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Research Article

The Effects of Two Chemo-attractants and Different First Feeds on the Growth Performances of African Catfish (*Clarias gariepinus*, Burchell, 1822) at Different Larval Stages

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Abstract: In this study, 4 days old African catfish larvae were fed with trout starter diet, minced beef liver and fresh water mussel, dried tubifex, DL-alanine and betaine supplemented trout starter, artemia nauplii and a combined diet consisting of boiled chicken egg yolk, minced mussel and dried tubifex as the first feed after yolk absorption for a week. At the end of the first period, it was observed that DL-alanine and betaine supplementation did not improve the larval growth and survival compared to other feeds. In the second stage of this study, ten days old larvae of *Clarias gariepinus* were fed with trout starter, artemia and trout starter, only DL- alanine, only betaine and both DL- alanine and betaine supplemented trout starter diets for 30 days. In contrast to first stage, DL-alanine and betaine supplementation improved the growth and survival rates. These results showed that DL-alanine and betaine did not have any attracting role in the pre-larval stage; however, in the post-larval stage, they had a very strong effect on the survival and growth rate of the African catfish, *Clarias gariepinus*.

Key Words: *Clarias gariepinus*, larvae, starter feeds, attractant, DL-alanine, betaine

İki Atraktant Madde ve Bazı Başlangıç Yemlerinin Farklı Larval Dönemlerde Karabalık (*Clarias gariepinus*, Burchell, 1822)'ların Büyüme Performansları Üzerine Etkisi

Özet: Bu çalışmada 4 günlük karabalık (*Clarias gariepinus*) larvaları bir hafta boyunca alabalık başlangıç yemi, kıyılmış sığır karaciğeri ve tatlı su midyesi, kuru tubifeks, DL-alanin ve betain katkılı alabalık başlangıç yemi, artemia nauplii ve kaynamış yumurta sarısı, kıyılmış midye eti ve kuru tubifeksten oluşan karma bir yemle beslenmiştir. Birinci aşamanın sonunda, DL-alanin ve betain ilavesinin larvaların büyüme ve yaşama oranlarında iyileştirme sağlamadığı gözlenmiştir. Çalışmanın ikinci aşamasında ise 10 günlük karabalık larvaları 30 gün boyunca alabalık toz yemi, artemia ve alabalık yemi, sadece alanin, sadece betain ve hem alanin hem de betain katkılı alabalık yemi ile beslenmiştir. Birinci aşamanın aksine, alanin ve betain ilavesi büyüme ve yaşama oranlarını arttırmıştır. Bu sonuçlar DL-alanin ve betainin erken larval dönemde herhangi bir etkiye sahip olmamasına rağmen, bu maddelerin post-larval dönemde oldukça etkili olduğunu göstermektedir.

Anahtar Sözcükler: *Clarias gariepinus*, larva, başlangıç yemleri, atraktant, DL-alanin, betain

Introduction

In fish larvae-culture, weaning problems from live food to dried diets have not been solved completely yet. It has been almost compulsory to feed with live food during a definite period and not possible to use only dry diets as a first feed for most cultured fish species (1). However, to a large extent these problems have now been overcome by the development of an artificial diet and specialized nursery techniques (2). Most endeavors to rear *C. gariepinus* larvae have relied on natural food organisms as a first feed (3,4). The most important live food organisms used today are micro-algae, rotifers (*Brachionus plicatilis*) and the brine shrimp (*Artemia* spp.). Use of artemia nauplii has increased the production costs of larvae-culture. For this reason, attempts have continued to find new food sources that can substitute for artemia and good results have been reported for most cultured fish species. *Moina* genus from the *Cladocerans* has been known to be an appropriate live starter food for milkfish (*Chanos chanos*) and *Clarias macrocephalus.* Tubifex worms have also been used successfully in culture of European catfish (*Silurus glanis*) larvae. Also, tubifex worms have been used in artificial diets of *Clarias gariepinus* and *Heterobranchius longifilis* larvae and it was reported that they can be used instead of artemia nauplii for Mekong catfish (*Pangasius bocourti*) larvae

(5). Another alternative food source for larvae culture is dry diets. Dry diets containing balanced and suitable feed ingredients could be prepared economically compared to live foods (6). Therefore, it is very important to decrease live food period or feed with dry diets completely. Although dry feeds have met the nutritional requirements of fish larvae and have been prepared their proper sizes with the aid of today's technology, attractiveness and digestibility of these feeds for the larvae have not been provided completely (7). Dabrowski and Poczyczynski (8) suggested that the inhibition of fish enzymes by proteinase inhibitors present in dry diets as an explanation for the consistent lower growth rates found in the larvae fed with formulated feeds. For this reason, success in weaning to a dry diet in early stages has been limited. Moreover, this low success of dry diets was attributed to insufficient feed intake, digestion and absorption due to absence of a functional digestive system in early larval stage. Verreth et al. (9) indicated that a functional digestive system has been completed on day 5 after the start of exogenous feeding in *Clarias gariepinus* larvae. Also, Verreth and Van Tongeren (10) fed *Clarias gariepinus* larvae with artemia during 0, 2, 3, 5, 7 or 10 days at the onset of exogenous feeding and they determined that the final weights and growth rates of the larvae weaned after 5 and 7 days did not differ significantly. Thus, in the present study, one week trial was found enough to determine the effects of DL-alanine and betaine for the pre-larval stage of *Clarias gariepinus*.

Some chemical compounds have been used to stimulate the feed intake, decrease feed waste and to prevent the deterioration of the water quality. These attractant substances help to improve dietary food intake and at the same time promote quicker food intake, minimizing the time the feed remains in water and thereby the leaching of water soluble nutrients, and providing additional nutrients for protein and energy metabolism (11). Behavioral studies demonstrated that some water-soluble amino acids (like L-amino acids) have a potential for stimulation of food intake and act as chemical cues (12). Betaine and L-amino acids are highly soluble substances and when feeds are wetted they spread rapidly in water (13). Some amino acids have been known to have attractive role on rainbow trout (*Oncorhynchus mykiss*) (14,15), sea bass (*Dicentrarchus labrax*) (16) and European eel (*Anguilla anguila*) (17). Also some amino acids and related substances (like betaine) are nutritional stimulators for penaeids including crustaceans (18).

Lots of chemical compounds have been described as attractant. Some of these stimulate olfactory and others gustatory receptors of fish. The olfactory system is responsible for fish behavior; for example schooling, courtship, migration, recognition of individuals and their own broods (19), while the gustatory system is directly associated with feeding behavior (20). In recent years, olfactory and gustatory studies in fish have focused largely on feeding problems in aquaculture. It has been understood that L-amino acids and betaine were used more widely as grow-out diets than larval feeding exists nutritional problems and what's more the effects of these attractant substances on the growth performances of *Clarias gariepinus* larvae has not been examined before. Starter feeds (i.e. tubifex and fresh water mussel) were selected as natural food organisms and due to nutritional potential (i.e. chicken egg yolk, tubifex, artemia, beef liver) for feeding larvae of African catfish, *Clarias gariepinus.* Effects of these starter diets and DL-alanine and betaine on the growth and survival of *Clarias gariepinus* were investigated at different stages.

Materials and Methods

Experimental fish and conditions

The present study was carried out Aquaria unit at Fisheries and Aquaculture Faculty of Mustafa Kemal University, Hatay, Turkey. All the aquaria sizes (80 x 40 x 40 cm) used in both pre-and postlarval stages were the same. Larvae of *Clarias gariepinus* were obtained through artificial reproduction (21). On the final day of yolk absorption, 150 larvae were stocked in each 30-L aquaria. Larvae for the second trial were fed with only artemia nauplii until onset of the experiment. In the postlarval stage, 100 larvae were stocked in each 60-L aquaria. Water temperature was maintained 25 ± 1 ^{0}C in aquaria during the experiments. Feces and waste feed siphoned after each feeding operation and dead larvae were removed, and also continuous aeration was supplied except during feeding times.

Feeds and Feeding

In the first stage, just yolk sac absorbed African catfish larvae (4 days old) were fed with commercial trout starter (TS); supplemented with DL-alanine and betaine

trout starter (ABSD); minced beef liver (MBL); artemia nauplii (AN); minced fresh water mussel (MM) (*Unio terminalis*); dried tubifex (DT) and a combined diet consisting of boiled chicken egg yolk, dried tubifex and minced mussle (EMT). ABSD were prepared by spraying 3 g alanine betaine mix (1:1) on 100 g trout starter diet. MM, MBL, EMT were prepared by homogenizing in a kitchen food processor and DT was ground finely with a grinder. This feeding period continued for one week after yolk absorption and larvae were fed by hand five times a day up to satiation level. Larvae were bulk weighed and placed randomly in the aquaria (80 x 40 x 40 cm).

In the second stage (postlarval stage), the larvae were fed with trout starter (TS); both trout starter and artemia (ATS); supplemented with only DL-alanine (AS), only betaine (BS) and both DL-alanine and betaine trout starter diet (ABSD). AS, BS and ABSD were prepared by dissolving 2 g of each in 50 ml distilled water and spraying on 100 g trout starter diet. In the artemia and trout starter feeding groups, hatched artemia and TS were given to the larvae two and three times a day respectively for one week, thereafter only TS was used during the remaining 23 days. Three aquaria were used for each treatment. Larvae were fed to satiation five times a day and aquaria were cleaned and dead larvae were removed daily for 30 days.

Chemical Composition of Larvae and Statistical Analysis

At the end of first stage, chemical composition of the larvae fed different feeds were not analyzed. At the end of the second stage (postlarval stage), chemical composition of larvae of *Clarias gariepinus* and feeds were analyzed. All larvae were harvested and the required amount was used for the proximate analysis at the end of the experiment. The dry matter, ash, lipid and protein content of whole body of the larvae were analyzed.

One way ANOVA was used to compare means of weight, length, specific growth rate and body composition of *Clarias gariepinus* larvae. Differences between feeding groups were assessed by Duncan's (22) multiple range test. Statistical analyses were considered significant at $P < 0.05$.

Results

At the end of the first stage, from yolk absorption (four days old) to ten days old, the mean weight gain of larvae fed with different feeds were found different (Table 1). The highest weight gain was obtained in the artemia fed group, while the lowest weight gain was observed in the dried tubifex fed group. No significant difference were detected among mean weight gains of the larvae fed with DT, TS and ABSD ($P > 0.05$). Also, differences between mean weights of larvae fed with EMT, MM and MBL were not found different ($P > 0.05$). Artemia fed group was found different from all feeding groups with respect to weight, length and specific growth rates ($P < 0.05$) (Table 2).

In the post-larval stage, fish fed with both alanine and betaine supplemented trout starter diet showed the best growth, but the survival was low compared to fish fed only betaine supplemented diet. Differences between betaine and both alanine and betaine supplemented feeding groups were not different with respect of weight and specific growth ($P > 0.05$). However, mean weight of fish fed with only alanine or betaine supplemented trout starter diet were different each other ($P < 0.05$)

Table 1. The chemical composition of the feeds at the different larval stages*.

Feed	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)
TS	56	15	7	10
BEY	16.16	34.1	49.22	1.65
MBL	20	3.85	68.99	1.34
DT	60	6.5	6	-
AN	60	24	8.5	4.4
MM	8.18	1.61	86.88	3.57

* Each sample was analyzed in duplicate, TS- trout starter; DT- dried tubifex; BEY- boiled egg yolk; MM- minced mussel; MBL- minced beef liver; AN- artemia nauplii.

Table 2. The mean body weight, length (mean ± SE), specific growth and survival rates of *C. gariepinus* fed with different feeds at the end of the first feeding stage.

Feeding groups	Initial weight (mg)	Final weight (mg)*	Final length (mm)*	Survival (%)	SGR (%d^{-1})*
TS	2.89±0.18	9.35±0.61^{a}	10.23±0.28^{a}	80	16.86^{a}
DT	3.21±0.12	8.35±0.51^{a}	10.05±0.20^{a}	90	13.71^{b}
ABSD	3.11±0.16	9.65±0.46^{a}	10.74±0.22^{ab}	79	16.28^{a}
EMT	3.03±0.04	13.35±0.49^{b}	11.85±0.16^{c}	98	21.14^{c}
MM	3.47±0.11	13.65±0.88^{b}	11.92±0.23^{c}	96	19.57d
MBL	3.36±0.19	14.35±1.29^{b}	11.27±0.30^{c}	83	20.71^{cd}
AN	3.31±0.15	20.80±0.84^{c}	14.00±0.16^{d}	100	26.29^{e}

* Means within the same column not sharing a common superscript letter are significantly different ($P<0.05$). TS- trout starter; DT- dried tubifex; ABSD- alanine and betaine supplemented diet; EMT- containing of egg chicken yolk, mussel and tubifex; MM- minced mussel; MBL- minced beef liver; AN- artemia nauplii; SGR: specific growth rate. SE: standard error

(Table 3). These results showed that betaine was more effective than DL-alanine on the growth and survival of *Clarias gariepinus* larvae in the post-larval stage. Additional feeding with artemia for a week did not increase the growth, but increased the survival and the lipid content of the fish (Table 4).

Discussion

In the first stage, it was observed that DL- alanine and betaine supplementation had not any attractive role on larval feeding behavior. On the other hand, wet feeds were very effective and especially fresh water mussel (*Unio terminalis)* and combination of tubifex, chicken egg yolk and minced mussel gave significantly high survival rates. In spite of improved growth rate, larvae fed with minced beef liver had a relatively low survival rate, because aquaria given minced beef liver were polluted rapidly and this situation required the continuous filtering or renewing the water. If this could be provided, good results would be obtained. In dry feeding groups, larvae were fed with dried tubifex had a good survival, but lower growth rate. This situation might be due to insufficient nutrient composition of dried tubifex and if it was supplied with nutrient rich moist feeds, growth of larvae could be increased. Dried ground tubifex had also attracted the larvae and most of the feed given was consumed by the larvae rapidly. The trout starter and DL-alanine and betaine supplemented trout starter feeding groups gave poor growth and survival. These results showed that DL-alanine and betaine did not have any attractive role in the first feeding period.

In the second stage, effects of DL-alanine and betaine on the weaning (from live food organism to a dry feed) period of *C. gariepinus* were examined. For this purpose, some authors advised different weaning periods. Appelbaum and Van Damme (23) reported an experimental duration of 46 and 50 days, including postlarval period, while Verreth (24) determined that the maximum growth was attained when the larvae received artemia for 7 days before weaning. Our observation indicated that betaine and DL-alanine together had a good effect on the growth and survival of ten days old larvae. Similar results were obtained from the larvae fed with only betaine supplemented trout starter diet at the end of the second stage. These findings showed that African catfish larvae did not have any chemo-reception like olfaction and gustation due to undeveloped sensory organs in the pre-larval stage. On the other hand, larvae were attracted well by betaine or alanine and this increased the feed consumption and growth in the second stage. There was no difference between growth and survival of the larvae fed artemia and only trout starter for 30 days. Therefore, one week artemia feeding seems to be adequate because extra one week feeding with live food and then weaning to a dry diet did not give any additional growth.

Although maximum lipid content was found from the larvae fed with artemia and trout starter for 30 days,

Table 3. Mean growth (mean ± SE) and survival rates of ten days old larvae fed with DL-alanine, betaine and both DL-alanine and betaine supplemented diets.

Feeding groups	Initial weight (mg)	Final weight (mg)	Survival (%)	SGR (%d^{-1})*
TS	8.92±0.58	165.70±15.08[a]	9.77[a]	50.83
ATS	9.17±0.76	144.40±9.75[a]	9.20[a]	63.05
AS	8.21±0.44	301.06±31.92[b]	12.0[b]	72.22
BS	8.64±0.61	458.03±44.82[c]	13.27[c]	84.44
ABSD	8.11±0.57	466.37±61.21[c]	13.50[c]	73.61

TS- trout starter; ATS- artemia and trout starter; AS- alanine supplemented;
BS- betaine supplemented; ABSD- alanine and betaine supplemented diet
Means within the same column not sharing a common superscript letter are significantly different ($P < 0.05$). SE: standard error

Table 4. The chemical composition of the larvae fed with different diets at the end of the second feeding period.*

Feeding groups	Dry matter (%)	Ash (%)	Lipid (%)	Protein (%)
TS	29.35[a]	3.09[a]	7.90[a]	19.06[a]
ATS	27.12[b]	4.02[b]	11.11[b]	15.3[b]
AS	23.38[c]	2.97[c]	5.31[c]	14.77[c]
BS	23.80[cd]	2.91[c]	6.23[cd]	13.94[cd]
ABSD	22.93[c]	3.07[a]	6.50[d]	13.64[d]

* Each sample was analyzed in duplicate. Results are expressed as the percentage of the wet weight. Different superscripts in a column are significantly different each other ($P < 0.05$).

chemical composition of the larvae in other feeding groups were not significantly different. Only DL-alanine and betaine supplemented diets gave less lipid content for the second stage could be attributable to the lipotrophic characteristic of betaine (15). From the current results, it could be concluded that DL-alanine and betaine had the similar lipotrophic effect on the body composition of the African catfish, *Clarias gariepinus* larvae.

In conclusion, it was understood that DL-alanine and betaine did not improve the growth and survival *Clarias gariepinus* larvae in the first stage, but they had a strong effect in the second feeding period. Larvae fed with only betaine supplemented diet showed the faster growth and better survival than larvae fed with alanine supplemented diet. Therefore, there is no need to feed the larvae with alanine in the second stage.

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