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TOTAL REPLACEMENT OF FISHMEAL WITH AN ORGANICALLY CERTIFIED YEAST–BASED PROTEIN IN PACIFIC WHITE SHRIMP (*Litopenaeus Vannamei*) DIETS: LABORATORY AND FIELD TRIALS

E. McLean, B. Reid, D. Fegan, D. Kuhn, S. Craig

Summary

The feasibility of totally replacing the fishmeal component of marine shrimp (*Litopenaeus vannamei*) diets was examined both in the laboratory setting and during a full–scale commercial trial. Animals were fed either a traditional fishmeal–based diet or one in which complete replacement of fishmeal, on a per protein basis, was manufactured using a yeast–based product, NuPro®. Laboratory studies determined that irrespective of diet fed, no difference in shrimp performance (weight gain, survival and SGR) occurred. A field trial was thus activated to determine whether lab–scale studies were transferable to the commercial setting. Trials were conducted in earthen ponds from mid–June to early November 2005. Ponds were initially stocked with PL12–16 shrimp at a rate of 100,000 per hectare. At trial end, ponds receiving the NuPro®–based feed had equivalent growth to that of shrimp fed the traditional, fishmeal–based diet. Percent increase in weight from initial values and survival for the NuPro® ponds was 296, 269 and 275%, and 78, 76 and 85% respectively, whereas that for the fishmeal–based diet was 305% and 80% respectively. Noteworthy was that within pond size variation of *L. vannamei* was lower in NuPro® fed animals (±2.3 g) when compared against animals receiving the traditional feed (±4.1 g). Overall observations from the field trial

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indicate the importance of the »bioreactor« pond with respect to the supply of energy to sustain shrimp growth potential.

Key words: organic, sustainability, yeast

INTRODUCTION

Shrimp farming represents an important industry, especially in many developing nations. At present, global production of farmed shrimp exceeds 1 million tons per year (F A O, 2004) and production rates on a per annum basis increase at approximately to 2–4%. Major concerns however, face the industry, especially with regard to environmental and sustainability issues (Burford *et al.*, 2003). This has forced the shrimp aquaculture industry to respond to the severe criticisms from the environmental lobby (Naylor *et al.*, 1998) by adjusting traditional production practices. For example, significant reductions in water discharge during shrimp production have led to enhanced effluent quality and concomitant reduction in hypernutrification and eutrophication of receiving waters (Sandifer and Hopkins, 1996; Browdy and Moss, 2005). Another serious concern not only with regard to shrimp culture, but for aquaculture in general, is the industry's dependence upon fishmeal as a feed ingredient (Craig and McLean, 2005). Obviously there is a need to couple non–conventional culture practices with aquafeeds that do not rely upon fishmeal as the major protein source. This becomes inevitable especially when considering sustainability and the production of organically certifiable seafood products. Currently, twenty percent of the $8 billion US seafood deficit is represented by imported shrimp (Caro and Leites, 2005). This has declined slightly over the last 2 years, when shrimp imports of 500 million kilograms, valued at $3.8 billion, accounted for approximately 40% of the seafood trade imbalance. Of all seafood consumed in the US, 56% is derived from aquaculture. Since 2002 shrimp has been the nation's most popular seafood item with a per capita consumption rate of over 2 kg per year (www.nfi.org, 2006). This trend shows no indication of decline.

A fundamental component of diets used during the culture of shrimp, like that for carnivorous fishes, is fishmeal. However, the rising cost of fishmeal, when married to increasing global demands, has led to the search for alternative sources of protein for use in shrimp diets. Feed represents the largest single operational variable during shrimp production (Molina–Poveda and Morales, 2004) and during semi–intensive shrimp culture this may amount to 28% of total costs (Treece, 2000). In efforts to reduce the expenditures associated with fishmeal incorporation, many studies have examined whether alternative protein sources may be used in shrimp feeds (Sudaryono *et al.*, 1995; Cruz–Suarez *et al.*, 2001; Beiping *et al.*, 2005). However, while significant progress has been made in replacing a proportion of fishmeal in shrimp aquafeeds many alternative feeds employ animal by–products and meals. Here an issue of concern to the aquafeed and aquaculture industries is the potential of zoonotic entry into the human food chain (Craig and McLean, 2006). A broad variety of studies have demonstrated that the fishmeal component of shrimp and fish diets can be replaced by as much as 40%, or even greater levels, using plant–based proteins (Molina–Poveda and Morales, 2004). This is so even for high level carnivores such as cobia (Craig and McLean, 2006). Even given proof–of–principle however, there is resistance, both by the aquaculture and aquafeed industries, to move completely away from fishmeals, even when this is biologically possible. The reasons for this reticence remain obscure. Nevertheless, continued pressure upon fishmeal supplies by competing users, including other agrifeed industries and a burgeoning human population, will inevitably reduce its overall availability (Lunger *et al.*, 2006). The search for alternative protein sources that provide comparable performance to fishmeals must therefore, continue.

Soybean meal has been effectively incorporated into many formulations for carnivores such as the salmonids. The new interest in organic aquaculture and the organic market in general, further drives the need to replace fishmeal with organically certified protein sources. Such products however, are presently relatively few and mostly expensive. In previous studies (McLean and Craig, 2004), NuPro®, an organically certifiable yeast–based protein, was found to represent a highly suitable alternative protein to fishmeal for Nile tilapia; 100% replacement of fish meal by NuPro® was achieved, without detriment to any production–related characteristic. Preliminary trials with *L. vannamei* in the summer of 2004 (Reid *et al.*, 2004) determined that it was possible to replace 67% of dietary fishmeal with the same product. The current studies were instigated to determine the feasibility of replacing 100% of the fishmeal component in a commercial shrimp feed. Due to the success of described laboratory studies, a commercial–scale field trial was undertaken in order to determine whether laboratory observations translated into success in the »real world«.

MATERIALS AND METHODS

Laboratory Studies

System and husbandry

Six AHAB benchtop systems (Aquatic Ecosystems, Apopka, FL, USA) were employed, comprising 4 10–L aquaria, a 38 L/min magnetic driven pump, a 35L aerated sump with Siporex™ Biomedia, a 10 inch 50 micron canister filter, a 10 inch canister carbon filter, a 25 watt UV sterilizer, and a 250 watt submersible heater. Evaporative loss was recovered using reverse osmosis water (3–stage Pinnacle Series RO unit; Seachem Laboratories Inc., Madison, GA, USA). Certified, specific pathogen–free (SPF) postlarval shrimp were supplied by the Oceanic Institute (Kailua–Kona, Hawaii, USA). After acclimation to experimental conditions for seven days, salinity was lowered from 18 ppt to approximately 2.0 ppt. The shrimp were maintained at this salinity for an additional 10 days prior to the start of the experiment and were conditioned on a 35% protein, ground shrimp feed (Melick Aqua Feeds, Catawissa, PA, USA).

Diets, experimental design, and water quality

Animals were fed one of two diets: (Diet 1) a commercial shrimp feed with 35% crude protein or (Diet 2) NuPro® (Alltech Inc., Nicholasville, KY, USA), which replaced the fishmeal component on a unit protein basis. Shrimp were fed for a period of 40 days. Diets were fed *ad libitum*, but a surplus of feed was available for consumption during a 24 hour period. Every 24 hours, all feed was completely removed from the shrimp aquaria by siphoning. Dietary groups were randomly dispersed into each of the six AHAB benchtop systems. Diets were fed to triplicate groups of aquaria which were initially stocked with five juvenile *L. vannamei* with a mean weight of 26.3±1.3 mg. Survival was monitored every 48 hours and final weights were measured for all surviving shrimp in each aquarium at the end of the feeding trial. Shrimp were patted dry with Kim Tech Wipes (Kimberly–Clark, Roswell, GA, USA) and weighed using an A&D HM–202 analytical balance (A&D Engineering Inc., Milpitas, CA, USA). All treatments were conducted in 2.0 ppt salinity derived using synthetic sea salt (Crystal Sea, Marineland, Baltimore, MD, USA). Water quality was monitored for nitrite, nitrate, and total ammonia by spectrophotometry (HACH DR/2400 Spectrophotometer, HACH Co., Loveland, CO, USA). Salinity, temperature, and DO were measured using a YSI model 85 probe (Yellow Springs Inc., Yellow Springs, OH, USA). A HI 9024 pH meter (HANNA Instruments, Woonsocket, RI, USA) was used to measure pH.

Field Evaluation

Trials were conducted at the Organic Aquaculture Institute's facilities in Imperial, Texas, between mid–June and early November 2005. The trial employed Pacific white shrimp under organically certified production practices. Four, 1.8 hectare ponds were stocked with $PL_{12–16}$ *L. vannamei* obtained from a commercial hatchery at a rate of 180,000 PL/pond (i. e. 100,000 per hectare). All four ponds were inoculated with endemic rotifer and copepod populations collected from the Pecos River, Texas. On June 13, 2005, three ponds were randomly assigned to the diet containing NuPro®. The remaining pond was designated as a control. This pond was stocked on June 20, 2005. Ponds did not receive prepared feed during the first 30 days of the trial but were provided with compost derived from a local dairy farm. Compost was applied at a rate of 51 kg ha^{-1} every two weeks, a protocol that was continued for the duration of the trial. Ponds received prepared feeds commencing July 16, 2005. Initially feed was applied at 18 kg d^{-1} as two equal feedings until October 1, when 14 kg was dispensed as a single feeding until harvest later that month. Throughout the trial, temperature recordings were collected and cast net samples were taken weekly in order to monitor growth. Following harvest, total production per pond, increase from initial weight, feed conversion ratio values, specific growth rates and survival were calculated.

Statistical Analyses

Statistical analysis was performed using SAS v13.0 for windows (SAS Institute Inc., Cary, NC, USA). Differences in water quality were considered significant when $P<0.05$. A one–way ANOVA was employed with a Duncan's Multiple Range Test to test significant differences ($P<0.05$) between treatments for both experimental and field trials.

RESULTS

During laboratory studies no differences were recorded in survival, final mass attained, or specific growth rates (SGR) of Pacific white shrimp fed either dietary formulation (Table 1). Measured water quality parameters also did not differ between treatments (Table 2), indicating that fishmeal was not prerequisite to maintain shrimp growth. Accordingly, field evaluations were undertaken. At the end of the commercial–scale production trial, ponds receiving the NuPro®–based feed had equivalent growth to that of Pacific white shrimp

Table 1. Final mass, specific growth rates and survival of experimental shrimp fed either a fishmeal–based control or NuPro®–containing diet over a 40 day period in tank systems. No differences were observed between treatments (P < 0.05).
Tablica 1. Završna masa, specifični dnevni prirast i preživljenje račića hranjenih ribljim brašnom kao kontrolom i hranom koja sadrži NuPro® u pokusu tijekom razdoblja od 40 dana. Između tretmana nisu zapažene razlike (P < 0,05).

Treatment Tretman	Final mass Završna masa [mg]	SGR Spec. dnevni prirast [d^{-1}]	Survival Preživljenje [%]
Control	493.4	7.33	73
NuPro®	438.6	7.04	67
Pooled error Grješka uzor.	16,740		237.0
P > F	0.9110		1.000

Table 2. Mean water quality parameters of AHAB benchtop systems measured throughout the 40 day experimental period. No differences in any parameter were recorded.
Tablica 2. Srednje vrijednosti parametara vode mjerenih tijekom razdoblja pokusa od 40 dana. Ni u jednom parametru nisu zapažene razlike.

System Sistem	Ammonia–N, total [mg/L]	Dissolved oxygen DO [mg/L]	Nitrate–N [mg/L]	Nitrite–N [mg/L]	pH	Salinity [ppt]	Temperature [°C]
	n = 11	n = 13	n = 9	n = 9	N = 11	n = 16	n = 28
Control	0.020 (0–0.040)	5.18 (4.76–5.61)	16 (5.9–26)	0.022 (0–0.077)	8.48 (8.35–8.62)	1.96 (1.85–2.06)	29.4 (27.7–31.1)
Control	0.022 (0.002–0.041)	5.20 (4.82–5.57)	18 (5.5–31)	0.029 (0–0.11)	8.50 (8.37–8.62)	1.97 (1.87–2.06)	29.1 (27.9–30.4)
Control	0.021 (0–0.048)	5.06 (4.73–5.39)	20 (6.7–33)	0.032 (0–0.14)	8.53 (8.37–8.68)	2.15 (1.91–2.39)	30.0 (28.3–31.6)
NuPro®	0.018 (0–0.045)	5.21 (4.87–5.55)	15 (5.5–25)	0.020 (0–0.072)	8.45 (8.37–8.53)	1.88 (1.60–2.17)	29.8 (28.5–31.1)
NuPro®	0.025 (0–0.065)	5.16 (4.85–5.47)	12 (5.9–18)	0.017 (0–0.049)	8.45 (8.24–8.65)	1.86 (1.39–2.32)	29.4 (28.0–30.7)
NuPro®	0.029 (0–0.070)	5.10 (4.71–5.50)	18 (5.9–31)	0.012 (0–0.024)	8.49 (8.34–8.65)	2.11 (1.97–2.26)	29.5 (27.7–31.3)

fed the traditional, commercial shrimp diet. Percent increase in weight between week 9 and the end of the trial, at which point animals attained approximately 4.5 g wet weight, and survival for the NuPro® ponds was 296, 269 and 275%, and 78, 76 and 85% respectively. Percent increase in weight and survival for the control pond was 305% and 80%. Cumulative SGRs for the three ponds fed the NuPro®–based diets were 1.64, 1.70 and 1.89 respectively, whereas that for the control was 1.67 (Table 3). Weekly weight SGRs for the experimental groups versus that of control shrimp from week 9 of the trial are presented in Fig. 1. All groups expressed positive growth throughout this period, with, as may be expected, greatest SGRs being expressed between weeks 9 and 11, followed by a gradual reduction, which was allied to the decreasing temperatures observed during the growing period (Fig. 1). Noteworthy was that 2–3 weeks prior to harvest morning temperatures dropped to 23 °C and below. Because water temperatures were only recorded twice daily (05.00 and 18.00) it remains impossible to determine the number of hours that higher daytime temperatures were experienced. Total harvest weights and feed conversion ratios for the NuPro®–based and control diets are presented in Table 3. Highest harvest yields were recorded in NuPro® pond#3, which also returned the highest cumulative SGR. Remarkable was that within pond size variation was lower in NuPro® fed animals (± 2.3 g) when compared to the control animals (± 4.1 g).

Table 3. Production characteristics of Pacific white shrimp fed an experimental NuPro®–based or control (fishmeal–based) diet during a 12 week trial.
Tablica 3. Značajke proizvodnje račića hranjenih hranom s NuPro® i ribljim brašnom kao kontrolom u razdoblju pokusa od 12 tjedana.

Treatment Tretman	Harvest Izlov (kg)	Harvest Izlov (kg ha^{-1})	Harvest Izlov kg $(m^2)^{-1}$	FCR[1]	Cumulative SGR[2]
Control	2654	1474	.147	0.51	1.67
NuPro® 1	2558	1421	.142	0.53	1.64
NuPro® 2	2508	1393	.139	0.54	1.70
NuPro® 3	2792	1551	.155	0.49	1.89

[1] Food conversion ratio: kg fed/kg wt. Gain — Konverzija hrane: kg hrane/kg prirasta
[2] Specific growth rate/ Specifični dnevni prirast:
SGR = $(\ln W_f - \ln W_i)*100/t$
Where/gdje je::
$\ln W_f$ = the natural logarithm of the final weight / prirodni logaritam završne težine
$\ln W_i$ = the natural logarithm of the initial weight / prirodni logaritam početne težine
t = time (days) between $\ln W_f$ and $\ln W_i$ /
t = vrijeme (dani) izmedu $\ln W_f$ i $\ln W_i$

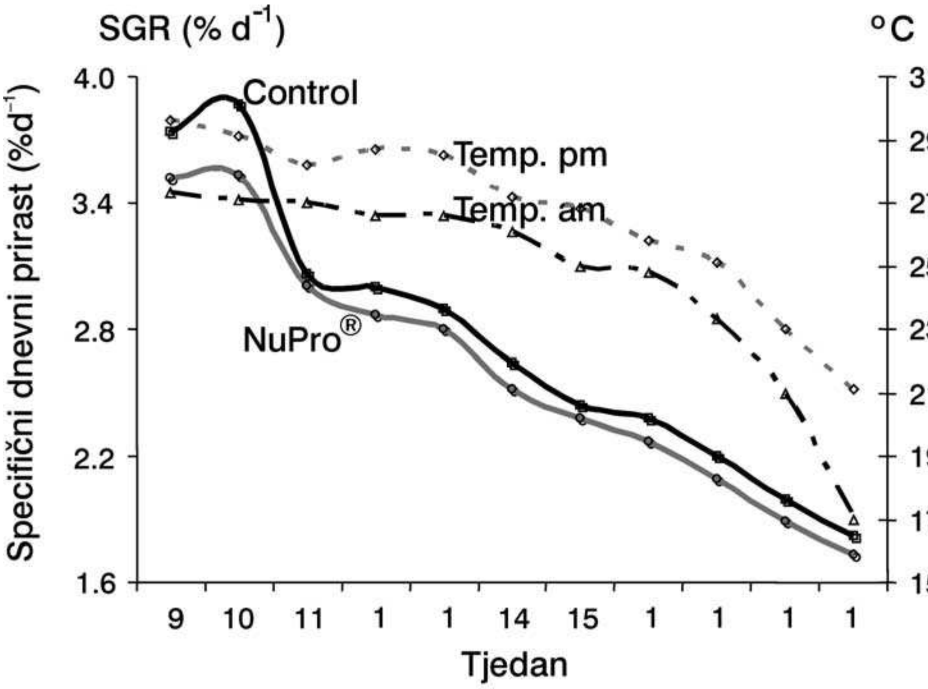

Figure 1. Specific growth rates of Pacific white shrimp fed an experimental NuPro®–based or control (fishmeal–based) diet during the last 11 weeks of a commercial–scale field trial, at which point animals achieved approximately 4.5 g wet weight. Temperature decline for the period is also presented.
Slika 1. Dnevni prirast pacifičkih račića hranjenih hranom s NuPro® i ribljim brašnom kao kontrolom tijekom posljednjih 11 mjeseci u komercijalnim uvjetima, pri čemu su životinje postigle prosječno 4,5 g tjelesne težine. Vrijednosti temperature također su prikazane.

DISCUSSION

The lack of organically certified, alternate protein sources represents a critical obstacle to the growth of the organic aquaculture sector (Craig and McLean, 2005). Discussion continues to surround the verification and certification of by–catch from commercial fisheries, and by–product and processing waste from fish and meat processing industries and aquafarming, as organic aquafeed ingredients. Furthermore, issues concerning the palatability and amino acid availability of such products must be addressed (Li *et al.*, 2004). Challenges are also met when considering plant protein sources due to the presence of anti–nutritional factors and imbalances in essential amino acid profiles and reduced digestibilities (Hardy, 1996; Francis *et al.*, 2001). These problems are augmented further with organically certified plant proteins where postponed field operations, poor soil humidity, weeds, and reduced mineralization of organically–certified composts during plant production cycles, may negatively impact crop production and nutritive value. Additionally, risks of contamination of organic crops by traditional and genetically modified harvests are worrying (Hanson *et al*., 2004). The manufacture of single cell–based products by fermentation technologies overcomes all the above problems while also providing security in production. The present investigation illustrates that growth and survival obtained by the juvenile shrimp reared under laboratory–based conditions, and fed yeast–based diets derived from biosecure fermentation systems were similar to that attained by animals maintained on fishmeal–based feeds. Moreover, both groups of shrimp performed similarly to previous reports (Ponce–Palafox *et al*., 1997). Laboratory investigations therefore, demonstrated the biological possibility of completely replacing the fishmeal component of manufactured marine shrimp diets with NuPro®.

Often the transfer of technologies that have performed successfully in the laboratory environment to full commercial production is fraught with difficulties. However, observations made during the described field trial served to confirm the utility of the test protein even in an industrial setting. The pond–based trial lends support to the idea that natural pond production provides a significant nitrogen source for growing shrimp (Hopkins *et al*., 1995; Burford *et al*., 2003; Yanbo *et al*., 2005). This fact is highlighted not only by survival of shrimp (80% overall compared to 50% for Texas as a whole for the 2005 season) during the first month of production, wherein feed was not added to ponds, but also by the feed conversion ratios obtained: 0.5 kg prepared diet resulted in 1 kg shrimp production. Clearly use of organic compost provided adequate fertilization which likely resulted in the production of in–pond bioflocs and supported growth of phytoplankton, protozoa and other aquatic food sources. Many studies suggest that high protein inputs improve shrimp production (Akiyama *et al*., 1992; Pascual *et al*., 2004). However, results herein indicate that provided natural pond productivity is maintained, protein inputs during shrimp farming do not necessarily correlate with pond output in terms of production (see Martinez–Cordova *et al*., 2003). This has obvious economic consequences.

A negative aspect of the present field trial was that due to pond availability, the study commenced approximately 6 weeks into the Texas shrimp growing season. Accordingly, this shift in experimental period resulted in a significant reduction of growth rates over the last 3 weeks of the trial where temperatures declined well below the 27 °C optimum for Pacific white shrimp (Fig. 1; Wyban *et al*., 1995). At temperatures ~ 23 °C reduced growth and feeding can be anticipated. Nevertheless, average harvest weight of individual shrimp was 18 g. With an appropriate study starting date this average weight could have been in the 22–23 g range. The latter represents the premium size, and thus value, for cultivated Pacific white shrimp. Under organically certified production methods the current trial with NuPro®–based feeds produced animals that did not vary in size as much as those fed a traditional commercial shrimp feed. From a processor and retail perspective this issue cannot be undervalued and could result in a higher farm gate price for the product.

In conclusion, the described commercial–scale feeding trial established, beyond any reasonable doubt, that non–traditional shrimp cultivation practices, including total removal of dietary fishmeal, can be effectively and economically utilized to produce organically certified, and thus, high value marine shrimp.

Sažetak

ZAMJENA RIBLJEG BRAŠNA S ORGANSKIM PROTEINOM NA BAZI KVASCA U HRANI ZA PACIFIČKE BIJELE RAČIĆE (*Litopenaeus vannamei*): LABORATORIJSKI I PRIRODNI UVJETI

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Mogućnost potpune zamjene ribljega brašna u prehrani morskih račića (*Litopenaeus vannamei*) istraživana je u laboratorijskim i komercijalnim uvjetima. Životinje su bile hranjene uobičajenom hranom s ribljim brašnom i hranom u kojoj je riblje brašno u potpunosti zamijenjeno proteinom na bazi kvasca NuPro®. U laboratorijskim uvjetima nije zapažena razlika kod račića glede prirasta, preživljenja i dnevnoga prirasta. Pokusi u prirodnim uvjetima provedeni su da bi se rezultati dobiveni u pokusnim uvjetima mogli potvrditi u komercijalnom uzgoju. Pokus je postavljen u zemljanim ribnjacima od sredine lipnja do ranoga studenoga godine 2005. Ribnjaci su nasađeni s PL12–16 račića u gustoći od 100 000 komada/hektaru. Na kraju pokusa rast račića hranjenih objema hranama bio je jednak. Postotak povećanja težine od početnih vrijednosti i preživljenje račića hranjenih s NuPro® bio je 296, 269 i 275%, odnosno 78, 76 i 85%, dok je u onih hranjenih ribljim brašnom bio 305%, odnosno 80%.

Ključne riječi: organski, održivost, kvasac

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