Aquaculture 296 (2009) 51–57

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed

David D. Kuhn [a,*], Gregory D. Boardman [a], Addison L. Lawrence [b], Lori Marsh [c], George J. Flick Jr. [c]

[a] Department of Civil and Environmental Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061, USA
[b] Texas AgriLife Research Laboratory, Texas A&M System, Port Aransas, TX 78373, USA
[c] Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

ARTICLE INFO

Article history:
Received 26 August 2008
Received in revised form 21 July 2009
Accepted 22 July 2009

Keywords:
Alternative protein
Biomass
Bacteria
Biofloc
Sustainable
Recirculating aquaculture systems

ABSTRACT

Microbial flocs produced in suspended growth bioreactors could offer the shrimp industry a novel alternative feed. In this study, microbial flocs were produced in sequencing batch reactors (SBRs) using tilapia effluent and sugar as a growth media. It was determined that 1 kg of microbial floc could be produced per 1.49 kg of sucrose. These microbial flocs were tested as an ingredient for shrimp feed over a 35 day feeding trial. Two control diets (absent of microbial flocs) were compared against three dietary treatments (microbial floc inclusion). Control 1 and microbial floc diets (diets 1–3) were formulated to be equivalent for levels of crude protein, total fat, crude fiber, calcium, magnesium, phosphorus, potassium, and sodium. Controls 1 and 2 did not contain microbial flocs and differed slightly from each other in soybean oil, krill meal, and mineral/salt levels. For diet 1 (microbial floc 7.8%) and diet 2 (microbial floc 15.6%), soybean protein isolate on a protein basis was replaced with microbial flocs at a 7.8 and 15.6% inclusion level on a dry matter basis. For diet 3, fishmeal was replaced with microbial flocs at 7.8% and fish oil at 0.50% (microbial floc 7.8% + fish oil). Four juvenile *Litopenaeus vannamei* were stocked per tank and each dietary treatment was tested in 12 replicates over a 35 day feeding trial. No differences were observed between final survival rates (93 to 100%) between any of the dietary treatments. Growth (weight gain per week) for control 1, control 2, diet 1, diet 2, and diet 3 were respectively 1.09 ± 0.14, 0.88 ± 0.14, 1.64 ± 0.03, 1.61 ± 0.03, 1.63 ± 0.04 g/week. The total gain in weight for the three diets containing microbial floc of 8.07 to 8.18 g in five weeks with an initial weight of 0.44 ± 0.005 g is truly exceptional. Tukey's HSD (Honestly Significant Differences) test revealed that each of the three microbial floc diets significantly ($P<0.01$) outperformed each control in terms of weight gain per week with no differences in survival.

1. Introduction

Traditional Penaeid shrimp culture has a long proven history using pond culture in tropical climates. The global shrimp market has expanded from less than $1 billion to $5.8 billion (US) from 2000 to 2005 (FAO, 2008). To meet this growing demand, the shrimp industry is shifting from extensive rearing systems to more intensive rearing systems. Presently, shrimp production in earthen ponds using cutting edge technology in tropical zones can produce two to four crops per year while only one crop per year can be produced per year in a temperate zone. To combat limitations in temperate areas, indoor recirculating aquaculture systems could be implemented to simulate a tropical environment. Therefore, more than one crop could be produced per year. Moreover, there are numerous drawbacks and concerns regarding outdoor intensive systems. Drawbacks include stressed animals, increased disease, increased oxygen demands, and decreased water quality. Generally, these risks can be reduced, while maintaining a high density of animals, when a controlled indoor environment is used (e.g. recirculating aquaculture systems). Recirculating aquaculture systems use numerous technologies to clean water for reuse within the culture system (Timmons et al., 2002) or even from one animal to another (Kuhn et al., 2007). Recirculating systems often include the following technologies; nitrification (e.g. fluidized sand filters), oxygenation (e.g. speece cone), disinfection (e.g. ultraviolet sterilization), and solids removal (e.g. drum filters) (Skjølstrup et al., 2000; Menasveta, 2002; Timmons et al., 2002).

Clear water recirculating systems have numerous benefits over outdoor intensive pond systems, especially in temperate climates. However, implementation of recirculating aquaculture systems has not yet translated into a solution for intensive shrimp culture. This is because numerous studies have demonstrated that shrimp reared in clear water using initial stocking densities above 300/m^3 have a significantly lower growth rate than those reared in dirty water using much lower initial stocking densities. This becomes increasingly evident when shrimp tested in clear water are compared directly to shrimp in systems with a high productivity of natural organisms (e.g. algae, bacteria, and other natural biota) (Tacon et al., 2002; Izquierdo

* Corresponding author. Tel.: +1 540 231 6325.
E-mail address: davekuhn@vt.edu (D.D. Kuhn).

0044-8486/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2009.07.025

et al., 2006; Moss et al., 2006). McLean et al. (2006) reported that shrimp can grow equally as well when fed a yeast based diet compared to a fishmeal diet. These naturally occurring organisms may contribute nutritionally and serve as a pre-/probiotic and/or unknown growth promoter. For these reasons, it was hypothesized that production of microbial flocs in sequencing batch reactors (SBRs) using effluent from a tilapia production facility and a carbon source (e.g. sugar) could produce a viable alternative ingredient for shrimp feed.

Biological treatment of aquaculture effluent using suspended growth processes has been demonstrated with (Schneider et al., 2006; Schneider et al., 2007) and without (Sharrer et al., 2007) carbon supplementation. Carbon supplementation is necessary if nitrogen removal is not efficient. Microbial flocs produced in biological reactors would be different from naturally occurring organisms found in pond systems because they would be produced externally from shrimp while treating a fish effluent in dark reactors. Microbial flocs produced in SBRs could be dried and incorporated into a pelleted feed for shrimp. If this alternative feed proved to be successful, it could offer the shrimp industry a new culture option in clear water recirculating aquaculture systems. A very significant further justification is the need to have alternative lower cost ingredients replacing marine animal meals and traditional plant meals. For these reasons, this study investigated if it would be possible to produce microbial floc in SBRs as a potential ingredient for replacing fish meal and soybean meal in shrimp feed.

2. Materials and methods

2.1. Experimental design

Microbial flocs generated in SBRs by Virginia Tech researchers at a shrimp pilot facility (Virginia Shrimp Farms, Martinsville, Virginia, US) were used as a test ingredient in shrimp feed, over a 35 day feeding trial. Two control diets (absence of microbial flocs) were compared against three dietary treatments (microbial floc inclusion). Each dietary control/treatment consisted of 12 replicates (each tank was a replicate) over six systems in a randomized block design. Each 20 L tank was stocked with four juvenile shrimp. This feeding trial was conducted indoors at the Texas A&M AgriLife Research Mariculture Laboratory (Port Aransas, Texas, US) using indoor recirculating systems with seawater renewal. This is essentially a clean or clear water system with no natural productivity present.

2.2. Sequencing batch reactors used to produce microbial flocs

Wastewater was diverted from a tilapia farm where recirculating aquaculture systems are used. This effluent was pumped to an aerated well-mixed equalization tank (11,300 L). Wastewater in the equalization tank was monitored for five different days over a one week period to determine water quality in terms of nitrate (95.7 ± 10.9 mg/L), nitrite (0.56 ± 0.11 mg/L), pH (7.09 ± 0.02), total ammonia nitrogen (TAN, 10.8 ± 2.7 mg/L), and soluble total organic carbon (TOC, 20.8 ± 3.1 mg/L). Effluent was manually drained into two commercially available, 5100 L SBRs (Model CA-15d, Cromaglass Corp., Williamsport, Pennsylvania, US). Atmospheric air and water pumps were used to aerate and agitate the tilapia effluent with microorganisms (microbial flocs) which reduce dissolved organic matter and assimilate and/or oxidize ammonia and nitrite. Carbon (Granulated white sugar, i.e. sucrose, Kroger Co., Cincinnati, Ohio, US) was supplemented at a target rate of 80 mg carbon/L every 24 h. The SBRs were operated inside a building and had manhole covers to prevent light penetration. Well-mixed aerobic batch tests were performed with and without carbon supplementation at 19 °C over a six hour period. These batch tests were repeated to determine consistency.

Total ammonia nitrogen was determined spectrophotometrically using methods approved by the US Environmental Protection Agency for the analysis of wastewater (HACH Co., Loveland, Colorado, US). Soluble chemical oxygen demand (COD), soluble TOC, and volatile suspended solids (VSS) were measured in accordance with APHA (2005). Microbial floc levels were measured as VSS (Metcalf and Eddy, 2003).

2.3. Microbial flocs as an ingredient for shrimp feed

Nutritional composition reported for microbial flocs (Table 1) was analyzed by A&L Eastern Laboratories, Inc. (Richmond, Virginia, US). Two independent sampling events (14 days between events) were performed to determine microbial floc nutritional consistency. Microbial flocs were harvested as a dietary ingredient for the shrimp feeding trial during the first sampling event. Settled microbial flocs were harvested from SBRs by siphoning and were air dried in 5 cm layers to solids levels greater than 86%. Microbial flocs were subsequently ground into fine material using a stand mixer with grain mill attachment (KitchenAid® Professional 600 Series, Saint Joseph, Michigan, US).

2.4. Shrimp

Postlarvae of less than 1 mg/shrimp were obtained from Oceanic Institute (OI) and were free of pathogens listed by the US Marine Shrimp Farming Program (USMSFP, 2006), including taura syndrome virus (TSV), white spot syndrome virus (WSSV), yellow head virus (YHV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and infectious myonecrosis virus (IMNV). These shrimp were from OI's "Kona" line which is a reference strain of shrimp that originated from Sinaloa, Mexico. This shrimp line has been domesticated for over 15 years and is used as a positive control in disease-challenge studies because of its consistent performance over time (Hennig et al., 2004). These 20 day old postlarvae were reared in a recirculating aquaculture system with a high sea water exchange rate of 17.5%/day. Water quality parameters were ideal. Dissolved oxygen, salinity, and temperature averaged 6.01 mg/L, 31.7 ppt, and 28.1 °C, respectively. For the first week, postlarvae shrimp were feed artemia and crumbled commercial feed (Rangen 45/10, Buhl, Idaho, US). For the remaining 16 days, prior to experiment initiation, shrimp were fed the Rangen 45/10 diet only until the average shrimp weighed 435 mg.

2.5. Experimental systems for shrimp

Six systems using recirculating technology were used to culture shrimp. Each system comprised of two rows (front and back) with 12

Table 1
Composition of microbial flocs on dry matter basis, mean values with standard errors, as determined by laboratory analysis ($n = 2$).

Parameter	Microbial flocs
	[g/100 g]
Crude protein	49.0 ± 1.5
Carbohydrate[a]	36.4 ± 0.9
Total ash	13.4 ± 0.6
Crude fat	1.13 ± 0.09
Crude fiber	12.6 ± 0.1
Calcium	1.28 ± 0.07
Phosphorus	1.29 ± 0.08
Sodium	1.27 ± 0.03
Potassium	0.75 ± 0.13
Magnesium	0.41 ± 0.05
	[mg/kg]
Zinc	181 ± 1
Copper	92.5 ± 3.0
Manganese	35.0 ± 0.5

[a] Calculated value (Merrill and Watt, 1973): carbohydrate = 100 − (ash + crude protein + moisture + total fat).