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Evaluation of bioflocs derived from confectionary food effluent water as a replacement feed ingredient for fishmeal or soy meal for shrimp

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ABSTRACT

It is important to explore the use of alternative ingredients for soybean and fishmeal in aquaculture feeds because the demand and cost for those ingredients are expected to increase in the near future and long-term. Meanwhile, the food processing industry produces large quantities of wastes that often contain organic solids and nutrients (e.g. nitrogen waste and phosphorus) which can be converted in microbial protein (bioflocs) using suspended growth biological reactors. Bioflocs that were collected from such a reactor that treats confectionary food processing effluent water were dried and in shrimp feed as a replacement for soybean and fishmeal. A control diet (without bioflocs) was compared to three diets that replaced soybean (10, 20, and 30% biofloc inclusion) and two diets that replaced fishmeal (10 and 20% biofloc inclusion). The control and biofloc diets were formulated to be equivalent for levels of crude protein, total fat, crude fiber, calcium, magnesium, phosphorus, potassium, and sodium. Five juvenile shrimp were stocked per tank and each dietary treatment was tested using 8 replicates over a 35 day feeding trial. Dietary treatments had some impact on shrimp performance. No differences ($P > 0.05$) in shrimp performance were observed between the control and the diets that included bioflocs for survival (97.5 to 100%), growth (2.16 to 2.40 g/wk), harvest biomass (687 to 732 g/m^2), or food conversion ratio (1.50 to 1.66). These results indicate the bioflocs harvested from a suspended growth biological reactor that treats food effluent water can successfully be used in shrimp diets.

Statement of relevance: Alternative & sustainable protein source for shrimp culture.

1. Introduction

In order to meet the growing demand for global consumption of shrimp the aquaculture industry needs to explore new and innovative practices to maintain and increase sustainable growth. Since feed represents over 50% of the cost for aquaculture producers (FAO, 2009) this is an important economic area that can be improved upon. Two primary protein ingredients typically utilized in shrimp diets are soybean and fish meal. The dietary requirement of fish meal and soybean protein to shrimp is well defined and understood (Cuzon et al., 2004; Fox et al., 2006). However, the price of fish meal and soybean ingredients has risen over the years. For example, fish meal price has increased from around $400 per metric ton in the year 2000 to over $2200 per metric ton in 2014. Meanwhile, soybeans have increased from around $175 to over $500 during the same period of time (Index Mundi, 2015). Even though both of these ingredients will continue to be a mainstay in regard to economic importance it is also critical to explore alternative ingredients to these protein sources that may be more economical without compromising sustainability.

The food processing industry produces large quantities of wastes that often contain organic solids and nutrients (e.g. nitrogen waste and phosphorus) which can be detrimental to the environment if it is not treated properly before being discharged (Sharrer et al., 2007). In many countries, this type of wastewater is regulated and requires treatment before being discharged into the environment because it can contribute to eutrophication (Wetzel, 2001) and can be toxic to aquatic fauna (Boardman et al., 2004; Carmargo et al., 2005). Food processors typically send this waste into the municipal sewer system while others install biological reactors onsite to treat the effluent prior to discharging the wastewater. Suspended-growth biological reactors are often employed to remove accumulated solids and nutrients from effluent waters. People in the wastewater community call the suspended solids in the bioreactor mixed liquor volatile suspended solids (Metcalf and Eddy, 2003). Meanwhile, in the aquaculture community this material is referred to as bioflocs (Avnimelech et al., 2014).

Bioflocs are a consortium of microorganisms, micro/macro invertebrates, filamentous organisms, exocellular polymers, and uneaten feed. This nutritious mixture has often proven to be beneficial for

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shrimp production. When bioflocs are included in shrimp diets, either directly from the water column or in a processed feed, shrimp often exhibit positive responses such as improved health and growth rates. Bioflocs might also reduce the amount of feed required and/or replace a particular ingredient. Previous research by Kuhn et al. (2009, 2010a, 2010b) have demonstrated the bioflocs derived from treating fish effluent is a practical replacement for fishmeal and soy meal in shrimp diets. The work presented herein is different from previous research because bioflocs that were derived from the treatment of confectionary food processing effluent water were evaluated as a feed ingredient for shrimp, not bioflocs produced while treating aquaculture wastewater.

2. Materials and methods

2.1. Experimental design

Bioflocs were collected from a suspended growth biological reactor that was used to treat confectionary food processing effluent water. A 35 day feeding trial was conducted to evaluate this biofloc material as a potential ingredient for replacing soybean or fish protein in shrimp diets. A control reference diet was challenged against five experimental diets with biofloc inclusion. Each dietary treatment consisted of eight replicates (eight tanks) in two indoor recirculating aquaculture systems (RAS) with seawater renewal at the Texas A&M AgriLife Research Mariculture Laboratory (Port Aransas, Texas, US). Each of the 26 L tanks was stocked with five juvenile shrimp. These culture units were maintained as clear water systems that were essentially absent of natural productivity (e.g. algae).

2.2. Bioflocs

Bioflocs were produced in a suspended growth biological process for treating confectionary food processing wastewater. The chemical oxygen demand, biological oxygen demand, and total suspended solids in the effluent water respectively averaged (±, standard deviation) 68.4 ± 54.5, 5.9 ± 2.2, and 87.4 ± 64.2 mg/L over a three month period. Meanwhile, total nitrogen, total phosphorus, and mixed liquor volatile solids in the biological reactor averaged 4.5 ± 2.9, 19.7 ± 7.3, and 132.9 ± 30.3 mg/L over the same period. This facility disposes of 20 metric tons (~44,000 lb) of biofloc material on a dry weight basis every month of the year.

Biofloc material that was harvested from the biological reactor was air dried in a greenhouse in 5-cm layers until moisture levels were reduced to levels less than 12%. The dried material was subsequently ground into fine material using a stand mixer with a grain mill attachment (KitchenAid® Professional 600 Series, Saint Joseph, Michigan, US). These bioflocs were stored at −20 °C until experimental diets were made. Nutritional and amino acid profiles for bioflocs are provided in Table 1.

2.3. Shrimp

Post larval (PL < 15 days) shrimp were obtained from Shrimp Improvement Systems (SIS, Islamorada, Florida, US) that were certified to be free of the following diseases: 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). Postlarval shrimp were initially cultured on *Artemia* followed by Rangen 45/10 crumble feed in a recirculating seawater system with approximately 20% daily water exchange. Shrimp were raised to an initial stocking weight of 3.0 g for the experimental feeding trial.

Table 1
Nutrient composition (dry-weight basis) of bioflocs collected from food processing suspended growth biological reactor.

Parameter	Bioflocs
Proximate and mineral levels [g/100 g dry matter]	
Crude protein	38.3
Carbohydrate[1]	19.0
Total ash	31.6
Crude fat	0.42
Crude fiber	16.6
Calcium	1.07
Sodium	1.55
Phosphorus	1.37
Potassium	0.36
Magnesium	1.81
Essential amino acid levels [g/100 g dry matter]	
Leucine	2.28
Valine	2.38
Threonine	1.66
Isoleucine	1.46
Arginine	3.62
Phenylalanine	1.98
Lysine	1.45
Methionine	0.46
Histidine	0.93
Tryptophan	0.54
Nonessential amino acid levels [g/100 g dry matter]	
Glutamic acid	3.66
Aspartic acid	2.91
Alanine	2.06
Glycine	1.73
Serine	1.35
Proline	1.27
Tyrosine	1.64
Cystine	0.30
Calculated trace element levels [mg/100 g dry matter]	
Manganese	125
Zinc	532
Copper	211

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

2.4. Experimental system

Two RAS with 100 tanks each (24 tanks per system were dedicated to this study) were used for the nutritional trial. The control and five experimental diets were split evenly amongst both systems resulting in a randomized complete block (RCB) design. The control and five experimental diets were then randomly distributed into tanks (30.7 L tanks (bottom area of 0.1 m^2) within each of the recirculating system. These systems were outfitted with an aerated nitrification reactor, 100-μm filtration, and a 12 h photoperiod using fluorescent lamps. Seawater renewal in these systems was 154%. Seawater was sourced from the Corpus Christi Shipping Channel (27° 50′ 22.3116″, −97° 4′ 23.2854″). Seawater was then pumped through a series of pressurized sand filters (50, 25, and 1 μm respectively) and was stored in three 53,000-L opaque fiberglass reservoirs prior to use.

Water quality in the culture systems was monitored daily for dissolved oxygen (DO), salinity, and temperature using a YSI 85 m (YSI Inc., Ohio, US). Nitrate, nitrite, pH, and total ammonia nitrogen (TAN) were measured three times a week (Spotte, 1979). More details for nitrate, nitrite, and TAN procedures can be found in Strickland and Parsons (1972); Mullin and Riley (1955), and Solorzano (1969), respectively.

2.5. Diets

A control diet, absent of bioflocs, was evaluated against experimental diets that were formulated with bioflocs. All diets were formulated to be isonitrogenous, isolipid, and equivalent for calcium and phosphorus.