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NOTE

Appendage deformity syndrome—a nutritional disease of *Macrobrachium rosenbergii*

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ABSTRACT: Culture of the freshwater prawn *Macrobrachium rosenbergii* as an alternative to penaeid shrimp has recently increased in coastal areas of southern India in order to avoid numerous problems, particularly with white spot syndrome virus (WSSV). However, *M. rosenbergii* culture is now threatened by a new disease, appendage deformity syndrome (ADS), that also results in high mortality. Analysis of ADS prawns for viruses such as WSSV, monodon baculovirus (MBV) and infectious hypodermal and hematopoeitic necrosis virus (IHHNV) gave negative results. ADS prawns were also negative for bacterial pathogens and affected animals did not respond to antibiotic therapy. A study of potential nutritional deficiency revealed that carotenoid supplementation in the diet led to a significant decrease in ADS prawns.

KEY WORDS: Cultured freshwater prawn · *Macrobrachium rosenbergii* · Nutritional deficiency · Carotenoids

INTRODUCTION

Culture of the freshwater prawn *Macrobrachium rosenbergii* contributes a significant proportion to total world aquaculture production. The area devoted to its culture in India has increased in response to the high prevalence of various disease problems in penaeid shrimp culture. Recently, a new disease with unusual gross signs has been observed in cultured *M. rosenbergii* in the Nellore district, Andhra Pradesh, India, which has not been reported elsewhere. This disease has been named appendage deformity syndrome (ADS) because of the physical appearance of affected prawns. Its prevalence is very high, affecting almost 50% of the culture area. It is usually observed after 4 to 5 mo of culture and is associated with high mortality. Herein, we describe attempts to identify the causative agent of this disease.

MATERIAL AND METHODS

Experimental animals. Prawns from culture ponds (approximately 4 to 5 mo post stocking, 15 g weight) with external signs of ADS were brought to the laboratory for experimental analysis in polythene bags filled one-third full with water from the same ponds water. They arrived at the laboratory within 30 min and were transferred to 80 l glass aquaria containing tap water (7 ppt) and were maintained at room temperature (28–32°C). The animals were acclimatized in the laboratory for a period of 3 d and were fed with commercial pelleted feed (Chaitanya Aqua Feeds) (Table 1). The aquaria were provided with adequate aeration using an aquarium air pump. Physical appearance and behaviour were recorded during this acclimatization. Prawns were maintained under these conditions for all experiments.

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Table 1. Composition of commercial feed used by farmers

Ingredient	Grams per 100 g feed
Jawala	15.0
Fish meal	5.0
Fish	10.0
Rice bran	11.0
Soya bean meal	11.0
Soya lecithin oil	2.0
Groundnut oil cake	5.0
Broken rice	5.0
Gluten meal	5.0
Wheat flour	3.0
Bajra	5.0
Jowar	5.0
Fish oil	1.0
Mineral mix	2.0
Vitamin C	1.0
Dicalcium phosphate	1.0
Vitamin premix	1.0
Binder	12.0

Bacterial isolation and challenge trials. To determine whether bacteria were the cause of ADS, the hemolymph and hepatopancreas were examined from 20 diseased prawns collected from 6 different ponds. Hemolymph was collected from the ventral sinus of diseased animals using a sterile 1.0 ml syringe, and 100 µl of hemo-lymph was spread on tryptone soya agar medium (TSA) without dilution. The hepatopancreas was removed aseptically with sterile forceps, crushed in 5.0 ml of sterile saline and 100 µl of suspended material was spread on TSA plates. The TSA plates were incubated at 37°C for 24 h to allow growth of bacterial colonies. To obtain pure cultures, predominant colonies with different morphologies were selected from the spread plates, streaked on identical medium and incubated for 24 h at 37°C. A total of 10 pure cultures were isolated and maintained.

Bacterial culture suspensions were prepared individually for each isolate in sterile saline (carrier solution) at a concentration of 10^4 cells ml^{-1}. Each suspension was injected (0.1 ml dose) into the muscle of the 6th abdominal segment of 12 healthy prawns. The same number of uninjected prawns served as the control group. After injection, the prawns were inspected for a period of 15 d for the occurrence of ADS symptoms. For antibiotic therapy studies, prawns were exposed to antibiotics (4 ppm concentration) every day by bath treatment for 30 min. Antibiotics tested were ciprofloxacin, pefloxacin and oxytetracyclin. Aquaria water was changed daily (100%). For each antibiotic test, 12 diseased prawns and 12 normal prawns (15 g) collected from the same culture pond were used. The animals were observed for a period of 15 d after commencement of treatment. They were fed with the same commercial feed.

Cohabitation studies. To determine whether ADS is contagious, healthy and diseased prawns from the same or different culture ponds were cohabited in different combinations (diseased males with healthy males; diseased males with healthy females; diseased females with healthy males; diseased females with healthy females) for a period of 15 d. For all cohabitation studies 12 diseased animals and 12 healthy animals were used.

PCR detection of shrimp viruses. For PCR analysis, 16 ADS prawns from 4 different ponds (4 from each pond) were tested for white spot syndrome virus (WSSV) and for infectious hypodermal and hematopoietic necrosis virus (IHHNV) using the basic PCR technique described by Lightner (1996), and for monodon baculovirus (MBV) as originally described by Lu et al. (1993) and also by the commercial method of Hsu et al. (2000).

Nutritional tests. Since the ovaries of ADS prawns were white instead of the usual orange-red of normal females, carotenoid supplementation was tested for its efficacy in preventing ADS. Carotenoids (Granny Foods) were added (1 g kg^{-1} feed) to the commercial pelleted feed by top dressing, using soya lecithin oil as the binding agent. Diseased animals (12 females) were fed with this feed for a period of 21 d and the same number of diseased female prawns were maintained on the commercial feed without carotenoid supplementation. All prawns were examined every 3 d for

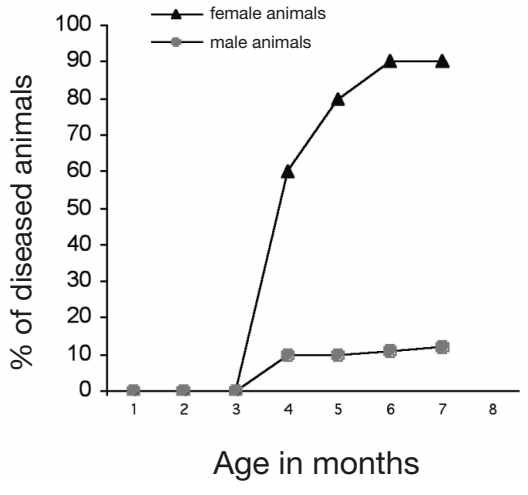
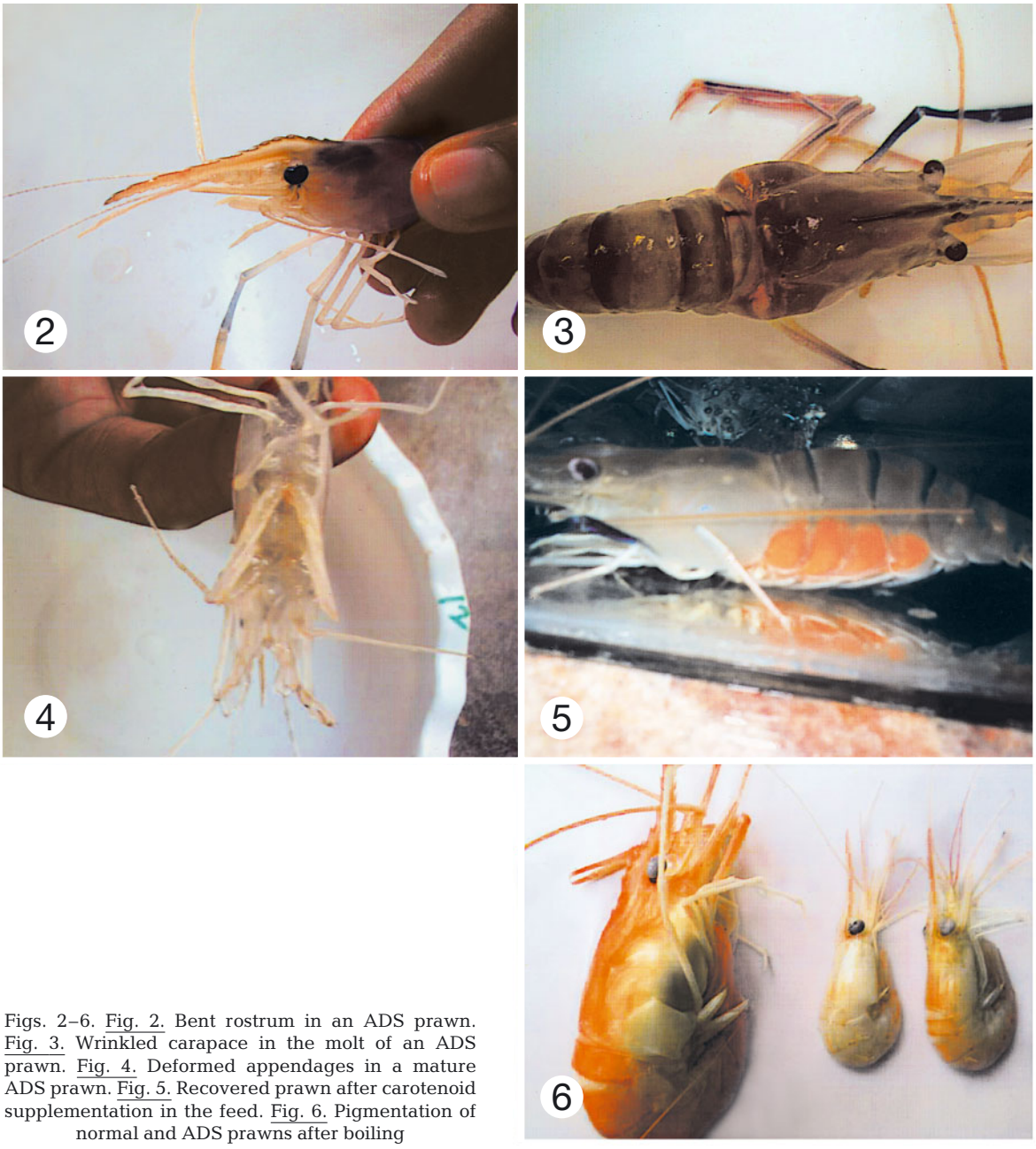


Fig. 1. Comparison of appendage deformity syndrome (ADS) incidence in male and female prawns



Figs. 2–6. Fig. 2. Bent rostrum in an ADS prawn. Fig. 3. Wrinkled carapace in the molt of an ADS prawn. Fig. 4. Deformed appendages in a mature ADS prawn. Fig. 5. Recovered prawn after carotenoid supplementation in the feed. Fig. 6. Pigmentation of normal and ADS prawns after boiling

gross visual signs of recovery. The presence or absence of carotenoids in dead prawns and in prawns surviving to Day 21 was tested by boiling the muscle tissue, as it has been reported that carotenoids (astaxanthin) are responsible for the red color of boiled prawns (Okada et al. 1994).

Field trials. Field trails of carotenoid supplementation were conducted in 8 selected ponds of 1 ha with equal stocking density (4 prawns m^{-2}) by top dressing the feed (1 g kg^{-1}) for a period of 1 mo.

RESULTS AND DISCUSSION

ADS is a chronic disease associated with late mortality particularly at 4 to 6 mo of culture when prawns have reached maturity (Fig. 1). The gross signs of ADS are more prevalent in females than males. The prominent features of the disease are appendage deformity, broken or bent rostrums, cut antennae, wrinkled carapace and mortality (Figs. 2, 3 & 4, Table 2). In addition, affected prawns are restless and continuously collide with ob-

jects, the probable cause of the broken or bent rostrums. Molting and feed intake are however normal.

In bacterial challenge studies, none of the 10 pure culture isolates tested caused any mortality for up to 30 d after challenge, nor did any of the challenged prawns develop signs of ADS. There was no mortality in the unchallenged control groups. Furthermore, none of the ADS prawns treated with antibiotics in bath exposure tests recovered within the 15 d observation period. These data suggest that ADS is not caused by bacterial pathogens.

During 15 d cohabitation tests the numbers of ADS and normal prawns did not change. This was the case whether the prawns were from the same or from different ponds, and whether the prawns were all male, all female or mixed.

In tests for the presence of possible viral agents, 16 diseased prawns from 4 ponds were all found to be negative for the known shrimp viruses WSSV, IHHNV and MBV.

After finding no association with pathogens in the initial tests, nutritional tests were carried out using carotenoid-supplemented feed. Supplementation resulted in complete recovery from ADS in all the animals within 21 d (Fig. 5, Table 3). The diseased animals fed with normal feed (without carotenoid) all died within 18 d. In support of this observation, the light pigmentation of boiled ADS prawns compared to the darker red pigmentation of healthy prawns (Fig. 6) suggests that ADS prawns have lower levels of carotenoid pigments. The results obtained from our laboratory trials agree with those from field trials where total recovery was achieved within 3 wk after commencement of carotenoid supplementation.

Carotenes and carotenoids are known to play a role in the coloration of crustaceans (Britton et al. 1981). The various gross signs of ADS, such as progressive mortality and loss of color from antennae and the ovary, support the proposition that ADS is a nutritional disorder related to carotenoid pigments. Carotenoids have also been reported to play a role in the fecundity, hatchability and egg quality of carp and in the ovarian development of *Macrobrachium rosenbergii* (Kaushik 1993, Sigurgisladottir et al. 1994, Izqueierdo et al. 1997). This supports our hypothesis that pale ovarian color in ADS prawns is due to carotenoid deficiency.

Table 2. Gross signs of ADS in *Macrobrachium rosenbergii* from different culture ponds (25 prawns per pond)

Culture pond	Atrophied ovary	Bent/broken rostrum	Deformed appendages	Crooked carapace	Pale antennae
Pond-I	+	+	+	+	+
Pond-II	+	-	+	+	+
Pond-III	+	+	+	+	+
Pond-IV	+	+	-	+	+
Pond-V	+	+	+	+	+
Pond-VI	+	-	+	+	+

Table 3. Effect of carotenoid supplementation on ADS prawns

Days of feeding	Appearance with commercial feed	Appearance with commercial feed plus carotenoids (1 g kg^{-1})
1	ADS	ADS
3	ADS	ADS
6	ADS	Slight improvement in body color
9	ADS	Normal body color
12	ADS	Molted
15	ADS	Wrinkle-free carapace
18	Dead	Regeneration of appendages
21	-	Total recovery from ADS (normal)

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