Effect of bioethanol waste flour on feed for the growth and blood profile of vannamei shrimp (*Penaeus vannamei*)

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Abstract. With the increasing productivity of vannamei shrimp (*Penaeus vannamei*) and the high prices of essential feed ingredients, alternative ingredients that are cheap and available in large quantities are needed. Waste flour from making bioethanol is a siding material, but has good nutritional value: 32% protein, 11% fiber, 9% fat, vitamins, and minerals. This research evaluates waste flour from bioethanol (DDGS) as a fish and soybean meal substitute to optimize shrimp growth and health. The research consisted of treatments A (commercial feed), B (5% bioethanol waste flour in feed), C (10% bioethanol waste flour in feed), and D (15% bioethanol waste flour in feed). Feeding was carried out 4 times per day. The vannamei shrimp used had a weight of 1 g. The growth was carried out in hapa nets, with a density of 120 individuals m^{-3}. The research results showed that it was necessary to add 10-15% bioethanol production waste flour to the feed to obtain a specific growth rate, absolute length, and total hemocytes of 3.79-3.82%, 6.84-6.86 cm, and $1.066\text{-}2.188 \times 10^7$ cells mL^{-1}, respectively. The current study offers valuable information regarding some aspects of shrimp quality after feeding with DDGS to partially replace the inclusion of soybean meal.
Key Words: distiller's dried grains with solubles, growth, health, soybean meal.

Introduction. Vannamei shrimp (*Penaeus vannamei*) is one of the leading business commodities in aquaculture. This is due to the large number of requests from Indonesia and abroad. This large number of requests makes many people compete in vannamei shrimp cultivation activities, and the production of vannamei shrimp in Indonesia continues to increase throughout the year. The growth in farmed shrimp, including vannamei shrimp, is primarily driven by increasing worldwide demand due to population growth and rising incomes, while wild supply remains stagnant (Asmild et al 2024). Proper management practices are essential for successful vannamei shrimp cultivation to ensure economic viability and sustainability. An efficient Pacific white shrimp production system requires cost-effective diets that meet particular dietary requirements to enhance growth, health, and profitability throughout the production cycle (Lim & Akiyama 1995; NRC 2011; Sookying et al 2013; Molina-Poveda 2016; Ayisi et al 2017).

The feed cost is a significant factor affecting the economics of shrimp production. In Indonesia, the feed cost for vannamei shrimp can range from 0.84 to 0.9 USD per kg. The high cost of imported shrimp feed in Indonesia can impact the profitability of shrimp farming (Rubel et al 2019). Highly nutritious feed is essential to ensure good growth and high survival rates in the early stages of the life cycle. However, the high cost of imported feeds and the limited availability of locally produced high-quality feeds can hinder optimal growth and productivity in shrimp farming (FAO 2024).

The challenges in making feed are finding alternative ingredients that are cheap and available in large quantities, but still meet the nutritional needs of vannamei shrimp. Corn is a significant source of feed energy widely used in Asia and America, with a portion of it being utilized for ethanol production. The high ethanol production from corn results in valuable by-products that can be used as animal feed. The ethanol industry not only produces fuel, but also generates various feed products that contribute to feeding livestock and poultry. These co-products, such as distiller grains and corn gluten meal, provide essential nutrients for animals (U.S. Grains Council 2012). Diogenes et al (2018) note that ethanol production waste is widely used in animal feed. For fish feed, it has only been tested on several fish species, such as rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), and European seabass (*Dicentrarchus labrax*). Bharathi et al (2020) added that, unlike soybean meal, bioethanol production waste does not have anti-nutrients, making it an excellent alternative ingredient to replace soybean meal and fish meal. Waste flour from bioethanol (DDGS) has garnered considerable attention in the shrimp industry as a feed additive due to its nutritional profile and cost-effectiveness (Rhodes et al 2015; Gyan et al 2022; Novriadi et al 2022). Therefore, the present study aims to evaluate DDGS as a substitute for fish and soybean meals to optimize shrimp growth and health.

Material and Method. This research was conducted at Marine Science Techno Park, Diponegoro University, Jepara, Central Java, Indonesia in 2022.

Experimental diets. The treatments in this research were A (without adding DDGS to the feed composition), B (5% DDGS to the feed composition), C (10% DDGS to the feed composition), D (15% DDGS in feed composition). The formulation was based on Novriadi et al (2023). The composition of the feed used in the research can be seen in Table 1.

Table 1

Composition of diets

Ingredients (%)	*Diet*			
	A	*B*	*C*	*D*
Soybean meal[a]	25.0	22.5	20.0	17.5
Poultry by-product meal[b]	20.3	20.3	20.3	20.3
Fish meal[c]	8.0	8.0	8.0	8.0
Corn DDGS[d]	0.0	5.0	10.0	15.0
Tuna hydrolysate[e]	2.0	2.0	2.0	2.0
Squid liver powder[f]	6.0	6.0	6.0	6.0
Wheat flour[g]	31.85	29.32	26.78	24.22
Soya lecithin[h]	1.5	1.5	1.5	1.5
Fish oil[i]	1.0	1.0	1.0	1.0
MCP[j]	1.8	1.8	1.8	1.8
L-lysine HCl[k]	0.00	0.04	0.09	0.14
DL-methionine[l]	0.19	0.18	0.17	0.17
L-threonine[k]	0.08	0.08	0.08	0.09
Mineral premix[m]	1.20	1.20	1.20	1.20
Vitamin premix[m]	0.41	0.41	0.41	0.41
Magnesium sulphate[n]	0.35	0.35	0.35	0.35
Choline chloride[k]	0.20	0.20	0.20	0.20
Anti-mold[o]	0.12	0.12	0.12	0.12

Note: [a]Dehulled Solvent Extracted Soybean Meal, Argentina; [b]Griffin Industries, USA; [c]Foodcorp, S.A., Chile; [d]US Grains Council, USA; [e]SPF Diana, Thailand; [f]Hana Industrial Co.Ltd, South Korea; [g]PT Agristar Grain, Indonesia; [h]Shandong Maowei International Trade Co. Ltd., China; [i]Pasquera La Portada S.A., Chile; [j]Sinochem YunLong, Co.Ltd, China; [k]PT Dian Cipta Perkasa, Indonesia; [l]Evonik Nutrition & Care GmbH, Hanau, Germany; [m]PT DSM Nutritional Products, Indonesia; [n]PT Jannisika Sumber Jaya, Indonesia; [o]PT Tienyen International, Indonesia; DDGS - waste flour from bioethanol.

Preparation of the media. 4 ponds were used, measuring 25x10x1.8 m. Prior to the experiment, the pond was sterilized and maintenance work was carried out. 16 hapa nets measuring 2x3x1 m with a mesh size of 0.5x0.5 cm were used. The hapa nets were placed in the pond and tied to support poles. The pond was filled with water to a height of 1.8 m, to anticipate the decrease in water during and after treatment. The water was sterilized using 30 ppm chlorine. Chlorine will be neutral after aeration for 30 hours, followed by 15 ppm saponification. Every pond had one waterwheel for oxygenation.

Preparation of shrimp. Vannamei shrimp measuring 1 g were obtained from P.T. Riz Samudera (Jepara, Central Java, Indonesia). White vannamei shrimp were kept in hapa nets at a density of 120 individuals m^{-3} per hapa net.

Feeding. Feeding was done four times daily, at 07.00, 11.00, 14.00, and 17.00 WIB. This research was conducted for 60 days.

Specific growth rate. Specific growth rate (SGR) was calculated using the following formula (Martini 2017):

$$SGR = [(\ln W_t - \ln W_0)/t] \times 100$$

Where: SGR - specific growth rate (% per day); W_t - final weight of fish (g); W_0 - initial weight of fish (g); t - maintenance time (days).

Absolute length growth. Absolute length growth was determined using the following formula (Zulfahmi et al 2018):

$$Pm = Pt - P0$$

Where: Pm - absolute length growth; Pt - average length of individuals at the end of rearing; P0 - average length of individuals at the beginning of rearing.

Total hemocytes. According to Satyantini et al (2016), the shrimp hemolymph can be collected from the base of the pleopod in the abdominal segment near the genital opening, using a 1 mL syringe moistened with anticoagulant solution. 3 shrimps were sampled from each treatment. This is also the procedure used in this paper. The total number of hemocytes was counted using a hemocytometer. Calculation of total hemocytes was carried out using the following formula (Jannah et al 2018):

$$THC = (\text{The number of cells counted/Number of fields of view}) \times 10^7 \times DF$$

Where: THC - total hemocyte count; DF - dilution factor.

Water quality. Water quality measured includes DO, pH, temperature, and salinity. The water quality meters used are a pH tester and a DO meter. Water quality observations were conducted every day, twice in the morning and evening at each pool.

Data analysis. Observational data were analyzed using analysis of variance (ANOVA) to determine the effect of the treatments on the observed parameters. If a difference is observed, the Duncan test is further used to determine between which treatments the differences are significant.

Results. The results of SGR for vannamei shrimp are presented in Figure 1. Based on Figure 1, the SGR of vannamei shrimp from treatment A was not different from the SGR of vannamei shrimp from treatment B. However, the SGR of vannamei shrimp from treatment A was significantly different from vannamei shrimp from treatments C and D. Treatment D produced the highest SGR among treatments (3.82±0.08%).

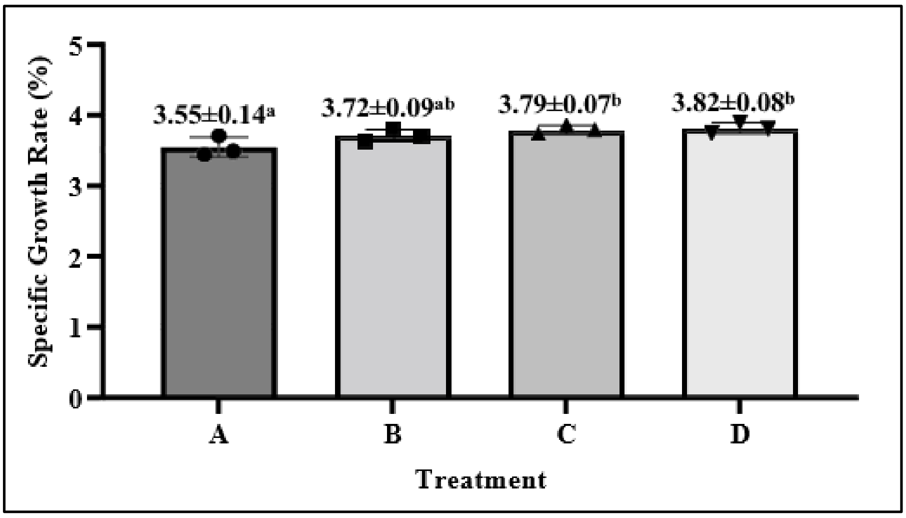

Figure 1. The specific growth rate of *Penaeus vannamei*; values with different superscripts are significantly different ($p<0.05$).

Absolute length. A histogram of absolute length results was obtained for vannamei shrimp, as presented in Figure 2.

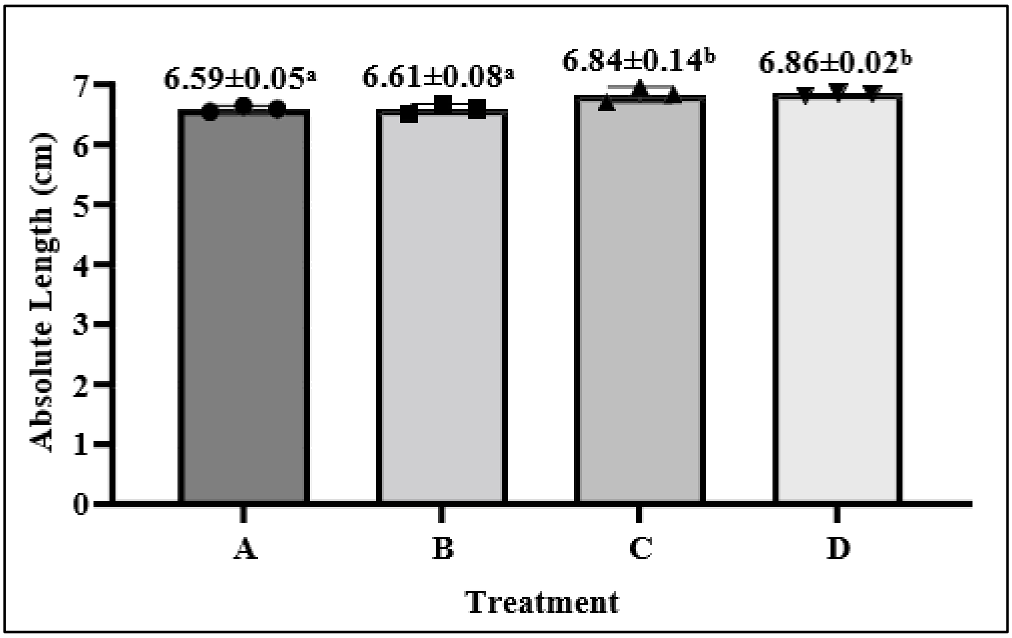

Figure 2. Absolute length of *Penaeus vannamei*; values with different superscripts are significantly different ($p<0.05$).

Figure 2 shows that the absolute length of shrimp from treatment A was not significantly different from that of treatment B, but was significantly different from those treatments C and D. Treatment D produced the highest absolute length among treatments (6.86±0.02 cm).

Total hemocytes. Figure 3 shows that the total hemocyte count of shrimp in treatment A was not significantly different from that of treatment B, but was significantly different from those of treatments C and D. Treatment D produced the highest total hemocyte count (2.12±0.03 $x10^7$cells mL^{-1}).

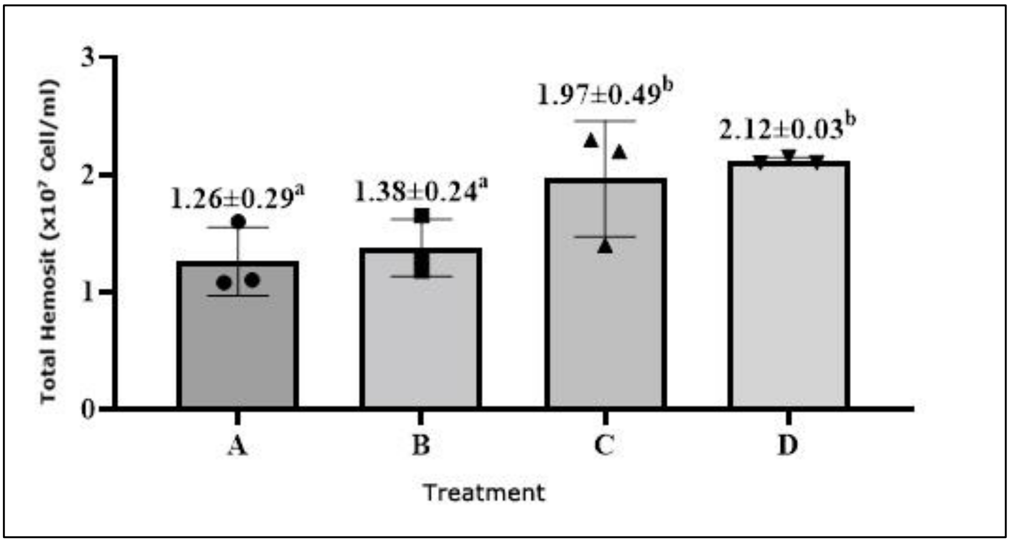

Figure 3. Total hemocyte count of *Penaeus vannamei*; values with different superscripts are significantly different ($p<0.05$)

Water quality. The results of water quality determined in this study are presented in Table 2.

Table 2

Water quality during the maintenance period

No	*Variable*	*Morning*	*Evening*	*Optimum value*
1	Temperature (ºC)	29.01±0.36	28.80±0.39	27–31*
2	DO (mg L^{-1})	5.97±0.49	6.03±0.51	4–6**
3	Salinity (ppt)	34.18±0.85	34.25±0.83	15–25***
4	pH	7.76±0.14	7.81±0.12	7.4–8.9*

Note: DO - dissolved oxygen; *- Sahrijanna & Septiningsih (2017); ** - Anita et al (2017); *** - Arsad et al (2017).

Discussion. The SGR of vannamei shrimp shows an increasing trend and reaches a maximum point when given DDGS with a feed composition of 10%. Lipid-extracted DDGS at a concentration of up to 20% is recommended as a replacement for using soybean meal in the diet of the shrimp species *P. vannamei* (Rhodes et al 2015). The research of Gyan et al (2022) further reveals that replacing fish meal, as the most preferred source of protein for aquafeeds, with 8% DDGS is able to boost the development performance of *P. vannamei*, suggesting that this is the most effective way to achieve this goal. Qiu et al (2017) stated that applying waste flour from producing bioethanol to aquatic animal feeds has become a potential alternative to soybean meal, being able to replace it up to 40%. However, in the case of Gyan et al (2022), the alterations in amino acids content that occurred following the addition of lysine, methionine, arginine, and threonine to the diet may have contributed to the improved growth of shrimp that were treated with DDGS. In addition to the formulation of feeds that can fulfill the specific nutrient requirements of shrimp, the absence of anti-nutritional factors and the presence of a high fiber content in DDGS, which helps to increase the energy of the diet, could be sufficient to explain the superior growth that was achieved in the long-term culture period and an outdoor setting in comparison to a controlled environment.

The increasing trend in absolute length growth occurred because the treatment feed had a supplementation of lysine and threonine. Although DDGS has a high protein value of 30-32%, the concentration of lysine, threonine, and tryptophan in corn from DDGS is relatively lower (Sandor et al 2021). Allam et al (2020) also noted that, in some cases, it is necessary to supplement feed with lysine, methionine, and threonine with DDGS.

Hemocyte cells consist of granular (hyaline) hemocytes, semi-granular and granular cells. The purpose of analysing the total hemocyte count is to determine indicators of stress levels and shrimp health (Prayitno et al 2022). The total hemocyte value in vannamei shrimp reared during the research showed a better trend with the addition of 10% DDGS. According to Febriani et al (2018), shrimp weighing 11-12 g present a healthy status when the total hemocyte count is approximately $1.8\pm9.28\times10^{7}$ cells mL^{-1}. It has been suggested that the utilization of DGGS as an alternate component might have an impact on the metabolic processes, immunological functions, and intestinal health of aquatic species (Rahman et al 2015; Gyan et al 2021; Zhu et al 2022). Based on the findings of Ceseña et al (2021), Sakai (1999), Sohn et al (2000), and Zhang et al (2018), it has been demonstrated that the yeast protein accounts for 5.3% of the total protein content in DDGS, which also contains B-complex and ß-glucan (Webster & Thompson 2015), and has the potential to enhance immune response in shrimp and fish significantly. Numerous studies demonstrated that β-glucans, which are among the compounds found in yeast protein, possess the capability to stimulate the activation of hemocytes in crustaceans (Ceseña et al 2021; Gyan et al 2021; Perveen et al 2021). Good water quality can also support good growth and a good immune system.

Conclusions. Adding DDGS in feed can increase the specific growth rate, absolute length growth, and total hemocytes in vannamei shrimp. To achieve optimal results for these three parameters, DDGS requires a 10-15% addition to the feed.

Conflict of Interest. The authors declare that there is no conflict of interest.

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