On demand feeding and the response of Pacific white shrimp (*Litopenaeus vannamei*) to varying dietary protein levels in semi-intensive pond production.

By

Leila Strebel

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Approved By

D. Allen Davis, Chair, Professor School of Fisheries, Aquaculture, and Aquatic Sciences
James Stoeckel, Assistant Professor School of Fisheries, Aquaculture, and Aquatic Sciences
Luke A. Roy, Associate Extension Professor School of Fisheries, Aquaculture, and Aquatic Sciences

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Abstract

Feed is one of the primary costs associated with commercial production of Pacific white shrimp (*Litopenaus vannamei*). The cost is the combined outcome of feed cost and feed management. As feeding technology evolves, specifically the use of passive acoustic monitoring (PAM) feeding systems, it is vital to reevaluate the optimal protein levels in diets for the best production outcomes. The use of acoustic monitoring adds another level of complexity to how shrimp respond to feed because it has the potential to automatically adjust feed offerings based on protein because of the shrimp's response. In this research, four diets with various protein levels (40, 35, 30, and 25%) were fed to shrimp which were stocked (0.045 g, 25 shrimp/m^2) into 16 ponds (0.1 ha) and cultured for an 85-day production cycle. Shrimp were fed using the AQ1 passive acoustic monitoring system. Final individual weights were significantly smaller for shrimp fed the 25% diet (31.22 g) compared to all other diets. The total biomass of all ponds ranged from 7,037- 7,878 kg/ha for shrimp offered the 25%- 40% diets, respectively. Analysis of this and all other production data showed no differences between treatments ($p>0.05$). Whole-body analysis revealed significant differences in fat ($p=0.0002$), copper ($p=0.018$), and apparent net protein retention ($p=0.0025$). Analysis of economic values indicated a statistically significant difference between treatments for feed cost ($p=0.02$). The significantly lower individual weights from shrimp fed the 25% diet and the notably lower total biomass resulted in a subsequent difference in class size distribution. This ultimately led to a difference in the market value of the shrimp ranging from \$60,383 to \$71,247. However, the 40% protein diet was significantly higher in cost and showed no differences in production or economic outcomes compared to the other diets. Therefore, these results indicate that a 30-35% protein diet would be the most efficient for use in pond production of Pacific white shrimp under the culture conditions examined in this study.

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1. Introduction

Marine shrimp are the leading aquaculture production species by value and their culture is essential for meeting consumer demand for shrimp (Cai et al., 2019). Pacific white shrimp were the top produced shrimp species in 2020 with a total of 5.8 million tonnes (FAO, 2022) accounting for ~61% of shrimp cultured (Liddel & Yencho, 2020). The majority of shrimp aquaculture takes place in semi-intensive and intensive aquaculture systems, which rely on the input of nutritionally complete feeds for efficient and successful production. Feeds are the largest expense associated with production, accounting for 40-60% of production costs and are the primary contributor of waste in the system. Therefore, improving diet formulations and feed delivery mechanisms will undoubtedly save time, money, and could help reduce the environmental impact of aquaculture (Naylor et al., 2009; Chatvijitkul et al., 2017).

Fish meal has traditionally been the preferred protein source for shrimp feeds due to its digestibility, nutrient content and palatability (Miles & Chapman, 2006). However, fish meal has limited availability, and a high price (Naylor et al., 2009; Hardy, 2010). There are several proposed alternatives for fish meal including different animal by-products, plant-based sources as well as insect and single cell protein sources (Bae et al., 2020; McLean et al., 2020; Sánchez-Muros et al., 2020; Soares et al., 2020; Luthada-Raswiswi et al., 2021). The increased use of alternatives will allow aquaculture to continue to expand and improve sustainability (Olsen & Hasan, 2012). Soybean meal as a complete and partial replacement with other alternatives, such as poultry meal, have been widely investigated as viable options to serve as fish meal alternatives (Allen Davis & Arnold, 2000; E. A. Amaya et al., 2007). These ingredients tend to be less expensive and have greater availability compared to fishmeal. The nutrient profile of soybean meal in combination with other meals is a suitable replacement to fish meal. Previous studies have demonstrated that complete or partial replacement of fish meal with soybean meal and other nutrient sources does not affect the growth of Pacific white shrimp, making it an ideal alternative protein source (Allen Davis & Arnold, 2000; E. Amaya et al., 2007; E. A. Amaya et al., 2007; Bae et al., 2020; Sánchez-Muros et al., 2020; Hussain et al., 2022).

In addition to diet formulation, the delivery method is important for maximizing growth and feed intake of the shrimp. Research suggests that daily protein intake, which is a combination of dietary protein and feed intake, is a limiting factor for shrimp growth (Weldon et al., 2021). Variable feed inputs are likely one of the reasons that a wide range of protein levels in the feed have been reported. Although tolerant of a range of dietary protein levels, too much protein can lead to poor water quality conditions without significant improvements to growth (Kureshy & Davis, 2002; Martinez-Cordova et al., 2003). Hence, determining the best protein level in feeds that allows for the highest growth and most economical nutrient conversion is important for optimizing resources as protein is one of the highest cost ingredients in feeds.

As technology advances, specifically with the use of passive acoustic monitoring (PAM) for feed management, the ideal protein level for feeds should continue to be reevaluated. The use of PAM has been demonstrated to be an effective feed management strategy for feeding shrimp, due to proven improvements in feed conversion ratio (FCR), average body weight (ABW), yield, and economic return (Napaumpaiporn et al., 2013; Ullman et al., 2019; Reis et al., 2021; Reis et al., 2022). However, PAM adds another layer of complexity to determining ideal protein level as daily feeding is based on shrimp feeding response.

PAM monitors shrimp feeding activity by "listening" to the sound made by the shrimp's mandibular occlusion while eating using an underwater microphone, otherwise known as a hydrophone. Based on the response after a "test spin", which distributes a small amount of feed to the pond, an algorithm created by the AQ1 System determines if the shrimp should be offered more feed based on the feeding response. Shrimp have a relatively small stomachs and a short digestive tract, hence, they can only eat small amounts at one time. On-demand feeders allow for feed to be dispersed in small amounts many times throughout the day, in accordance with the shrimp's feeding activity. More feedings over the day have shown to improve production outcomes in pond production of *L. vannamei* (Ullman et al., 2019; Reis et al., 2021). Therefore, since protein level also impacts production outcomes, the use of acoustic feeding systems could change how shrimp respond to different protein levels.

When feeding variable protein levels, the lower protein content in a diet can be potentially compensated for by increased consumption. The use of on-demand feeding could help realize this difference because it uses a feedback mechanism to determine the amount of feed to be fed to the shrimp. Shrimp being fed higher protein feed will be satiated quickly and require less feed compared to those fed a diet with lower protein inclusion, hence, the amount of total protein consumed by the shrimp will be similar which will yield similar results (Venero et al., 2007; Weldon et al., 2021). Past research has demonstrated this by altering feeding rates of diets with variable protein levels. Kureshy and Davis (2002) applied this principal using 16, 32 and 48% diets. However, the higher protein diets were shown to have better feed efficiency and shrimp were not able to eat enough of the 16% protein diet to compensate for the low protein level in the feed.

There has been few investigations on demand feeding and its interaction with protein levels. Therefore, this experiment had the following objectives:

1. Analyze response of shrimp with on demand feeding to four diets with variable dietary protein levels
2. Identify differences in production outcomes, water quality, and basic economic values in shrimp fed different dietary protein levels.

2. Materials and Methods

The pond trial was conducted at the Alabama Department of Conservation and Natural Resources, Claude Peteet Mariculture Center (CPMC) (Gulf Shores, AL, USA). The post-larvae (PL) shrimp were provided by Homegrown Shrimp USA (Indiantown, FL, USA). The PLs were brought to CPMC and acclimated into six, 6000 L outdoor nursery tanks. Days 1 through 5 the PLs were fed 25% of their body weight (BW) Zeigler Bros Raceway 1 (50% protein, 15 %lipids). Days 6-8 the PLs were fed a combination of Raceway 1 and 2 at 25% of their body weight. During days 9-11 the PLs were fed Raceway 2 (50% protein, 15 % lipids) at 15% BW. On day 12 the PLs were fed a combination of raceway 2 and 3 at 5%BW. Finally, on day 13 the PLs were fed Raceway 3 (50% protein, 15% lipids) at 5% BW. 14 days after receiving the PLs, juvenile shrimp were stocked into 16, 0.1 ha ponds at 25 shrimp/m^2.

2.1 Ponds

Lined ponds (1.52 mm high-density polyethylene) with a 25 cm sandy-loam soil bottom were tilled and then filled using water from the intercoastal waterway in Gulf Shores, AL (14 ppt). The water was filtered for organic matter using a 250-micron mesh sock. Prior to stocking with shrimp, the ponds were fertilized with inorganic fertilizers (1687 mL of 32-0-0 and 303 mL 10-34-0 for 5.70 kg/ha of N and 1.03 kg/ha of P).

2.1.1 Feed formulation

Four diets with various protein (25, 30, 35, and 40%) and lipid (5,6,7, and 8%) levels were formulated using soybean and poultry by-product meal as main protein sources. Diets were produced by Zeigler Bros. Inc. (ZBI, Gardners, PA, USA) as extruded (2.4 mm) sinking feeds. All diets were analyzed for proximate composition and amino acid profile by the University of Missouri Agricultural Experiment Station Chemical Laboratory (ESCL) according to established techniques. Additionally, all diets were sent to Midwest Laboratories (Omaha, NE, USA) to confirm the crude fat levels using acid hydrolysis methods and to determine mineral content.

2.1.2 Feed management

During the first 16 days after stocking into the ponds, shrimp were hand fed twice per day with Zeigler Bros (ZBI, Gardners, PA, USA) 1.5 mm commercial diet (40% crude protein, 9% crude lipids) with daily amounts increasing weekly from 1.0 kg to 1.5 kg. Beginning on day 17, feed was delivered via a timer feeder, with each pond receiving their randomly assigned treatment diet. From day 17-22, 3.0 kg was offered daily and the amount was increased to 6.0 kg from days 23-29. On day 30 of production, the AQ1 Systems sonic feeder (AQ1 Feeder, AQ1 Systems Pty. Ltd., Tasmania, Australia) was implemented. The ponds were switched to sonic feeding after it was determined that the minimum activity required by AQ1 Systems was consistently met. A hydrophone placed in each pond collected acoustic activity made by the shrimp's mandibular occlusion while eating, which allowed the AQ1 system to determine when and how much more feed to apply to the pond after each test spin. The shrimp were allowed to feed from 8 am to 10 pm with a maximum daily limit of 160 kg/ha (16 kg/pond) to avoid detrimental water quality effects.

2.1.3 Water Quality management

Water quality, including dissolved oxygen (DO), salinity, and temperature were monitored twice daily at sunrise (5:15- 5:45 am) and sunset (7:30-8:00 pm) using a YSI ProPlus Meter (Yellow Springs Instrument Co., Yellow Spring, OH, USA). The AQ1 system continuously monitored dissolved oxygen via probes placed in the water and was programmed to stop feeding when DO dropped below 3.5 mg/L and resume feeding after reaching 4.5 mg/L. In addition to ceasing feeding, the AQ1 system initiated automatic aeration controls when DO dropped below 3.5 mg/L and turned off at 4.5 mg/L. Due to technical difficulties, several ponds without working aeration controls had an aerator turned on manually each night. For all ponds, if the DO dropped below 6mg/L before 10pm, a second aerator was turned on to ensure that DO would not drop to lethal levels overnight. Aeration was administered to ponds with one 1-HP Air-O-Lator (Kansas City, MO, USA) as the primary source and one 2-HP Aire-O2 (Aire-O2, Aeration Industries International, Inc., Minneapolis, MN, USA) for secondary use as needed.

Total ammonia nitrogen (TAN) was measured once per week every week and twice per week during weeks 8, 9, and 11 using an ion-selective electrode (Orion 4-Star Plus pH/ISE, Thermo Fisher Scientific, Waltham, MA, USA). The pH was measured once per week using a YSI EcoSense pH10A handheld meter (Yellow Springs Instrument Co., Yellow Spring, OH, USA). A photometer (WaterLink Spin TouchFF, LaMotte, Chestertown, MD) was used during weeks 0, 2, 4, 6, 10, 11, and 12 to analyze water for pH, ammonia, nitrite, nitrate, alkalinity, calcium, phosphate, and magnesium. Secchi depth was measured once weekly. All samples for water quality analysis were taken in the morning and certain parameters, especially pH, reflected this.

2.1.4 Sampling and Harvest

Beginning at day 15, shrimp were sampled using cast nets and weighed weekly. Approximately 60 shrimp, with a limit of ten throws, were sampled each week from every pond. After weighing the total biomass of the sample, all shrimp were counted as they were put back into the pond and average individual weight was calculated.

All ponds were harvested on days 86-88. Pond water levels were lowered the night before and drained the remaining amount just before harvesting. All shrimp were removed from the ponds and were weighed in baskets. 150 shrimp were randomly selected from each pond and individually weighed. A random sample of 6 shrimp from each pond were dried at 90°C, homogenized and sent to Midwest Laboratories (Omaha, NE, USA) for whole body proximate and mineral analysis. Following the receival of the results of the whole-body analysis the apparent net protein retention was calculated. This was calculated using $\text{ANPR}\ (\%) = [(\text{Wf} \times \text{BPf}) - (\text{Wi} \times \text{BPi}) / \text{TPC}] \times 100$, where Wf = final weight; BPf = final body protein; Wi = initial weight; BPi = initial body protein; TPC = total protein offered. Copper retention and phosphorus retention were also calculated using this formula using their respective values.

2.2 Statistical analysis

All data was analyzed in Statistical Analysis System for Windows (V9.4, SAS Institute, Cary, NC, USA). Production and economic data were analyzed using one-way analysis of variance (ANOVA), with all assumptions met, in order to determine statistical significance between treatments ($P>0.05$). After the ANOVA, a Tukey's honest significant difference (HSD) multiple range test was performed to show which treatments were significant from each other. A nested model was also used to analyze all individual weights taken at harvest to determine significant differences between treatments. All water quality data were analyzed using time series analysis. Shrimp values were calculated using market prices in Latin America for October 2022 from Seafood Price Current (Urner Barry, Toms River, NJ, USA). Shrimp pricing was for headless shrimp and a 60% dressed weight was used in these calculations. During production, large die offs occurred in 3 ponds due to low DO events paired with failed aeration. These ponds were removed from statistical analysis including one replicate from each of the 40%, 30% and 25% protein diets. During harvest, two ponds experienced a mortality event due to low water levels, possible low DO, and aerator issues. This included one pond in the 40% protein treatment, and one pond in the 35% protein treatment. The mortalities in the ponds were estimated using pictures and average individual weight from shrimp in these ponds. These estimated weights were included in the final biomass of the pond and all other production and economic values for statistical analysis.

3. Results

The diet formulations for all experimental diets and the analyzed results for crude protein, crude fat, crude fiber, moisture, and ash are all found in Table 1. The analyzed amino acid profile for all experimental diets is found in Table 2.

The mean water quality parameters of DO, salinity, temperature, pH, TAN, ammonia, nitrite, nitrate, alkalinity, magnesium, calcium, and phosphate are summarized in Table 3. Dissolved oxygen was found to be significantly different between treatments in the morning(p=0.0007). Dissolved oxygen in the 25% protein treatment (4.09 mg/L) was significantly higher than the 30% (3.92 mg/L) and the 35% (3.88 mg/L), while no differences were observed in the 40% treatment (4.00 mg/L). Salinity was significantly lower (p=0.0001) in the 40% treatment (10.72 g/L) compared to all other treatments (10.99 -11.01 g/L). Total ammonia nitrogen (TAN) was significantly higher (p=0.0001) in the 40% (1.07 mg/L) and 35% (0.81 mg/L) treatments compared to the 30% (0.39 mg/L) and 25% (0.38 mg/L) treatments. Figure 1 shows the TAN and average daily feed input each week during production. Nitrate was significantly lower (p=0.012) in the 40% (0.61 mg/L) and 35% (0.61 mg/L) treatments than in the 25% (1.00 mg/L) treatment. Nitrite was significantly higher (p=0.0008) in the 40% (0.6 mg/L) treatment compared to the 30% (0.05 mg/L) and the 25% (0.07 mg/L) treatments. Additionally, a significant interaction between date and treatment was revealed for TAN, nitrate, nitrite, phosphate, and calcium measurements.

All production results and economic analyses can be found in Table 4. There was a significant difference in individual weights between treatments (p=0.0001). The 30% (34.2 g), 35% (33.6 g), and 40% (33.9 g) treatments were all significantly higher compared to the 25% (31.2 g) protein treatments. Figure 2 displays the average individual weight recorded weekly over the production cycle. Figure 3 shows how the individual weights translated into the different size classes per treatment. No statistically significant results were found between treatments for growth per week, feed input, survival, total biomass, or FCR ($p>0.05$). To help visualize feed inputs, we pooled daily feed input data in Figures 4, 5 and 6. As one would expect, feed inputs increased until about day 60, which was 30 days after initiating on demand feeding. Feed inputs then leveled out as upper limits of maximum allowable feed inputs (160 kg/ha/day) were consistently being fed. The data was also pooled by two-week intervals to allow better visualization of the data (Figure 4). In the first two weeks of on demand feeding, the 25% diet was offered significantly less than the others and all other diets were not different from each other. However, over the next three two-week intervals or the total feed offered over the production cycle there were no differences between treatments.

The final total biomass ranged from 7,037 kg/ha to 7,878 kg/ha and feed inputs ranged from 8,193kg/ha to 8,806 kg/ha. The only economic value that was significant between treatments was feed cost ($p=0.02$) where cost of the 40% protein diet ($15,960) was significantly higher than the 25% protein diet ($12,559). The value of shrimp ranged from $60,382 to $71,247 but there were no statistical differences. Total biomass and value of shrimp are shown in Figure 7. No other differences in basic economic values including cost per kilogram of shrimp and partial income were significant ($p>0.05$). There was a statistically significant difference found for apparent net protein retention (ANPR) between treatments ($p=0.0025$). The 25% protein diet (66.11%) and 30% protein diet (57.18%) had a high ANPR compared to the 40% protein diet (43.03%). Phosphorus and copper retention were not found to have any statistical differences ($p>0.05$). Total protein fed was statistically significant between treatments (p=0.0001). The shrimp fed the 25% diet received the least amount of protein (2,115 kg/ha), while shrimp fed 30% (2,659 kg/ha) and 35% (2,984 kg/ha) protein were significantly higher but were not different from each other. Further, shrimp fed the 40% protein diet (3,679 kg/ha) received the most protein between treatments.

Results from whole-body proximate analysis are found in Table 5. The whole-body analysis showed a statistically significant difference in copper content (p=0.018) between treatments. The copper content in the shrimp fed the 25% protein diet (112.75 ppm) was significantly lower than the shrimp fed 35% protein (131.50 ppm). Additionally, a significant difference was shown in fat content between treatments (p=0.0002). The shrimp fed the 25% diet had a significantly lower fat content (8.13%) compared to the 30% (9.57%), 35% (10.60%), and 40% (10.48%) protein diets.

4. Discussion

The quantity of dietary protein input in pond production of shrimp not only impacts the feed costs but also water quality and shrimp growth (Venero et al., 2007; Jescovitch et al., 2018). Consequently, it is important to carefully examine the implications of each protein level and what may be best for practical production settings. There has been a wide range of recommended dietary protein levels for Pacific white shrimp (25-40%), However, with the advancement of technology these may continue to change (Xu & Pan, 2014; Yun et al., 2016; Ayisi et al., 2017) or require re-evaluation. The use of on demand feeding technologies adds complexity to interpreting the biological and economic impact of dietary protein level since all feed inputs rely on the shrimp's feed response. As the industry moves toward automated feeding systems, there is a clear need to develop data on the interaction of nutrition and feed management. In this work, we assessed four levels of dietary protein in combination with acoustic feeders which allowed shrimp to feed on demand.

First, to understand the biological data, we must look at the feed and protein inputs between treatments to further understand the production outcomes. In the first two weeks of on demand feeding, the 25% diet was offered at significantly lower levels than the other feeds. However, over the next three two-week intervals there were no differences between treatments. This lower consumption at the beginning of demand feeding is likely due to the level of protein and consequently the quantity of attractants in the feed producing a weaker signal compounded with the smaller size of shrimp. It is known from previous research that hydrophones can effectively pick up different acoustics signals from shrimp based on different diet characteristics (Silva et al., 2019; Peixoto et al., 2020). Walsh et al. (2022) demonstrated how the inclusion of different feed effectors in shrimp diets resulted in different responses from the shrimp and consequently significant differences in feed input. However, the overall input of feed was not affected by dietary protein level (Table 4). One possible explanation of this is that during the last four weeks of production the maximum daily input of 160 kg/ha feed was a limitation for shrimp fed the higher protein diets (Figure 4, 5, and 6). Limiting inputs may have allowed shrimp fed the low protein treatment to catch up which in turn resulted in no difference in total feed amounts over the 12-week production cycle. If the feed limit was higher and/or the shrimp had been able to feed *ad libitum*, a difference in total feed may have been realized between treatments.

Although there were no differences in total feed inputs there were significant differences in the total amount of protein fed to shrimp. The difference in offered protein did not statistically affect total biomass of shrimp; however, the mean weight of shrimp fed the 25% diet were significantly smaller. Figure 2 shows how the 25% diet had numerically smaller shrimp over much of the 12 weeks of production. These results indicate that a 25% diet would not be adequate for the shrimp to achieve maximum growth. These findings are consistent with previous research such as Shahkar et al. (2014) where in a clear water experiment the optimum protein level for *L. vannamei* was found to be higher than 25% and around 33.4% protein. Furthermore, Yun et al. (2016) concluded that protein could be reduced from 40 to 35% when shrimp were grown in a biofloc system without a reduction in growth and performance of *L. vannamei*. This implies that in general, reducing dietary protein level from 40% to 30% will not decrease the production outcomes.

Evaluating whole- body analysis of shrimp offers insight into how dietary protein level impacted biological response. First, the analysis revealed significantly lower fat content in shrimp fed the

25% protein diet. This could have been due to the lower fat content of this diet or limited nutrient intake. The 25, 30, 35, and 40% diets were formulated to have 5, 6, 7, and 8% lipids respectively. Diet proximate analysis confirmed these percentages showing that the 25% diet had 6.1% fat content while the 30% had 7.54% and both the 35% and 40% had over 8% fat. Additionally, shrimp fed higher protein diets received a more nutrient dense feed which has previously been reported to lead to increased fat deposition (Ullman et al., 2019). This would indicate that the shrimp had lower energy reserves and nutrient intake may be limiting (Li et al., 2017). Since both nutrient density and fat content of the diets changed, it is not possible to distinguish which variable impacted fat deposition more in the shrimp. Although it is not clear which had a greater effect on fat deposition, all diets were formulated with an acceptable lipid content for Pacific white shrimp (Wang et al., 2014). Whole-body proximate analysis also revealed a difference in copper content. This could be due to slight differences in diet formulations which ranged from 95.4- 114 ppm. There was no difference in copper retention between treatments, and retention was consistent with previously reported data with similar dietary copper levels (Zhou et al., 2017). The ANPR results showed increasing ANPR as protein in the diet and daily intake decreased. Shrimp fed the higher protein diets also had a higher daily intake and are thus more likely to have used protein as an energy source and therefore had lower protein retention. Previous research suggests that as the dietary protein level and/or daily intake increases, protein efficiency decreases (Yaemsooksawat et al., 2009). Additionally, the presence of natural productivity in the ponds is associated with an increase in protein retention of shrimp as protein offered decreases (Xu et al., 2012). These results are also supported by past research such as Weldon et al. (2021) where *L. vannamei* in a biofloc tank system were offered various feeding rates. These different feeding rates resulted in different amounts of total protein offered to shrimp, and the ANPR increased as protein offered decreased.

This indicates that efficiency of protein use by shrimp decreases as dietary protein intake increases. Therefore, the use of a high protein diet such as 40% may not be the most efficient for growth of *L. vannamei*.

Further, now that we have established dietary protein level and intake had an impact on the biological response of shrimp, we can discuss how protein level influenced the culture environment. Dietary protein level had a clear impact on pond water quality. This is observed by differences in nitrogen and carbon input which increased with dietary protein level. Significant differences in water quality parameters, most notably nitrogen, were observed between treatments. Total ammonia nitrogen (TAN) and nitrite measurements (Table 3) revealed that the highest values were in the ponds fed the 40% diet and the lowest were in the 25% dietary treatment. Since feed conversion rates (FCR) ranged from 1.13-1.18 in all treatments, it is unlikely that shrimp were being overfed and this result was from excess feed. Instead, it is potentially due to the higher concentration of nitrogen in the 40% protein diet being processed by the shrimp.

With the use of automatic feeding systems, total feed inputs are increased which in turn increases the risk of water quality issues. Hence, it is important to monitor TAN closely because of the susceptibility of shrimp to stress with high ammonia levels, especially at low salinities (Li et al., 2007; Valencia-Castañeda et al., 2018). Jescovitch e al. (2018) reported a maximum feed input of 5,280 (upper limit of 120 kg/ha/day) kg/ha using AQ1 and concluded that the increased feed amounts compared to timer or hand feeding using a standard rate caused there to be spikes in nitrogen especially towards the end of the 16-week production cycle. In comparison, ponds in this study were fed an average of 8,806 kg/ha (upper limit of 160 kg/ha/day) of the 40% protein diet, making this the maximum nitrogen that was loaded out of any of the treatments. Hence, it is reasonable for there to be significantly higher nitrogen measurements here not only due to a higher feed input compared to past research but also because of increasing amounts of dietary protein in the feeds.

Collectively, the assessment of various feed inputs, biological, and water quality data suggest a 40% diet may be inefficient and a water quality risk, while a 25% dietary protein diet may not maximize shrimp growth but does minimize water quality risks. Therefore, we should also examine the basic economic outcomes to see if they support a similar conclusion. The final value of the shrimp fed the 25% diet was \$60,382/ha, while the 30-40% diets ranged from \$67,368-\$71,247/ha. This pronounced difference in value is visualized in Figure 7, where the value is overlayed on top of total shrimp biomass. The value of the shrimp is a result of both total biomass and class size, and the value rises as shrimp fall into larger size classes. Since shrimp fed the 25% protein diet had both significantly lower individual weights and the total biomasses were lower compared to other treatments, this resulted in a lower economic value. To visualize distribution of size classes, Figure 3 shows each of these categories broken down. As the graph shows, the 25% diet had less shrimp in the 16-20 and 21-25 count per pound and many more in the 26-30 count per pound compared to the other treatments. Hence, to get both larger and higher value shrimp, a 30-40% protein diet would lead to the best outcomes based on the results of this study.

Differences in feed cost cannot be ignored when considering cost analysis. As the cost per unit of feed increased with dietary protein level, total feed inputs also showed the highest protein diet (40%) had a significantly higher cost compared to that of the lowest protein diet (25%). The 40%

diet may have resulted in the highest shrimp value, but once feed cost is considered, the margin between the 40, 35, and 30% diets narrows even further. After factoring in feed cost, the value of the shrimp or partial income for the 30-40% protein diets ranges from $53,698/ha to $55,287/ha. Like the shrimp value, the 25% diet still trails behind the others when feed cost is factored in ($47,823/ha). Taking into consideration feed cost suggests that a 40% diet is not worth the higher price since a diet containing 30 - 35% protein will yield nearly identical economic outcomes. Since cost is generally the driving factor of shrimp production, these results may have the most impact on the recommendation of dietary protein for production of Pacific white shrimp when employing an on demand feed management strategy.

In conclusion, a dietary protein level of 30-35% appears to be the most suitable for pond production of *L. vannamei* using on demand feeding under the culture conditions evaluated in this study. The shrimp fed a 40% protein diet had similar total biomass and value compared to all other dietary treatments, meaning its significantly higher cost and decreased water quality in ponds receiving this treatment was not mitigated by significantly increased production outcomes. Alternatively, even though the 25% protein diet didn’t produce a significantly lower total biomass, differences were pronounced enough, and the shrimp were significantly smaller, hence, the cost of a lower protein diet is not worth the sacrifice in productivity. Other results such as ANPR and class size distribution of shrimp also confirmed that diets containing 30-35% dietary protein will be the most logical for use in pond production of Pacific white shrimp with on demand feeding. Further investigation into optimal protein levels in feeds for production of *L. vannamei* in semi-intensive ponds with on-demand feeding should be explored, especially as it relates to diets containing protein within the range of 30-35%.

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TABLE 1. Formulation (g/100g) of four diets formulated to contain 25, 30, 35 and 40% protein which were commercially-extruded (Zeigler Bros, Inc. Gardners, Pennsylvania, USA) as a 2.4 mm sinking feed.

Diets	25:5	30:6	35:7	40:8
Soybean meal (47.5% Protein)	33.00	43.00	49.00	57.00
Whole Wheat	52.10	37.60	27.93	15.23
Poultry-By Meal (67% Protein)	2.00	4.00	6.00	8.00
Corn gluten meal (60% Protein)	2.00	4.00	6.00	8.00
Dicalcium phosphate	3.13	3.13	2.00	2.00
Fish oil	3.00	3.50	4.30	4.00
Squid meal	2.00	2.00	2.00	2.00
Bentonite	1.50	1.50	1.50	1.50
Lecithin	1.00	1.00	1.00	1.00
Vitamin Premix[a]	0.12	0.12	0.12	0.12
Mineral Premix[a]	0.12	0.12	0.12	0.12
Stable C (35% active)	0.02	0.02	0.02	0.02
Copper Sulfate	0.01	0.01	0.01	0.01
Crude Protein	26.55	31.99	32.77	40.57
Moisture	8.44	7.52	8.35	7.75
Crude Fat	6.10	7.54	8.53	8.72
Crude Fiber	2.61	3.12	3.00	2.84
Ash	7.45	7.93	7.3	7.65
Cu	101	95.4	102	114
P	1.26	1.25	1.11	1.2
Cost ($/kg)	1.52	1.58	1.66	1.74

[a] Vitamin and mineral premixes are proprietary products and therefore the composition is not listed.

TABLE 2. Proximate composition and amino acid profiles of the test diets. Analysis was performed by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). Results are expressed as g/100g as is.

Dietary Protein	25%	30%	35%	40%
Amino Acid				
Alanine	1.23	1.53	1.75	2.1
Arginine	1.67	2.06	2.28	2.7
Aspartic Acid	2.43	3.05	3.42	4.04
Cysteine	0.41	0.46	0.49	0.56
Glutamic Acid	5.11	5.91	6.48	7.4
Glycine	1.24	1.48	1.65	1.96
Histidine	0.64	0.77	0.86	1.01
Hydroxylysine	0.02	0.02	0.03	0.03
Hydroxyproline	0.23	0.25	0.27	0.28
Isoleucine	1.13	1.37	1.54	1.81
Lanthionine §	0.04	0.04	0.05	0.07
Leucine	2.07	2.57	2.95	3.47
Lysine	1.48	1.79	2	2.36
Methionine	0.43	0.5	0.57	0.66
Ornithine §	0.03	0.03	0.03	0.05
Phenylalanine	1.28	1.55	1.75	2.04
Proline	1.56	1.8	1.98	2.29
Serine	1.14	1.38	1.51	1.75
Taurine §	0.22	0.19	0.17	0.17
Threonine	0.96	1.17	1.32	1.55
Tryptophan	0.33	0.38	0.41	0.46
Tyrosine	0.94	1.17	1.31	1.54
Valine	1.23	1.47	1.65	1.96
Sum of AA	25.82	30.94	34.47	40.26

TABLE 3. Summary of water quality parameters during 12 weeks of pond production of Pacific white shrimp (*L. vannamei*) where juveniles (0.045 g, 25 shrimp/m^2) were stocked in 16 ponds (0.1 ha). Values are shown as the mean ± standard deviation and the minimum and maximum values below in parenthesis for dissolved oxygen (DO), salinity, total ammonia nitrogen (TAN), and temperature.

	25%	30%	35%	40%	Type III SS P-value
Morning DO[1] (mg/L)	4.09 ± 0.81^{a} (1.14, 7.47)	3.92 ± 0.87 ^{b} (0.73, 5.92)	3.88 ± 0.95 ^{b} (0.38, 7.4)	4.00 ± 0.88 ^{ab} (0.65, 7.01)	0.0007
Evening DO[1] (mg/L)	8.31 ± 2.31 (1.1, 13.5)	8.36 ± 2.35 (0.71, 17.23)	8.27 ± 2.48 (0.56, 16.26)	8.35 ± 2.49 (3.06, 19.54)	0.9480
Salinity (g/L)	11.01 ± 1.84 ^{a} (8.15, 14.19)	11.02 ± 1.80 ^{a} (7.29, 14.11)	10.99 ± 1.79 ^{a} (8.32, 14.05)	10.72 ± 1.81 ^{b} (7.22, 16.94)	0.0001
TAN[2]* (mg/L)	0.38 ± 0.87^{b} (0.002, 4)	0.39 ± 0.55^{b} (0.009, 2.06)	0.81 ± 0.99^{a} (0.01, 5)	1.07 ± 1.15^{a} (0.008, 4)	0.0001
Temperature (ºC)	31.04 ± 1.94 ^{ab} (25.6, 35.1)	31.01 ± 1.91^{ab} (25.6, 34.9)	31.17 ± 1.92 ^{a} (25.6, 35.5)	30.89 ± 1.88 ^{b} (25.5, 34.7)	0.0032
Secchi depth (cm)	36.8 ± 20.6	38.81 ± 20.6	34.58 ± 18.73	34.02 ± 18.9	0.4043
pH	7.07 ± 1.03	7.10 ± 0.44	7.11 ± 0.49	7.07 ± 0.34	0.9405
Nitrate*	1.00 ± 0.67^{b}	0.68 ± 0.67^{ab}	0.61 ± 0.83^{a}	0.61 ± 0.62^{a}	0.0120
Nitrite*	0.07 ± 0.19^{b}	0.05 ± 0.07^{b}	0.34 ± 0.52^{ab}	0.60 ± 0.99^{a}	0.0008
Phosphate*	1.21 ± 1.02	1.47 ± 1.10	1.32 ± 1.01	0.99 ± 0.96	0.4120
Calcium*	141.99 ± 39.13	141.36 ± 43.02	130.00 ± 54.81	138.64 ± 41.57	0.2464
Magnesium	384.79± 75.92	380.93 ± 72.50	370.61 ± 74.54	373.46 ± 71.71	0.2345
Alkalinity	44.14 ± 19.84	44.75 ± 11.31	46.39 ± 12.73	45.96 ± 18.83	0.5734

[1] DO: dissolved oxygen

[2] TAN: total ammonia nitrogen

* Indicates that there was a statistical interaction between date and treatment

TABLE 4. Production results for *L. vannamei* reared in 0.1 ha semi-intensive ponds over a 12-week culture period fed four diets containing 25-40% protein. Diets were extruded by Zeigler Bros Inc. (Gardners, PA, USA). Juvenile shrimp (0.045 g) were stocked at a density of 25 shrimp/m^2.

	25%[1]	30%[2]	35%[1]	40%[1]	*P*-value	PSE[3]
Growth (g/week)	2.49	2.72	2.68	2.73	0.27	0.074
Weight (g)	31.22^{b}	34.20^{a}	33.59^{a}	34.25^{a}	0.0001	14.24
Total Feed Fed (Kg/ha)	8,193	8,593	8,658	8,806	0.42	36.60
Total Protein Fed (kg/ha)	2115^{c}	2659^{b}	2984^{b}	3679^{a}	0.0001	11.86
Survival (%)	88.57	87.22	91.46	83.56	0.93	6.024
Yield (kg/ha)	7,037	7,492	7,704	7,878	0.78	57.91
FCR	1.18	1.17	1.13	1.17	0.99	0.0678
ANPR (%) [4]	66.1^{a}	57.2^{a}	54.8^{ab}	43.0^{b}	0.0025	0.028
Copper Retention (%)	19.0	19.9	21.3	19.5	0.66	1.40
Phosphorus Retention (%)	19.1	30.4	21.6	19.8	0.62	1.39
Electric Use (kwh/ha)	15,937	15,303	15,478	16,640	0.88	124.20
Electrical Cost ($)	2,231	2,142	2,167	2,330	0.88	17.38
Feed Cost						
$/kg shrimp	1.81	1.86	1.89	2.04	0.48	0.1052
$/ha	$12{,}559^{b}$	$13{,}670^{ab}$	$14{,}447^{ab}$	$15{,}960^{a}$	0.02	58.96
Shrimp value ($/ha)	60,382	67,368	69,141	71,247	0.5832	552.2
Partial Income ($/ha) [5]	45,746	50,863	51,708	51,798	0.79	4845.4

[1] n=3

[2] n=4

[3] PSE: Pooled Standard Error

[4] Apparent Net Protein Retention

[5] Shrimp value minus feed cost minus electrical cost

TABLE 5. Means of whole-body composition for *L. vannamei* grown in 0.1 ha ponds over a 12-week production cycle fed four protein variable diets (25-40%) extruded by Zeigler Bros Inc. (Gardners, PA, USA). Samples were analyzed by Midwest Laboratories (Omaha, NE, USA) and are all reported on a dry weight basis other than moisture.

Composition (dry weight basis)	25%	30%	35%	40%	*P*-value	PSE
Moisture %	5.82	6.16	5.53	6.12	0.288	0.499
Dry Matter %	94.18	94.47	93.84	93.88	0.288	0.499
Protein DW %	77.45	75.88	75.60	76.50	0.191	0.952
Fat DW %	8.13^{b}	9.57^{a}	10.60^{a}	10.48^{a}	0.0002	0.568
Fiber DW%	5.26	5.75	5.55	5.43	0.324	0.360
Ash DW %	10.80	11.08	10.58	10.70	0.598	0.395
Sulfur %	0.76	0.75	0.76	0.77	0.570	0.013
Phosphorus %	1.21	1.27	1.22	1.19	0.154	0.038
Potassium %	1.22	1.19	1.18	1.19	0.624	0.032
Magnesium %	0.27	0.28	0.28	0.27	0.328	0.009
Calcium %	2.31	2.41	2.32	2.3	0.832	0.174
Sodium %	0.72	0.68	0.70	0.71	0.472	0.030
Iron (ppm)	96.73	93.35	88.85	86.88	0.950	25.37
Manganese (ppm)	6.95	5.85	6.80	6.68	0.784	0.784
Copper (ppm)	112.75^{b}	119.50^{ab}	131.50^{a}	120.75^{ab}	0.018	6.512
Zinc (ppm)	74.28	64.43	71.05	69.15	0.228	3.912

FIGURE 1. Average weekly feed and total ammonia nitrogen (TAN) in 16 semi-intensive production ponds of pacific white shrimp over 12 weeks. Shrimp were fed four diets with different protein levels (25-40%) and juveniles (0.045 g) were stocked into the 0.1 ha ponds at 25 shrimp/m^2. The right side of the graph refers to the average feed per treatment (kg/ha/week) and corresponds with the line. The left side of the graph refers to the average TAN per treatments each week and corresponds with the bars.

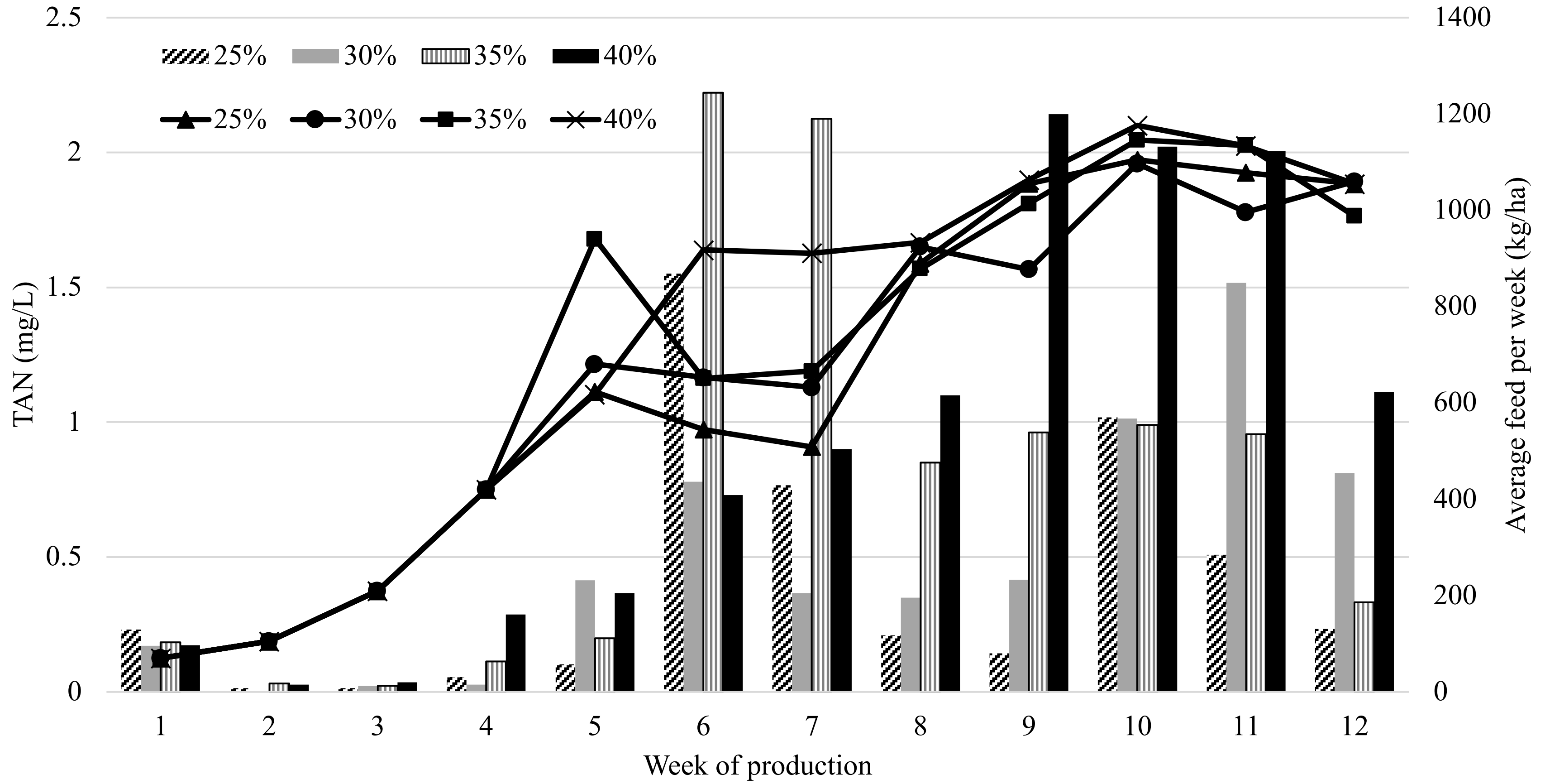

FIGURE 2. Growth of Pacific white shrimp reared in 16 ponds (0.1 ha) where juveniles (0.045 g) were stocked at 25 shrimp/m^2 and fed four protein variable diets (25-40%) over 12- week production cycle.

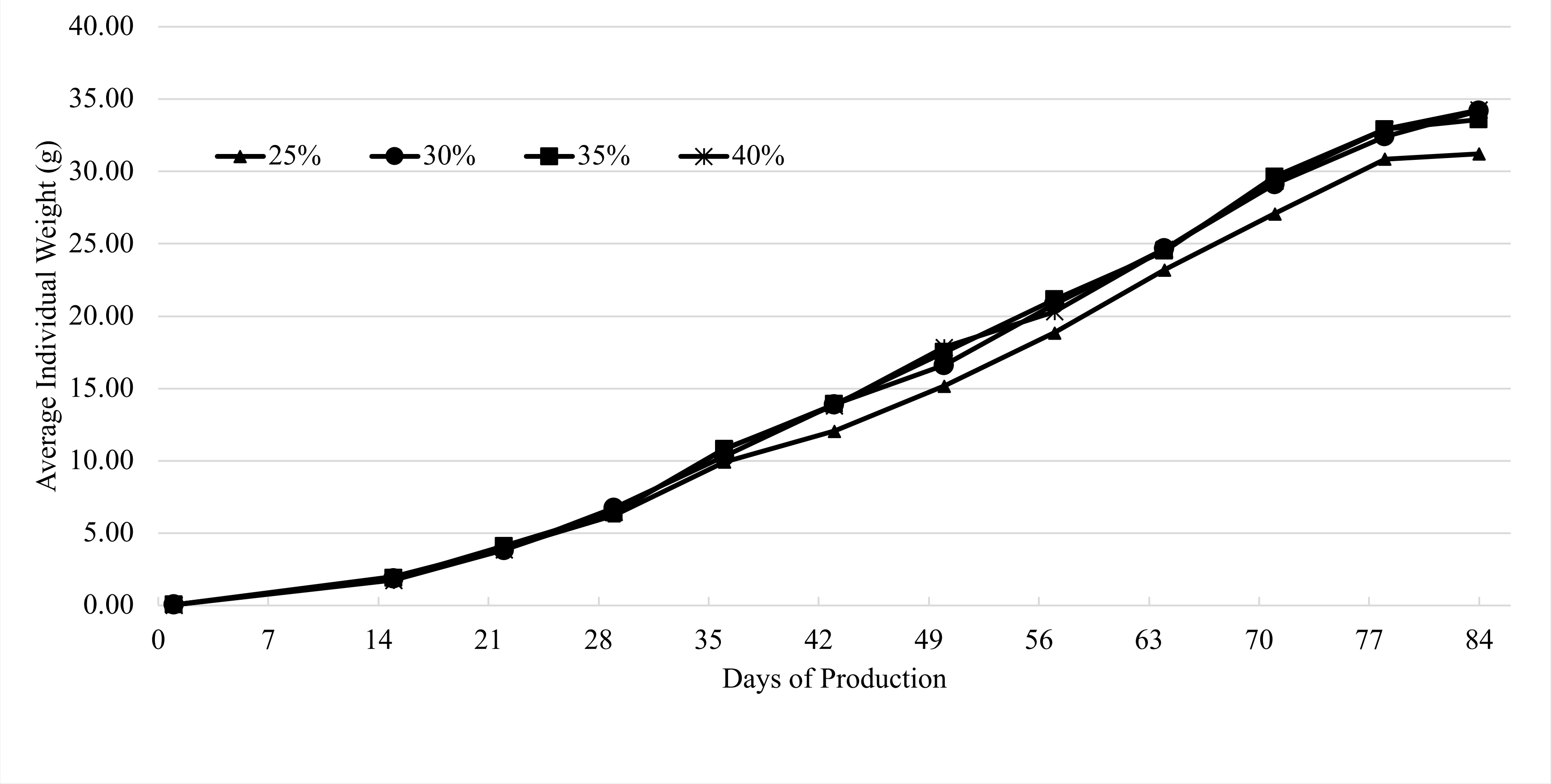

FIGURE 3: Break-down of percentages of each class size in total yields of Pacific white shrimp (head-off) after 12 -week production cycle in 16 ponds (0.1 ha) where juvenile shrimp (0.045 g) were stocked at 25 shrimp/m^2 and fed four protein variable diets (25-40%).

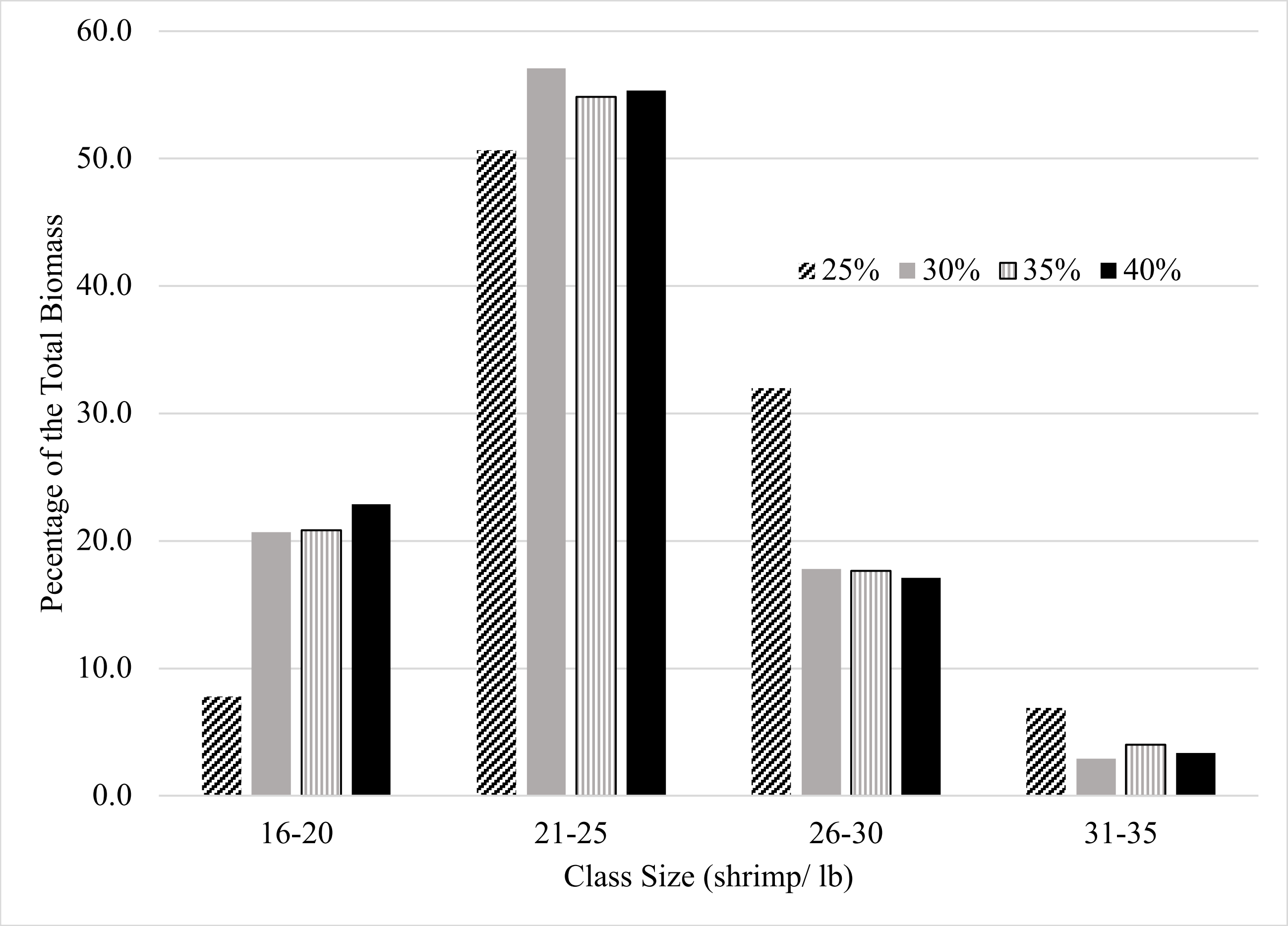

FIGURE 4. Average daily feed input by treatment of Pacific white shrimp (*L. vannamei*) where juveniles (0.045 g) were stocked in 16 ponds (0.1 ha) at 25 shrimp/m^2 and fed 4 protein variable diets (25-40%) over a 12-week production cycle.

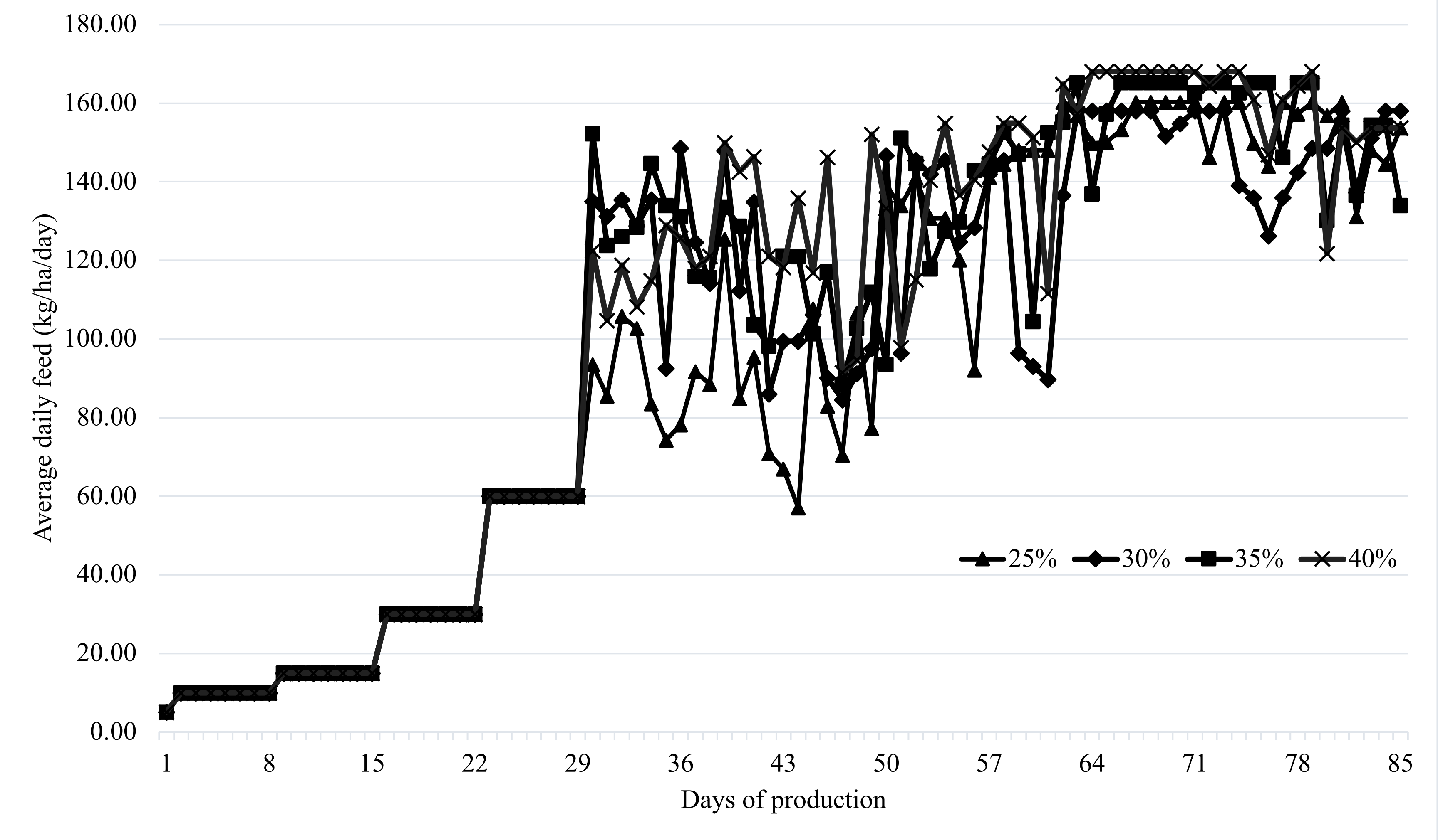

FIGURE 5. Average daily feed input each week by treatment of Pacific white shrimp (*L. vannamei*) where juveniles (0.045 g) were stocked in 16 ponds (0.1 ha) at 25 shrimp/m^2 and fed 4 protein variable diets (25-40%) over a 12-week production cycle.

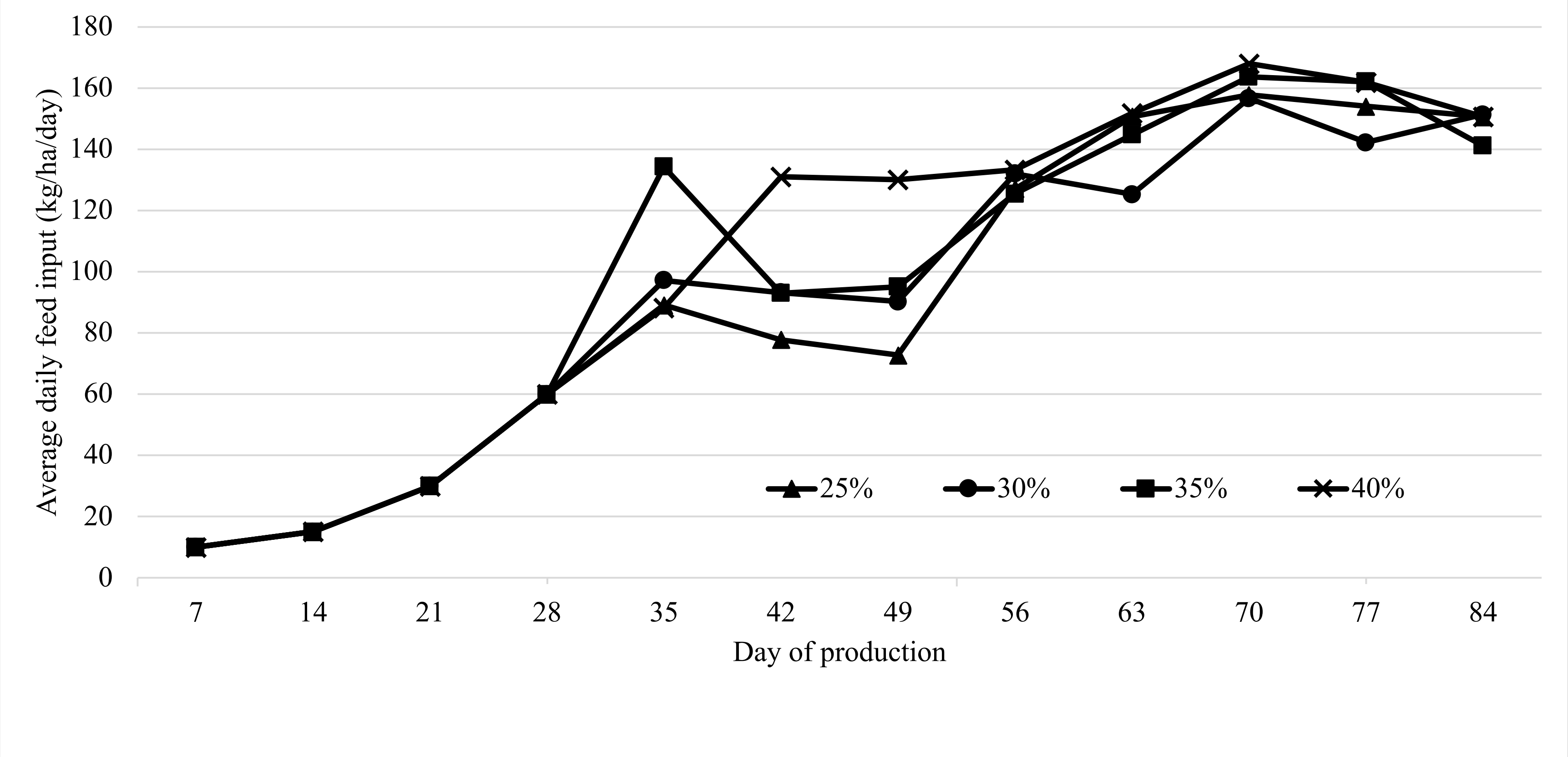

FIGURE 6. Two- week averages of feed applied to pond production of Pacific white shrimp (*L. vannamei*) fed four different protein diets (25-40%) using the AQ1 acoustic monitoring on demand feeding system. Juvenile shrimp (0.045 g) were stocked into 16 ponds (0.1 ha) at 25 shrimp/m^2

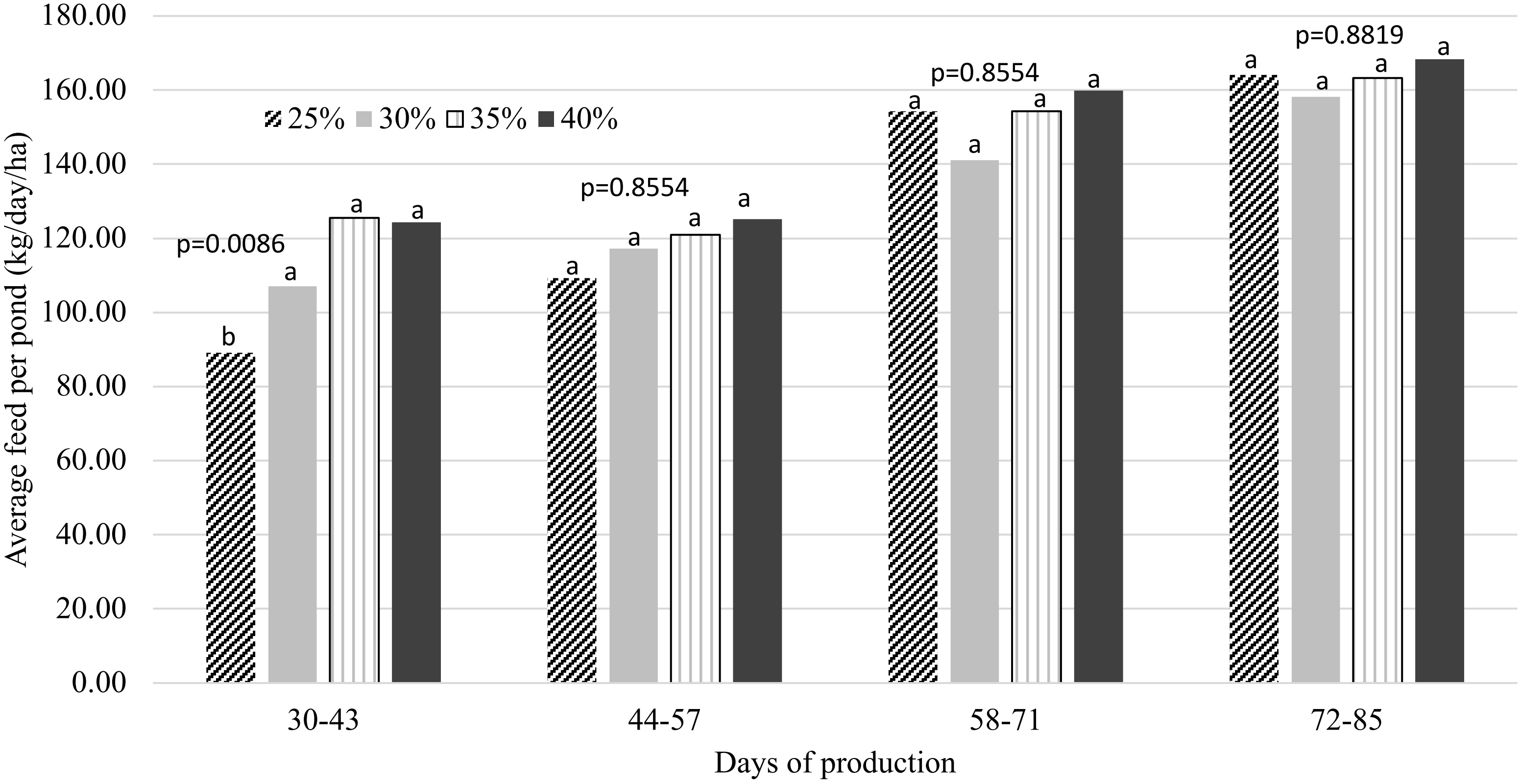

FIGURE 7. Total biomass and value of Pacific white shrimp after 12-week production cycle in 16 ponds (0.1 ha) where juvenile shrimp (0.045 g) were stocked at 25 shrimp/m^2 and fed four protein variable diets (25-40%).