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RESEARCH ARTICLE

Nutrient-enriched live lobster cockroach, *Nauphoeta cinerea*, enhances growth and pigmentation of the pearl arowana, *Scleropages jardini*

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Abstract

This is the first report on the use of live cockroaches as feed for farmed arowanas. Firstly, the nymphal development period, reproductive potential, longevity and nutrient profiles of the various development stages of the lobster cockroach, *Nauphoeta cinerea*, were determined. The fecundity of each female cockroach averaged 82.9 nymphs. An increase in body crude protein and lipid levels was observed as nymphs metamorphosised into later instars. The nutrient content of the adult stage was different from the nymphal stages with distinct differences evident between sexes. Secondly, in the feeding trial of pearl arowana, *Scleropages jardini*, three groups of live cockroaches were used; adult males with low lipid and high protein levels (3% and 30.1%, respectively; A-LLHP, control group), adult males with mid-level lipid and high protein levels (6.1% and 29.7%, respectively; A-MLHP) and late nymphs with high lipid and lower protein levels (10.3% and 22.9%, respectively; N-HLLP). Each group of cockroaches was fed to eight replicate individually-housed fish once daily for 12 weeks. A-LLHP and N-HLLP cockroaches were fed a commercial rat feed, whereas A-MLHP cockroaches were fed a specially formulated feed with added astaxanthin (80 mg/kg) to produce nutrient-enriched cockroaches. Arowana growth was highest in the N-HLLP-fed group followed by A-MLHP and A-LLHP ($P < 0.05$). The best feed utilization efficiency was observed in the N-HLLP group. Intraperitoneal fat and muscle lipid content was significantly higher in fish fed the nymphs than those fed adult cockroaches. Scales of arowana fed A-MLHP cockroaches showed the highest melanophore and xanthophore counts. Xanthophores were not present in the scales of the N-HLLP group. Late nymphal stages are optimal for growth enhancement and adult cockroaches for pigmentation of fish. This study showed that live cockroaches can be nutrient-enriched to enhance arowana growth and pigmentation.

Keywords

aquaculture – cockroach – insect feed – live foods – ornamental fish

1 Introduction

Live foods, including live insects, are the main nutrient input used in the captive farming of arowana. Arowanas have been called the "most expensive fish in the world" (Faber, 2017) and are in high demand as a status symbol in many Asian communities. Arowana farming has been rapidly expanding in many Asian countries to meet global demands from the aquarium fish trade (Yue *et al.*, 2020). The Asian arowana, *Scleropages for-*

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mosus, is the most well-known and popular but the pearl arowana, *S. jardini*, has also experienced increased demand. Very little information is known about the pearl arowana, which has been described as an opportunistic carnivorous fish, feeding on aquatic and terrestrial insects, crustaceans and small fish in their natural habitats (Merrick *et al.*, 1983). Despite the availability of commercial arowana feeds, the nutritional requirements of arowanas are still mostly unknown (Darias *et al.*, 2015). Using pelleted feeds has led to poor growth, low feed palatability, bone deformation and poor pigmentation in arowanas (Yue *et al.*, 2020). Arowana farmers and hobbyists prefer to use live shrimp, fish, frogs, worms and insects (Faber, 2017). The high reproductive rate and low maintenance costs of insects make them a cost-effective live food in arowana aquaculture. Fish eating insects as live prey in their natural habitats are well documented (Henry *et al.*, 2015; Nogales-Merida *et al.*, 2019) and arowanas have been reported to preferentially forage on insects (Merrick *et al.*, 1983; Torres del Castillo *et al.*, 2012). In Malaysia, the second largest arowana producer, cockroach farms can sometimes be found alongside commercial arowana farms. Live cockroaches are raised and used as live food. Nevertheless, as far as we know, there is currently no comprehensive research nor published report on the use of live cockroaches in arowana feeding.

To further investigate the potential of cockroaches, we focused on the lobster cockroach, *Nauphoeta cinerea* (Olivier). This tropical and subtropical species has a wide distribution and is easy to maintain as it does not glide despite having wings in its adult stage. It belongs to the family Blaberidae and is the sole representative of its genus. Willis *et al.* (1958) and Cornwell (1968) reported early research on its basic biology and development. Females are highly fecund and are ovoviviparous, incubating the ootheca (egg capsules) in the abdomen until the nymphs emerges. Nymphs undergo incomplete metamorphosis (eight instar stages) before emerging as adults, bypassing the pupal stage. Nymphs have thin exoskeletons and are wingless. In order to develop a sustainable mass production system, we conducted a comprehensive study to determine parameters such as fecundity, longevity and development periods of each life stage of the lobster cockroach. For the first time, the nutrient composition of the various life stages of the lobster cockroach was determined. The protein and lipid content of insects are known to be influenced by the type of food they receive and their life stage (Nogales-Merida *et al.*, 2019). Both the nymphal and adult stages, with different nutritional profiles, were used in the present study. The production of nutritionally enhanced insects for use in aquafeeds is a research area of great interest and commercial potential (Idris Zainab *et al.*, 2022). An attempt to produce "tailor-made" cockroaches in terms of protein and lipid content for the pearl arowana was investigated. Furthermore, since body coloration highly influences the price of arowanas, lobster cockroaches enriched with carotenoids (astaxanthin) were also used to evaluate their impact on fish pigmentation. Fish are not able to synthesize carotenoids *de novo* and must be introduced through their diets (Garcia-Chavarria and Lara-Flores, 2013). We hypothesized that live cockroaches could be used as a vehicle/vector to transport carotenoids to enhance skin/scale chromatophores in pearl arowana. Thus, this study investigated the effects of feeding live lobster cockroaches of different nutrient profiles and life stages on the growth performance, feed utilization efficiency, body indices and scale pigmentation in pearl arowana.

2 Materials and methods

Cockroach culture and biology

Lobster cockroaches, *Nauphoeta cinerea*, were reared at the Vector Control Research Unit of Universiti Sains Malaysia in polyethylene containers measuring 28 × 36 × 23 cm with perforated lids. All containers were placed in a 27.1 ± 0.1 °C room with a relative humidity of 80.7 ± 0.6% and 12:12 photoperiod. To ensure that the cockroaches do not crawl out from the containers during sampling when the lids are opened, a layer of petroleum jelly (Vaseline®, Unilever Holdings, Malaysia) was applied at the top of the container's inner surface. Rolled-up cardboards (8 × 8 cm) were placed in the containers as harbourage. Cockroaches were provided with free access to water and commercial rat pellet feeds (Gold Coin Feedmills Ltd., 702P, Malaysia). These containers provided the stock culture of cockroaches from which subsequent studies relied upon.

To study the development of the nymphal stages, 20 nymphs from the same pair of parent cockroaches were randomly isolated and transferred to individual polyethylene containers measuring 14.0 × 8.5 × 7.5 cm. A total of 20 replicate containers (n = 20) were used. Cockroach rearing conditions were as described earlier. Cockroach development was observed daily and the number of days for each instar stage to emerge was recorded (based on moulted outer cuticle). Once the adult stage

was achieved, the percentage survival and sex of the cockroaches were also determined.

To investigate the biology of the adult cockroach, five pairs of newly emerged adult male and female cockroaches were placed in a polyethylene container (28 × 36 × 23 cm) with culture conditions as previously described. Each female cockroach was marked with the number 1 to 5 on its pronotum using non-toxic white paint, respectively. A total of 20 replicate containers were used giving us 100 male and female cockroaches. The pre-oviposition period (the period between the hatching of an ootheca until the emergence of the next one) and the egg incubation period (the duration from the observation of the ootheca until the emergence of nymphs) were determined, and the number of aborted oothecae was also recorded. The number of cockroach nymphs hatched per ootheca per adult female was counted and the total nymphs produced per female was tabulated. We also recorded the longevity of the adult cockroaches from the day it became an adult stage to the day it died. All cockroaches that died before 20 days were not used to determine the average longevity of the adult cockroach.

TABLE 1 Protein and lipid composition (% wet weight) of live lobster cockroaches fed to pearl arowana, *Scleropages jardini*

Composition (%)[1]	Lobster cockroach[2]		
	A-LLHP (control)	A-MLHP	N-HLLP
Moisture	66.1 ± 0.4	65.0 ± 0.8	65.2 ± 0.0
Dry matter	33.9 ± 0.4	35.0 ± 0.8	34.8 ± 0.0
Crude protein	30.1 ± 0.5	29.7 ± 0.9	22.9 ± 0.2
Crude lipid	3.0 ± 0.1	6.1 ± 0.2	10.3 ± 0.3

[1] Mean ± standard deviation of three replicate values (n = 3).

[2] Cockroaches were fed either a commercial rat feed (Gold Coin Feedmills Ltd.; crude protein: 21.8%; crude lipid: 2.9%; crude fibre: 2.8%; ash: 6.9%) or a formulated cockroach feed (CF) containing 80 mg astaxanthin/kg (crude protein: 20.0%; crude lipid: 19.7%; crude fibre: 3.4%; ash: 5.6%).

A-LLHP = adult male cockroaches fed the rat feed which resulted in body composition containing low lipid and high protein levels; A-MLHP = adult male cockroaches fed CF which resulted in body composition containing mid-level lipid and high protein levels; N-HLLP = late nymphal cockroaches fed the rat feed which resulted in body composition containing high lipid and lower protein levels.

Nutrient composition of lobster cockroach

To determine the proximate composition of the lobster cockroach, samples from the stock culture were taken according to life stages and divided into three nymphal groups and two adult groups. Sixty early nymphs (first and second instar, 0.5-1.0 cm) and middle nymphs (third, fourth and fifth instar, 1.1-2.0 cm), respectively, were sampled. Thirty late nymphs (sixth, seventh and eighth instar, 2.1-3.0 cm), adult males (2.4-2.6 cm) and adult females (2.8-3.0 cm), respectively, were also sampled for proximate analysis.

For the present study, one group of cockroaches was fed commercial rat feed (21.8% protein, 2.9% lipid; Gold Coin Feedmills Ltd., 702P, Malaysia), the common practice in laboratory culture of cockroaches. Another group of cockroaches was fed our formulated cockroach feed (CF; 20.0% protein, 19.7% lipid) which allowed us to increase the lipid content of adult male cockroaches without affecting body protein content compared to the control group fed the rat feed. This proprietary CF also contained 80 mg astaxanthin per kg feed as a carotenoid source (CAROPHYLL® Pink, DSM Nutritional Products Ltd., Bangkok, Thailand). The analysed proximate composition of the rat feed and CF is shown in Table 1 footnote. Each cockroach population was fed their respective diet for at least four weeks before being used as live food for the arowana. Rearing conditions were as described earlier.

Arowana husbandry and feeding of live cockroaches

The handling and use of fish were conducted according to guidelines obtained from the USM Institutional Animal Care and Use Committee, Universiti Sains Malaysia. Pearl arowana, *S. jardini* (mean weight = 32 g), was purchased from a local ornamental fish shop and transferred to the Aquaculture Research Center, Universiti Sains Malaysia. As arowanas are highly aggressive territorial fish, all fish were individually-housed in an aquarium (95 L) with its own water filtration and aeration systems. Each aquarium was tightly closed at the top with a wire netting. About 30% of the water in the aquarium was replaced with fresh water every three days. A total of 24 fish were used. During the one-week acclimation period, all fish were fed five live cockroaches (middle nymphal stage) daily from the stock cockroach population fed the rat feed.

The arowana feeding trial comprised three dietary treatments consisting of three different groups of live lobster cockroaches. The first dietary treatment used adult male cockroaches fed the rat feed, resulting in body composition containing low lipid and high protein levels (3% and 30.1%, respectively; A-LLHP). The A-LLHP cockroaches were considered the control diet. The

second group of arowanas was fed adult male CF-fed cockroaches with mid-level lipid and high protein levels (6.1% and 29.7%, respectively; A-MLHP). The third group of fish was fed late nymphal cockroaches with high lipid and lower protein levels (10.3% and 22.9%, respectively; N-HLLP); these cockroaches were fed the rat feed. The protein and lipid content of all three groups of live cockroaches are as described in Table 1. Each group of cockroaches was fed to eight replicate fish once daily. Cockroaches were fed manually to each fish until apparent satiation and the number and weight of prey recorded. The feeding trial was conducted for 12 weeks.

Sample collection and proximate analysis

At the end of the feeding trial, all fish were starved for 24 h to empty their gut, killed by an overdose of tricaine methanesulphonate (MS-222; Sigma-Aldrich, St. Louis, Missouri, USA), and their individual weight and total body length measured. Blood samples were then collected into heparinized capillary tubes to determine hematocrit values. The fish was dissected and the liver, viscera and intraperitoneal fat collected and weighed to determine hepatosomatic index (HSI), viscerosomatic index (VSI) and intraperitonial fat value (IPF). Muscle samples were also collected and stored frozen at –20 °C until proximate analysis.

Proximate analysis of the feeds used, cockroaches and fish muscle tissues included the moisture, ash, crude protein and crude lipid content, and was completed according to the standard methods of AOAC (1997), as described by Ng *et al.* (2019).

Fish scale chromatophore analysis

The fourth fish scale counted from the head on the lateral line was carefully dislodged and placed on a glass slide with a drop of glycerine gel sufficient to cover the scale, secured with a microscope cover slip and allowed to dry. The slide was examined under a light microscope (Olympus S240) and chromatophores images of 800 μm × 800 μm dimensions were captured using a colour camera (JVC K-F55B) and processed with AnalySIS Docu 3.1 Image Analysis System software. The location of the scale where the chromatophores were counted was the same for all fish. The number of xanthophores (yellow pigment cells) and melanophores (black pigment cells) were counted. The melanophores were manually scored according to the degree of pigment dispersal based on the Melanophore Index (MI, Hogben and Slome, 1931), as visually depicted in Svensson and Skold (2011). Fully aggregated melanosomes were grouped into MI = 1 and fully dispersed melanosomes were grouped as MI = 5.

TABLE 2 Nymphal development time of the lobster cockroach, *Nauphoeta cinerea*[1]

Instar	Minimum number of days		
	Male (n = 8)	Female (n = 12)	Total (n = 20)
First	9.1 ± 0.4	10.5 ± 0.7	10.0 ± 0.5
Second	8.5 ± 0.4	8.5 ± 0.4	8.5 ± 0.3
Third	8.5 ± 0.5	9.3 ± 0.4	9.0 ± 0.3
Fourth	11.3 ± 0.6	10.7 ± 0.6	10.7 ± 0.4
Fifth	10.8 ± 0.3	9.9 ± 0.6	10.4 ± 0.4
Sixth	8.1 ± 1.2	8.3 ± 0.9	8.3 ± 0.7
Seventh	9.5 ± 1.1	10.7 ± 0.8	10.2 ± 0.6
Eighth	17.9 ± 1.7	30.1 ± 2.2	25.2 ± 2.0
Total days	83.6 ± 0.7[a]	98.0 ± 2.4[b]	92.0 ± 2.0

[1] Mean ± S.E values with different superscripts are significantly different ($P < 0.05$) using a pairwise T-test. Survival of cockroaches was 100% with a male-to-female ratio of 1:1.5.

Statistical analysis

The results were expressed as mean ± standard error. Statistical analysis was conducted using one-way ANOVA to detect significant differences among the various treatments at the significance level of 0.05. Tukey HSD test or T-test was used to compare the treatments when there was a significant difference among the means. Statistical analyses were performed with SPSS statistics 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

3 Results

Lobster cockroach development and reproduction

In the present study, since the cockroaches were being used as live food, we first need to establish some fundamental data on the nymphal development period and female reproductive potential. The lobster cockroach nymphs underwent eight instar stages and when raised in isolation, took an average of 92 ± 2 days before becoming adults (Table 2). All 20 cockroaches survived and upon achieving adulthood, it was observed that the ratio of male: female cockroaches was 1:1.5. Males were easily identified by their fully developed wings and prominent cerci. Female lobster cockroaches took significantly ($P < 0.05$) longer time (additional 15 days) to become adults compared to their male counterparts. From the first to the seventh instar, the development period was similar between the sexes, but at the eighth instar, female cockroach nymphs required an average

TABLE 3 Reproductive performance of the female lobster cockroach, *Nauphoeta cinerea*[1]

Ootheca	n	Pre-oviposition period (days)	n	Incubation period (days)	n	Nymphs per ootheca	n	Aborted ootheca (%)
First	87	13.1 ± 0.1[a]	62	34.8 ± 0.3[b]	62	30.0 ± 0.8[a]	83	25.3 ± 0.2
Second	78	10.2 ± 0.2[b]	77	33.4 ± 0.5[b]	77	29.7 ± 0.4[b]	78	1.3 ± 0.0
Third	78	10.3 ± 0.2[b]	75	32.9 ± 0.3[b]	75	29.5 ± 0.7[b]	78	3.9 ± 0.0
Fourth	72	12.8 ± 0.7[a]	34	31.4 ± 0.4[c]	34	21.0 ± 0.3[c]	58	41.4 ± 0.2
Fifth	34	10.9 ± 0.6[b]	2	43.0 ± 8.2[a]	2	31.0 ± 0.0[a]	19	89.5 ± 0.3
Sixth	5	14.6 ± 3.5[a]	1	27.0	1	13.0	4	75.0 ± 0.0
Mean		12.0 ± 0.7		33.8 ± 1.3		25.7 ± 2.9		–

[1] Mean ± SE values with different superscripts within the same column are significantly different ($P < 0.05$).

development time of 30.1 ± 2.2 days compared to only 17.9 ± 1.7 days for males (Table 2) before becoming adults.

In a mixed-sex cockroach population, it was observed that female cockroaches lived significantly longer compared to males, 186.3 ± 0.7 days (n = 85) versus 132.1 ± 2.9 days (n = 73), respectively. During its lifespan, the female cockroach was observed to have a maximum of six pre-oviposition occurrences (Table 3). Each pre-oviposition development period resulting in ootheca formation took 10.2 to 14.6 days (mean 12.0 ± 0.7 days) with the formation of the sixth ootheca taking the longest time. Inside the oothecae, the egg incubation period was between 31 to 43 days, with a mean of 33.8 ± 1.3 days (Table 3). The average number of nymphs produced per ootheca was 25.7 ± 2.9. The fourth, fifth and sixth ootheca showed high incidences of unsuccessful incubation due to the high percentage of aborted ootheca before the eggs hatch: 41.4 ± 0.2%, 89.5 ± 0.3% and 75%, respectively (Table 3). Only one female adult cockroach successfully carried and incubated the sixth ootheca, producing only 13 nymphs. The average fecundity of one female cockroach was estimated to be 82.9 nymphs.

Lobster cockroach body composition

Body moisture content varied from 63.1 ± 0.1% (adult female) to 68.6 ± 0.1% (early nymphs) (Table 4). Adult male cockroaches contained the highest crude protein content at 29.2 ± 0.1% wet weight basis or 84.3 ± 0.1% on a dry weight basis. The lowest body protein content was observed in the early nymphs (20.4 ± 0.1%) increasing to 24.4 ± 0.1% in the late nymphs. Similar to protein levels, crude lipid levels also increased as the nymphs grew, from an initial 7.0 ± 0.1% to 10.1 ± 0.4% in late nymphs on a wet weight basis. On a dry weight basis, crude lipid levels were equivalent to 26.5 ± 0.2% in late nymphs. Female adult cockroaches contained higher lipid content (5.6%) than males (3.5%). Crude fibre levels ranged between 0.6 to 1.3% and ash levels varied between 0.4 to 0.7% of wet body weight (Table 4).

Based on these initial body protein and lipid results, we decided to use the adult male cockroach (A-LLHP) and the late nymphs (N-HLLP), both of which were fed the rat commercial feed, as two of the live food groups for the present study. As previously described, the third group of cockroaches was fed a newly formulated cockroach feed (CF) which allowed us to modify the lipid content of the adult males (A-MLHP). The use of adult males and late nymphs allowed a distinct differentiation between "low", "mid-level" and "high" lipid dietary levels. The proximate composition of these three groups of live cockroaches was as previously described in Table 1.

Arowana growth and feed utilization efficiency

The source of live cockroaches had a significant effect on the final body weight (FBW), weight gain (WG) and specific growth rate (SGR) of pearl arowana (Table 5). The FBW, WG and SGR were significantly higher ($P < 0.05$) in fish fed the N-HLLP cockroaches compared to the control A-LLHP group. Arowanas fed the A-MLHP cockroaches were not significantly different in growth performance compared to the A-LLHP or N-HLLP groups. Feed conversion ratio (FCR), food intake (number of cockroaches eaten per day) and protein efficiency ratio (PER) were not significantly different ($P > 0.05$) among the different dietary groups (Table 5). The best FCR and PER were observed in fish fed the N-HLLP cockroaches. Only one fish died during the feeding trial in the A-LLHP group but this was not related to dietary treatment.

TABLE 4 Proximate body composition (% wet weight) of nymphal and adult stages of the lobster cockroach, *Nauphoeta cinerea*[1]

Parameter (%)[3]	Lobster cockroach stage[2]				
	Early nymphs	Middle nymphs	Late nymphs	Adult male	Adult female
Moisture	68.6 ± 0.1 (31.4 ± 1.4)	68.0 ± 0.2 (32.0 ± 0.2)	63.2 ± 0.3 (36.8 ± 0.3)	65.7 ± 0.7 (34.3 ± 0.7)	63.1 ± 0.1 (36.9 ± 0.1)
Crude protein	20.4 ± 0.1 (64.0 ± 0.2)	21.0 ± 0.1 (63.2 ± 0.3)	24.4 ± 0.1 (63.7 ± 0.2)	29.2 ± 0.1 (84.3 ± 0.1)	29.1 ± 0.1 (78.5 ± 0.2)
Crude lipid	7.0 ± 0.0 (21.9 ± 0.3)	8.3 ± 0.2 (25.1 ± 0.6)	10.1 ± 0.4 (26.5 ± 0.2)	3.5 ± 0.0 (10.0 ± 0.0)	5.6 ± 0.0 (15.2 ± 0.1)
Crude fiber	1.0 ± 0.0 (3.2 ± 0.1)	1.1 ± 0.0 (3.4 ± 0.0)	0.6 ± 0.0 (1.5 ± 0.1)	0.9 ± 0.0 (2.6 ± 0.0)	1.3 ± 0.1 (3.6 ± 0.2)
Ash	0.4 ± 0.2 (1.2 ± 0.7)	0.5 ± 0.0 (1.4 ± 0.8)	0.5 ± 0.1 (1.3 ± 0.7)	0.7 ± 0.2 (2.1 ± 1.2)	0.7 ± 0.1 (1.9 ± 1.1)
NFE[4]	2.6 ± 0.1 (9.7 ± 0.3)	1.1 ± 0.1 (6.4 ± 0.2)	1.2 ± 0.1 (6.5 ± 0.2)	0.0 ± 0.1 (1.0 ± 0.3)	0.0 ± 0.0 (0.7 ± 0.1)

[1] Mean ± SD of pooled sample analysis (n = 3).
[2] Early nymphs = first and second instar (0.5-1.0 cm); middle nymphs = third, fourth and fifth instar (1.1-2.0 cm); late nymphs (sixth, seventh and eighth instar (2.1-3.0 cm.
[3] Dry weight (%) is presented in parenthesis below the % wet weight value for easy comparison.
[4] Nitrogen-free extract = 100 – (% crude protein + % crude lipid + % crude fiber + % ash).

TABLE 5 Growth performance and feed utilization efficiency of pearl arowana fed live lobster cockroaches for 12 weeks[1]

Parameter	Lobster cockroach[2]		
	A-LLHP (control)	A-MLHP	N-HLLP
Average initial weight (g)	31.52 ± 0.94	31.60 ± 0.91	33.74 ± 0.79
Average final weight (g)	57.47 ± 4.26^{a}	67.49 ± 8.69^{ab}	84.00 ± 8.32^{b}
Weight gain (%)[3]	81.22 ± 8.57^{a}	109.44 ± 18.27^{ab}	151.85 ± 23.10^{b}
Food weight (g/day)	1.48 ± 0.10	1.51 ± 0.11	1.43 ± 0.20
Cockroach (number/day)	3.84 ± 0.10	4.56 ± 0.43	3.86 ± 0.23
SGR[4]	4.01 ± 0.07^{a}	4.14 ± 0.10^{ab}	4.38 ± 0.10^{b}
FCR[5]	6.60 ± 0.91	5.94 ± 0.73	4.87 ± 0.76
PER[6]	0.57 ± 0.05	0.67 ± 0.09	0.81 ± 0.09
Survival (%)[7]	87.5	100	100

[1] Values are the mean ± S.E. of eight replicate fish. Mean values within same row with different superscripts are significantly different ($P < 0.05$).
[2] See Table 1 footnote for live cockroach description.
[3] Weight gain (%) = [(FBW – IBW)/IBW] × 100.
[4] Specific Growth Rate (% BWday^{-1}) = ([ln(FBW) – ln(IBW)]/feeding days) × 100.
[5] Feed Conversion Ratio = total dry feed intake (g)/wet weight gain (g).
[6] Protein Efficiency Ratio = weight gain (g)/total protein intake (g).
[7] Survival (%) = (Final fish number – Initial fish number) × 100.

Haematocrit, body-organ indices and muscle composition

Dietary treatments did not significantly affect the haematocrit value, condition factor, HSI and VSI (Table 6). The IPF value of fish increased with increasing lipid content of the cockroaches fed to them. Fish fed N-HLLP showed significantly higher IPF compared to control group. Similarly, muscle lipid content was highest in fish fed the N-HLLP cockroaches and significantly higher than in the other two groups (Table 6). Muscle protein content did not vary significantly among the different groups.

TABLE 6 Haematocrit, condition factor, body-organ indices and muscle proximate composition of pearl arowana fed live lobster cockroaches for 12 weeks[1]

Parameter	Lobster cockroach[2]		
	A-LLHP (control)	A-MLHP	N-HLLP
Haematocrit (%)[3]	30.44 ± 1.99	32.88 ± 3.29	34.93 ± 3.54
Condition factor[4] (K)	1.02 ± 0.05	1.15 ± 0.19	1.39 ± 0.15
HSI[5]	1.92 ± 0.19	1.76 ± 0.19	1.87 ± 0.19
VSI[6]	4.09 ± 0.08[a]	6.57 ± 1.00[b]	3.51 ± 0.52[a]
IPF[7]	0.71 ± 0.20[a]	1.36 ± 0.57[ab]	3.05 ± 0.89[b]
Muscle composition (% wet weight)			
Moisture	76.3 ± 0.8[a]	75.6 ± 0.5[ab]	74.0 ± 0.8[b]
Crude protein	16.9 ± 0.3	17.8 ± 0.5	17.7 ± 0.1
Crude lipid	1.6 ± 0.2[a]	1.5 ± 0.2[a]	3.1 ± 0.3[b]

1 Values are the mean ± S.E. of 7-8 replicate fish. Mean values within same row with different superscripts are significantly different ($P < 0.05$).
2 See Table 1 footnote for live cockroach description.
3 Haematocrit (%) = (Length of haemoglobin/total length of plasma and haemoglobin) × 100.
4 Condition factor (g cm^{-3}) = weight of fish/(length of fish)^3 × 100.
5 Hepato-stomatic Index = [liver weight (g)/FBW (g)] × 100.
6 Viscero-somatic Index = [viscera weight (g)/FBW (g)] × 100.
7 Intraperitoneal Fat = [body fat weight (g)/FBW (g)] × 100.

TABLE 7 Number of chromatophores on the scales of pearl arowana fed live lobster cockroaches for 12 weeks[1]

Cockroach[2]	Melanophore Index (MI)[3]						Xanthophores
	1	2	3	4	5	Total	
A-LLHP	37.8 ± 15.1	32.0 ± 14.2	37.2 ± 23.5	6.0 ± 3.7	2.8 ± 2.8	115.8 ± 16.3	85.6 ± 26.8[a]
A-MLHP	60.8 ± 38.5	29.5 ± 10.6	47.0 ± 32.6	4.5 ± 4.5	0.0 ± 0.0	141.8 ± 24.2	101.0 ± 22.6[a]
N-HLLP	3.5 ± 1.9	11.5 ± 3.2	95.8 ± 10.7	20.8 ± 9.5	4.0 ± 2.3	135.5 ± 18.5	0.0 ± 0.0[b]

1 Values are the mean ± S.E. of five replicate fish. Mean values within the same column with different superscripts are significantly different ($P < 0.05$).
2 See Table 1 footnote for live cockroach description.
3 According to Hogben and Slome (1931).

Scale chromatophore type and numbers

More melanophores in category MI = 1 was observed in the scales of arowanas fed A-LLHP or A-MLHP whereas more melanophores in category MI = 3 was observed in fish fed N-HLHP (Table 7). However, these numerical differences were not significantly different. Scales of arowana fed the A-MLHP cockroaches had the highest count of melanophores. There was a significant difference among the treatments in terms of the total xanthophores counts (Table 7). Only the scales of fish fed the adult cockroaches (A-LLHP or A-MLHP) exhibited these yellow pigment cells. Scales of fish fed A-MLHP cockroaches showed the highest xanthophore count but were not significantly higher than the A-LLHP group. Xanthophores were not present in the scales of the N-HLLP group.

4 Discussion

Research on the use of cockroach meals, derived from various cockroach species, in fish feeds has only very recently been reported (Garcia-Perez *et al.*, 2022; Long *et al.*, 2022; Tubin *et al.*, 2023). Whether cockroaches will become the next market-relevant insect type for the aquafeed industry remains to be seen. In the present study, pearl arowana fed live lobster cockroaches at the late nymphal stages (N-HLLP) showed the best growth performance and feed utilization efficiency. Despite the lower protein content of the late nymphs (22.9%) compared to the adult male cockroaches (A-LLHP) with a protein content of 30.1%, pearl arowana showed higher growth, possibly due to the protein-sparing action of the high 10.3% lipid content in N-HLLP. The impor-

tance of dietary lipids was further confirmed by the A-MLHP group, which showed better growth (not significant) despite being fed adult cockroaches of similar protein content but double the lipid content compared to the A-LLHP group. High lipase activity had been detected in the gut, pancreas and stomach of the Asian arowana (Natalia *et al.*, 2004). This is not unexpected since substantial lipase would be required by the carnivorous arowanas in their natural habitats to digest live prey such as insects, worms, small fish, etc., which typically contain high body fats. In contrast, Darius *et al.* (2015) reported that silver arowana (*Osteoglossum bicirrhosum*) showed better growth when fed commercial pelleted feeds with higher protein levels (45 and 48%) and lower lipid content (8%) compared to fish fed a diet with 40% protein and 14% lipid. Apart from species-specific and nutrient-content differences, our study which used live cockroaches instead of non-arowana commercial feeds (Darius *et al.*, 2015), might more accurately reflect the natural nutrient requirements and metabolism of arowanas.

Another potential reason for the better growth and FCR of arowana fed cockroach nymphs (N-HLLP) might be their higher nutrient digestibility compared to adult cockroaches. Fontes *et al.* (2019) reported that the apparent digestibility coefficients of adult lobster cockroach meal for Nile tilapia were 61.7 and 69.6% for dry matter and crude protein, respectively. Studies conducted with insect meals in fish diets, especially at higher dietary levels, frequently attributed the reduction in nutrient digestibility to the presence of chitin in the cuticles of insects (Shiau and Yu, 1999; Piccolo *et al.*, 2017). The chitin content of adult lobster cockroaches was reported to be 24.4% on a dry matter basis (Fontes *et al.*, 2019), which was relatively high. However, based on the results reported by Finke (2007), the measurement of chitin in this case may need to be reconfirmed as it may be an overestimation. The chitin content of lobster cockroach nymphs had not been reported but we presumed less chitin is present due to a thinner cuticle and the absence of wings. Finke (2013) estimated that the chitin content of Turkestan cockroach (*P. lateralis*) nymphs to be only 6.7 g/kg (0.67% "as is" basis). According to Fontes *et al.* (2019), the larval meals of beetles (*Zophobas morio* and *T. molitor*) showed higher dry matter, energy, protein and chitin digestibility compared to adult insect meals of cockroaches (*N. cinerea*, *Gromphadorhina portentosa*) and crickets (*Gryllus assimilis*) due to their lower chitin content. Similarly, Hoffman *et al.* (2021) reported that sea trout fed diets with 20% insect meal from the larval stage (mealworm and black soldier fly) outperformed fish fed diets containing insect meal from the adult stage (cockroach and cricket) possibly due to the higher chitin content in adult insect meals thereby reducing nutrient utilization. Interestingly, in Nile tilapia fed various insect meals, Fontes *et al.* (2019) observed that lipid digestibility was generally high and was not correlated with chitin levels. Lipid digestibility was at 91.6% in Nile tilapia fed adult lobster cockroach meals. Based on the results of the present study, it is recommended that late nymphal stages of the lobster cockroach be used for better growth and feed utilization efficiency in arowanas and possibly for other fish species as well.

All live cockroaches were well received and stimulated the predatory feeding habits of the arowana. The lack of negative impact on the haematocrit value, condition factor and body-organ indices such as HSI and VSI seems to indicate that the live cockroaches did not harbour any major anti-nutritional factors detrimental to fish health. Accumulation of fatty deposits was observed in the intraperitoneal cavity and in the muscle tissue which were correlated with dietary lipid content of the cockroaches that were fed. Similarly, Darius *et al.* (2015) reported higher fat in the livers and guts of silver arowana fed higher dietary lipids and lower dietary proteins. Fat deposition is often reported in farmed fish fed live insects or insect meals due to the high lipid content of insects (Henry *et al.*, 2015; Ng *et al.*, 2001). Even though high fat deposition in food fish might be problematic due to reduction in shelf life and meat quality, such deposition in ornamental fish may be less of a concern.

Colour is one of the major determinants of the selling price of arowanas. In general, the main chromatophores present in fish are melanophores, erythrophores, xanthophores, leucophores and iridophores which are cells containing various types of pigments (Fujii, 1993). Coloration of fish comes from pigments such as melanin and carotenoids, which cannot be biosynthesized and must be provided in the diets (Garcia-Chavarria and Lara-Flores, 2013). In the present study, pearl arowana fed the A-MLHP astaxanthin-enriched cockroaches showed the highest melanophore and xanthophore cell counts on their scales. Similarly, in a preliminary study, Sukarman *et al.* (2023) reported that Asian arowana (super red var.) fed live crickets enriched with astaxanthin increased the total carotenoid content in the caudal fin leading to significantly enhanced fin colour. However, body coloration was not affected possibly because the crickets were fed the astaxanthin-enriched feed for only two hours before being fed to the fish. In

the present study, cockroaches were fed their respective feeds for at least four weeks before being used as live prey, possibly allowing the added astaxanthin to bioaccumulate throughout the body of the A-MLHP cockroaches. Interestingly, only the scales of arowana fed the adult cockroaches showed the presence of xanthophores, irrespective of whether the cockroaches were fed added astaxanthin or not in their diets.

For the first time, cockroaches were shown to be able to contribute to fish pigmentation by virtue of the natural pigments present in their cuticles. The dark brown and black pigmentation observed on the cuticles of mid instars to adult stages of cockroaches is attributed to the melanin pathway (Lemonds *et al*., 2016). The thinner cuticle of the nymphal group (N-HLLP), presumedly with less pigments present, probably resulted in the absence of xanthophores in arowanas fed these nymphs. Nevertheless, melanophores were still observed in the scales of arowanas fed the cockroach nymphs. Finke (2013) reported that no carotenoids were detected in the nymphs of Turkestan cockroaches. Therefore, it is recommended that adults and not the nymphal stages of lobster cockroaches be used as live feed for arowanas when the objective is to enhance fish coloration. Carotenoid-enriched adult cockroaches can be used to further enhance arowana pigmentation.

The whole life cycle of the lobster cockroach was determined in the present study which included important parameters such as fecundity, longevity and development periods of each life stage. These latest data will complement the pioneering work done by Willis *et al*. (1958) and Cornwell (1968) to increase our understanding of its biology and development. The duration of each instar and nymphal development period in lobster cockroaches reared alone was similar to the results reported by Willis *et al*. (1958). In addition, Willis *et al*. (1958) reported that nymphs reared in groups matured faster than when reared in isolation. In contrast, Willis *et al*. (1958) reported that the longevity of female and male cockroaches was 344 and 365 days, respectively, which were markedly higher than the 186 and 132 days, respectively, observed in the present study. The reasons for this discrepancy are unknown and might be due to differences in the feed given (Haydak, 1953) and laboratory conditions. Nevertheless, it should be pointed out that the adult lifespan data by Willis *et al*. (1958) was based on only 16 cockroaches compared to the relatively large numbers used in the present study. The preoviposition development and egg incubation periods as well as the nymphs produced per ootheca were similar to that reported by Willis *et al*. (1953). The high fecundity of the female cockroaches observed in this study makes them a potential source of insect meals for the aquafeed industry as well as live prey for the ornamental fish industry. The various sizes of the cockroach nymphs at different development stages are well suited to cater for ornamental fish of different mouth sizes.

As mentioned by Cerreta *et al*. (2022), there is currently a paucity of published information on the nutritional profile of cockroaches at different life stages and between the sexes. They went on to report the nutritional profile of nymphs and adults of four species of cockroaches but did not include the lobster cockroach in their study. They also lumped the nymphal stages as a single group for proximate analysis. In the present study, we divided the nymphs into early, middle and late nymphal stages (Table 4). As far as we know, the present study is the first report on the nutrient composition of the various development stages of the lobster cockroach. We observed a distinct increase in body crude protein and lipid levels as the nymphs metamorphosised into later instars. The nutrient content of the adult stage was also different from the nymphal stages with distinct differences evident between sexes as similarly observed by Cerreta *et al*. (2022) for other cockroach species. The adult male lobster cockroach had the highest body protein (84.3%) and lowest body lipid levels (10%) among all groups in the present study. These cockroach body nutrient profiles should be useful in better targeting of nutrient intake in the formulation of feeds or as live food for farmed fish.

The company, Nutrinsecta (Betim, MG, Brazil), commercially sells dried *N. cinerea* that are fed with fruit and vegetable remains and reported to contain 59.8% protein, 21.3% lipid, 6.8% fibers and 5.1% ash on a dry weight basis (De Oliveira *et al*., 2017). In contrast, Tubin *et al*. (2023) analysed the cockroach meal (*N. cinerea*) purchased from Nutrinsecta and reported a nutrient composition of 66.8% protein, 6.1% lipid, 4.8% ash and 5270 kcal/kg gross energy (dry weight basis). The development stage of the lobster cockroaches sold by Nutrinsecta was not specified. In the present study, protein levels ranged from 63.2 to 84.3% of dry body weight depending on life stage, which is much higher compared to that reported by De Oliveira *et al*. (2017). Cockroaches were fed a commercial rat feed with 21.8% protein and 2.9% lipid in our study which probably accounted for the differences in nutrient profile. The lipid content of cockroaches in the present study was high in the nymphs (21.9 to 26.5%) but low (10 to15.2%) at the adult stage. It is not known whether the considerable variation in lipid content of lobster cockroaches sold by

Nutrinsecta (De Oliveira *et al*., 2017; Tubin *et al*., 2023) was due to the use of different life stages or cockroaches being fed different diets.

Specific diet modifications can change the nutrient profile of insects to improve their body composition to target specific fish species. In this study, we attempted to produce "tailor-made" cockroaches by feeding them a formulated cockroach feed with higher lipid levels and added astaxanthin (A-MLHP; Table 1) which altered their nutrient profile. This resulted in arowanas showing higher growth performance and markedly more chromatophores on their scales. Fish were also observed to store more body fat which may be physiologically important during the breeding season. This study also showed that live cockroaches can be carotenoid-enriched to enhance fish pigmentation.

Author contributions

W.-K. Ng sourced research funding, conceptualized and supervised the study, and wrote the manuscript. C.-Y. Lee conceptualized and supervised the study, and edited the manuscript. K.-T. Koay conducted the experiments and analysis. All authors read and approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

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