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The effects of methionine supplementation to soybean meal (SBM)-based diets on the growth and whole body-carcass chemical composition of tilapia (*T. zillii*)

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Abstract: The effects of supplementing soybean meal-based diets with methionine on the growth and carcass-whole body chemical composition of *T. zillii* were studied during a 62-day feeding trial. A control diet prepared for *T. zillii* was supplemented with five different levels of free methionine, 0.5%, 1%, 1.5%, 2% and 2.5% (the same amounts of α-cellulose were removed). At the end of the study, the best growth rate and FCR were determined in the group fed a diet supplemented with 0.5% methionine (total 1.38% methionine+cystine). Statistically significant effects of methionine supplementation on whole body protein content were determined ($p<0.05$). No significant effects of control and test diets were detected on carcass dry matter and protein content; however, a significantly low ($p<0.05$) carcass lipid content in the group fed a diet not supplemented with methionine (control group) was determined.

Key Words: *Tilapia zillii,* methionine, soybean meal, whole body-carcass chemical composition

Soya fasulyesi küspesi (SFK) katkılı yemlere yapılan metiyonin ilavesinin, tilapialarda (*T. zillii*) gelişme ve tüm vücut-karkas besin madde bileşenleri üzerine etkileri

Özet: Soya fasulyesi küspesi katkılı yemlere yapılan metiyonin katkısının tilapialarda (*T. zillii*) gelişme ve tüm vücut-karkas besin madde bileşenleri üzerine etkileri, 62 gün süren bir çalışma ile araştırılmıştır. Çalışmada, *T. zillii* için hazırlanan bin rasyona (kontrol) beş farklı düzeyde serbest metiyonin (%0.5, %1, %1.5, %2, %2.5) eklenmiştir (aynı oranlarda α-cellulose ile yerdeğiştirilmiştir). Çalışma sonunda, en yüksek gelişme oranı ve yem değerlendirme oranı, %0.5 metiyonin ilave edilmiş (toplam %1.38 düzeyinde metiyonin+sistin içeren) rasyonla beslenen grupta saptanmıştır. Rasyonlara yapılan metiyonin ilavesinin, kontrol rasyonuna göre tüm vücut protein oranlarını istatistiksel olarak önemli oranda ($p<0.05$) etkilediği saptanmıştır. Metiyonin ilave edilmiş rasyonların, karkas kuru madde ve protein oranları üzerine, kontrol rasyonundan farklı bir etkisi saptanamamıştır. Ancak, metiyonin ilavesi yapılmayan kontrol rasyonu ile beslenen gruptaki balıkların karkas lipit oranları, diğer gruplardaki balıkların karkas lipit oranlarına göre önemli derecede düşük bulunmuştur ($p <0.05$).

Anahtar Sözcükler: *T. zillii,* soya fasulyesi küspesi, metiyonin, tüm vücut-karkas besin madde bileşenleri

Introduction

There is still a lack of information on supplementary diets used in intensive or semi-intensive tilapia culture. It has been determined that finfish have an essential requirement of 10 amino acids (1). Deficiencies in these amino acids cause anorexia, poor growth and low food conversion rate in general (2). Methionine is the most limiting amino acid with lysine in natural feed stuff, especially in plants. Because of that, diets are supplemented with crystalline methionine according to the requirement of cultured species. Additional supplementation of methionine naturally depends on feed ingredients used in diets. Methionine deficiency usually causes cataract in trout, *O. mykiss,* (3). In the mean time, the excess of methionine (3 to 4 times more than the requirement) cause low feed intake and poor growth in land animals (4).

Diets for tilapia can be prepared with a variety of low protein sources. Supplementation of free amino acids is especially necessary when alternative plant protein sources are added to a diet as a fish meal replacer. Studies have shown that soybean meal (SBM) processed prior to usage can be used as a fish meal replacer in aquafeeds (5). SBM is known to be one of the best readily available protein sources with a good essential amino acid profile, except for the absence of methionine. Thus, methionine supplementation is usually required in SBM-based diets used in fish nutrition. Several studies have shown that SBM can replace up to 75% of fish meal in *O. niloticus* (6), *O. mossambicus* (7) and tilapia hybrids (8). *T. zillii* is able to consume a variety of foods in ponds, including benthic organisms (9), and can be cultured in high salinity (10). Like other fish, it has an essential requirement of 10 amino acids (11). The methionine and lysine requirements of some tilapia specise were determined (12); however,

there is still a lack of information on methionine requirements and the effects of methionine supplementation in SBM-based diets on the growth and carcass-whole body chemical composition of *T. zillii*. In this study, an attempt was made to determine the optimum supplementation ratio of methionine in a standard soybean based diet and the effects of different levels of methionine supplementation on the carcass and whole body chemical composition of *T. zillii*.

Material and Methods

This research was carried out at the Experimental Fish Culture and Research Station, Faculty of Fisheries, University of Çukurova. A total of six diets (control and five test diets) were prepared for this research. For this aim, a standard diet (control) was prepared from several ingredients including low methionine. Diets were soybean meal based. In the control diet, 2.5% α-cellulose was added and there was no methionine supplementation in the control diet. The test diets were then supplemented with methionine at levels of 0.5%, 1%, 1.5%, 2% and 2.5% of the total diets. At the mean time, the same amount of α-cellulose was removed from the diets. Ingredients, composition and the proximate contents of the experimental diets are shown in Table 1.

After all ingredients were finely ground, and maize and wheat middling were treated with hot water to improve digestibility. Then oil and other ingredients were added and mixed untill a stiff dough resulted. This was placed on a clean platform in a thin layer and air dried. Then they were ground with middlings and sieved into vonvenient pellet sizes.

Fingerling *T. zillii* (hatched at he same time and in the same batch) were supplied from the Freshwater Experimental Station at the Faculty of Fisheries. All fish were acclimated to experimental conditions for 30 days. Twelve glass aquaria (each 80 l) were used and 15 fish

Table 1. Ingredients, composition and the proximate contents of standard (Control) and test diets used in the study

Ingredients (g/100 g diet)	Diet No 1)
White Fish Meal	10.5
Soybean meal	40.0
Maize	25.5
Wheat middling	6.0
Oil (sunflower)	9.0
L-methionine	-
L-lysine	1.5
DCP	2.0
Min. mix[1]	1.0
Vit. mix[2]	1.0
NaCl	1.0
α-cellulose	2.5
Composition of standard diet	**(Diet 1)**
Dry matter[3]	91.34
Crude protein[3]	26.86
Crude lipid[3]	11.72
Crude ash[3]	5.30
ME (kcal/kg)[4]	2821
Met+cys[4]	0.88
Ca[4]	0.64
P[4]	0.41

Test diets	Methionine supplementation (g/100 g diet)	Estimated Met-Cys Ratio, % of diet	Crude protein, % [3]
Diet 2	0.5	1.38	27.2
Diet 3	1.0	1.88	27.6
Diet 4	1.5	2.38	28.1
Diet 5	2.0	2.88	28.5
Diet 6	2.5	3.38	28.9

[1] Remineral 1C (per kg) Choline Chloride 200.000 mg, Manganese 80.000 mg, Iron 30.0 mg, Zinc 60.000 mg, Copper 5.000 mg, (cobalt 500 mg, 2.000 mg, Selenium 150 mg, Calcium Carbonate 74, 784 mg.
[2] Jackson et al. (12); [3] Analysed; [4] Estimated

randomly stocked in each (9.95±0.41 g mean body weight). Each diet was assigned to duplicate groups of fish. Each aquarium was supplied with 0.3 1/min water from the main water tank and aired with an air stone. Water quality and temperature were recorded during the experiment. Oxygen (measured daily) and temperature (measured 3 times a day) ranged between 6.8-8.2 ppm and 23.8-27°C, respectively. pH (measured daily) ranged between 7.2 and 8.4. Each diet was randomly assigned to duplicate fish groups. The fish were fed three times a day for 62 days at a ratio of 3% of live weight and weighed biweekly during the experiment after being anesthetized with MS-222 solution. At the end of the research, the food conversion ratio (FCR) was calculated as total feed fed/wet weight gain. A 12: 12 dark-light photoperiod was used during the experiment.

At the end of the research, all fish in research groups were removed for individual weighing. The mean of each group was calculated and the groups were statistically analysed by Duncan Multiple Range Teste (13). Then 10 fish (five for whole body analysis, five for carcass analysis) were randomly taken from each aquarium. Fillets of each fish were taken for carcass analysis. Whole body and fillets were homogenized with a mixer and stored at -20°C until chemical analysis.

Chemical analysis

Dry matter and crude ash analysis of test diets and whole body-carcass samples were conducted at 103°C and 550°C to constant weight. Crude protein analysis were conducted by the Kjeldahl method (14). Lipids were determined by a modification of the extraction method (water-chloroform-methanol) of Bligh and Dyer, 1959 (15).

Results

Performances of fish

Increase in live weight, FCR and mortality ratios of *T. zillii* fingerlings fed control and test diets supplemented with different level of methionine are presented in Table 2.

As seen in Table 2, fish fed the 2nd diet (supplemented with 0.5% methionine) had statistically higher live weight gain ($p<0.05$). Methionine supplementation at a level above 1.0% (total 1.88 met+cys) of the total diet not improve live weight gain, and had results similar to those of the control diet (Diet 1). These results show that excess supplementation of methionine above 0.5% of the total diet has no positive effect on the growth of tilapia. It is also clear that addition of methionine up to 2.5% of diet has no toxic effects, but that it does have a depressive effect in comparison to the 2nd diet (supplemented with 0.5% methionine). Supplementation of 0.5% methionine (Diet 2) caused statistically significant reduction in FCR in comparison to Diets 1 (control), 4, 5 and 6.

Body and carcass composition

The effects of different diets on carcass quality and whole body chemical composition are other important criteria in fish nutrition. The results of the analysis of whole body and carcass chemical composition are given in Table 3.

A significant ($p<0.05$) effect of methionine supplementation on whole body protein content was determined. No significant effect of diets on carcass dry matter and protein content was found; however, fish fed

Table 2. Final growth data (mean final weight, g), total mortality (number of dead fish) and feed conversion ratio (FCR)

Diet no.	Supplementation of Met. (% of diet)	Number of Fish	Initial weigth, g	Mean final weigth, g	Weight gain (%)	Total Mortality in two replicates	FCR
1	-	15	9.95±0.41	23.92±0.97^{a}	140	5	3.49^{a}
2	0.5	15	9.95±0.41	28.72±0.48^{b}	189	2	2.82^{b}
3	1.0	15	9.95±0.41	26.38±0.88^{bc}	165	1	2.97^{bc}
4	1.5	15	9.95±0.41	25.22±0.74^{ac}	154	3	3.26^{ac}
5	2.0	15	9.95±0.41	25.50±0.62^{ac}	156	1	3.36^{ac}
6	2.5	15	9.95±0.41	25.73±0.36^{ac}	159	3	3.18^{c}

Table 3. The mean dry matter, ash, crude protein and lipid composition of whole body and carcass of *T. zillii* fed diets supplemented with different levels of methionine.

WHOLE BODY					
Diet no.	Suppl. Met (%)	Dry matter (%)	Ash (%)	Crude Protein (%)	Lipid (%)
1	-	26.30±0.89	3.56±0.70	15.08±0.25^{a}	5.78±0.16
2	0.5	26.66±0.41	3.56±0.20	16.19±0.20^{ab}	5.75±0.22
3	1.0	26.50±0.30	3.65±0.36	16.29±0.43^{ab}	4.90±0.23
4	1.5	26.47±0.63	3.83±0.18	16.53±0.18^{ab}	5.18±0.13
5	2.0	26.67±1.41	4.11±0.56	16.02±0.38^{ab}	4.84±1.25
6	2.5	27.79±0.69	4.13±0.61	17.44±0.38^{b}	4.83±0.13
CARCASS					
Diet no.	Suppl. Met (%)	Dry matter (%)	Ash (%)	Crude Protein (%)	Lipid (%)
1	-	25.93±0.36	1.57±0.10^{a}	22.10±0.10	1.06±0.25^{a}
2	0.5	25.53±0.10	1.53±0.16^{a}	21.56±0.16	1.54±0.12^{b}
3	1.0	24.82±0.35	1.44±0.21^{a}	21.13±0.21	1.45±0.10^{b}
4	1.5	25.80±0.57	1.84±0.52^{b}	21.70±0.52	1.56±0.30^{b}
5	2.0	25.36±0.65	1.53±0.28^{a}	21.16±0.28	1.47±0.15^{b}
6	2.5	25.54±0.46	1.55±0.36^{a}	21.67±0.36	1.52±0.12^{b}

with non-methionine supplemented diet (control group) were found to have significantly low carcass lipid contents. Carcass ash contents of fish fed Diet 4 (1.5% methionine supplemented) were significantly higher ($p<0.05$) than in other groups.

Discussion

The growth rate in terms of weight increase was affected by supplementation of methionine to an SBM-based diet. Addition of free methionine at a level of 0.5% (1.34% total met+cys) significantly improved the weight gain, but the additon of excess amounts of methionine (more than 0.5%) did not cause a statistically significant increase in live weight gain. It means addition of excess methionine (more than 0.5% of tilapia diet) depressed the growth rate in comparison to the diet supplemented with methionine at a level of 0.5% of total diet. Methionine supplementation had been shown to cause an increase in free methionine sulphoxide (MSO) in serum in *O. mykiss* (16) and *D. labrax* (17). It seems there is a pathway of methionine degradation to MSO in some fish species. Lovell (1984) (18) stated that the depressive effect of excess methionine is probably due to inhibitory effect of MSO on glutamine syntheses, which also has a role in detoxification of NH_3. Murai et al. (19) also reported the depressive effect of excess methionine in the diet on serum glutamine concentration of *C. carpio.*

The other important point is that there must be a balance between cystine and methionine in fish diets. Cystine can be obtained from methionine, and thus the level of cystine in diet has a sparing effect on methionine requirement. Because of that methionine and cystine usually thought together in fist diet formulations. Murai et al. (19) reported that the level 1.30% methionine +cystine (1.05% methionine +cystine from ingredient and 0.25 free methionine is supplemented) yielded the best growth performance in *C. carpio.* They also found that a dietary level of 1.55% methionine + cystine (1.05% methionine + cystine from ingredient and 0.50% free methionine is supplemented) had no positive effect on growth and yield to Taurine and MSO in serum. Jackson and Capper (1982) researched the same topic in tilapia (*S. mossambicus*), finding the optimum dietary requirement of this species to be 0.127% (0.53% methionine + 0.74% cystine). They also determined an inhibitory effect of excess free methionine on growth rate. Hidalago et al. (1987) reported the methionine and cystine requirements of sea bass (*Dicentrarchus labrax*) to be 2.20% of the total diet. In our study, no toxicity effects were determined, but the addition of methionine at a level of more than 1% significantly depressed growth in terms of weight, in comparison to the diet supplemented with 0.5% methionine (Diet 2). The best results in weight gain were found in the diet supplemented with 0.5% methionine (total methionine-cystine was 1.34% of the total diet). These results show that the requirement of methionine+cystine of *T. zillii* is similar to the requirements of *C. carpio* and *S. mossambicus.* It seems the requirements of methionine for fish species living in similar ambients such as freshwater or marine environments are similer.

As seen in Table 3, lipid ratios in whole body ranged between 4.83% and 5.78%. The carcass lipid ratios ranged between 1.44% and 1.82%, respectively. It seems there is no clear effect of supplementation of different levels of methionine on the whole body lipid contents of *T. zillii.* However, supplementation of methionine at all levels resulted in an increase of lipid carcass levels, as compared to the diet not supplemented with methionine (control). The lowest whole body protein content was found in the control group. It seems that the insufficient methionine content of the diet caused low protein synthesis in the whole body. It was also interesting that the lowest lipid content in the carcass was found in the control group (the reasons are unknown). However, no systematic effects of methionine supplementation on carcass protein content were found.

In recent years, plant protein sources have been in use as fish meal replacers in tilapia feed. Free amino acid supplementation is usually necessary for such diets. Each plant source has different digestibility and usage in each fish species. Thus, the same subject must be studied with different digestibility and usage in each fish species . Thus, same subject must be studied with different alternative protein sources, such as cotton seed meal, sunflower meal and sesame meal. In addition, further research is required to determine the requirements of other essential amino acids and their effects on the whole body and carcass chemical quality of *T. zillii.*

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