

Report of the color/stress trial

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PROJECT/TASK:

Evaluation of the impacts of Motiv on coloration in a RAS stress model

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ABSTRACT:

A controlled trial was conducted to evaluate the effects of Motiv, a value-added feed ingredient, on shrimp coloration and stress resistance in a recirculating aquaculture system (RAS). Juvenile *Litopenaeus vannamei* were fed diets containing 0%, 7.5%, and 12.5% Motiv for 50 days. Motiv replaced Peruvian fishmeal on a weight-for-weight basis. Shrimp performance metrics, pigmentation (astaxanthin concentration and color scores), and stress tolerance under zero-salinity conditions were assessed. Results showed that shrimp fed the 7.5% Motiv diet achieved significantly higher final weights, average daily gain (ADG), and feed conversion ratio (FCR) compared to the control. Astaxanthin levels in shrimp muscle increased with Motiv inclusion, correlating with improved color scores. Stress resistance was enhanced in both Motiv-fed groups, with significantly lower mortality under zero-salinity challenge. These findings suggest that Motiv improves shrimp pigmentation and resilience under stress, offering potential value for commercial aquaculture.

BACKGROUND

Motiv is a value-added feed ingredient developed specially to enhance the productivity of shrimp. Based on laboratory and commercial trials, Motiv has demonstrated improvements in growth, survivability, as well as improvements in shrimp coloration. Among the negative impacts of stress on shrimp include: poor productivity, morbidity, mortality and potentially decrease ability of astaxanthin uptake. Astaxanthin uptake in shrimp directly impacts the coloration and value the shrimp in the market, thus understanding Motiv's ability to mitigate the impacts on coloration would be valuable to our customers.

OBJECTIVES

To evaluate the impacts of different levels of Motiv inclusion in the diets of shrimp on shrimp coloration after a stress event.

MATERIALS AND METHODS

Trial location: RAS system at house in Hoc Mon District – Ho Chi Minh City – Vietnam

Animals: *L. vannamei* weighing approximately 10g/pcs appearing in healthy conditions will be selected for the trial. Shrimp were sourced from SIS super-growth hatchery postlarvae and reared on common commercial diet consisting of 42% protein at commercial farm in Vietnam. 10-g shrimp were acclimated for 10 days with the control diet (to reach appropriate 15-g shrimp) and then 15 15-g shrimps in similar-size looking were weighted for randomly arranged to each replicate of the treatment.

Treatments: Treatments consist of a basal diet (control), a MOT7.5 diet with 7.5% Motiv, and a MOT12.5 diet with 12.5% Motiv included. Motiv inclusion was accomplished by replacing Peruvian Fishmeal and a wt./wt. basis in the diets. Diets were manufactured at ShrimpVet Lab using small extrusion machine and analyzed for nutrient content at UpScience Lab. Dietary formulas both ingredients and nutrients can be found in the following Table 1 and Table 2.



Figure 1. Experimental design with buckets served as replicates (3 buckets per each treatment).

Ingredient Description	Origin	Control	Motiv 7.5%	Motiv 12.5%
Fishmeal (Anchovy)	Tasa, Peru	320.000	245.000	195.000
Motiv (Cargill US)	USA	0.000	75.000	125.000
Soybean meal, 48% CP, solvent extracted	USA	280.000	265.000	258.000
Fish oil, Tuna oil, Co-products	China	16.000	19.000	20.000
Wheat, flour	Vietnam	280.000	280.000	280.000
Wheat gluten meal, 78% CP	Vietnam	60.000	60.000	60.000
Choline chloride, 60% choline	Japan	2.000	4.000	6.000
Soy lecithin	Korea	18.000	20.000	21.000
Cholesterol	China	0.000	0.250	0.425
Mono calcium phosphate, MCP, Ca(H ₂ PO ₄) ₂	China	6.500	8.000	8.000
Salt, NaCl	Vietnam	6.000	8.000	10.000
Rovimix-stay-C 35, ascorbyl-monophosphate	DSM, France	2.000	2.000	2.000
Shrimp Premix	China	5.000	5.000	5.000
DL-Methionine	France	2.000	2.800	3.000
L-Threonine	Japan	1.000	2.075	2.000
L-Lysine	Japan	0.625	3.000	3.700
Carboxymethylcellulose (CMC)	Turkey	0.000	0.000	0.000
Mold inhibitor (calcium propionate)	China	0.750	0.750	0.750
BHT/BHA	Spain	0.125	0.125	0.125
Total		1000.000	1000.000	1000.000

Table 1. Ingredient formulas of the three diets.

Each diet was arranged for three replicates, consisting of a floating bucket (42 x 64 x 50 cm) with 15 shrimp per bucket as a replicate. All buckets were arranged in a RAS system connected with a middle tank for ammonia and nitrite treatment with probiotics and molasses to limit total ammonia (TAN) bellows 0.5 mg/L and Nitrite (NO₂-N) bellows 1 mg/L. Each bucket was assigned for an air-stone plus the aero-tube aeration in the RAS tank to secure at least 5 mg/L dissolved oxygen.

Daily water exchange was done at 5% of the total tank to fill in the siphoning loss. Salinity at 10 ppt was produced by mixing the seawater and freshwater (tap-water) and water disinfected using Virkon (Bayer) at 1.2 ppm and/or H₂O₂ (50%) at 6 ppm and aerated for at least 48 hours before using disinfected water to fill in the loss water due to siphoning.

Control diet and Motiv diets were sampled for astaxanthin measurement before arranging the shrimp for the trial. Shrimp was fed 4 times a day at an average of 3% – 2.5% of bodyweight and feeding adjustment basing on daily feed consumption for a satiation.

Shrimp was feeding for a period of 7 weeks (50 days), including a week for feed acclimation.

Analytical Parameters	Unit	Control	Motiv 7.5%	Motiv 12.5%
Cholesterol	ppm (mg/kg)	1185	1179	1263
Cystine	g/100g	0.51	0.55	0.59
Aspartic acid	g/100g	3.65	3.46	3.37
Methionine	g/100g	1.04	1.05	1.06
Threonine	g/100g	1.69	1.7	1.69
Serine	g/100g	1.87	1.89	1.97
Glutamic acid	g/100g	7.97	8.36	8.59
Glycine	g/100g	2.05	1.87	1.79
Alanine	g/100g	2.03	2.08	2.14
Valine	g/100g	1.90	1.89	1.87
Isoleucine	g/100g	1.64	1.63	1.65
Leucine	g/100g	2.94	3.33	3.6
Phenylalanine	g/100g	2.00	2.04	2.07
Histidine	g/100g	1.06	1.02	1.03
Lysine	g/100g	2.52	2.56	2.58
Arginine	g/100g	2.37	2.23	2.17
Proline	g/100g	2.31	2.61	2.84
Amino Acids Profile	g/100g	37.57	38.28	39.02
Astaxanthin	ppm (mg/kg)	0.50	3.28	5.28
Moisture	g/100g	10.75	10.04	8.82
Crude fiber	g/100g	1.04	1.11	1.24
Protein	g/100g	42.16	41.84	42.62
Ash	g/100g	9.72	8.81	8.76
Hydrolyzed fat	g/100g	6.51	7.07	7.51

Table 2. Nutrient analysis of the three diets (UpScience Lab).

- pH was measured 2 times a day using pH meter (LAQUAtwin – made in Japan).
- Alkalinity will be measured once week (using CP kit). Mineral mixture and lime (CaCO₃) were daily added at 50g to maintain alkalinity higher than 100 mg/L.
- Ammonia and Nitrite were measured daily (using Sera kit).
- Salinity was fixed at 10 ppt (using Hydrometer).
- DO was measured once week (using YSI – Pro20).

Feed consumption: daily feed observation and collection of uneaten feeds to estimate the uneaten feed amount by counting the uneaten pellets. 1g of each feed was counting three times for average number of pellets per g and using this average number of pellets per g to estimate the uneaten feed per day.

FCR: total feed consumption per live shrimps will be total feed consumption – the feed eaten by death shrimps. Feed eaten by death shrimp will be calculated as averaged feed consumption per shrimp until the date shrimp died. FCR is the average amount of feed per live shrimp divided by average weight growth per shrimp.

ADG is average weight growth per live shrimp divided by 50 days.

Salinity-stress test: 10 shrimps were pulled from each treatment replicate and directly stocked in free-chlorine tap water (zero-salinity water) with aeration and recorded mortality at 1 hour, 2 hours, 6 hours and 12 hours.

Pigmentation (astaxanthin): shrimp after the test were pulled from each treatment to have at least 150g per diet and sent to UpScience Lab for astaxanthin measurement. 10 shrimp pulled from each diet also boiled for 3 minutes and then directly added to ice water and wait for another 5 minutes to measure color scores using Salmon color pan.

RESULTS

1. WATER QUALITY

- Shrimp were reared in RAS system with the following water parameters:
- Ammonia (TAN) : controlled bellows 0.5 mg/L using probiotics and molasses in a middle tank.
- Nitrite (NO₂-N): controlled bellows 1 mg/L using probiotics and molasses in a middle tank.
- pH was measured and the ranges varied between 7.6 to 8.3 during the experiment.
- Alkalinity was measured and varied between 100 to 130 mg/L.
- Dissolved oxygen was measured and varied between 6.2 to 6.7 mg/L.
- Salinity was fixed at 10 ppt during the experiment.

2. SHRIMP PERFORMANCES

Treatment	Initial weight (g)	Final weight (g)	ADG (g/day)	FCR	Survival rate
Control	14.69 ± 0.30a	32.53 ± 0.32a	0.65 ± 0.01a	1.33 ± 0.03a	77.78 ± 3.85a
Motiv 7.5%	14.51 ± 0.20a	34.14 ± 0.20b	0.68 ± 0.00b	1.27 ± 0.02b	84.44 ± 3.85a
Motiv 12.5%	14.53 ± 0.29a	33.24 ± 0.52a	0.66 ± 0.01a	1.29 ± 0.01a	84.44 ± 3.85a

(Stats, Duncan Test, 5% Significant level, SPSS 25 – Values are presented as mean ± standard deviation. The same letters on the same column are presenting for not significantly different (or P > 0.05).

Statistical analysis indicates the means of initial weight was not significantly different and results after 50 days of feeding period resulted in significant differences (P < 0.05) for means of final weight, ADG, and FCR but it was not significant differences for the survival rates. The best final weight, ADG and FCR was obtained for the shrimp fed on Motiv 7.5% diet while there were not significant differences for final weight, ADG, FCR and survival rates for the control diet and Motiv 12.5% diet.

3. PIGMENTATION (COLOR SCORES)

Astaxanthin (ppm)	Control diet	Motiv 7.5%	Motiv 12.5%
In diet	0.5	3.28	5.28
In shrimp at harvest	0.3	0.58	0.92



Color scores evaluation

Control (1): white-pink color and scored at around 22 scores
Motiv 7.5% (2): yellow-red color and scored at around 23-plus scores (23+)
Motiv 12.5% (3): red color and scored at around 24-plus scores (24+)

Results of both astaxanthin in shrimp muscles and color scores is matched with astaxanthin measured in the diets as astaxanthin increases from the control diet (0.5 ppm) to Motiv 7.5% (3.28 ppm) and Motiv 12.5% (5.28 ppm), the astaxanthin has also increased in the shrimp muscles (0.3 ppm, 0.58 ppm and 0.92 ppm, accordingly). Color scores has also increased in shrimp fed on the control diet (22 scores), Motiv 7.5% (23+ scores) and Motiv 12.5% (24+ scores). **In conclusion, Motiv has improved color scores for shrimp.**

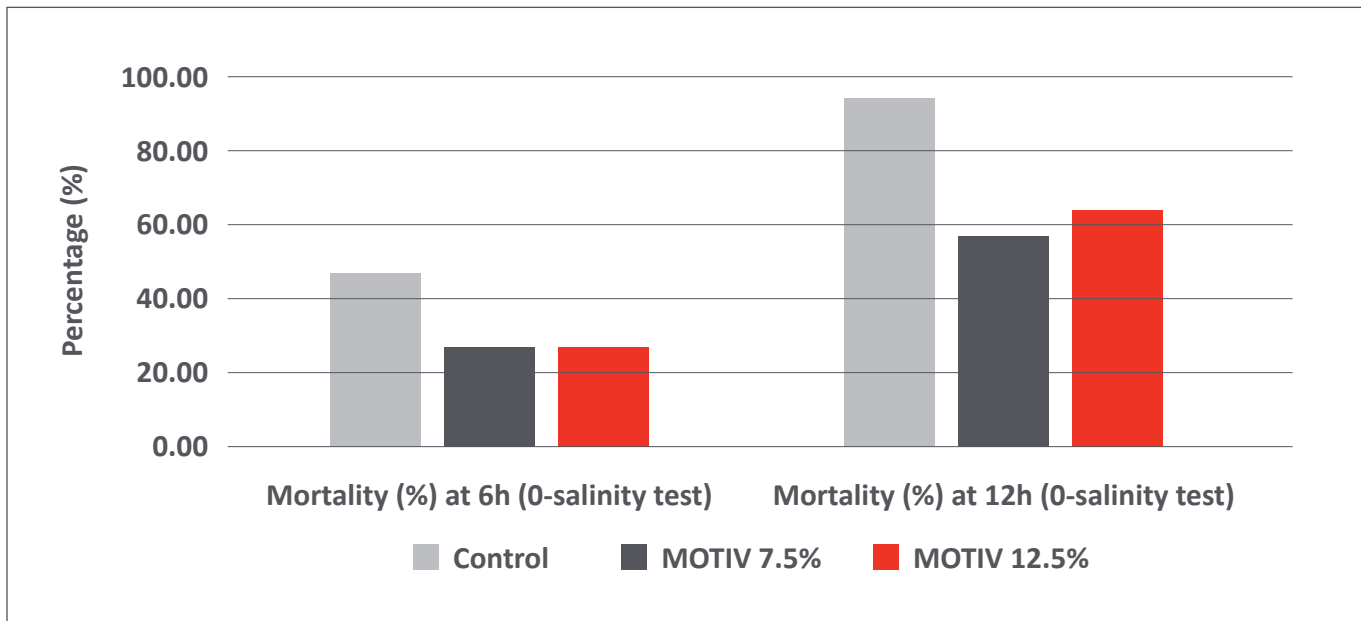
4. STRESS TEST AT 0-SALINITY

Shrimp was not death at 1 hour and 2 hours during the zero-salinity stress test and therefore mortality was recorded at 6 hours and 12 hours (shrimp started to die after 4 hours).

Mortality (%) at 6h & 12h at zero-salinity stress test (STATS, DUNCAN TEST, 5% Significant level, SPSS 25)

Treatment	Mortality (%) at 6h (0-salinity test)	Mortality (%) at 12h (0-salinity test)
Control	46.67 ± 5.77a	93.33 ± 11.55a
Motiv 7.5%	26.67 ± 5.77b	56.67 ± 5.77b
Motiv 12.5%	26.67 ± 5.77b	63.33 ± 5.77b

(Stats, Duncan Test, 5% Significant level, SPSS 25 – Values are presented as mean ± standard deviation. The same letters on the same column are presenting for not significantly different (or $P > 0.05$).



Statistical analysis indicates there is no significant differences for mortality at 6 hours and 12 hours between the shrimp fed on Motiv 7.5% diet and Motiv 12.5% diet while there is significant differences for mortality of shrimp feed on both Motiv diets (7.5% & 12.5% Motiv) vs the shrimp fed on the control diet for both 6 hours and 12 hours of zero-salinity test. **In conclusion, Motiv improves stress resistances through challenged directly to zero-salinity water.**