CC BY

Functional amino acids supplementation for shrimp increases water stress resistance under low oxygen and low salinity acute challenges

Pratoomchat B.[1], Kersanté B.[2*], Pereira R.T.[3], Le Reste G.[4]

Received: December 2024 Accepted: February 2025

Abstract

Two consecutive trials were conducted to evaluate the effects of functional amino acid mix obtained from poultry keratin in white shrimp (*Litopenaeus vannamei*) diets. Three diets were formulated, a control and two diets containing the supplementation of 0.50% (KFAA0.5%), and 1% (KFAA1%) of functional amino acids mix. For the low oxygen acute challenge, shrimp were suddenly transferred from 4.2 mg/L of dissolved oxygen into a 0.5 mg/L of dissolved oxygen (no aeration) 20 L tanks. For the low salinity acute challenge, shrimp were suddenly transferred from 15 ppt salinity into 0 ppt salinity 20 L tanks. Shrimp fed KFAA1% had significantly higher hemocyanin levels (+29.4%) and higher levels of plasmatic protein (+2.1%) through the 16 days of feeding. Differences in hemocyanin and plasma protein levels were detected mainly at 16 days of feeding. Shrimp under low oxygen acute challenge and fed KFAA1% resisted significantly longer to lose balance (+18.3%) and hemocyanin and plasma protein levels significantly decreased (-25.3% and -4.8%, respectively). Similarly, shrimp under low salinity acute challenge and fed KFAA1% resisted significantly longer to lose balance (+79.1%) and plasmatic protein significantly decreased during the stress (-4.2%). These results suggest the shrimp supplemented the functional amino acids mix had a more prepared physiological state which later, during the acute challenge, shrimp could benefit from to achieve higher water stress resistance. For challenge-oriented nutrition strategies, this study encourages discussion on understanding amino acids and their role in physiology above and beyond the traditional approaches as essential and dispensable.

Keywords: Sustainability, Functional amino acids, Water stress resistance

1- Department of Aquatic Science, Faculty of Science, Burapha University, Bangsaen, Chonburi, Thailand.
2- BCF Life Sciences, Boisel, 56140 Pleucadeuc, France.
3- Raquel Pereira Consultoria LTDA, 13087605 Campinas, Brazil.
4- Halieutica, 49000 Angers, France
*Corresponding author's Email: pkersante@bcf-lifesciences.com

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

Introduction

In aquaculture, the utilization of amino acids is commonly considered according to the essential and non-essential amino acids categories and in the perspective of equilibrating physiological requirements in essential amino acids (AA) through addition of crystalline amino acids in diets. Progressively and mainly thanks to knowledge evolution in the animal nutrition industry, the gap between traditional AA classification and physiological importance has led to the emergence of the "Functional amino acids" concept. Functional AAs are defined as those which participate and regulate key metabolic pathways to improve growth and health in mammals and fish. This AA group encompasses arginine, cysteine, glutamine, glutamate, glycine, leucine, proline, and tryptophan regardless of their designation as dispensable or indispensable (Wu, 2013, 2010; Wu *et al*., 2014).

The functional AA concept has also led to a paradigm shift regarding the classification of AA as nutritionally dispensable or indispensable (Wu, 2013, 2014). It has been proposed that animals have dietary requirements for not only indispensable, but also dispensable AAs to achieve maximum growth and also health (Pereira *et al*., 2017; Xing *et al*., 2024). Indeed, this approach encourages discussion regarding whether AA requirements in animals could be underestimated under certain conditions and/or if ratios between dispensable or indispensable AAs have been taken into account, as they should (Xinyu *et al*., 2021; Li *et al*., 2009). The keratin, a major component of poultry feathers, constitutes a circular-economy with significant potential for aquafeed applications (Li *et al*., 2009). The keratin-derived free amino acids mix (KFAA) have emerged as a promising supplementation strategy to address amino acid challenge-oriented nutrition. KFAA consists of a soluble and ready-to-be-absorbed source of 17 amino acids (Wangkahart *et al*., 2023; Wangkahart *et al*., 2022; Khoklang *et al*., 2024). Previous studies highlighted the effects of supplementing KFAA on higher disease resistance in whiteleg shrimp challenged with *Vibrio parahaemolyticus* and WSSV (Kersanté *et al*., 2021) and on feed attractability or improved growth performance (Le Reste *et al*., 2019).

The present study was designed to evaluate the dose-response supplementation of KFAA for grow-out whiteleg shrimp (*Litopenaeus vannamei*) set as two consecutive trials. The first trial aimed to evaluate oxygen transportation and plasmatic protein levels under homeostasis through the time of 16 days of feeding. The second trial aimed to assess resistance to water stress based on oxygen transportation and plasmatic protein levels under the acute challenge of low oxygen and low salinity at day 16 of feeding.

Materials and methods

Experimental diets

The feeding trial was conducted in an indoor semi-recirculating system at the Department of Aquatic Science, Faculty of Science, Burapha University,

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

Bangsaen, Chonburi, Thailand. Experimental diets were formulated to meet the nutritional requirements of *L. vannamei* whiteleg shrimp. Subsequently, the diets were supplemented with two levels of functional amino acids mix (KFAA, Kera-Stim®50 from BCF Life Sciences (Pleucadeuc, France): 5g/kg (KFAA 0.5%) and 10g/kg (KFAA 1.0%), in comparison with a control feed (Table 1). KFAA inclusions were chosen according to a previously published work (Kersanté *et al*., 2021; Le Reste *et al*., 2019).

Table 1: Formulation (% dry matter) and chemical composition of experimental diets.

Ingredients	% dry matter
Wheat flour	24.8
Soybean meal	19.5
Fishmeal 60% Protein	15.8
Poultry meal 64% Protein	11.4
Rice bran	6.6
Shrimp by Product	4.8
Squid by product	3.5
Fermented soy bean	3.4
Lecithin	2.0
Fish hydrolysate	2.0
Squid liver oil	2.0
Squid liver paste	1.7
Corn protein concentrate	1.4
Wheat gluten	1.1
Chemical composition %	
Dry matter	91.2
Crude Protein	38.5
Moisture	8.8
Crude Fat	7.6
Crude Ash	5.2
Crude Fiber	2.9

The KFAA is obtained from a biotechnological process that promotes extensive hydrolysis of poultry keratin, leading to the complete denaturation of the protein chain to achieve a state of free amino acids. Initially developed for extracting cystine (Cys) and tyrosine (Tyr) for pharmaceutical and human nutrition purposes, this industrial process also yields a mixture containing mainly free amino acids and mineral salts, and little content of small peptides. The KFAA is unique product particularly rich in free-form AA, with a typical amino acid profile resulting from the combination of raw material, partial extraction and purification steps of into single AA (Tables 2 and 3) produced by BCF Life Science, France (Kera-Stim®50; https://www.bcf-lifesciences.com).

Table 2: Proximate composition of the KFAA (Kera-Stim®50).

Items	Value
Dry matter	98.4%
Total amino acids (CE 152/2009)	50.4%
Free amino acids (CE 152/2009)	47.3%
Crude ash	43.8%

The KFAA was dissolved in distilled water (20 gDW/kg feed) and top-coated on the feed for 15 minutes using a mixer. The control feed followed the same process but it was sprayed with distilled water only. After drying at room temperature for 24 hours, the experimental feeds were coated with cod liver oil sprayed at 20 g/kg feed. Following an additional 24 hours of drying at room temperature, batches of the feeds were individually stored in sterile bags and kept in a refrigerator at 4°C for the duration of the trial.

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

Table 3: Amino acids profile of KFAA (Kera-Stim®50) with the proportion of each amino acids under free form.

Amino acid	Mean value (g/100g KFAA)	Free AA/total (%)
Serine	6.60	100
Proline	5.84	100
Glutamic acid	5.43	96
Glycine	4.48	98
Valine	4.07	73
Leucine	3.96	95
Aspartic acid	3.67	99
Arginine	3.40	94
Alanine	2.53	98
Phenylalanine	2.49	97
Threonine	2.48	100
Isoleucine	2.44	82
Cystine	1.04	71
Lysine	0.97	93
Histidine	0.32	89
Methionine	0.30	96

Feeding trial I

This first trial aimed to evaluate oxygen transportation and plasmatic protein levels under homeostasis. The Ethics Committee of Animal Welfare of the Faculty of Science, Burapha University, Bangsaen, Chonburi, Thailand approved all the procedures used in this study.

Healthy *L. vannamei* shrimp were acquired from an intensive pond from a commercial shrimp farm, with an average weight of 14.12±0.35 g and measuring 11.67±0.22 cm. The individuals were acclimated in the laboratory for five days before their utilization in the study. Following this, 405 shrimp were held at 100 ind/m^2 with 45 shrimp per tank and three replicates were randomly assigned to each group. The shrimp were housed in rectangular plastic tanks (250 L; 0.55m x 0.82m x 0.5m) filled with water at a salinity of 15 ppt for 16 days. All the tanks were randomly placed in the same room. Shrimps were maintained under natural photoperiod and room temperature.

Shrimp were fed with a control diet or either one of the KFAA supplementary feeds at two inclusion levels: 5 kg/T (KFAA 0.5%) and 10 kg/T (KFAA 1%). The shrimp were fed four times daily (08:00, 12:00, 16:00, and 20:00 h) at 5% of the biomass. To control sunlight and temperature, a blue-coloured plastic covered the tanks. To determine the survival rate, the number of dead shrimp in each tank was daily checked. The temperature was maintained at 28-30°C, pH 7.8-8.2, dissolved oxygen (DO) 6-7 mg/L, and ammonium under0.50 mg/L. Water quality parameters such as salinity, temperature, DO, alkalinity, nitrite, and ammonia were monitored twice a week. Every two days, 10% of the water was replaced with new water and 50% of the water was replaced every six days.

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

Hemolymph sampling and analysis through time

The shrimp used for hemolymph analysis were standardized as those in the early premoult stage, the proecdysis (D0-D1), when shrimp start preparing for moulting according to the method described by Pratoomchat *et al*. (2002), by observing the uropod features under light microscopy.

Hemolymph samples were collected from three shrimp of each replicate (n=9 shrimp/treatment) for Oxygen-binding hemocyanin (OxyHc) and plasma protein (PP) determination at 4, 8, 12, and 16 days of the feeding trial.

For OxyHc measurement, 10 µL hemolymph of each sample was diluted immediately with 990 µL of deionized water in a 10-mL quartz cuvette, and absorption was measured at 335 nm spectrophotometer (Hagerman , 1986; Chen and Cheng, 1995) was followed. The extinction coefficient (E^{mM}) used was 17.26, which was calculated from $E^{1\%}$=2.83 (Nickerson and Van Holde, 1971). OxyHc concentrations were calculated based on a functional subunit of 74,000 (Coates and Decker, 2016; Zhang *et al*., 2006).

For PP protein determination, 300 µL of hemolymph was mixed with 30% trisodium citrate at ratio of 1:1 for anticoagulant then centrifuged at 10,000 g, 4°C for 20 minutes. The plasma was subsequently collected in a 1 µL eppendorf tube and stored at -40^{O}C. The determination of PP was quantified using the method described by Lowry *et al*. (1951), with bovine serum albumin as standard reference for quantifying the concentration of proteins in the sample.

Challenge trial II

This second trial aimed to evaluate resistance to water stress based on oxygen transportation and plasmatic protein levels under acute challenge of very low oxygen and salinity. From feeding trial I, after 16 days of feeding, a total of 45 shrimp (15 shrimp/treatment), average body weight of ~20 g, were selected to a low oxygen and low salinity acute challenge performed separately. Each challenge trial was carried out in two different 20 L aquaria, temperature of 29±0.01℃, pH of 8.0±0.01, 4.2±0.03 mg/L and salinity of 15 ppt.

For the low oxygen acute challenge, shrimp were suddenly transferred from 4.2 mg/L of dissolved oxygen aquaria to 0.5±0.01 mg/L of dissolved oxygen (no aeration), and the time needed (minutes) for complete loss of balance in which shrimp were immediately returned to well-oxygenated water (6.8±0.05 ppm) for recovery.

For the low salinity acute challenge, shrimp were suddenly transferred from 15 ppt salinity aquaria to 0.0 ppm salinity tanks (containing freshwater), and the time needed (minutes) for complete loss of balance in which shrimp were immediately returned to 15ppt-salinity water for recovery. The complete loss of balance reached was classified as stage III, characterized by a complete loss of equilibrium and no reaction to touch stimuli (Rotllant *et al*., 2023) (Fig. 1).

[DOI: 10.22034/INJBIR.3.1.121] [Downloaded from injbir.com on 2026-02-22]

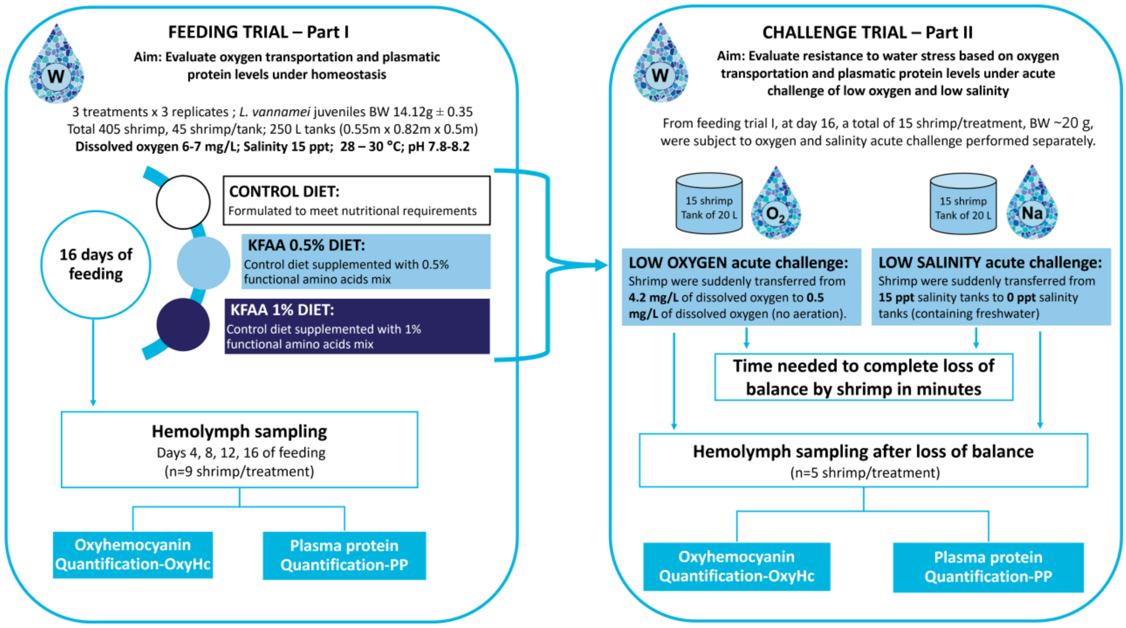

Figure 1: Schematic summary of feeding trial I and challenge trial II of shrimp fed two levels of functional amino acids mix (KFAA).

Hemolymph sampling after loss of balance

Hemolymph samples were collected from 5 shrimp/treatment for quantification of oxygen-binding hemocyanin (OxyHc) and plasma protein (PP) of those shrimp that have reached a loss of balance. Both lab assays were performed as already described in feeding trial I.

Statistical analysis

All data were analyzed using SPSS software. One-way ANOVA was performed and, the model included fixed effects of diets on hemolymph parameters. Normality and homogeneity of variance were evaluated. The significance level was set at $p<0.05$ and Ducan's test compared differences among means. Results were reported as least square means with standard deviation mean (S.D).

Results

Feeding trial I

Overall, the average of OxyHc and PP concentrations throughout 16 days were significantly higher in shrimp fed the diets containing 1% KFAA, +29.4% and +2.1% respectively (Fig. 2), compared to control. The survival rates were similar among all treatments ranging from 95.3% to 98.5%.

When sampling times were analyzed inside each dietary treatment, no differences were observed until the 16th days of feeding for all treatments for OxyHc concentration in the hemolymph. For PP, no differences in control for any sampling time, meanwhile shrimp fed the KFAA at 1% were significantly higher only on day 16, despite increases starting to appear on day 12 (Fig. 3 a, b).

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

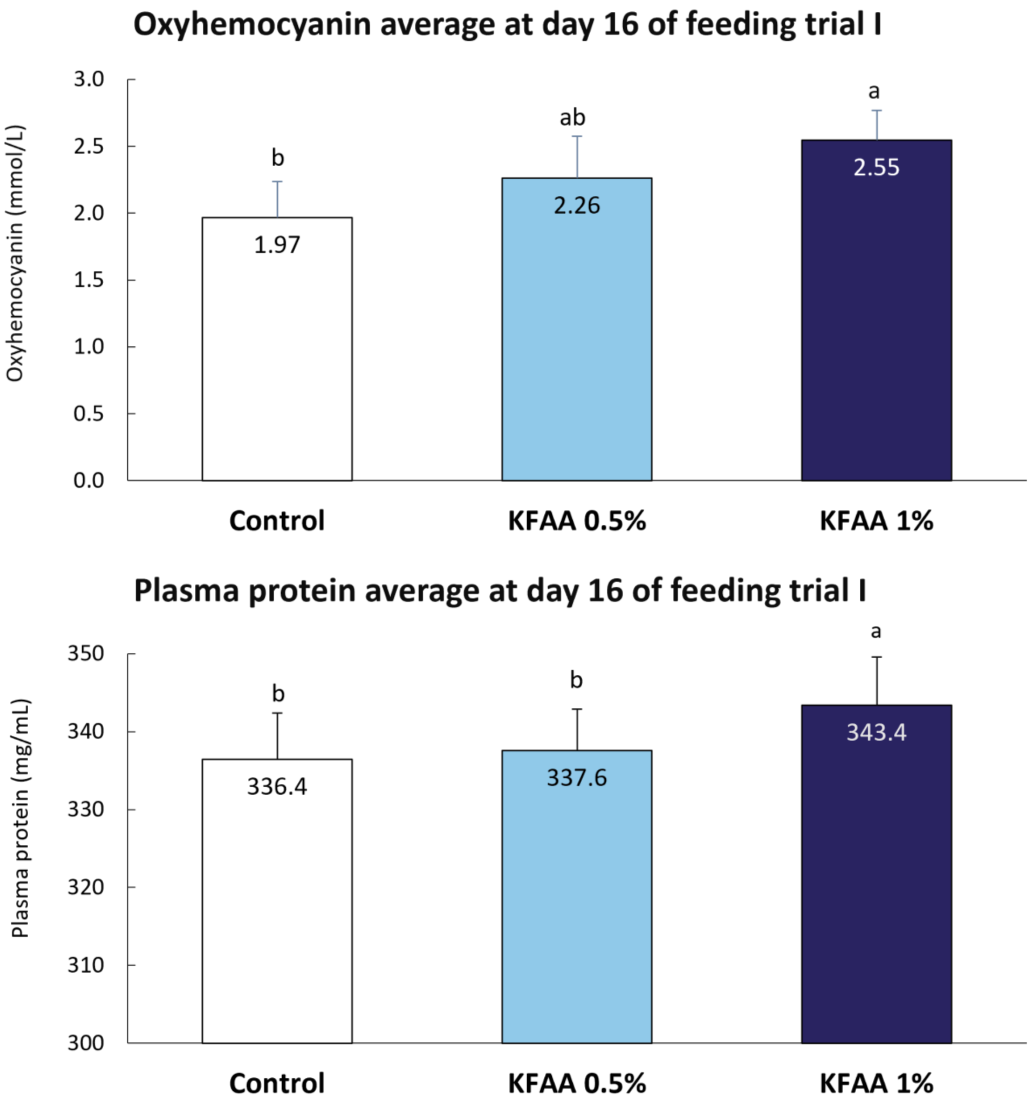

Figure 2: Charts of Oxyhemocyanin and plasma protein quantification of shrimp fed two levels of KFAA at day 16, feeding trial I. Whiteleg shrimp juveniles (Litopenaeus vannamei) initial body weight 14.12±0.35 g. Results were obtained from One-way ANOVA and Duncan's test. Different superscript letters indicate significant differences (*p*<0.05). Results were reported as least square means with standard deviation mean (S.D).

In the case of comparison among dietary treatments within each sampling day, for both parameters, the OxyHc concentration and PP were significantly higher at day 16 in shrimp fed the 1%, followed by 0.5% and lastly by control, +82.5% and +4.2% respectively compared to control (Fig. 3 c, d).

These results underline a dose-dependent effect of KFAA supplementation on these physiological parameters.

Challenge trial II

The recovery rate of loss of balance for all shrimp groups was 100% with afterward survival rate also of 100% following recovery from both water stress challenges, low salinity and low dissolved oxygen levels.

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

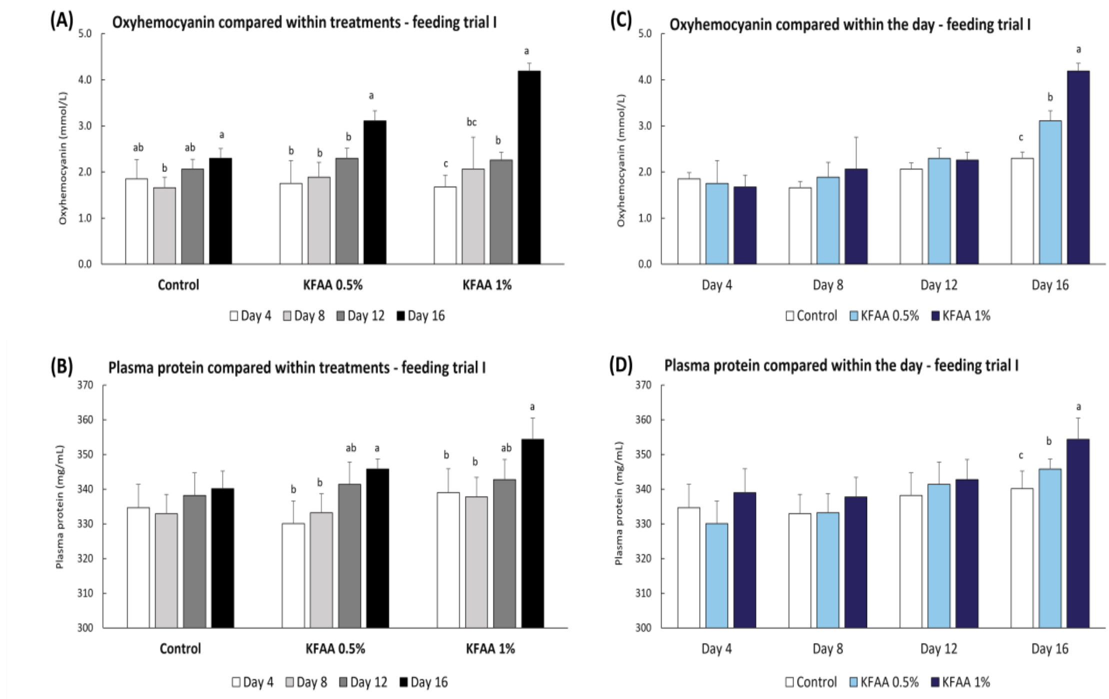

Figure 3: Charts of Oxyhemocyanin (a, c) and plasma protein (b, d) quantification of shrimp fed two levels of KFAA for 16 days, feeding trial I. Charts a and b compared among dietary treatments and charts c and d compared within the day. Whiteleg shrimp juveniles (Litopenaeus vannamei) initial body weight 14.12±0.35 g. Results were obtained from One-way ANOVA and Duncan's test. Different superscript letters indicate significant differences (*p*<0.05). Results were reported as least square means with standard deviation mean (S.D).

Shrimp fed the KFAA 1% showed a significantly longer resistance to loss of balance in both acute challenges, low oxygen and low salinity (Fig. 4).

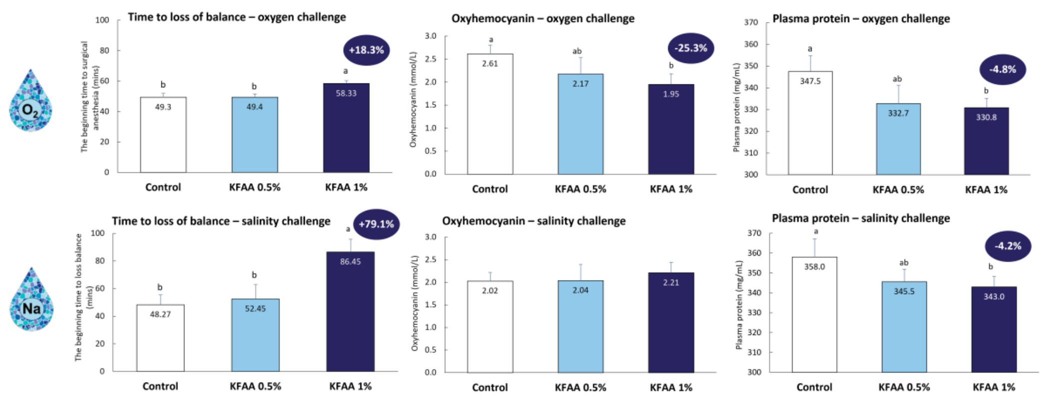

Figure 4. Low oxygen and low salinity acute challenges, time to loss of balance in minutes, oxyhemocyanin and plasma protein quantification in shrimp fed two levels of KFAA, challenge trial II. Whiteleg shrimp juveniles (Litopenaeus vannamei) initial body weight ~20g. Results were obtained from One-way ANOVA and Duncan's test. Different superscript letters indicate significant differences (*p*<0.05). Results were reported as least square means with standard deviation mean (S.D).

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

The time needed to lose balance was increased by 18.3% (58.3 min) in shrimp fed KFAA 1% compared to the other treatments during the low oxygen challenge. Interestingly, OxyHc concentration and PP were significantly decreased in shrimp fed the KFAA 1% supplemented diet (-25.3% and -4.8%, respectively), suggesting it would have been used during the challenge.

For the low salinity challenge, likewise, shrimp fed the KFAA 1% showed a significantly longer resistance to loss of balance compared to the other treatments, +79.1% (86.45 min) compared to control. The PP of shrimp fed the KFAA 1% was the lowest (-4.2%), followed by KFAA 0.5% and lastly control. No differences were observed in OxyHc concentrations during the low salinity challenge (Fig. 5).

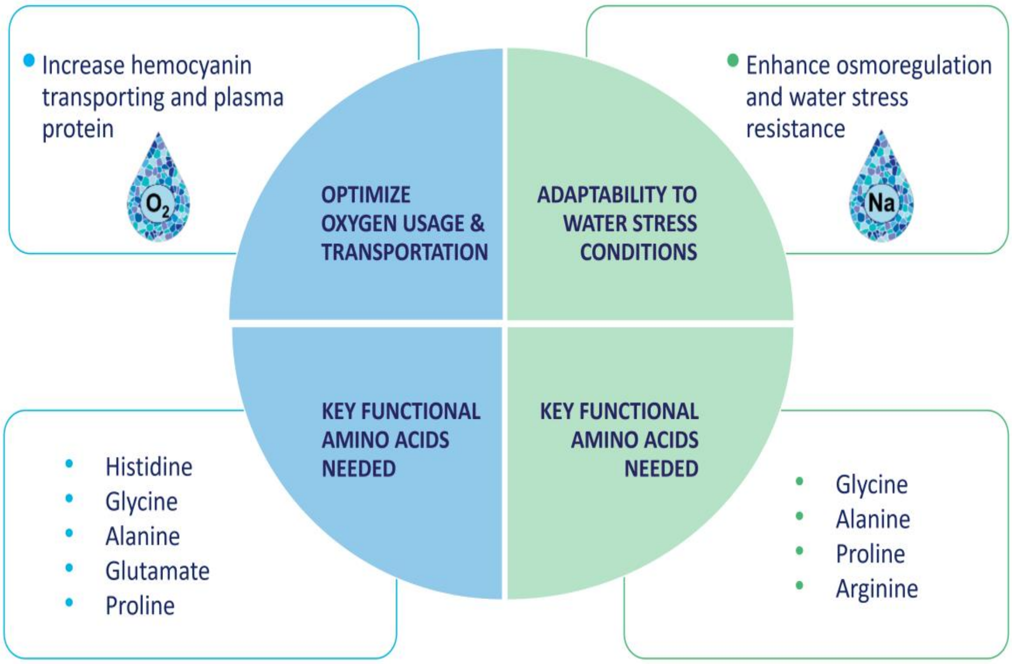

Figure 5: Schematic of functional amino acids needed for oxygen transportation and osmoregulation in shrimp (adapted from Wu, 2021).

Discussion

These results suggest the high levels of oxygen-binding hemocyanin and plasma protein shrimp had before (feeding trial I) the challenge served as a more prepared physiological state which later, during the acute challenge (challenge trial II), shrimp could benefit from for more nutrient resources to achieve higher water stress resistance. The key-functional amino acids needed for hemocyanin transporting and plasma protein include glycine alanine, proline, and arginine meanwhile the main amino acids involved in osmoregulation include histidine, glycine, alanine, glutamate, and proline (Xinyu Li *et al*., 2021).

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

Shrimp fed with 1% KFAA exhibited the highest OxyHc and PP values, with peak concentration at day 16 of the feeding trial. However, even shrimp fed with 0.5% KFAA exhibited higher OxyHc and PP values compared to the control group. These findings corroborate previous research indicating a positive correlation between dietary amino acids levels and hemolymph protein concentrations in shrimp (Ozbay and Riley, 2002).

Although the KFAA ingredient contributes a minor portion to the overall protein content of KFAA diets (5g/kg and 10g/kg of an ingredient with 50.4% protein, equaling 0.25g and 0.5g of protein per kilogram of feed respectively), nevertheless the main advantage of this ingredient could be attributed to the excellent digestibility of free amino acids since they are ready to be uptaken by gut cells (Le Reste *et al*., 2019) and its evolvement as functional amino acids in the key health metabolic pathways of *L. vannamei* juveniles.

Hemocyanin, the primary respiratory pigment in Arthropoda, constitutes 80–95% of the total hemolymph protein content measured at 102.8–217.5 mg/mL in *L. vannamei* (Pascual *et al*., 2003). It binds oxygen (OxyHc), serving as an indicator of oxygen-carrying capacity (Cheng *et al*., 2003), thus reflecting the health status of aquatic organisms. Shrimp fed with KFAA at 1% had a better ability to resist water stress, probably due to the increased OxyHc and PP levels in the hemolymph. Previous studies have demonstrated that water stress challenges based on exposure to higher concentrations of ammonia and nitrite which led to decreased hemolymph OxyHc, protein, and OxyHc to protein ratio in shrimp, such as *Marsupenaeus japonicus* (Cheng *et al*., 2013) and *Penaeus monodon* (Chen and Cheng, 1995; Cheng and Chen, 2002), attributed to nitrite's interference with the oxygen-binding mechanism in the hemolymph (Cheng *et al*., 2013; Chen *et al*., 1994).

Shrimp fed with 1% KFAA demonstrated longer resistance to loss of balance under low salinity and low dissolved oxygen stress tests, indicating KFAA's role in balancing shrimp physiology beyond the traditional amino acids formulation in the diet, especially in fluctuating environmental conditions common in shrimp culture (Chen *et al*., 1994; Chen and Cheng, 1995). The ability of shrimp fed KFAA-supplemented feed to maintain lower OxyHc values suggests enhanced resilience to oxygen shortages, contributing to their survival under adverse conditions. Higher PP values indicate improved hemolymph protein levels, crucial for supporting oxygen capacity and body biological processes, enhancing resistance to environmental stressors (Wu *et al*., 2003).

Lower OxyHc values observed in shrimp fed 1% KFAA feed (1.93 mg/L) suggest their heightened ability to endure oxygen scarcity. Shrimp exhibit better resistance to loss of balance even at lower oxygen concentrations in their hemolymph compared to the control, which had a higher OxyHc value (2.61 mg/L) at loss of balance. This resilience

[Downloaded from injbir.com on 2026-02-22]
[DOI: 10.22034/INJBIR.3.1.121]

allows shrimp to thrive longer in extremely low oxygen conditions. Similarly, the PP values of shrimp fed 1% KFAA feed (330.8 mg/µL) surpassed those of the control group. Plasma protein values are closely linked to OxyHc levels in this study, indicating that higher protein levels in the hemolymph enhance the production of hemocyanin, thus bolstering oxygen-carrying capacity in biochemical processes (Xie *et al*., 2015). This leads to higher resilience to environmental stressors, as amino acids are readily available for use.

During a salinity stress test using freshwater, shrimp fed 1% KFAA exhibited a significantly longer time before losing balance (86.5 mins) alongside the lowest PP values (342.9 mg/µL). This suggests their enhanced ability to maintain osmoregulation and support life under stressful conditions, remaining conscious even at the lowest protein levels in their hemolymph. This underlines the efficient utilization of energy sources from supplemented mixed amino acids to favor osmoregulation. Moreover, amino acids are likely utilized directly to maintain osmotic and ionic balance, particularly during critical conditions leading to the lowest PP concentration. However, OxyHc values did not differ among the groups.

In a salinity stress test, shrimp likely allocated similar oxygen consumption rates to support osmoregulation while prioritizing equilibrium in the osmoregulatory system to maintain iso-osmotic conditions by expelling water and retaining ions. Amino acids are then used as an emergency resource for osmotic and ionic balance. Hemolymph osmotic pressure regulation in crustaceans primarily relies on inorganic ions such as Na+, K+, and Cl-, with Na+ and Cl- contributing significantly to hemolymph osmolality. Changes in salinity can induce oxidative stress, potentially harming animals (Pan *et al*., 2007). Shrimp demonstrate adeptness in balancing osmotic pressure under low salinity cultures, possibly by adjusting hydrostatic and colloid osmotic pressure related to water economy. Therefore, 1% KFAA in feed serves as a valuable protein source, reducing stress associated with osmotic or ionic imbalances, critical for sustaining physiological processes and promoting growth (Gilles and Péqueux, 1981).

Xie *et al*. (2014) reported that dietary glycine levels influence Na+/K--ATPase activity in *L. vannamei* following acute salinity changes, with higher glycine levels potentially enhancing porphyrins and hemocyanin synthesis (Wang *et al*., 2013). Glycine also plays a crucial role in the osmoregulatory responses of fishes and shellfish, potentially enhancing survival after rapid salinity changes. Additionally, glycine's antioxidative capacity may contribute to survival following acute salinity changes (Xie *et al*., 2014). Proline, present in high proportion in evaluated KFAA (12.25% of total AA), improves amino acid constituent, antioxidative capacity, immune response, and NH_3 stress tolerance of juvenile *L. vannamei* (Xie *et al*., 2015). KFAA seems to contribute to

[Downloaded from injbir.com on 2026-02-22]
[DOI: 10.22034/INJBIR.3.1.121]

crucial physiological functions including osmoregulation, neurotransmission, antioxidation, and immune response (Wu, 2021).

Conclusion

The functional amino acids mix obtained from extensive poultry keratin hydrolysis consists of a readily-absorbable and sustainable source of nutrients for shrimp to overcome challenges at the farm conditions. Both trials highlighted the promising benefits of supplementing functional amino acids in shrimp diets.

Overall, the average of OxyHc and PP concentrations throughout the period of 16 days were significantly higher in shrimp fed the diets containing 1% KFAA, +29.4% and +2.1% respectively, compared to control. Shrimp fed the KFAA 1% showed a significantly longer resistance to loss of balance in both low oxygen and low salinity acute challenges. The time needed to lose balance significantly increased in shrimp fed 1% in both challenged water stress conditions.

Therefore, for challenge-oriented nutrition strategies, this study encourages discussion on understanding amino acids and their role in physiology above and beyond the traditional approaches as essential and non-essential.

Acknowledgements

This study was carried out at the Department of Aquatic Science, Faculty of Science, Burapha University, Bangsaen, Chonburi, Thailand under Dr. Boonyarath Pratoomchat guidance. We thank the intellectual collaboration and financial support from BCF Life Sciences that made possible the accomplishment of this study. This work is part of R&D projects from BCF Life Science that aim to generate a scientific background on amino acids supplementation in diets for shrimp as a sustainable and profitable approach for modern aquaculture.

References

Chen, J.C., Cheng, S.Y. and Chen, C.T., 1994. Changes of haemocyanin, protein acid levels in the haemolymph of exposed to ambient ammonia and free amino *Penaeus Japonicus*. *Camparative Biochemistry Physiology*, 109A, 339–347.

Chen, J.C. and Cheng, S.Y., 1995. Hemolymph oxygen content, oxyhemocyanin, protein levels and ammonia excretion in the shrimp *Penaeus monodon* exposed to ambient nitrite. *Journal of Comparative Physiology B,* 164, 530–535.

Cheng, S.Y. and Chen, J.C., 2002. Joint Action ofelevated ambient nitrite and nitrate on hemolymph compounds and nitrogen excretion oftiger shrimp monodon. *Comparative Biochemistry and Physiology Part C*, 303–314, DOI:PII:S1532-0456Ž02.00004-2.

Cheng, S.Y., Shieh, L.W. and Chen, J.C., 2013. Changes in hemolymph

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

oxyhemocyanin, acid-base balance, and electrolytes in *Marsupenaeus japonicus* under combined ammonia and nitrite stress. *Aquatic Toxicology*, 130–131, 132–138, DOI:10.1016/j.aquatox.2012.12.015.

Cheng, W., Liu, C.H. and Kuo, C.M., 2003. Effects of dissolved oxygen on hemolymph parameters of freshwater giant prawn, *Macrobrachium rosenbergii* (de Man). *Aquaculture*, 220, 843–856. DOI:10.1016/S0044-8486(02)00534-3.

Coates, C.J. and Decker, H., 2016. Immunological properties of oxygen-transport proteins: Hemoglobin, hemocyanin and hemerythrin. *Cellular and Molecular Life Sciences*, 74, 293–317.

Gilles, R. and Péqueux, A., 1981. Cell volume regulation in crustaceans: Relationship between mechanisms for controlling the osmolality of extracellular and lntracellular fluids. *Journal* of *Experimental Zoology*, 215–351.

Hagerman, L., 1986. Haemocyanin concentration in the shrimp *Crangon crangon* (l.) after exposure to moderate hypoxia, 85.

Kersanté, P., Le Reste, G., Diringer, B., Quimi, J., Sergheraert, R. and Duperray, J., 2021. Free amino acids mix made of poultry keratin improves survival of whiteleg shrimp post larvae (*Litopenaeus vannamei*) challenged with acute hepatopancreatic necrosis disease and white spot syndrome virus. *Aquaculture International*, 29, 879–890, DOI:10.1007/s10499-021-00665-x.

Khoklang, A., Kersanté, P., Nontasan, S., Sutthi, N., Pakdeenarong, N., Wang, T. and Wangkahart, E., 2024. Insights into the functional properties of a natural free amino acid mix: Effect on growth performance, nutrient metabolism, and immune response in a carnivorous fish, asian seabass (Lates Calcarifer). *Fish and Shellfish Immunology*, 144, DOI:10.1016/j.fsi.2023.109232.

Le Reste, G., Kersanté, P. and Duperray, J., 2019. Free Amino-acids mix made of poultry keratin as a new functional ingredient for white shrimp (*Litopeaneus vannamei*) feed. *Universal Journal of Agricultural Research*, 7, 203–209, DOI:10.13189/ujar.2019.070602.

Li, P., Mai, K., Trushenski, J. and Wu, G., 2009. New developments in fish amino acid nutrition: Towards functional and environmentally oriented aquafeeds. *Amino Acids*, 37, 43–53.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193(**1**), 265–275.

Nickerson, K.W. and Van Holde, K.E., 1971. A comparison of

[DOI: 10.22034/INJBIR.3.1.121] [Downloaded from injbir.com on 2026-02-22]

molluscan and arthropod hemocyanini-I. Circular dichroism and absorption spectra. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 39B, 855–872.

Ozbay, G. and Riley, J.G., 2002. An analysis of refractometry as a method of determining blood total protein concentration in the american lobster *Homarus americanus* (Milne Edwards). *Aquaculture Research*, 33, 557–562, DOI:10.1046/j.1365-2109.2002.00675.x.

Pan, L.Q., Zhang, L.J. and Liu, H.Y., 2007. Effects of salinity and pH on ion-transport enzyme activities, survival and growth of *Litopenaeus vannamei* postlarvae. *Aquaculture* 2007, 273, 711–720, DOI:10.1016/j.aquaculture.2007.07.218.

Pascual, C., Gaxiola, G. and Rosas, C., 2003. Blood metabolites and hemocyanin of the white shrimp, *Litopenaeus vannamei*: The effect of culture conditions and a comparison with other crustacean species. *Marine Biology*, 142, 735–745, DOI:10.1007/s00227-002-0995-2.

Pereira, R.T., Rosa, P.V. and Gatlin, D.M., 2017. Glutamine and arginine in diets for Nile tilapia: Effects on growth, innate immune responses, plasma smino scid profiles and whole-body composition. *Aquaculture*, 473, 135–144, DOI:10.1016/j.aquaculture.2017.01.033.

Pratoomchat, B., Sawangwong, P., Guedes, R., De Reis, M.L. and Machado, J., 2002. Cuticle ultrastructure changes in the crab *Scylla serrata* over the molt cycle. *Journal of Experimental Zoology*, 293, 414–426, doi:10.1002/jez.90002.

Rotllant, G., Llonch, P., García del Arco, J.A., Chic, Ò., Flecknell, P. and Sneddon, L.U., 2023. Methods to induce analgesia and anesthesia in crustaceans: A supportive decision tool. *Biology (Basel)*, *12*.

Wang, W., Wu, Z., Dai, Z., Yang, Y., Wang, J. and Wu, G., 2013. Glycine metabolism in animals and humans: Implications for nutrition and health. *Amino Acids*, 45, 463–477, DOI:10.1007/s00726-013-1493-1.

Wangkahart, E., Kersanté, P., Lee, P.T., Sanbut, O., Nontasan, S. and Chantiratikul, A., 2022. Effect of Kera-Stim®50, a feed additive containing free amino acid mix on growth, antioxidant and immune responses, digestive enzymes, and fatty acid composition in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 551, DOI:10.1016/j.aquaculture.2021.737874.

Wangkahart, E., Kersanté, P., Phudkliang, J., Nontasan, S., Pholchamat, S., Sunthamala, P.,

[Downloaded from injbir.com on 2026-02-22]

[DOI: 10.22034/INJBIR.3.1.121]

Lee, P.T., Chantiratikul, A., Soonngam, L. and Pakdeenarong, N., 2023. Effects of a dree amino acid mixture in replacing dietary fishmeal and reducing Nile tilapia (*Oreochromis niloticus*) production costs. *Aquaculture Reports*, 32, doi:10.1016/j.aqrep.2023.101739.

Wu, G., 2010. Functional amino acids in growth, reproduction, and health. *Advances in Nutrition*, 1, 31–37. DOI:10.3945/an.110.1008.1.

Wu, G., 2013. Functional amino acids in nutrition and health. *Amino Acids*, 45, 407–411.

Wu, G., 2014. Dietary requirements of synthesizable amino acids by animals: A paradigm shift in protein nutrition. *Journal of Animal Science* and *Biotechnology*, 5.

Wu, G., Bazer, F.W., Dai, Z., Li, D. and Wu, Z. , 2014. Amino acid nutrition in animals: Protein synthesis and beyond. *Annual Review of Animal Biosciences.,* 2, 387–417, DOI:10.1146/annurev-animal-022513-114113.

Wu, G., 2021. Amino acids in nutrition and health acids in the nutrition of companion, zoo and farm animals, second., springer: College station, TX, USA, Vol. 1285, ISBN 978-3-030-54461-4.

Wu, H.C., Chen, H.M. and Shiau, C.Y., 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International*, 36, 949–957. DOI:10.1016/S0963-9969(03)00104-2.

Xie, S.W., Tian L.X., Jin Y., Yang H.J., Liang G.Y. and Liu, Y.J., 2014. Effect of glycine supplementation on growth performance, body composition and salinity stress of juvenile pacific white shrimp, *Litopenaeus vannamei* fed low fishmeal diet. *Aquaculture*, 418–419, 159–164. DOI:10.1016/j.aquaculture.2013.10.023.

Xie, S.W., Tian, L.X., Li, Y.M., Zhou, W., Zeng, S.L., Yang, H.J. and Liu, Y.J., 2015. Effect of proline supplementation on anti-oxidative capacity, immune response and stress tolerance of juvenile pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, 448, 105–111. DOI:10.1016/j.aquaculture.2015.05.040.

Xing, S., Liang, X., Zhang, X., Oliva-Teles, A., Peres, H., Li, M., Wang, H., Mai, K., Kaushik, S.J. and Xue, M., 2024. Essential amino acid requirements of fish and crustaceans, a meta-analysis. *Reviews in Aquaculture*, 16, 1069–1086.

Li, X., Han, T., Shixuan Zheng, S. and Wu, G., 2021. Nutrition and functions of amino acids in aquatic, Guoyao Wu, Ed., Springer Nature:

[DOI: 10.22034/INJBIR.3.1.121] [Downloaded from injbir.com on 2026-02-22]

College Station, Texas, USA, Vol. 1285, ISBN 978-3-030-54461-4.

Zhang, Y., Wang, S., Xu, A., Chen, J., Lin, B. and Peng, X., 2006. Affinity proteomic approach for identification of an IgA-like protein in *Litopenaeus vannamei* study on its agglutination characterization. *Journal of Proteome Research*, 5, 815–821, DOI:10.1021/pr0503984.

[DOI: 10.22034/INJBIR.3.1.121]
[Downloaded from injbir.com on 2026-02-22]