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Comparing effects of feeding crystalline or coated methionine to soybean meal based diets on the Nile tilapia (*Oreochromis niloticus*) growth performance and protein quality

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ABSTRACT

A sixty days feeding trial was conducted to investigate the influence of including different forms of dietary methionine on growth performance and plasma methionine levels in Nile tilapia diets *(Oreochromis niloticus).* One hundred and thirty two Nile tilapia fingerlings *(Oreochromis niloticus)* mono sex of mean initial body weight 24.32 ± 0.31g were randomly distributed into 12 closed system 120 liter tanks. Fish in each three tanks were fed one of four diets. 1) Control positive (C+) (fish meal as main protein source covering methionine requirement for Nile tilapia), 2) Control negative (C-) (soybean meal as primary protein source deficient in methionine), 3) soybean meal supplemented with crystalline DL methionine (CRM), or 4) soybean meal supplemented with coated methionine (COM). Before the end of the experiment by 48h., blood samples were obtained at 1.5, 3.5, 5.5, 12.5 and 24h after feed administration to measure methionine uptake in blood plasma. Results revealed that supplying fish diet free of animal protein source with coated methionine (COM) had comparable final body weight(FBW), weight gain (WG), feed conversion ratio (FCR) and specific growth rate (SGR) with the control group (P>0.05). The poorest growth performance and feed utilization was exhibited by the group fed the negative control (C-) diet. Apparent protein digestibility were significantly higher (P<0.05) in groups fed diets supplemented with either coated or crystalline methionine compared with groups fed un-supplemented diets (C+ and C-). Dorsal muscle protein and total essential amino acids (ΣEAA %) content were not significantly affected by dietary treatments. Plasma methionine concentration was significantly influenced by both protein source and methionine form, where the highest plasma methionine concentration throughout the measuring period was revealed by fish fed diet supplemented with crystalline methionine (CRM), while the lowest plasma methionine concentration resulted from group fed (C-). Both group of fish fed (C+) or (COM) had moderate plasma methionine concentration. Based on these results, it appeared that coated methionine effectively slow the release of free methionine in a way to be near the animal bounded protein. Accordingly, it could be recommended to supplement soybean- based diets with coated methionine which had shown no adverse effect on Nile tilapia growth performance and protein utilization efficiency.

INTRODUCTION

The development of commercial aqua-feeds has been traditionally based on fish meal (FM) as the main source of animal protein, due to its high protein content and balanced essential amino acids (EAA) profile. FM is also an excellent source of essential free fatty acids (EFFA), digestible energy, minerals and vitamins (EL-Sayed, 1999).

Now a days, aqua-feed production is one of the fastest growing aquaculture industries, world aquaculture production of fish accounted 44.1 percent of total production from capture fisheries and aquaculture (FAO, 2016).

However, the production of fish meal, which is the primary source of protein in aquafeeds has decreased extremely, due to exhausting of wild fisheries resource which fish meal production relies on (FAO 2016). As a result, there is a constant search for alternative protein sources for aqua-feeds production. Plant proteins are considered to be the most viable alternative protein sources to replace fish meal for economic fish production in most developing countries. Many attempts have been made by different authors to make partial or total replacement of fish meal protein by plant protein (El-Sayed ,1999 and Muin.*et al*., 2015 and Al-Thobaiti, *et al*. 2018). So far soybean meal (SBM) is considered to be one of the most nutritious of all plant protein feedstuffs that is used to replace fish meal partially or totally in tilapia diets (shiau *et al*., 1989 ; and Sharda *et al*, 2017).

In fact, soybean is one of the richest plants proteins in essential amino acids that cover most of fish requirements (NRC, 2011), however, soybean is deficient in Methionine (NRC, 2011) this required supplementing fish diet with methionine to cover the nutritional requirements of the studied species. Authors have tried using crystalline methionine to overcome such deficiency. Shiau *et al* (1989) reported that supplementing soybean diets with methionine has no significant effect on growth of Nile tilapia fry compared with those fry fed un-supplemented soybean diet. On the contrary, Ajani, *et al*., (2016) reported that soybean meal could either partially or totally replace fish meal when supplemented with methionine. In the same trend Espe *et al*. (2006) stated that supplementing wheat and corn gluten meal basal diet with amino acid mixture have improved growth of Atlantic Salmon but not to the same extend for fish fed diet contained fish meal.

According to many authors supplementing plant protein based diet with amino acids have improved growth performance of fish compared with other groups fed diets containing plant proteins as the only source of dietary protein. However, this performance is still below the performance of those fish fed FM based diets (Mazid *et al*., 1978; Viola *et al*., 1994; Davies and Morris 1997, and Bodin *et al*. 2012).

It is generally accepted that, the apparent reduced utilization efficiency of free amino acid (FAA) relative to amino acids obtained from digested protein is related to the rapid rate of absorption of purified FAA which may result in excessive amino acid catabolism and reduced utilization efficiency (Lovell, 1991).

Accordingly, reducing the rate of release of FAA to transport sites in the intestinal mucosa might improve FAA utilization efficiency. By coating FAA with a digestion –resistant compound, release of purified FAA at a rate similar to the rate of release of AA from intact protein during digestion could be accomplished.

Microencapsulation and micro coating of FAA have been used to improve delivery of FAA in aquatic feeds (Villamar and Langdon, 1993). Alam *et al*., (2002, 2004 and 2005) have reported that supplementation of coated methionine and lysine was effective in improving the nutritional value of soy-protein for Kuruma shrimp compared with group of shrimp fed the uncoated methionine and lysine.

The aim of the present study is to investigate the influence of adding two forms of methionine (crystalline or coated) in Nile tilapia *(Oreochromis niloticus)* diets on growth performance and plasma methionine concentration.

MATERIALS AND METHODS

Experimental system and fish:

Mono sex Nile tilapia *(Oreochromis niloticus)* fingerlings were brought to Fish Experimental Unit in Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt, from a fresh water commercial farm in Damietta Governorate. The experimental work was carried out in accordance with the national guidelines for care and use of laboratory animals and fish had received the utmost human care.

One hundred and thirty two mono sex Nile tilapia fingerlings *(Oreochromis niloticus)* of mean initial body weight 24.32 ± 0.31g from the stocked fish were randomly distributed into a 12 closed water recycle system (120 liter tanks), where each tank contained 11 fingerlings. Each three tanks (triplicate) represented one experimental treatment. The first 15 days of the experiment were considered as habituation period and thereafter the growth trials were carried out for 60 days. Diets were randomly assigned to the experimental units. Fish were hand fed the experimental diets at 3 % of body weight for six days weekly, three times per day **(**Jauncey and Ross, 1982 and Coche**,** 1982**)**.

The water flow rate out of each aquarium was 2L/min into a submerged bio-filter after passing through a mesh net to remove solid impurities. Water was then collected in a common reservoir from which the filtered water was pumped up to the rearing units. Water used in the system was tap water stored for 24h to be sure that water is free of chlorine. Water was continuously aerated by a blower. Five percent of the total water volume was renewed in the early morning to compensate the loss in water volume resulted from evaporation. A thermo-controlled electric heater was used to adjust water temperature about 24±1ºC. All the experimental treatments were conducted under an artificial photo period equal to natural light/darkness period (12h light:12h darkness).

Experimental Diets

Four experimental diets were formulated to contain approximately 32% crude protein and 17.09 MJ estimated gross energy according to NRC, 2011 (Table 1). The first diet (control positive, C+) was formulated to contain fish meal as the primary protein source. Three experimental diets were formulated to contain soybean meal as the main protein source and were designed to cover Nile tilapia requirements from essential amino acids except methionine according to NRC, 2011 (Table 2). One of these diets was the control negative (C-) with no added methionine and the other two experimental diets were supplemented with either crystalline DL methionine (CRM) (purity 99%, supplied from Orkila Egypt company, Egypt) whilst the other was supplemented with coated methionine (COM) (purity 55%, hydrogenated vegetable oil as the main coating ingredients supplied ADISSEO company, France) to a level equivalent to methionine in C+.

Calcium and phosphorus were adjusted using mono calcium phosphate. Vitamins and trace minerals were added according to manufacturer requirements (Table 1). Silica was used as indicator to calculate the protein apparent digestibility.

Table 1: Composition (g/100g) and proximate analysis of the experimental diets (as fed).

Item	(C+)	(CRM)	(COM)	(C-)
Fish meal[1] (FM 65% CP)	45.00	------	------	------
Soybean meal[2] (SBM 46% CP)	-----	60.00	60.00	60.00
Yellow corn	52.40	34.40	34.01	35.40
Corn gluten[3] (CG 60% CP)	------	2.00	2.00	2.00
Soybean oil[4]	1.00	1.00	1.00	1.00
Vit & min. premix[5]	0.10	0.10	0.10	0.10
Mono calcium phosphate	1.50	2.00	2.00	2.00
Methionine (crystalline CRM, or coated COM)[6]	------	0.50	0.89	------
Total	100	100	100	100
Determined chemical composition (% DM)				
Crude protein (%)	31.80	31.90	31.80	32.00
Ether extract (%)	6.21	2.48	2.47	2.51
Crude fiber (%)	1.53	4.00	3.99	4.02
Ash (%)	9.43	6.32	6.31	6.33
NFE (%)[7]	39.03	44.30	44.43	44.14
Dry matter (%)	88	89	89	89
Silica (%)[8]	0.36±0.04	0.515±0.045	0.52±0.04	0.40±0.05
Estimated energy				
Gross energy MJ/Kg[9]	17.14	17.05	17.04	17.05
Digestible energy (MJ/kg)	12.85	12.79	12.78	12.79
Protein energy ratio (mg/kcal)[10]	103.5	104.38	104.12	104.64

[1]Delta Vet Trading Co, Alexandria, Egypt. Imported from China.

[2]Soybean meal solvent extracted, Cargill, Egypt. Imported from Argentina.

[3]Corn gluten, Sunrise biotech, Imported from China.

[4]Soybean oil (99%), soy oil factory, Agriculture Research Center, Giza, Egypt

[5]Vitamin and mineral premix for fish (supplied from Multivita, Egypt) at 1.0% of the diet supplies the following per kg of the diet: vit.A 10000 IU, Vit.(D3) 2000 IU, vit. E 50mg, vit. K 10mg, vit. B_1 10mg, vit. B_2 10mg, vit. B_6 15mg, vit. B_{12} 20mcg, niacin 50mg, pantothenic acid 40mg, folic acid 5mg, biotin 500 mcg, Mn 25mg, Fe 75mg, Zn 50mg, Cu 15mg, I 3mg, Se 0.3 mg, and Co 0.2mg.

[6] Crystalline DL methionine (99%) supplied from Orkila Egypt Company, coated methionine (purity 55%, hydrogenated vegetable oil as the main coating ingredients supplied ADISSEO Company, France)

[7]NFE = 100 – (% protein + %EE + %ash + % Fibre).

[8] values are presented as mean ±SE (n=3);

[9]Gross energy content was calculated using the values 5.65, 4.2 and 9.45 Kcal/ gm for protein, carbohydrate and lipid, respectively and applying the coefficient of 0.75 to convert gross energy to digestible energy according to Hepher *et al.* (1983). The Kcal was transferred to Joule by multiplying by 4.184.

[10] Protein energy ratio (P/E ratio) = crude protein x 10000 / digestible energy, according to Hepher *et al.*(1983).

Analytical procedures

Throughout the feeding trial, fish were weighed every two weeks to monitor growth rate and re-adjust daily feed ration. Near the end of the experimental period, fecal samples were collected in a 6 days period in the morning before introducing fed to fish to be sure that there is no feed residual from the previous day. The samples were collected using the siphoning technique to determine the apparent digestibility of protein according to Riche and Brown, (1996). Likewise, five fish were randomly taken from each tank, and then frozen at -20˚C to determine both protein and amino acid content of the dorsal muscle (Eya and Lovell, 1997).

Plasma amino acids

Prior the end of the experiment by 48h., blood samples were obtained at 1.5, 3.5, 5.5, 12.5 and 24h. For each sample one fish from each tank was removed and killed by a blow to the head (Rolland *et al*., 2015). Within minute blood samples were taken from the caudal vein using a 1-ml heparinized syringe (three samples per treatment). Blood samples were centrifuged for 10 min at 4000 rpm at 4°C. Plasma was transferred to a new vial and frozen at -18°C until further analysis.

The proximate analyses and dry matter of the experimental diets were performed according to AOAC, (2016) standard methodology. Samples of diets, fish dorsal muscles and feces were dried to a constant weight at 105 °C and Protein was determined by measuring total nitrogen of dried samples (N×6.25) using the Kjeldahl method after acid digestion using an auto Kjeldahl System. Lipids content was determined gravimetrically by ether extraction using a Soxhlet apparatus, and ash by combustion at 550 °C for 4h using a muffle furnace. Silica in both diet and fecal samples were determined according to AOAC, (2016) standard methodology using the gravimetric method. Amino acids in both experimental diets and fish dorsal muscles were analyzed according to AOAC, (2016), where amino acid concentrations were detected by using an amino acid analyzer. The digestible energy and protein energy ratio were calculated according to Hepher *et al.* (1983). Plasma samples were thawed to room temperature and deproteinised using 5- sulfosalicylic acid, then centrifuged for 10 min 3000 rpm at 4°C. Free methionine in plasma was determined according to Teerlink *et al* (1994) in a private lab using reversed-phase HPLC after pre-column derivatization.

Growth parameters and nutrient utilization:

Growth and nutrient utilization parameters were monitored and analyzed in term of final body weight (FBW), weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), apparent protein digestibility (APD) and protein efficiency ratio (PER). Mortality was recorded to calculate the mortality percentage at the end of the trial.

Statistical analysis:

Data are presented as means ± standard error (SE). The obtained data were subjected to one way analysis of variance (ANOVA) using the linear model (GLM) of SAS (SAS institute, 1991). Then means are compared adopting Duncan's new multiple range test ($P<0.05$).

RESULTS

Major nutrients composition of experimental diets:

Proximate analyses of experimental diets (Table1) showed limited variations among them, also; there was limited variation in amino acid content between control diets C+ and the C-. However, the largest tangible difference was between methionine content in diet C+ and diet C-, this was upgraded by supplementing either crystalline or coated methionine into both soybean meal tested dietary groups to the level equivalent to that in diet C+ (Table 2).

Table 2: Essential amino acids (EAA) content (% total, DM n=3) of the experimental diets.

AA	(C+)	(COM)	(CRM)	(C-)	Nile tilapia AA requirements[1]
Arginine	2.02	2.34	2.35	2.36	1.18
Histidine	0.84	0.89	0.90	0.90	0.48
Isoleucine	1.36	1.39	1.40	1.40	0.87
Leucine	2.60	2.57	2.57	2.57	0.95
Lysine	2.49	1.91	1.92	1.92	1.43
Methionine	1.04	1.04	1.04	0.55	0.75
Cystine	0.38	0.66	0.66	0.66	0.15
Phenylalanine	1.37	1.65	1.66	1.66	1.05
Tyrosine	1.09	1.28	1.29	1.29	0.50
Threonine	1.38	1.16	1.16	1.16	ND
Tryptophan	0.33	0.41	0.41	0.41	0.28
Valine	1.74	1.61	1.61	1.61	0.78

[1] (NRC, 2011).
ND: not determined

Growth performance:

After the 60 days feeding trial no mortality have observed among fish fed the four experimental diets (Table 3). It is clear that growth performance and feed utilization indices were significantly affected by adding either forms of methionine. Results showed that fish fed the SBM-based diet supplemented with coated methionine (COM) had comparable FBW (47.19g, 48.33g) WG (22.93g, 24.12g), FCR (1.72g/g, 1.58g/g) and SGR (1.65%/d, 1.72%/d) with positive control group ($P>0.05$). The poorest growth performance and feed utilization criteria were exhibited by the fish group fed the negative control diet (C-). Even though APDC were significantly higher ($P<0.05$) in fish groups fed diets supplemented with either coated or crystalline methionine compared with those fed un-supplemented diets (C+ andC-) yet the best PER was observed by fish groups fed the fish-based diet (C+) followed by fish group fed COM diet.

Table 3: Growth and feed utilization indices (mean ±SE, n=3) of Nile tilapia fed diets supplemented with either crystalline or coated methionine for nearly 9.weeks.

Index	Treatments				
	C+	COM	CRM	C-	P value
IBW (g)	24.21±0.27	24.26±0.31	24.08±0.18	24.14±0.13	>0.05
FBW (g)	48.33±1.95^{a}	47.19±4.33 ^{a}	39.87±0.17^{b}	37.60±0.75 ^{b}	<0.01
WG (g)	24.12±1.83 ^{a}	22.93±4.19 ^{a}	15.79±0.23 ^{b}	13.46±0.61 ^{b}	<0.01
SGR (%/d)	1.72±0.09 ^{a}	1.65±0.27 ^{a}	1.26±0.02 ^{b}	1.11±0.15 ^{b}	<0.01
FI (g)	38.03±0.61 ^{a}	38.52±1.05 ^{a}	35.00±0.19 ^{b}	34.50±0.57 ^{b}	<0.01
FCR (g/g)	1.58±0.21 ^{a}	1.72±0.31 ^{a}	2.23±0.06 ^{b}	2.57±0.07^{c}	<0.01
APDC %	88.82±1.29 ^{c}	93.07±0.57 ^{a}	91.69±0.80 ^{b}	85.37±0.57^{d}	<0.001
PER (g/g)	1.99±0.17 ^{a}	1.87±0.33 ^{a}	1.42±0.02 ^{b}	1.23±0.23 ^{b}	<0.01

Means with different superscripts letters in the same row are significantly different ($P<0.05$).
IBW Initial fish weight (g); FBW, Final body weight (g); WG Weight gain (g) = (FBW - IBM); SGR specific growth rate (SGR, % /d) = 100 × [Ln (FBW) – Ln (IBW)] / days of experiment; FI Feed intake (g)/ fish*day = air drying diet fed in g / (fish × day);; FCR Feed conversion ratio (g/g) = FI / WG; APDC Apparent protein digestibility coefficient (%)=APDC% = 100 – [100 (%Indicator $_{diet}$ / %Indicator $_{feces}$) x (%protein in $_{feces}$ / %protein in $_{diet}$)]; PER Protein efficiency ratio (g/g) = WG (g) / protein intake (g).

Table 4 shows that, even fish fed C- had the lowest dorsal muscle protein and total EAA contents comparing to the other dietary groups yet, in overall dorsal muscles protein and total EAA% content were not significantly affected by dietary treatments (P>0.05). Some of the essential amino acids profile of the dorsal muscles had significantly affected (P<0.05) by diet treatments. Where Leucine (leu.), Phenylalanine (Phe.), Histidine (His.), Lysine (Lys.) and Arginine (Arg.) content had significantly increased in the dorsal muscle of Nile tilapia that fed diet supplemented with Met. in either forms compared with fish groups fed C+ or C- diets (except for Arg. Which showed no significant variation among fish fed the CRM supplemented diet and both C+ and C- diets). However, other essential amino acid contents were not affected by dietary treatments (P>0.05).

Table 4: Protein and essential amino acids (EAA) content (% total) in dorsal muscles of Nile tilapia fed either crystalline or coated methionine supplemented diets.

Index	Treatments				
	C+	COM	CRM	C-	P value
Protein (%DM)	75.10± 1.1	78.00±0.5	78.00±0.5	73.97±2.73	>0.05
Arginine	4.61 ± 0.08^{b}	4.97 ± 0.01^{a}	4.63 ± 0.01^{b}	4.60 ± 0.14^{b}	<0.01
Histidine	2.11 ± 0.005^{d}	2.29 ± 0.03^{b}	2.46 ± 0.025^{a}	2.18 ± 0.045^{c}	<0.001
Isoleucine	3.60±0.12	3.71±0.01	3.63±0.045	3.41±0.20	>0.05
Leucine	6.03 ± 0.06^{bc}	6.39 ± 0.01^{a}	6.24 ± 0.035^{ab}	5.92 ± 0.24^{c}	<0.05
Lysine	6.63 ± 0.21^{b}	7.13 ± 0.13^{a}	7.07 ± 0.025^{a}	6.50 ± 0.13^{b}	<0.01
Methionine	2.29±0.08	2.50±0.02	2.50±0.03	2.36±0.12	>0.05
Phenylalanine	3.36 ± 0.09^{b}	3.68 ± 0.015^{a}	3.70 ± 0.07^{a}	3.45 ± 0.19^{b}	<0.05
Threonine	3.42±0.045	3.76±0.07	3.69±0.1	3.38±0.26	>0.05
Valine	3.55±0.2	3.33±0.12	3.67±0.01	3.55±0.36	>0.05
ΣEAA%	36.99±1.63	37.73±0.03	37.57±0.03	35.34±1.68	>0.05

[1]values are presented as mean ±SE (n=3); Mean values with different superscripts in the same row are significantly different (P<0.05).

Plasma methionine

Estimate values of Nile tilapia's plasma methionine at end of the feeding trial for positive control (C+), negative control (C-), SBM-basal dietary groups supplemented with either crystalline (CRM) or coated methionine (COM) are shown in Fig. 1. Plasma Methionine concentration was influenced by both protein source (FM or SBM and methionine form. Plasma methionine level decreased throughout the measuring period for fish fed Met-un-supplemented diet (C-) compared with those fed the fish meal- basal diet (C+). Supplementing SBM-diet with either crystalline or coated methionine showed a great elevation in fish plasma until 12.5h after feeding, then level started to decrease thereafter. Fish fed the diet supplemented with crystalline methionine (CRM) had the highest plasma methionine concentration throughout the measuring period as compared to the corresponding's for other dietary groups.

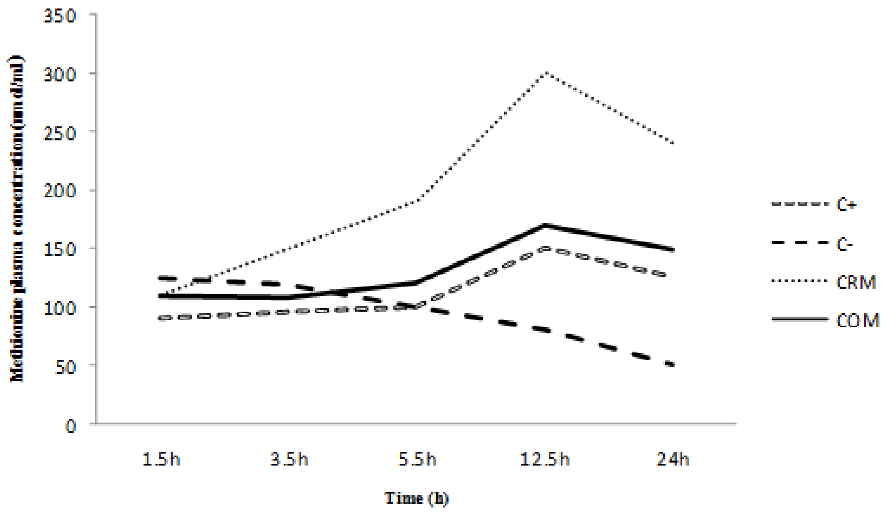

Fig.1: Estimated plasma methionine (nmol/ml) over time (h) in Nile tilapia fed diets supplemented with either crystalline (CRM) or coated methionine (COM) versus control positive (C+) and control negative (C-) diets.

DISCUSSION

Amino acids profile that fulfilling Nile tilapia requirements is assumed to maximize growth, feed utilization, nitrogen retention or other criteria related to amino acid utilization (NRC, 2011). Using plant protein source as major component in fish feed may cause limitation in one or more of their EAA content. In the present study, soybean meal was used as the primary protein source, in the negative control (C-), which caused a limitation in its' methionine content as compared to the fish meal control diet (C+). Therefore, according to the above concept by adding crystalline methionine to the level equivalent to that present in fish meal diet, this will cover fish requirements and so hypothetically will result in a fish performance equivalent to that of fish fed fish meal diet. Despite that, diet supplemented with CRM has shown a significant improvement in FCR and APDC values and slight improvement in fish FBW, WG, SGR and PER as compared with fish fed the un-supplemented diet (C-), values were still significantly inferior to the corresponding fish fed fish meal diet (C+). This agreed with the poorer performance reported by Mazid *et al*., (1978); Viola *et al*., (1994) and El-Saidy and Gaber, (2002) for tilapia ; Zarate and Lovell, (1997); Zarate *et al*., (1999) for channel catfish; Dabrowski *et al*.,(2003) for teleost fish; Bodin *et al*., (2012) for rainbow trout fish fed diets supplemented with crystalline amino acid (CAA) compared to all protein bound diets. On the contrary in the present study results showed that diet supplemented with COM recorded comparable FBW, WG, FCR and SGR value with those of the positive control fish (C+) (P>0.05) and highly significantly different from those fed the diet supplemented with CRM. This is consensuses with data reported by Yuan-Chao *et al*., (2011) that Chinese sucker (*Myxocyprinus asiaticus)* juveniles fed the coated lysine and methionine diet showed significantly higher growth performance than those fed the crystalline lysine and methionine diet (P<0.05). Jing Lu *et al*., (2014) reported that adding coating lysine and methionine significantly improved the weight gain and specific growth rate of black sea bream compared with un-supplemented diets. Furthermore, Chi *et al*., (2014) stated that coating techniques have enhanced AA utilization in the diets of Japanese flounder (*Paralichthys olivaceus*) (Deng *et al*., 2006, 2007), grass carp (*Ctenopharyngodon idellus*) (Liu *et al*., 1999, 2002), crucian carp (*Carassius carassius)* (Wang *et al*., 2006), and Chinese sucker (*Myxocyprinus asiaticus)* (Yuan *et al*., 2011). The technique delays the leaching of AA in water and/or improves AA utilization by slowing AA absorption in the intestine (Chi *et al*., 2014).

In the current research, the lowest dorsal muscle protein and total EAA content was observed in the muscles of fish fed (C-) diet. However, in overall, dorsal muscles protein and total EAA content were not significantly affected by dietary treatments ($P>0.05$). This finding agrees with those finding of Yuan *et al*., (2011) and Lu *et al*., (2014) who reported that the lowest protein level in dorsal muscles was observed in Chinese sucker (*Myxocyprinus asiaticus)* fish fed un-supplemented with FAA. The possible reason for the increase in protein-deposition is that balanced dietary amino acid profile usually promote protein synthesis rates in fish muscles (Furuya *et al*., 2004).In the present study most of the essential amino acids content had increased in the dorsal muscles of Nile tilapia that fed the diet supplemented with either COM or CRM as compared with those fish fed C+ or C- diets (except Val and His in COM dietary group). These results are in accordance with the findings of Lu *et al*., (2014), who reported a significant increase in Met-deposition within the dorsal muscles when fish fed a diet supplemented with high lysine and methionine levels in comparison to those fish fed the un-supplemented diets. Similar results were found for black sea bream by Zhou *et al*., (2011). From the above results it is clear that supplementing the SBM-based diet with either coated or crystalline methionine have improved the dietary balance of the amino acids and so decreased the deamination effect in fish metabolism Rolland *et al*., (2015).

Diet supplemented with CRM registered the highest fish plasma methionine concentration throughout the whole measuring period compared with either C+ or COM. Accordingly fish fed the CRM- added diet retained a relatively poorer growth performance than fish groups fed either bounded FM- protein (C+) or the SBM-protein based diet with coated methionine supplementation. According to Bodin *et al*.,(2012) the highest availability of amino acids derived from crystalline amino acids compared to bounded protein results temporal mismatch at the site of protein synthesis in rainbow trout (*Oncorhynchus mykiss* W.) , leading to decrease in growth and increase in ammonia excretion. Rolland *et al*., (2015) reported in addition to the rapid appearance of amino acid in the plasma, methionine from a crystalline source also remained elevated for up to 36h after meal ingestion.

In conclusion supplementation of the coated form of methionine was proved to be effective in improving the growth performance of monosex Nile tilapia in comparison to the crystalline form to the SBM-based diets. More studies could be done on the coating techniques and/ or coating material to improve methionine utilization in fish to approach a level similar to that of the FM- bounded protein.

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