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Use of Shrimp *Penaeus* spp. and Krill *Euphausisa* spp. in a Feed Training Diet for Striped Snakehead *Channa striata*

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Abstract
Snakehead *Channa striata* fry, with an initial average weight of 60 mg were trained to accept artificial feeds containing either freeze-dried krill powder, or pulverized dried shrimp. A third, more traditional diet, containing 36% fishmeal was used as a control. Training diets were given, which contained 100%, 75%, 50%, 25%, or 0% krill meal by weight, or the same rates of pulverized shrimp by weight. Feed training began with the 100% krill or shrimp diets, and they were lowered by 25% every two days until 0% krill and shrimp diets were reached. The control received a 36% protein containing powder feed throughout the study period. The experiment contained four replicates per treatment group. Each replicate contained 200 fry at the starting of the study. Fry were fed to satiation four times a day for 10 days. At harvest, survival of fish given the control diet (47.25 ± SD 13.34%) was lower than those given krill (84.13± SD 12.41%) or shrimp diets (87.5 ± SD 1.47%) ($P < 0.05$). The survival of fish which were given the krill or shrimp diets was similar ($P > 0.05$). Weight gain differed between treatments ($P = 0.0009$), being highest with the shrimp diet, intermediate with the krill diet, and lowest in the control. Condition factors were similar between the treatments.

Keywords: Snakehead, Feed training, Fish meal, Krill

1. Introduction
Currently, there are eight reported species of snakehead found in the Mekong basin. Only two of these species: the giant snakehead (*Channa micropeltes* Cuvier, 1831), and the chevron snakehead (*Channa striata* Bloch, 1793) are used in any significant capacity in aquaculture [1]. The chevron snakehead is the most common form of snakehead found in the lower Mekong basin. It is highly carnivorous, air breathing, and is capable of migrating over wet ground [2]. Due to its abilities to tolerate harsh conditions, poor water quality, fluctuating drought, and flood seasonal changes it has become a highly valuable species cultured in rice farming areas in Vietnam [2]. Furthermore, monoculture settings of this species can be achieved at very high densities, since it is capable of breathing air, which allows it to live in waters with low dissolved oxygen and minimal aeration [3].
Although the snakehead is a relatively hardy cultured species, a major obstacle presents itself particularly among the juveniles in the form of cannibalism [4, 5, 6]. Due to this constraint, survival rates in juveniles are low. In addition to low survival, a large variation in size can also be seen during the grow-out phase [7]. Juvenile snakeheads are capable of cannibalizing siblings almost two thirds their body lengths [7]. In a monoculture setting where snakehead are stocked between 40-80 individuals per square meter, cannibalism can result in average survivals ranging between 13-15% after a 9-11 month grow out period [8].
Feed training has been used in several different carnivorous species to reduce mortality and size variations due to juvenile cannibalism [5, 6]. Feed training protocols and formulated feeds have been used as part of several snakehead studies regarding growth and survival of larval snakehead [9], feed dependent cannibalism [4], food selection and growth [3], effects of temperature, stocking size and density on performance [7]. Increased availability of formulated feed reduced mortality by cannibalism from 83% to 43% when the daily feeding rate was 15% of the larger fish's body weight [4]. Decrease in size variance also resulted in a significant decrease in cannibalism. Various protocols have been evaluated for feed training snakehead using *Artemia*, zooplankton, fishmeal-based formulated feed, and pelletized feed [3, 9]. There has been limited success in feed training snakehead using a live feed such as *Artemia* or

Zooplankton as a starter diet; a transition feed composed of live feed and formulated feed; and finally a completely formulated pelletized diet [9]. The affinity, palatability and digestibility of the formulated diet is the primary concern when feed training a carnivorous fish. Formulated feeds do not have the characteristics of live food organisms and an attractant is needed to facilitate a feeding response. Therefore, fish require being more attracted toward the formulated feed relative to cannibalism [10].

Krill *(Euphausia* spp.*)*, either as whole freeze-dried krill or as krill meal has been used with some success in the feed training of largemouth bass *(Micropterus salmoides)* [5], and peacock bass *(Cichla* sp.*)* [6]. Krill has been used with Atlantic salmon, where fishmeal in formulated diets was replaced with krill meal [11]. Furthermore krill based starter diets showed better success in growth due to the 60% protein composition compared to fishmeal-based diets [11]. Krill based diets have also been used successfully in gradual feed ingredient transition (GFIT) studies where the krill meal has been replaced with fishmeal or a pelleted diet towards the end of the feed training transition period [12]. Therefore, a krill-based starter diet with a gradual transition to standard formulated pelleted feed could be used to feed train snakehead.

The primary disadvantage to using freeze dried krill or krill-meal based diets is the cost [13]. The 2011 cost for freeze-dried whole krill was about 70 USD per kg while the price of krill meal ranges between 30-36 USD per kg. Due to this relatively high cost, studies have been done to find a suitable replacement for krill to be used in starter diets. Commercial fish feed top coated with menhaden oil has been used with some success [14]. Shrimp *(Penaeus* spp.*)* has also been used with some success as a replacement for krill in largemouth bass feed training [13]. Its similar gustatory qualities to krill as well as its storage qualities make it an ideal replacement [13]. Furthermore, its cost is relatively lower than that of krill in the Asian subcontinent. The 2011 price of dried shrimp is between 16-18 USD.

The following study was conducted at the fisheries unit at Can-Tho University, Vietnam, in August 2011. The primary objective was to conduct an intensive feed training trial on snakehead fry with krill and shrimp based diets. Growth, survival, size variance, and condition factor between treatments were evaluated in a 10-day trial after a gradual feed ingredient transition.

2. Materials and Methods

Six snakehead nests containing approximately 10,000 fry each, with a mean length of 15.5 mm and a weight of 60 mg were obtained from a snakehead hatchery in An-Giang province, located in the Mekong Delta, in the southwestern part of Vietnam. Fry were placed in a 0.60 m diameter circular, fiberglass holding tank, with about 0.90 m^3 of de-chlorinated water. The water was kept static, and adequate aeration was provided. The fry were allowed to acclimate for 24 h prior to the start of the feed training. The fry were given a commercial powdered fry feed containing 36% protein made into a dough ball and stuck on the wall of the tank, during the 24 h of acclamation. The feed training study was conducted indoors, using 12 square (0.90 x 0.90 x 0.90 m) fiberglass tanks with a depth of 0.70 m filled with clean de-chlorinated water, and aerated with an air-stone. A random sample of fish were taken from the acclimation tank using a dip net, and 200 were counted and weighed in groups of 50. The lengths of 40 fish from these 200 fish were also measured and recorded. The 200 fish were then placed in one of the prepared square tanks. The procedure was repeated until the 12 tanks were stocked with 200 snakehead fry each. Each tank was randomly assigned to one of the three treatments to give four replicates per treatment. The water in each tank was kept static except for a two thirds exchange of its volume once a day. Following a regime modeled after Campbell and Phelps [13], and Kubitza and Lovshin [12], fish were fed to satiation four times a day during daylight hours at 09:00, 11:20, 13:40, and 16:00 hrs. Feeds made by mixing krill meal or shrimp meal with the commercially available feed powder to form a dough were given in proportions containing 100%, 75%, 50%, 25%, and 0% by weight. The dough was run through a fine extruder to form a 1 mm crumble-pellet, which had been sun dried for 6 h. The commercial feed was a 36% protein powder containing: fishmeal, rice bran, soybean meal, and a vitamin and mineral supplement. The control diet was 100% of this commercial feed powder by weight, similarly extruded and dried. All feeds during the experiment were stored in airtight plastic containers. The initial diet of 100% krill, or shrimp was given as crushed particles for the first two days, followed by the pelleted 75% diets for two days, the 50% diets for two days, the 25% diets for two days, and finally the 0% shrimp or krill (plain commercial feed) diets for the last two days. The controls received the pelleted fishmeal based commercial diet throughout the experiment.

After 10 days of feed training, all the fish were harvested. The total number of fish for each tank was counted and total weights taken. A sample of 40 fish was randomly picked from each tank, and individual lengths were measured, and total sample weight recorded. The data was used to calculate the average weight and length per fry as well as the condition factor. The data were analyzed using Prism statistical software [15] to determine the differences in survival, growth and weight gain, size variation, and condition factor, between the two diets and the control. All percentage data were arcsine transformed prior to analysis [16].

3. Results

3.1 Survival

The fish given the commercial diet had a lower survival (47.25 ± SD 13.34%) than fish given either the krill or shrimp meal based diets ($P<0.05$). Fish given the krill or shrimp meal based diets had similar survivals ($P>0.05$) (Table 1).

Table 1. Mean values for survival, weight, weight gain, and length for the snakehead fry at the end of the 10 day feed training. The superscripts indicate statistically significant differences between treatments. The values in the same row sharing a common letter are not significantly different.

Values at day 10	Krill based diet	Shrimp based diet	36% protein based control
Mean survival (%)	84.13%^{a}	87.5%^{a}	47.25%^{b}
Mean weight (mg)	155 mg^a	185 mg^b	125 mg^a
Mean weight gain (mg)Mean length (mm)	85 mg^a 24.66 mm^a	115 mg^b 25.86 mm^b	55 mg^c 22.15 mm^c

3.2 Growth and Weight

The mean weight of the snakehead fry was 60 mg at the start of the study. At day 10 the mean weight per fish was statistically different (P = 0.0009). The mean weight in the krill treatment was 155 ± 5.7 mg, the shrimp treatment 185 ± SD 23.8 mg, and the control 125 ± SD 5.7 mg (Table 1). Weight gain reflected a similar pattern with a significant difference between all three means. The mean weight gain per fish was 85 ± SD 5.7 mg for krill, 115 ± SD 23.8 mg for shrimp, and 55 ± SD 5.7 mg for the control (Table 1).

3.3 Length and Size variation

Fish fed the shrimp meal diet were significantly larger at harvest (25.86 ± SD 1.88 mm), ($P < 0.0001$) than those of the other treatments. Fish given the krill diet were significantly larger than those given the control diet, 24.66 ± SD 2.17 mm vs. 22.15 ± SD 1.83 mm respectively (Table 1). At the start of the study, there was not a significant difference between the coefficients of variance (CV) for length between treatments (P = 0.9674) with treatment means ranging from 8.05 mm to 8.26 mm. At the end of the 10-day trial, there was a larger variance in size in the control relative to the krill and shrimp diets. CV for lengths when compared between treatments showed no statistical difference in CV between the shrimp (CV = 14.39%) and krill diets (CV = 13.48%). The CV for the control diet (CV = 18.81%) was larger and significantly different from the krill and shrimp diets (Figure. 1). There were potential cannibals in all treatments.

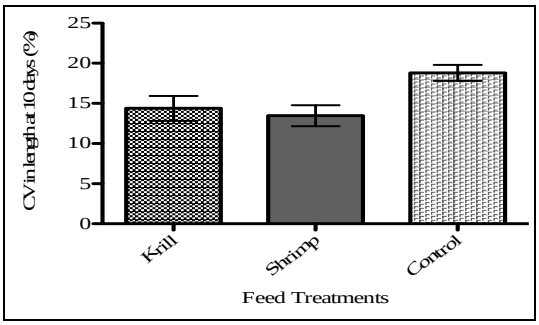

Fig 1: Mean values for Coefficient of Variance for length (+/-) SD between the krill meal, shrimp meal and control treatments at day 10.

3.4 Condition Factor

The mean condition factor for fish in the three different treatments were: 1.19 ± SD 0.122 for krill, 1.29 ± SD 0.085 for shrimp, and 1.34 ± SD 0.161 for the control (Figure. 2). An analysis of variance indicated no significant difference between any of the treatments for condition factor (P = 0.27).

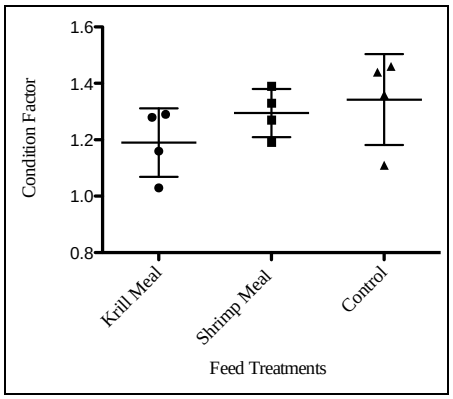

Fig 2: Mean values for Condition Factor (+/-) SD between the krill meal, shrimp meal and control treatments at day 10.

4. Discussion

Carnivorous fish are often difficult to culture due to their reluctance to accept formulated feeds. Such species must be trained to accept a formulated feed. When food is inadequate or of low quality, fish may resort to cannibalism [17]. Cannibalism rates of 50% or more have been reported in several species: African catfish *Clarias gariepinus* [18], sea bass *Dicentrachus labrax* [10], and yellow perch *Perca flavescens* [19].

Key elements in feed training of a carnivorous species are the attractability, palatability, and digestibility of the formulated diet. Before fish can consume formulated feed, they must be attracted to it. Adding Krill meal to formulated feeds for Japanese eel, *Anguilla japonica*, red sea beam *Pagrus major*, and striped mullet *Mugil cephalus* resulted in increased feed responses [20]. Krill has been used successfully as an attractant in the feed training of largemouth bass *Micropterus salmoides* [5]. Dried shrimp *(Penaeus* spp.*)* has been used with some success as a replacement for krill in largemouth bass feed training [13]. Its similar gustatory qualities to krill, as well as its storage qualities, make it an appropriate alternative [13].

The use of krill or shrimp, as a transition to a pelleted 36% commercial diet, improved snakehead survival over trying to train the fish to directly accept the commercial diet. The initial feeds that contained high percentages of krill and shrimp had very robust aromas, relative to the mild aroma of the control diet. The feeding behavior was also very lively in the treatments containing krill and shrimp and less so in response to the control diet.

Cannibalism is a common problem with snakehead, as they are able to consume fish of more than half their length [8]. Qin and Fast [4] were able to feed train snakehead and reduce the rate of cannibalism to 43% vs. 83% with non-feed trained fish. Qin *et al.* [9] were not able to get snakehead fry to directly accept a formulated diet, which resulted in no fish survival. However, after an evaluation of various transition feeding protocols using artemia, they were able to reduce cannibalism rates by15% to 25%. The use of dried krill and shrimp allows dry products to be given as a first feed in the feed training process and a gradual reduction of these ingredients in a formulated feed. Trying to feed train snakehead directly to the commercial diet resulted in an overall survival, after 10 days, of 47.25% ± SD 13.34%, while using krill or shrimp resulted in 84.13% ± SD 12.41%, and 87.5% ± SD 1.47% survivals respectively.

When only a portion of a population learns to accept formulated feed, the result can be a greater size variation with its ensuing opportunity for cannibalism. Folkvord and Ottera [21] have discussed how coefficient of variance (CV) in fish length could be an indicator for size-dependent cannibalism. In a 61 day study Qin *et al.* [9] have found snakehead length CVs ranging from 19.2% to 28% depending on the diet given with the greatest amount of cannibalism occurring where the CVs were the highest. In the 10 day study period the three feeding protocols tested resulted in similar coefficients of variation of length ranging from 13.48% for fish given the shrimp meal based diet to 14.39% for the krill diet. However, the CV for the 36% protein containing control diet was 18.81%, which was higher and significantly different from the other two diets. This along with the survival data indicates that there was a significantly higher occurrence of size dependent cannibalism in the control, while the shrimp and krill treatments showed significantly lower rates of size dependent cannibalism.

The krill and shrimp based diets not only improved survival but also growth. The shrimp based diet resulted in the most growth, the control the least, and the krill based diet an intermediate growth. The attract ability of the feed of the krill and shrimp based diets resulted in better growth vs. the control diet. The stability of the pellets may have been a factor contributing to the difference in growth between fish given the krill and shrimp based diets. The krill diet was not as stable in water as was the shrimp based diet. In the shrimp treatments, fry could be seen grazing on the leftover feed that had sunk to the bottom of the tank between feedings, while in the krill treatments this behavior was not seen. Therefore, having a longer period to feed on the shrimp may explain the size difference between these two treatments.

Condition factor is a measure of the overall physiological status or health of a fish [22]. It would be expected that in a treatment such as the control with the lowest snakehead survival and weight gain, the condition factor would also be lower. However, there was no significant difference in mean condition factors between the treatments. It is probable that cannibalism may have contributed enough nutrition to the surviving fish in the control treatment. Therefore, the condition factor in the control group is similar to the other treatments groups.

5. Conclusion

In designing this study, the primary practical consideration was the production cost of the feed. In addition to designing a feeding protocol that would increase snakehead survival and growth, it was important to develop a practical protocol that was not cost prohibitive. Both krill and shrimp based diets showed significant effects in lowering cannibalism, while the shrimp diet performed the best in growth. As krill meal cannot be found locally in Vietnam, it has to be imported from international suppliers. This increases production cost of the feed and further limits the access to this ingredient. Dried shrimp is a product that can be locally found in Vietnam, and it is relatively cheap. Since the shrimp based diet performed as well as the krill based diet in reducing cannibalism, and it also performed better than the krill based diet for growth, a shrimp-based gradual feed ingredient transition protocol is appropriate as a starting diet for snakehead culture in Vietnam.

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