## FINAL REPORT

**Project title:** Use of Animal By-Product meals in Zero Exchange Feeds for Shrimp

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**Goal & objective**: The goal and long term objective of this research project is to improve the economic viability and sustainability of marine shrimp culture operations within the U.S. and globally through the development of cost-effective fishmeal-free feeds using terrestrial animal by-product meals as the main protein source for the Pacific white shrimp *Litopenaeus vannamei*; total farmed shrimp production was reported as 3.275 million tonnes in 2007 and valued at over US $ 13.4 billion (FAO, 2009; http://www.fao.org).

**Project activity:** A 10-week feeding trial was conducted with white shrimp (*L. vannamei*) from juvenile to market size at the commercial shrimp diet testing facilities of a major aquaculture and animal feed manufacturer in Indonesia (PT. Luxindo Internusa: and current importer of US animal by-product meals).

**By-products tested:** *Poultry by-product meal - feed grade* (Fornazor International, Hillsdale, NJ)
Declared composition (Ampro Laboratories: 68.75% crude protein, 11.2% fat, 9.2% ash, 5.5% moisture, 2.3% fiber, 5.4% calcium); Analyzed composition (Luxindo Labs: 60.04% crude protein, 10.92% fat, 15.93% ash, 5.62% moisture); Dietary levels tested during this study: 15 to 30% ;

*Meat & bone meal – from pure beef* (Baker Commodities Inc., Vernon, CA)
Declared composition (51.04% crude protein, 11.99% fat, 27.64% ash, 2.82% moisture, 2.71% fiber, 9.12% calcium, 4.66% phosphorus); Analyzed composition (50.0% crude protein, 10.5% fat, 28.7% ash, 3.5% moisture); Dietary levels tested during this study: 0 to 5%

*Hydrolyzed feather meal* (Inno Resource, Carolina Byproduct, Winchester, VA)
Analyzed composition (83.7% crude protein, 5.3% fat, 1.20% ash); Dietary levels tested: 0 to 5% with and without supplemental limiting amino acids

*Blood meal - spray dried* (Jackson, New Zealand)
Analyzed composition (88.8% crude protein, 0.9% fat); Dietary levels tested during this study 0 to 2.5%

**Control protein:** *Peruvian fishmeal* (Austral)
Analyzed composition (65.0% crude protein, 7.4% fat, 15.0% ash); Dietary levels tested during this study 0 to 8%

**Methods used:** Experimental diets and formulations

13 experimental test diets were formulated, a control diet containing 8% fishmeal and 2% squid meal (successful cost-effective diet of the company), and 12 diets

containing various levels of fishmeal and marine protein/lipid replacement. All diets were formulated to contain 33-35% crude protein, 5.5-6.5% crude lipid, and 1.8 to 2.0% lysine, 0.75 to 0.81% methionine, and a minimum of 0.8% estimated available phosphorus (Table 1).

Table 1. Shrimp feed formulations and composition (values expressed on a percent as fed basis)

| Ingredient/diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Marine meals* | | | | | | | | | | | | | |
| Fishmeal | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Squid meal | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Krill meal | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 |
| Fish oil | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| *Animal meals* | | | | | | | | | | | | | |
| Poultry meal | 15 | 23 | 20 | 25 | 30 | 20 | 20 | 20 | 20 | 30 | 20 | 30 | 30 |
| M & B meal | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 10 | 0 | 0 |
| Blood meal | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.5 | 1 | 1.5 | 1 | 1 |
| Feather meal | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 2.5 | 0 | 0 |
| *Plant meals* | | | | | | | | | | | | | |
| Soybean meal | 16 | 16 | 25 | 20 | 9 | 20 | 14 | 15 | 20 | 10 | 10 | 10 | 10 |
| *Plant oils* | | | | | | | | | | | | | |
| Palm oil | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| *Amino acids* | | | | | | | | | | | | | |
| Methionine-HA | 0.25 | 0.25 | 0.22 | 0.19 | 0.16 | 0.22 | 0.25 | 0 | 0.22 | 0.22 | 0.25 | 0.22 | 0.22 |
| Lysine-HCL | 0.16 | 0.16 | 0 | 0 | 0 | 0 | 0.22 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ca/P* | | | | | | | | | | | | | |
| MDP 16/20 | 1.25 | 1.25 | 1.5 | 1 | 0.56 | 0.16 | 1.50 | 1.50 | 1.50 | 0.78 | 0 | 0 | 0 |
| *Vits/mins* | | | | | | | | | | | | | |
| Premix | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0 |
| Others[1] | 54 | 54 | 50 | 50.5 | 57 | 51.3 | 55.7 | 55.2 | 52.5 | 56.7 | 54.5 | 57.5 | 57.8 |
| Cost (Rp/kg)[2] | 6128 | 5936 | 5644 | 5779 | 5874 | 5733 | 5810 | 5611 | 5728 | 5671 | 5694 | 5652 | 5565 |
| <u>Composition</u> | | | | | | | | | | | | | |
| Moisture (%) | 10.7 | 9.7 | 9.7 | 9.5 | 9.1 | 9.1 | 9.2 | 9.1 | 9.2 | 9.3 | 9.5 | 9.5 | 9.7 |
| Crude protein | 35.5 | 35.6 | 33.6 | 33.6 | 34.3 | 35.1 | 35.3 | 34.5 | 34.5 | 34.8 | 34.1 | 35.4 | 35.7 |
| Crude fat | 6.0 | 7.4 | 6.6 | 6.6 | 6.8 | 6.6 | 5.9 | 6.0 | 5.7 | 5.5 | 6.4 | 6.9 | 6.6 |
| Crude fiber | 3.1 | 3.0 | 3.1 | 3.1 | 3.2 | 2.9 | 3.0 | 3.0 | 2.9 | 3.1 | 2.8 | 2.9 | 2.8 |
| Ash | 8.5 | 10.1 | 9.2 | 9.4 | 9.1 | 9.0 | 8.7 | 8.5 | 8.4 | 8.5 | 9.0 | 9.0 | 9.0 |
| Calcium | 1.8 | 2.8 | 2.1 | 2.5 | 2.2 | 2.1 | 2.0 | 2.0 | 1.8 | 2.1 | 2.3 | 2.4 | 2.4 |

[1]Others includes wheat flour and byproducts, rice bran, yeast, soy lecithin, anti-mold agents, antioxidants

[2]Raw material costs in Indonesian Rupiah per kg (June 2009)

Feed manufacture

All 13 experimental diets were produced at PT Luxindo Internusa in Jakarta (Indonesia) using a commercial shrimp feed mill (IDAH 53SA with triple conditioners), with 1,600 kg of each diet produced and observations taken during the production process so as to ascertain the effect of ingredient use on energy usage, ease of production, physical characteristics, water stability and handling (if any).

Shrimp and experimental culture conditions

Juvenile Pacific white shrimp (*L. vannamei*), of the same strain and size, were obtained from a local shrimp hatchery and stocked within 48 round black-coated fiberglass microcosm tanks ($1m^3$ water volume, with a conical bottom) at an initial stocking density of 75 shrimp $tank^{-1}$ (equivalent to a shrimp density of 75 $m^{-3}$ water volume), with three tanks allotted per dietary treatment. The tanks were situated within an outdoor plastic lined greenhouse structure as shown in Figure 1 and 2.

Figure 1. Experimental diet testing building – Global Innovation & Solutions Unit (GISU)


Figure 2. Experimental tank system used for conducting shrimp feeding trial

Water within the microcosm tanks was continuously mixed and aerated using a diaphragm air diffuser placed at the bottom of the tank (so as to keep all particulate matter in suspension) and a zero-water-exchange `green water' management system operated within the tanks for the duration of the 70-day culture trial. Air was continuously supplied to all experimental tanks using a 2HP air blower and freshwater added to tanks as required so as to replace evaporative losses.

In addition to the above mentioned 13 dietary treatments operated on a zero-water exchange management system, a control running water treatment was also implemented using diet 2 (Table 1).

Diurnal water temperature, dissolved oxygen, pH and salinity measurements were made within the experimental tanks throughout the study, together with an estimate of the quantity of suspended microbial `floc' present within the water column of the experimental tanks using a volumetric sedimentation column.

Feeding regime

Experimental shrimp were fed at regular 3h intervals over a 24-h period (8 feedings per day) by hand application using a feeding tube so that feed enters 10cm below the water surface. Shrimp were fed on a fixed daily feeding regime based on the average estimated body weight as described below:

| Shrimp body weight (grams, g) | Daily feeding rate (% bw/day) |
|---|---|
| 1 < 2 | 8.00 |
| 2 < 3 | 7.50 |
| 3 < 4 | 7.00 |
| 4 < 5 | 6.50 |
| 5 < 6 | 6.00 |
| 6 < 7 | 5.50 |
| 7 < 8 | 5.25 |
| 8 < 9 | 5.00 |
| 9 < 10 | 4.50 |
| 10 < 11 | 4.25 |
| 11 < 12 | 4.00 |
| 12 < 13 | 3.75 |
| 13 < 14 | 3.50 |
| 14 < 15 | 3.25 |
| 15 < 16 | 3.00 |
| 16 < 17 | 3.00 |
| 17 < 18 | 2.75 |
| 18 < 19 | 2.75 |
| 19 < 20 | 2.50 |
| 20 < 21 | 2.50 |

Feeding rates were adjusted on a weekly basis based on the results obtained during each major 2-week weighing and intermittent week sample weighing.

Shrimp weighing

All experimental animals were weighed individually at the start and end of the 70-day feeding trial, and by group weighing at bi-weekly intervals so as to determine average body weight and estimate shrimp survival.

Sample collections

Representative samples of shrimp were collected at the start and end of the 70-day feeding trial, and stored by freezing at $-20^{o}$C for subsequent proximate chemical analysis (if time allows).

Statistical treatment of results

Data obtained from the experiments, which had a completely randomized design with 3 replicates per treatment, were analyzed by one-way analysis of variance (ANOVA) to determine if significant differences existed among treatment means. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). Differences were considered significant at the 5% level of probability.

Start and end of the feeding trial

The feeding trial commenced on July 3, 2009 and ended on September 12, 2009.

**Results of trial:**

Water quality

Water quality within the experimental tanks as determined at 8.00 am and 4.00 pm over the course of the 10-week experiment varied as follows:

*Zero-exchange tanks (treatments 1 to 13)*

Water temperature: range 27.9 to 33.9°C, mean 29.2 °C (am) to 31.6 °C (pm)
Oxygen: range 5.04 to 6.89 mg/l, mean 5.90 mg/l (am) to 5.64 mg/l (pm)
Salinity: range 32 to 36 ppt, mean 34 ppt
pH: range 7.2 to 8.2, mean 7.7
Alkalinity: range 87 to 144 mg/l as $CaCO_3$, mean 116 mg/l
Total ammonia nitrogen (TAN): range 0 to 1 mg/l, mean 0.20 mg/l
Suspended solids (floc): 1.50 to 4.83 ml, mean 3.45 ml (Figure 3)

*Running water tank (treatment 14)*

Water temperature: range 26.0 to 30.3°C, mean 27.6°C (am) to 29.2°C (pm)
Oxygen: range 5.61 to 6.97 mg/l, mean 6.32 mg/l (am) to 5.96 mg/l (pm)
Salinity: range 30 to 35 ppt, mean 33 ppt
pH: range 7.4 to 8.2, mean 7.9
Alkalinity: range 81 to 140 mg/l as $CaCO_3$, mean 114 mg/l
Total ammonia nitrogen (TAN): range 0 to 1 mg/l, mean 0.13 mg/l

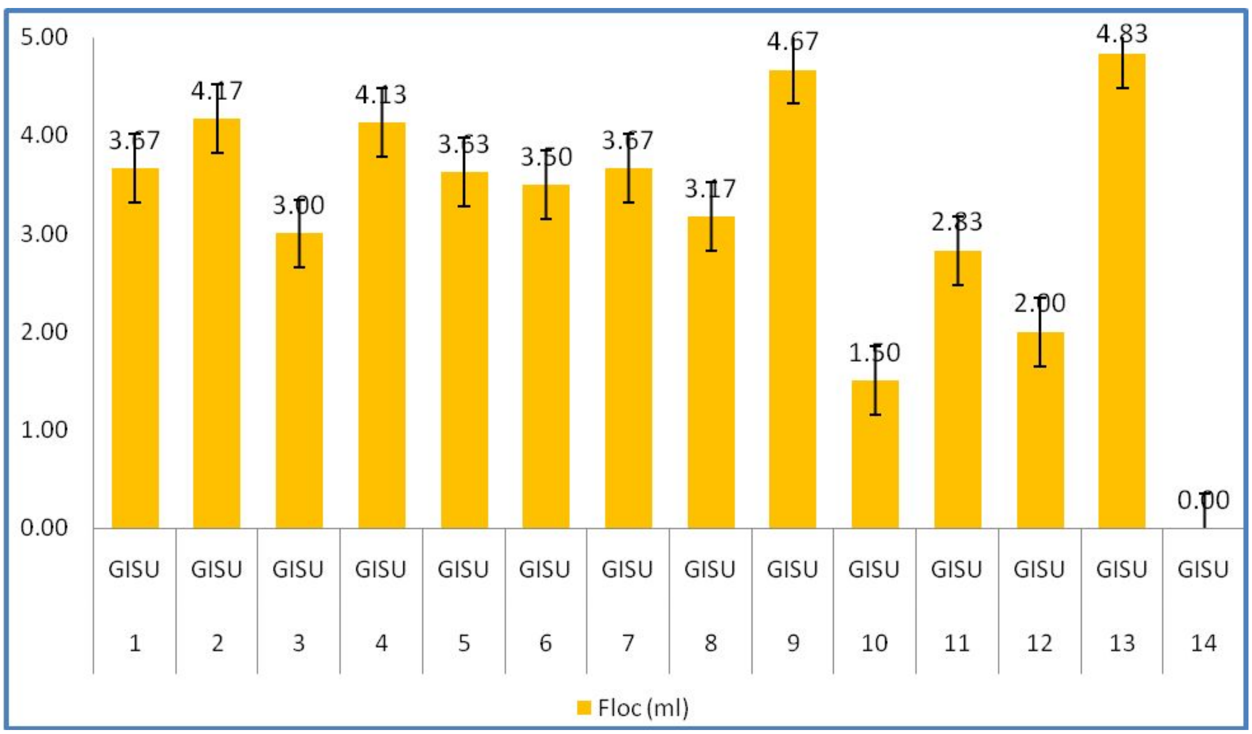

Figure 3. Mean suspended solids level (floc) within experimental tanks over the experimental period

<u>Shrimp growth and performance</u>

The growth performance of shrimp fed the experimental test diets is shown in Table 2 and Figure 4 to 7.

Table 2. Growth performance of shrimp fed the experimental diets over a 10-week feeding period

| Dietary Treatment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | SEM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Shrimp weight*** | | | | | | | | | | | | | | | |
| Mean initial body weight (gr)* | 2.75$^{b}$ | 2.70$^{b}$ | 2.70$^{b}$ | 2.65$^{b}$ | 2.49$^{b}$ | 2.68$^{b}$ | 2.61$^{b}$ | 2.83$^{b}$ | 2.50$^{b}$ | 2.65$^{b}$ | 2.72$^{b}$ | 2.70$^{b}$ | 2.73$^{b}$ | 1.98$^{a}$ | 0.04 |
| Mean final body weight (gr)** | 18.93$^{f}$ | 18.06$^{ef}$ | 17.80$^{ef}$ | 17.68$^{ef}$ | 17.33$^{de}$ | 15.97$^{bcd}$ | 17.78$^{ef}$ | 16.78$^{cde}$ | 17.90$^{ef}$ | 16.73$^{cde}$ | 15.59$^{bc}$ | 14.87$^{b}$ | 15.58$^{bc}$ | 11.16$^{a}$ | 0.31 |
| ***Shrimp growth response*** | | | | | | | | | | | | | | | |
| Mean daily weight gain (gr/d)** | 0.22$^{g}$ | 0.21$^{efg}$ | 0.21$^{efg}$ | 0.21$^{efg}$ | 0.21$^{defg}$ | 0.19$^{bcd}$ | 0.21$^{defg}$ | 0.19$^{cde}$ | 0.22$^{fg}$ | 0.20$^{cdef}$ | 0.18$^{bc}$ | 0.17$^{b}$ | 0.18$^{bc}$ | 0.13$^{a}$ | 0.003 |
| Mean weekly weight gain (gr/w)** | 1.57$^{e}$ | 1.49$^{de}$ | 1.47$^{de}$ | 1.46$^{de}$ | 1.44$^{de}$ | 1.29$^{bc}$ | 1.48$^{de}$ | 1.36$^{cd}$ | 1.50$^{de}$ | 1.37$^{cd}$ | 1.25$^{bc}$ | 1.18$^{b}$ | 1.25$^{bc}$ | 0.89$^{a}$ | 0.03 |
| Feed Conversion Ratio** | 1.67$^{a}$ | 1.87$^{ab}$ | 1.90$^{ab}$ | 1.67$^{a}$ | 1.87$^{ab}$ | 1.95$^{ab}$ | 1.88$^{ab}$ | 1.85$^{ab}$ | 1.65$^{a}$ | 1.85$^{ab}$ | 2.20$^{bc}$ | 2.52$^{bc}$ | 1.99$^{ab}$ | 2.41$^{bc}$ | 0.05 |
| ***Shrimp feed utilization*** | | | | | | | | | | | | | | | |
| Apparent feed efficiency (%)** | 60.1$^{de}$ | 53.6$^{cde}$ | 52.8$^{cde}$ | 59.6$^{de}$ | 53.7$^{cde}$ | 51.4$^{cde}$ | 54.6$^{cde}$ | 54.8$^{cde}$ | 60.9$^{e}$ | 54.7$^{cde}$ | 46.3$^{abc}$ | 39.7$^{a}$ | 50.4$^{bcd}$ | 41.7$^{ab}$ | 1.15 |
| ***Total shrimp production*** | | | | | | | | | | | | | | | |
| Total initial shrimp biomass (gr)* | 206.50$^{b}$ | 202.25$^{b}$ | 202.75$^{b}$ | 199.00$^{b}$ | 187.00$^{b}$ | 201.00$^{b}$ | 195.50$^{b}$ | 212.50$^{b}$ | 187.75$^{b}$ | 199.00$^{b}$ | 203.75$^{b}$ | 202.25$^{b}$ | 204.75$^{b}$ | 148.75$^{a}$ | 3.38 |
| Total final shrimp biomass (gr)** | 1205.00$^{e}$ | 1089.00$^{bcde}$ | 1037.00$^{bcd}$ | 1173.00$^{de}$ | 1051.70$^{bcde}$ | 1001.70$^{bc}$ | 1007.00$^{bcde}$ | 1075.70$^{bcde}$ | 1163.30$^{cde}$ | 1041.30$^{bcde}$ | 926.67$^{b}$ | 768.67$^{a}$ | 956.00$^{b}$ | 689.00$^{a}$ | 24.48 |
| Total biomass increase(gr)** | 998.50$^{c}$ | 886.75$^{bc}$ | 834.25$^{bc}$ | 974.00$^{c}$ | 864.67$^{bc}$ | 800.67$^{b}$ | 874.50$^{bc}$ | 863.17$^{bc}$ | 975.58$^{c}$ | 842.33$^{bc}$ | 722.92$^{b}$ | 566.42$^{a}$ | 751.25$^{b}$ | 540.25$^{a}$ | 23.67 |
| Total feed offered (gr)* | 1663.30$^{d}$ | 1656.70$^{d}$ | 1585.00$^{cd}$ | 1633.30$^{cd}$ | 1613.30$^{cd}$ | 1556.70$^{bcd}$ | 1610.00$^{cd}$ | 1570.00$^{bcd}$ | 1603.30$^{cd}$ | 1538.30$^{bcd}$ | 1555.00$^{bcd}$ | 1425.00$^{b}$ | 1491.70$^{cd}$ | 1291.70$^{a}$ | |
| Survival Rate (%)** | 84.9$^{b}$ | 80.4$^{b}$ | 77.8$^{a}$ | 88.4$^{b}$ | 80.9$^{b}$ | 83.6$^{b}$ | 80.4$^{b}$ | 85.3$^{b}$ | 86.7$^{b}$ | 83.1$^{b}$ | 81.8$^{b}$ | 68.9$^{a}$ | 81.8$^{b}$ | 83.1$^{b}$ | 0.97 |

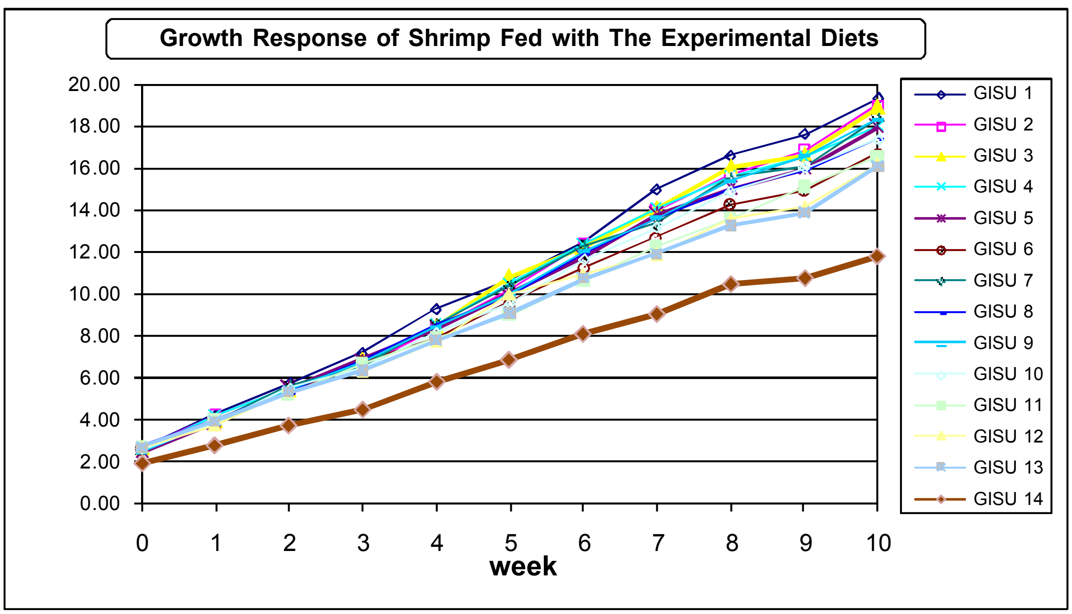


Figure 4. Mean weekly growth response of experimental shrimp fed experimental diets

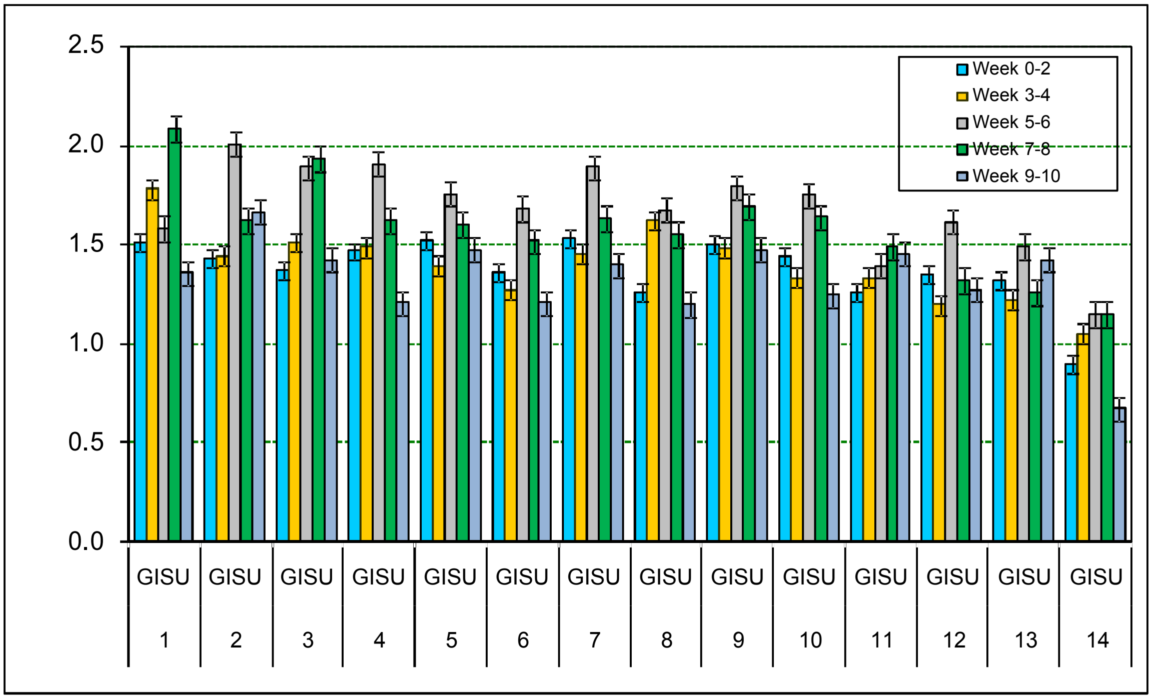


Figure 5. Mean biweekly growth response of experimental shrimp fed experimental diets

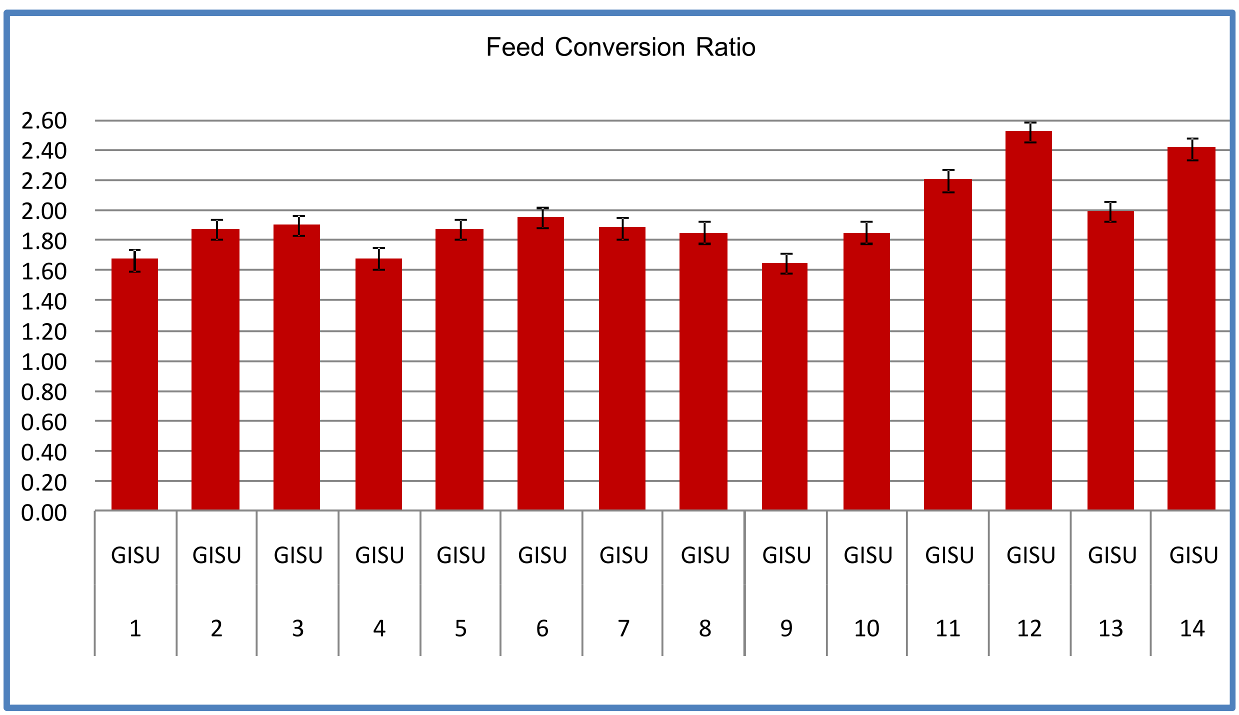


Figure 6. Final feed conversion ratio observed for shrimp fed the experimental diets after 10-weeks

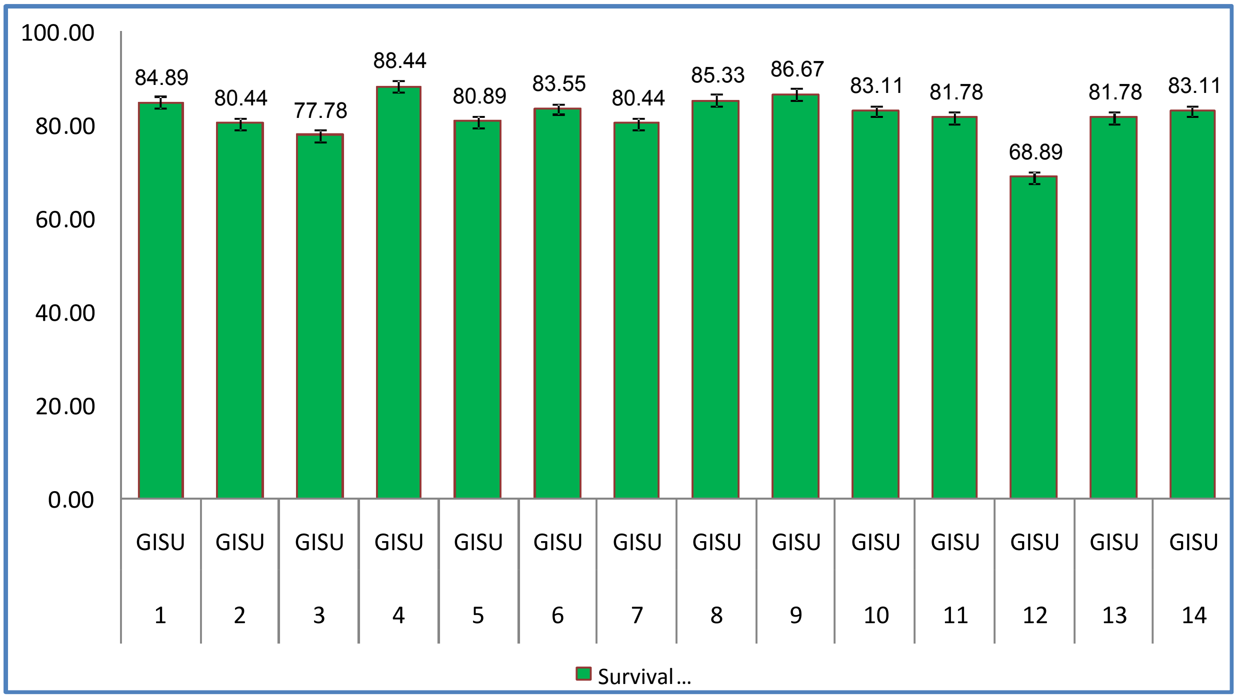


Figure 7. Final survival observed for shrimp fed the experimental diets after 10-weeks

Excellent shrimp growth and survival was observed over the course of the feeding trial, with animals reaching a final body weight of 17.6 to 18.9g (commercial size), with no significant difference observed between treatments 1, 2, 3, 4, 7 and 9 in terms of final body weight and treatments 1 through 10, and 13 (Table 2). Moreover, the best overall food conversion ratios were observed for treatments 1, 4 and 9, respectively; overall trial FCRs ranging from 1.65 (treatment 9) to 1.67 (treatment 1 and 4).

Economic performance

From Table 1 it can be seen that the most expensive diet in terms of raw material cost was the control fishmeal containing diet (treatment 1; 6128 Rp/kg), with the best equally performing diets being 7.9% cheaper (treatment 3; 5644 Rp/kg), 6.5% cheaper (treatment 9; 5728 Rp/kg), 5.7% cheaper (treatment 4; 5779 Rp/kg), 5.2% cheaper (treatment 7; 5810 Rp/kg) and 3.1% cheaper (treatment 2; 5936 Rp/kg).

**Discussion of results:** The results clearly show the nutritional and economic efficacy of totally replacing fishmeal and squid meal within commercial shrimp feeds by using:

Feed grade poultry byproduct meal (60/10.9/15.9/5.6 CP/EE/Ash/Moisture): up to a dietary inclusion level of between 20 and 25% of the total diet (treatment 3 and 4), with supplemental methionine, and increasing dietary soybean levels from 16% to between 20 and 25%, with ingredient cost savings of between 5.7 and 7.9% compared with respect to a similar diet containing 8% fishmeal and 2% squid meal.

Hydrolyzed feather meal (83.7/5.3/1.2 CP/EE/Ash): up to a dietary inclusion level of 5% (treatment 7) of the total diet, with supplemental lysine and methionine, and decreasing dietary soybean levels from 16% to 14% , with ingredient cost savings of 5.2% compared with respect to a similar diet containing 8% fishmeal and 2% squid meal. Surprisingly, shrimp fed the same diet with no supplemental amino acids (treatment 8) showed no significant differences in final body weight or feed efficiency with animal fed the supplemented diet, with consequent ingredient cost savings of 8.4% compared with a diet containing 8% fishmeal and 2% squid meal.

Spray dried blood meal (88.8/0.9 CP/EE): up to a dietary inclusion level of 2.5% of the total diet (treatment 9), with supplemental methionne, and increasing dietary soybean levels from 16% to 20%, with ingredient cost savings of 6.5% compared with respect to a similar diet containing 8% fishmeal and 2% squid meal.

Meat & bone meal from pure beef (50/10.5/28.7/3.5): results with 5% meat and bone meal (treatment 6) were disappointing, with shrimp displaying significantly lower final body weight ($P<0.05$) compared and poorer FCRs compared with the other treatments.

The results obtained with rations devoid of all marine protein and lipid sources (treatment 10 to 13) were disappointing, with the best results obtained with the diet containing 30% poultry byproduct meal and 1% blood meal with supplemental lysine; the other treatments displayed significantly reduced shrimp growth and feed efficiency compared the control diet (Table 2). Surprisingly, there was no significant difference between shrimp fed diet 12 and 13; the former containing a complete vitamin and trace mineral premix and the latter containing no vitamin or mineral premix. It is also important to note that treatment 13 also recorded the highest floc concentration within experimental tanks (Figure 3).

Finally, as expected, shrimp reared in running water (treatment 14) displayed the worst growth response and feed efficiency compared with shrimp fed the same diet under zero-water exchange conditions (diet/treatment 2). However, it is also important to note that the water temperature within the clear running water tanks were at least 2 degrees lower than tanks with a zero-water exchange management system.

**Recommendations:**

1. That the results of this study be published in a international aquaculture trade journal such as the Global Aquaculture Advocate or International Aquafeed;

2. That the study be repeated within earthen ponds under commercial shrimp farming conditions in Indonesia/Sulawesi (8,000-10,000 m ponds, stocking density 80/$m^2$) and or Mexico/Hermosillo (1,000 m ponds, stocking density 20/$m^2$);

3. That a similar feeding study be conducted concerning the use of rendered products within shrimp broodstock feeds, maturations feeds, and larval feeds, and by so doing closing the production cycle for the Pacific white shrimp;

4, To establish a routine laboratory protocol for routinely determining the apparent nutrient digestibility of rendered animal byproduct meals *in vivo* under conditions of maximal attainable growth in juvenile shrimp; and

5. To establish a interactive national database of rendered anpmal byproduct suppliers within North America, including standardized product specification sheets and relevant published scientific papers and supporting documentation.