acceptability and utilization of soybean meal by the aquaculture feed industry and its limited availability worldwide have resulted in high feed costs. Thus, there is a need to investigate the possibility of using other more economical leguminous seed protein sources with nutritive value comparable to that of the soybean.

Comparison of the composition of lupin and soybean meal suggests that there could be considerable potential for the use of lupin meal in feeds (van Barneveld and Petterson, 1994). Lupin is a legume, which contains high protein levels (32 – 36%) for the whole seed (Sudaryono *et al*., 1999), low market price (Orr, 1994) and has similar amino acid composition to that of soybean (Kyle, 1994). Production of lupin focuses primarily on *Lupins albus*, with significant tonnages being produced in Chile, Egypt, South Africa and Eastern Europe (Perry *et al*., 1998). It has been used as a key feed ingredient in diet formulation for terrestrial species; indeed this is the primary use of the grain (Petterson and Fairbrother, 1996). A notable difference, however, that has been identified in many fin fish species in particular, is a relatively higher dietary protein requirement than for terrestrial domestic species (NRC, 1993). This high need for dietary protein is to satisfy two primary nutritional needs, amino acids and energy. The aim of the present study is to evaluate the effect of replacing fish meal and soybean meal by sweet lupin with methionine on the productive performance of *Oreochromis aureus* fingerlings.

MATERIALS AND METHODS

Fish and Management

A number of 360 tilapia fingerlings (*Oreochromis aureus*) obtained from a private farm near Wadi Elmollak – Ismalia Governrate with an average initial weight of 3 g/ fish were used in the present experiment (8 weeks). Fish were randomly distributed in 18 twenty liter fiberglass tanks each containing 20 fish. The experimental tanks were a part of closed system in the Faculty of Agriculture - Ain Shams University, provided with biological and mechanical filter to keep equal water quality in all tanks. A central air blower aerated the experimental tanks via an air stone fixed in each tank. Water temperature was kept constant at 27° C through the experimental period (8 weeks) by an electrical thermo regulated heaters (4kW). During the experiment fish were weighed every 7 days to adjust feed amount to 7% of fish weight. A sample of another 100 fingerlings was randomly taken at the start of the experiment for analysis of initial whole body composition. At the end of the experiment, all fish in each tank were taken for the analysis of whole body composition.

Feeds and feeding

Sweet lupin used in the experiment was imported from Australia to be used in poultry diets. A sample of 50 kg was used to test possibility of using sweet lupin in fish diets. Chemical composition and amino acid profile of the soybean meal, fish meal and tested sweet lupin are presented in Table (1). Feed ingredients were first ground to small particle size, and then thoroughly mixed. Water was added to obtain a 30% moisture content. Diets were passed through a mincer with 0.6-mm diameter holes and dried at 55° C for 16 h using a dry oven. After drying, the diets were broken up and sieved into convenient pellet size. All diets were frozen at (-15° C) until feeding.

Table 1: Chemical composition and amino acid profile of sweet lupin used in the present experiment compared with soybean meal (44% crude protein) and fish meal

	Soybean meal	Fish meal	Sweet lupin
Chemical composition (% DM)			
Moisture (%)	10.47	8.1	9.91
Crude Protein (%)	43.97	65.5	30.11
Ether extract (%)	1.31	4.1	5.24
Crude fiber (%)	5.67	1.0	16.37
Ash (%)	6.51	14.8	3.32
NFE[1] (%)	42.54	14.6	44.96
GE[2] (Kcal/ kg diet)	38051	3613	4128
Amino acid profile[3] (% DM)			
Histidne	1.14	1.61	0.78
Isoleucine	2.63	3.1	1.69
Leucine	3.62	4.99	2.75
Lysine	2.79	5.04	1.91
Methionine	0.65	1.99	0.32
Phenylalanine	2.2	2.78	1.49
Threonine	1.72	2.41	1.42
Tryptophan	0.61	0.75	0.44
Valine	2.28	3.5	1.65

1) Calculated by difference
2) Calculated by the conversion factors: protein 4 Kcal/g, lipid 9 Kcal/g and carbohydrate 4 Kcal/ g (Jobiling, 1983)
3) Amino acid profile for soy bean meal; fish meal (NRC, 1993) and for sweet lupin (FAO/ WHO, 1990)

Experimental diets were formulated to contain 31% crude protein and 4150 cal gross energy/ kg (Table 2). Control diet included fish meal and soybean meal as main sources of crude protein. In the second group soy been meal protein was substituted by sweet lupin protein. In the rest groups (3, 4,5 and 6) fish meal protein was replaced by plant protein (sweet lupin). To compensate methionine level (the first limiting amino acid) in the first diet, 0.3% of DL-methionine was added in the fourth diet. Also in diets five and six, double and triple amount of synthetic DL-methionine (0.6 and 0.9%) were added, respectively. Chemical analysis and calculated amino acid content are presented in Table (2).

Table 2: Formulation, chemical analysis and amino acid profile of the experimental diets

	Diets					
	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6
Formulation						
Fish meal	20	20	--	--	--	--
Soybean meal	30	--	30	30	30	30
Sweet lupin	--	44	51	50.7	50.4	50.1
Wheat flour	33	22	12	12	12	12
Soy oil	5	5	5	5	5	5
Vitamin & mineral premix[1]	2	2	2	2	2	2
DL-Methionine	--	--	--	0.3	0.6	0.9
Chemical composition (% DM basis)						
Moisture (%)	4.26	3.93	4.71	5.52	5.18	5.90
Crude protein (%)	30.02	30.91	31.71	31.08	30.92	31.72
Ether extract (%)	7.60	7.65	6.91	6.78	6.85	7.60
Crude fiber (%)	3.42	7.49	10.13	10.65	10.80	10.52
Ash (%)	9.35	6.61	4.62	4.72	4.62	4.56
NFE (%)	49.61	47.34	46.63	46.77	46.81	45.60
GE (cal/ g DM diet)	4.529	4.656	4.708	4.687	4.692	4.748
Amino acid composition[2]						
Histidne	0.72	0.69	0.77	0.77	0.77	0.76
Isoleucine	1.52	1.42	1.71	1.70	1.70	1.69
Leucine	2.27	2.30	2.59	2.59	2.58	2.57
Lysine	1.86	1.84	1.84	1.84	1.84	1.82
Methionine	0.61	0.53	0.38	0.68	0.98	1.28
Phenylalanine	1.35	1.28	1.49	1.49	1.48	1.48
Threonine	1.13	1.20	1.28	1.28	1.27	1.27
Tryptophan	0.35	0.35	0.42	0.42	0.42	0.42
Valine	1.49	1.48	1.59	1.58	1.58	1.57

1) Each kg contains: Vit A 4.8 mIU; D_3 0.8 mIU; E 4 g; K 0.8 g; B_1 0.4 g; B_2 1.6 g; B_6 0.6 g; B_{12} 4 g; Pantothenic acid 4g; Nicotinic acid 8 g; Folic acid 400 mg; Biotin 20 mg; Cholin chloride 299 g; copper 4 g; Iodine 0.4 g; Iron 12 g; Manganese 22 g; Zinc 22g and Selenium 0.04g

2) Calculated from NRC (1993)

Diets were allocated to triplicate tanks. Daily feed allowances (7% of fish body weight) were fed at four equal portions at 8.00, 11.00, 14.00 and 17.00 h.

Chemical analyses

Triplicate sub samples of each homogenate prepared from the initial fingerlings and all fish remaining in each tank were analyzed for dry matter, crude protein, crude fat, and ash following the standard methods of AOAC (1990). The same methods of analysis for all the above parameters including crude fiber were employed for the diets. Diets nitrogen free extract was determined by difference.

Gross energy of the experimental diets and fish carcass was calculated from their chemical composition, using the factors 5.64, 9.4, 4.0 and 4.0 (cal GE/g DM) for protein, fat, fiber and carbohydrate, respectively (Jobiling, 1983).

Statistical analysis

The results obtained in the present experiment were statistically analyzed by one-way analysis of variance (ANOVA) using the MSTAT4 (Nissen 1987). Duncan's (1955) multiple-range test was used to compare differences among individual mean at 5% probability level.

RESULTS

Fish in all groups accepted the experimental diets, no residuals were found on the bottom of the tanks, also no mortality was observed during the experimental period except group 3, where fish were fed only plant protein source without supplementation with methionine. Daily water quality records made during the experiment showed that dissolved oxygen concentration did not fall bellow 5.6 mg/ l and pH remained between 7.2 and 7.6. Table (3) shows the performance parameters obtained in the present study. The highest significant ($P<0.05$) weight gain was observed in fish groups fed diet 1 and 2, where combinations of animal and plant proteins were provided. Dramatic decreases ($P<0.05$) in fish final body weight (less than 50%) were observed when protein source of the diet was only from plant protein (groups 3, 4, 5 and 6). All the experimental diets have almost the same amino acid profile except the essential amino acid methionine (Table 2). Fish group fed diet 3 (only plant protein source), which was not fortified with synthetic methionine showed the lowest growth performance. However, when synthetic methionine was added in diets 4, 5 and 6 (also only plant protein source), growth performance was slightly improved (Table 3). It was observed that adding 0.9% methionine (group 6) improved significantly ($P<0.05$) fish weight gain to about 180% of the gain of group 3 (plant protein source without adding methionine). This value is about 40% of the 1st fish group (fish meal + soybean meal) and 38% of the 2nd fish group (fish meal + lupin meal). The SGR values of fish groups fed plant protein failed to about one third of the SGR of the first and the second groups. Feed conversion ratio (FCR) of groups fed only plant protein (3, 4, 5 and 6) was high (5 g feed/ g gain). However, combined protein source proved the normal values of feed conversion ratio (2 g feed/ g gain). By adding ascending levels of DL-methionine the FCR of tilapia fingerlings was improved. The calculated amino acid profile of the experimental diets showed that diet 3 (only plant protein source without methionine supplementation) had the lowest methionine content (the first limiting amino acid). This group showed the lowest growth and poorest feed utilization parameters (Table 3). Values of PER and protein retention, as measured by PPV were negatively influenced when only plant protein sources were used in the diets. Group 6 (plant protein source provided with 0.9% methionine) had higher significant PER and PPV than group 3 where no methionine was added.

Table 3: Growth performance and feed utilization parameters of the experimental groups

	Group 1 (control)	Group 2	Group 3	Group 4	Group 5	Group 6
Initial body weight (g/fish)	3.08	3.08	3.07	3.09	3.07	3.08
Final body weight (g/fish)	10.68^{a}	11.10^{a}	4.73^{c}	4.84^{c}	5.40^{bc}	6.13^{b}
Weight gain (g/fish)	7.60^{a}	8.02^{a}	1.65^{c}	1.76^{c}	2.32^{bc}	3.05^{b}
Weight gain (%)	247^{a}	261^{a}	54^{c}	57^{c}	75^{bc}	98^{b}
SGR [1] (%/d)	2.96^{a}	3.04^{a}	1.03^{c}	1.08^{c}	1.34^{bc}	1.64^{b}
Feed fed (g/ fish)	12.84^{a}	13.40^{a}	9.37^{c}	9.80^{b}	9.70^{bc}	9.93^{b}
Feed/ gain [2]	1.69^{c}	1.67^{c}	5.67^{a}	5.57^{a}	4.18^{ab}	3.26^{b}
PER [3]	1.97^{a}	2.02^{a}	0.58^{c}	0.61^{c}	0.99^{b}	1.03^{b}
PPV [4]	32.09^{a}	30.60^{a}	12.42^{c}	12.01^{c}	19.89^{b}	22.01^{b}
EU [5]	21.03^{a}	20.37^{a}	5.54^{c}	5.40^{c}	8.59^{b}	10.92^{b}
Survival rate (%)	100^{a}	100^{a}	98^{b}	100^{a}	100^{a}	100^{a}

a, b...d. Means with the same superscript within each row are not significantly different ($P<0.05$).

1) Specific growth rate = {(ln fish weight at the end – ln fish weight at start)/ days} x 100
2) g feed/ g weight gain
3) Protein efficiency ratio (PER) = Weigh gain (g) / protein fed (g)
4) Protein productive value (PPV) = {Retained protein (g)/ protein fed (g)} x 100
5) Energy utilization value (EU)= {Retained energy (cal/ fish)/ Energy intake (cal/ fish)} x 100

Final body dry matter content was increased ($P<0.05$) when sweet lupin was supplemented with methionine. Also, body crude protein percentage was increased with increasing methionine supplementation levels (Table 3). However, final body fat content was highest ($P<0.05$) by feeding fish a combination of animal and plant protein in diets (groups 1 and 2). Ash content of fish body was decreased only when fish were fed plant protein sources without supplementation by methionine, whereas ash content was increased when methionine was added.

DISCUSSION

The good acceptance of all the experimental diets observed by all experimental groups was of interest, since a low intake of feeds containing increasing levels of some vegetable feedstuffs has been reported by many authors (Jackson *et al.*, 1982; Roselund, 1986). Reigh and Ellis (1992) reported that extremely low feed intake was associated with increasing soybean meal (SBM) in red drum diets. This may be related to the poor palatability of SBM (Fowler, 1980). The relatively poor feed intake of the lupin groups reported in the present experiment agrees with the results of Robaina *et al.* (1995). Lupin contains low levels of anti-nutritional factors, particularly the protease inhibitors and lectins (Petterson and Mackintosh, 1994; Petterson and Fairbrother, 1996; Petterson *et al.*, 1997). Thus feed intake in all experimental groups was affected by protein source (either animal or plant), as shown in Table (3).

Table 4: Fish body composition (% of DM) of the initial and at the end of the experimental period

	Initial	Groups					
		1	2	3	4	5	6
Moisture	74.35^{a}	68.43^{b}	69.06^{b}	67.65^{c}	68.87^{b}	65.97^{c}	66.41^{c}
Crude protein	55.47^{c}	56.99^{b}	55.37^{c}	56.79^{b}	57.68^{b}	59.83^{a}	58.15^{a}
Ether extract	11.41^{d}	23.44^{a}	24.22^{a}	15.01^{b}	14.11^{c}	13.96^{c}	15.95^{b}
Ash	21.94^{a}	15.78^{c}	14.60^{c}	19.84^{a}	18.58^{b}	18.21^{b}	17.18^{b}
Rest	11.18^{a}	3.79^{d}	5.20^{c}	6.16^{b}	9.42^{b}	8.01^{b}	8.48^{b}

a, b...d. Means with the same superscript within each row are not significantly different ($P<0.05$).

The significant reduction in weight gain and SGR observed when fish meal was replaced by plant protein in the present study, was similar to that obtained by Gomes *et al.* (1995). Several hypotheses have been suggested to explain these results; 1. sub-optimal amino acid balance; 2. antinutritional factors; 3. inadequate levels of phosphorus; and 4. inadequate levels of energy (Webster *et al.*, 1995). They also reported that when protein is below 30%, tilapia fish fed diets containing fish meal had higher weight gains compared with fish fed diets without fish meal. They added that amino acid profiles of the lower protein diets might not be adequate when plant protein replaced fish meal. In the present study incorporation of ascending levels of the first limiting amino acid (methionine) has improved weight gain and feed/ gain ratio. The best performance was obtained when soybean meal protein was replaced by sweet lupin protein. This may due to the low levels of anti-nutritional factors, particularly the protease inhibitors and lectins in lupin (Petterson and Fairbrother, 1996). These results are in accordance with those obtained by Jenkins *et al.* (1994) and Robaina *et al.* (1995) who found that lupin seed meal could be successfully replace soybean meal in fish diets. The high weight gain obtained for the combined protein groups were in agreement with the results of Robaina *et al.* (1995), who found that weight gain of sea bream fed combination of fish meal and lupin seed meal was better than fish fed fish meal as a sole protein source in the diet. Synthetic methionine added in diets 4, 5 and 6 to have similar or higher methionine level than diet 1; did improve the performance of fish fed such diets compared with diet 3 (no methionine was added). Murai *et al.* (1986) reported that nutritional value of soy flour was improved by addition of 0.4% methionine. It seems that 0.6% DL-methionine under the present study condition added to diet 4 had the same effect as 0.9%. Addition of supplemental methionine in fish diets has had variable success (Webster *et al.*, 1995). Tacon *et al.* (1994) stated that addition of synthetic methionine to a diet deficient in methionine did not improve fish growth. However, Shiau *et al.* (1987) reported for tilapia and Murai *et al.* (1986) for common carp that addition of supplemental methionine improved fish growth. This supports the results obtained in the present study.

Table 5 : Nutrients deposition in fingerlings body (g/ fish) as affected with the experimental diets

	Groups					
	1	2	3	4	5	6
Dry matter retained	2.06 ^{a}	2.20 ^{a}	0.60 ^{c}	0.57 ^{c}	0.89 ^{b}	1.09 ^{b}
Crude protein retained	1.18 ^{a}	1.22 ^{a}	0.35 ^{c}	0.35 ^{c}	0.57 ^{b}	0.65 ^{b}
Ether extract retained	0.58 ^{a}	0.63 ^{a}	0.12 ^{d}	0.11 ^{d}	0.14 ^{c}	0.21 ^{b}
Ash retained	0.28 ^{a}	0.26 ^{a}	0.10 ^{cd}	0.08 ^{d}	0.13 ^{c}	0.15 ^{c}

a, b...d. Means with the same superscript within each row are not significantly different (P<0.05).

The positive effect of fish meal diets on dry matter, protein and fat deposition in fish body (Table 5) may be due to the increase of the feed intake associated with high palatability of the diet which consequently increased fish growth. Mohsen and Lovell (1990) reported that addition of animal protein source to a soybean meal based diet improved diet palatability. Less feed consumption was observed in the present study when diets contained only plant protein sources such as soy bean meal or lupin, which are agree with the results of (Reigh and Ellis, 1992).

CONCLUSION

1- Sweet lupin can replace successfully soybean meal in tilapia diets.
2- A combination of animal and plant protein sources are necessary for adequate blue tilapia growth.
3- In plant protein diets (soybean meal or sweet lupin meal), addition of DL methionine improved productive performance of blue tilapia fingerlings.

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تأثير إحلال مسحوق السمك بالترمس الحلو المدعم بالمثيونين في العلائق على الأداء الإنتاجي لإصبعيات البلطي الأوريا (*Oreochromis aureus*)

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في تجربة أجريت في المعمل الرطب في كلية الزراعة – جامعة عين شمس تم استخدام ٣٦٠ اصبعية من البلطي الأوريا بمتوسط وزن بداية ٣ جم للسمكة. تم توزيع الأسماك عشوائيا على ١٨ حوض ٣٠ لتر من الألياف الزجاجية وبكل حوض ٢٠ سمكة . كانت الأحواض جزء من نظام مغلق مزود بمرشح بيولوجي وميكانيكي وذلك لتثبيت جودة المياه في جميع الأحواض . كانت درجة حرارة المياه ثابتة على ٢٧ درجة مئوية بواسطة سخان كهربائي (٤ كيلو وات) يتم التحكم فيه بواسطة ترموستات. تم تكوين ٦ علائق تحتوي على ٣١% بروتين خام و ٤١٥٠ كالورى لكل كيلو جرام مادة جافة بالعليقة حيث كانت العليقة الأولي (المقارنة) تحتوي على مسحوق سمك وكسب فول صويا كمصادر رئيسية للبروتين في العليقة الثانية تم استبدال بروتين كسب فول الصويا ببروتين الترمس الحلو ، أما العلائق ٣، ٤، ٥، ٦ والتي احتوت فقط على بروتينات نباتية (كسب فول صويا والترمس الحلو) فقد تم تزويدها ب صفر ، ٠,٣ ، ٠,٦ ، ٠,٩% DL مثيونين ، على الترتيب. كل عليقة من هذه العلائق كان يتم تغذيتها لثلاث أحواض بمعدل ٧% من وزن الأسماك بالحوض يوميا . خلال فترة التجربة (٨ أسابيع) كان يتم وزن الأسماك كل ٧ أيام لضبط كميات العليقة اليومية ، في بداية ونهاية التجربة تم عمل تحليل كيماوي لجسم الأسماك لتحديد الاختلافات في تركيب الجسم.

أظهرت النتائج أن مجموعات الأسماك التي تم تغذيتها على علائق تحتوي على بروتينات نباتية وحيوانية حققت أعلى نمو معنوي (P<0.05). عند استبدال البروتين الحيواني ببروتينات نباتية بدون دعم بالمثيونين انخفض الوزن النهائي للأسماك إلى أقل من ٥٠% من الوزن النهائي لمجموعة المقارنة . بإضافة المثيونين تحسنت قيم الإنتاج (النمو ، معدل النمو النسبي ، كفاءة التحويل الغذائي ، كفاءة الاستفادة من البروتين ، الكفاءة الإنتاجية للبروتين وكذلك الكفاءة الإنتاجية للطاقة) مع تصاعد نسب المثيونين المضاف في العليقة. لم يتأثر بروتين الجسم معنويا بتأثير نوع البروتين في العليقة . انخفض دهن لجسم عند إحلال الترمس الحلو في العليقة ، ولم يتأثر الرماد الخام بتغيير مصدر البروتين في العليقة. من نتائج التجربة يمكن القول أن استبدال كسب فول الصويا بالترمس الحلو في علائق البلطي لم يكن له أي تأثيرات سلبية على الأداء الإنتاجي للأسماك ، كما أن دعم الترمس الحلو بالحمض الأميني DL-Methionine يحسّن معدل النمو و كفاءة الاستفادة من الغذاء لإصبعيات البلطي الأزرق.