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Amino acid, nutrient digestibility and FCR of juvenile vannamei shrimp (*Litopenaeus Vannamei*) at various dosage tofu waste using mixed organism in feed

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Abstract. Tofu dregs are industrial waste that can be used as a source of carbohydrates. However, its use in feed formulations is limited because it contains high crude fiber and low quality. The fermentation process uses a mix of microorganisms, proven to improve the nutritional quality of tofu pulp. This study aims to determine the dosage of fermented tofu pulp in feed against feed amino acids, nutrient digestibility and juvenile food conversion ratio (FCR) of vaname shrimp. The study was designed in a completely randomized design with treatment of 4 doses of fermented tofu pulp in the feed, namely 5, 10, 15 and 20%. The feed was given as much as 10% of total body weight / day to juvenile vaname shrimp measuring 0.5 g per individual kept in a glass aquarium with 20ppt media passage for 60 days. Amino acid measurement results are within the range sufficient for the needs of vaname shrimp. Analysis of variance showed that treatment had a significant effect on total digestibility, protein, crude fiber, carbohydrates and fat, but not on FCR. The highest yield was shown at a dose of 15% protein digestibility (85.62 ± 1.576), crude fiber (87.30 ± 1.117), carbohydrates (90.67 ± 1.231) and a dose of 20% fat digestibility (98.75 ± 0.070) and total digestibility (79.70 ± 0.222), the FCR ranges (3.237 ± 0.35) to (4.141 ± 0.64). The dosage of fermented tofu dregs with mixed microorganisms can be used 15% in the feed formulation.

1. Introduction

Shrimp is one of the fishery commodities that has made a real contribution to increasing South Sulawesi's native income. In connection with this, the national "Shrimp Awakening Movement" initiated by the provincial government of South Sulawesi in 2008 was held [1]. This movement was developed due to indications that shrimp production in South Sulawesi has decreased production from year to year. Vanamei shrimp *L. vannamei* is a type of penaeid shrimp which has a higher immune system compared to tiger prawn species against virus attack. These healthy seeds can be selected by their ability to survive in poor environmental conditions and withstand changes in salinity [2]. In an intensive vanamei shrimp culture system in ponds, feed is one of the strategic components that determine the success of the business. Feed constitutes a very large part of the operational costs in crustacean cultivation [3]. In these activities, almost 60-70% of the total production costs are used for the purchase of feed [4].



This has become an obstacle to the vaname shrimp cultivator community, especially South Sulawesi, soaring feed prices and limited feed raw materials such as fish meal and soybean meal. Consequently, the search for alternative raw material sources that have high nutritional value and abundant availability is the main focus of attention for cultivators and nutritionists [5]. To overcome this problem, efforts are made to use feed raw materials which are not competitive with humans, cheap and easy to obtain, one of which is tofu dregs. In South Sulawesi, tofu dregs are sufficiently available, not competing with human needs (because they are not eaten). However, currently tofu dregs are also widely used for livestock feed such as pigs.

Tofu dregs can be used as feed ingredients because they contain quite high carbohydrates, namely 41.3%, crude protein around 28.6%, 18.3% fat and 17.6% crude fiber. Utilization of tofu dregs as feed raw material, in addition to increasing the economic value and quality of tofu dregs also reduces production costs in cultivation, but the utilization of tofu dregs is still low, due to its high crude fiber, low digestibility, low amino acids and fast properties. stale and smells bad if not handled immediately [6]. The use of high crude fiber is not good for digestion and can inhibit growth in shrimp. One way to increase the nutritional value of tofu pulp and reduce crude fiber is fermentation. Fermentation is an effort to improve nutritional quality, reduce and even eliminate the influence of certain feed ingredients which can be done by using microorganisms. Microorganism mix is an excellent fermenter to use because it is mixed with natural ingredients and contains 44 superior microbes which can produce various beneficial enzymes and increase the growth of cultivated organisms. This is in accordance with the research results obtained [7] stated that the use of mixed microorganisms 10 mL / 100 g and an incubation period of 6 days is good for use as feed raw materials.

Therefore, this research needs to be done to determine the effect of using fermented tofu dregs using mixed microorganisms on feed amino acids, feed nutrient digestibility and feed conversion ratio of vaname shrimp (*Litopenaeus vannamei*). The results of this study are expected to be able to contribute to reducing production costs which in turn can increase the income of vaname shrimp farmers

2. Materials and methods

This research was conducted for 60 days in the Mini Hatchery Unit, Department of Fisheries, Faculty of Marine and Fisheries Sciences, Hasanuddin University, Makassar. This research was conducted with experimental methods using a completely randomized design. The aspect studied was the effect of adding fermented tofu pulp using mixed microorganisms. in artificial feed with 4 treatments and 3 replications, in order to obtain 12 experimental units.

2.1. Feed

The feed used in this study was in the form of pellets. The process of making feed begins with the preparation of raw materials, drying, flouring, mixing of feed raw materials, printing feed, drying feed, and packing the feed. The feed raw materials used consist of fish meal, soybean meal meal, shrimp head meal, fermented tofu dregs, corn flour, bran, vitamin & mineral mix and fish oil. The tofu dregs are fermented at different doses using mixed microorganisms.

2.2. Shrimp juvenile

Each aquarium was filled with 15 test shrimp aged 22 days (PL) with a weight of ± 0.09 g. Before being given the treatment, the test shrimp were acclimatized for three days and fasted for one day prior to initial weighing to determine the initial weight.

2.3. Amino acid

Principles of analysis Amino acid analysis results can be improved by utilizing the precolomic reaction of amino acid groups with certain reagents to form a deviation that can absorb UV light or fluoresce [4]. To calculate the concentration of amino acids in the material, a standard chromatogram is made using ready-to-use standard amino acids that are subjected to the same treatment as the sample. The amino acid content in 100 grams of material can be calculated by the formula:

$$= \frac{\text{Sample Area} \times C \times f \times B \times M}{\text{Standard Area} \times \text{Sample Weight}(\mu\text{g})} \times 100\%$$

Description :

C = standard concentration of amino acids ($\mu\text{g} / \text{ml}$)

f = dilution factor

B = molecular weight of each amino acid (g / ml).

The conditions of the HPLC tool during amino acid analysis are as follows:

Temperature : 38 OC

HPLC column type : Pico tag 3.9 x 150 nm column

Eluent flow rate : 1 ml / minute

Pressure : 3000 psi

Mobile phase : 60% acetonitrile and 40% sodium acetate 1 M

Detector : UV

Wavelength : 254 nm

Brand : waters

2.4. Nutrient Digestibility

The nutrient digestibility of the tested animals was analyzed using the Cr_2O_3 indicator using the formula according to Takeuchi (1988)[8] :

Protein digestibility = $100 - [100 \times (a \times b') (a' \times b)]$

Fiber digestibility = $100 - [100 \times (a \times c') (a' \times c)]$

Digestibility of carbohydrates = $100 - [100 \times (a \times c') (a' \times c)]$

Fat digestibility = $100 - [100 \times (a \times e') (a' \times e)]$

Description :

a: % Cr_2O_3 in feed.

a': % Cr_2O_3 in feces

b: % protein in feed

b': % protein in feces

c: % fiber in feed

c': % fiber in feces

d: % carbohydrates in feed

d': % carbohydrates in feces

e: % fat in feed

e': % fat in feces

2.5. Feed conversion ratio (FCR)

Feed conversion ratio (FCR) The feed conversion was calculated using the Djajasewaka formula (1985)[9]:

$$FCR = \frac{F}{(W_t + D) - W_o}$$

Description :

FCR = Feed conversion ratio

W_o = weight of test animals at the beginning of the study

W_t = weight of the test animal at the end of the study

D = Number of fish that died

2.6. Data analysis

The data to be obtained was tested using Analysis of Variance (ANOVA) at a 95% level of confidence through SPSS statistical software. If the results of statistical analysis show a significant difference effect, a further test will be carried out to determine the best treatment.

3. Results and Discussion

3. 1. Amino acid

The composition of essential amino acids contained in the artificial feed given during the study is presented in Table 1.

Table 1. Amino acid composition at various feed levels (% g / 100 g feed)

Amino acid	A (5% tofu waste) (% w/w)	B (10% tofu waste) (% w/w)	C (15% tofu waste) (% w/w)	D (20% tofu waste) (% w/w)	Requirement (% inside the body)
Histidine	0.39	0.46	0.24	0.35	1.08
Threonine	0.78	0.82	0.60	0.70	1.3
Arginine	1.31	1.33	0.97	0.99	1.2
Methionine	0.14	0.27	0.08	0.03	2.4
Valine	1.41	1.20	1.05	1.09	4
Phenylalanine	1.21	1.11	0.92	1.01	1.27
I-leucine	1.16	1.02	0.89	0.90	3.5
Leucine	1.80	1.65	1.35	1.39	5.4
Lysine	0.79	1.47	0.19	0.76	5.3
Tryptophan	-	-	-	-	1.3

Description: -: Cannot be detected

Judging from the table above that essential amino acids such as Histidine, Threonine, Methionine, Valine, I-leucine, Leucine, Lysine and Tryptophan are not sufficient for the needs of vaname shrimp, but Phenylalanine has met the needs of vaname shrimp and Arginine for feed A and B is sufficient for shrimp needs. vaname, but C and D feed is not sufficient. It is suspected that feed A and B contain higher soybean meal than C and D, so that the amino acid value is also better, whereas in C and D feed, the content of soybean meal is very low and the content of tofu pulp is higher so that the resulting amino acids are not good.

Histidine is an essential amino acid, histidine is needed to maintain nitrogen balance in the body. Threonine amino acid is an essential amino acid that works on the digestive system and protects the liver. Arginine requirements are determined by digestible energy (Dietary energy) and protein content in feed. Besides playing a role in protein synthesis, arginine also plays a role in urea biosynthesis. Arginine is distributed mainly in the mitochondrial fraction, both in the kidney and liver of teleost fish.

Methionine is one type of essential amino acid, this methionine is needed for growth. This is in accordance with the statement [10] which states that methionine is an amino acid containing a sulfur group. Methionine plays a role in the formation of nucleic acids and tissues as well as protein synthesis, as well as a building block for other amino acids (cysteine) and vitamins (choline). Besides that, another important function of methionine is to help absorb fat and cholesterol.

Phenylalanine is a compound that functions as a messenger (neurotransmitter) and regulates the secretion of the thyroid gland. The amino acid leucine is essential for growth and maintaining nitrogen

balance. Leucine is also useful for the breakdown and formation of protein. Changes in serum concentrations of I-leucine, leucine and lysine are affected by an increase in feed protein levels.

An increase in one of these amino acids, for example leucine, will affect the I-leucine and lysine concentrates. Leucine is able to facilitate the body's tissues in the process of absorbing branched chain amino acids [10]. The same leucine content in each treatment feed was very different from the leucine content in the shrimp body, presumably indicating that the feed did not meet the needs of the fish. It is suspected that the test feed is difficult to digest in the vaname shrimp body. A good feed is feed that is close to the amino acid pattern of cultivated cultivants.

I-leucine is needed in the production and storage of protein by the body and the formation of hemoglobin, it also plays a role in metabolism and pituitary gland function. Lysine deficiency causes the content of essential amino acids in the feed to be unbalanced, thus affecting the value of protein for body tissue formation, because the protein in meat that is formed depends on the least essential amino acids.

The amino acid tryptophan is the least needed essential amino acid. The fact above shows that the role of the essential amino acid tryptophan is the smallest when compared to other amino acids. Tryptophan amino acid in this study could not be analyzed because tryptophan could not be analyzed together with other amino acids, it could only be measured by different and separate techniques.

The reduction of one or more amino acids in protein results in decreased growth and appetite [11]. Furthermore, stated that amino acid imbalance can lead to amino acid antagonism or amino acid toxicity[12]. Antagonism occurs when certain levels of amino acids exceed the required level. This is in accordance with the opinion of [13]who stated that if the proportion of feed amino acids differs by one or more the proportion of essential amino acids from the acid profile of fish and crustaceans, the consequence is an imbalance in feed nutrition and causes growth retardation.

3.2. Nutrient Digestibility of Vaname Shrimp

Nutritional digestibility of vannamei shrimp treated with various doses of tofu pulp flour is presented in Appendix 8, the average is presented in Table 2.

Table 2. Average nutrient digestibility of vaname shrimp during the study.

Feed (% tofu waste)	Digestibility				
	Total	Protein	Crude Fiber	Carbohydrate	Fat
A (5%)	67,87±7,536 ^a	72,69±2,217 ^a	75,63±1,888 ^a	74.71±7,699 ^a	97,25±0,075 ^a
B (10%)	71,83±0,396 ^{ab}	82,43±1,576 ^b	80,34±0,797 ^b	73.96±0,292 ^a	98,14±0,117 ^b
C (15%)	78,26±0,209 ^b	85,62±1,576 ^b	87,30±1,117 ^c	86.49±4,204 ^b	98,62±0,078 ^c
D (20%)	79,70±0,222 ^b	82,60±1,457 ^b	87,20±2,095 ^c	90.67±1,231 ^b	98,75±0,070 ^c

Description: Different superscript letters in the same column indicate a significant difference between treatments at the 95% confidence level (P <0.05).

The results of the analysis of variety showed that the dosage treatment of tofu pulp in artificial feed (5,10,15,20%) had a significant effect (p <0.05) on the total digestibility, protein, crude fiber, carbohydrates and fat. Digestibility value describes the amount of nutrients in the feed that can be digested. The influence of feed with various doses of tofu dregs flour on total digestibility, protein, crude fiber, carbohydrates and fat of vaname shrimp indicates that an increase in the composition of tofu dregs in the feed up to 20% can still be digested properly by vaname shrimp. But provided that the feed is supplemented with mixed microorganisms at a dose of 10 mL / 100 g of feed. The W-Tuckey test showed that the total digestibility at the dose of fermented tofu dregs in the feed was 5% different from the dosage of tofu pulp in the feed 15, 20% but not different from the 10% tofu dregs dose, while the 10% tofu dregs dose was the same as the other tofu pulp doses. Total digestibility indicates the total digestibility of nutrients as a source of energy, namely protein, fat and

carbohydrates[14]. Differences in the composition of ingredients and nutrients in feed will affect the total digestibility value of the feed.

The W-Tuckey test showed that the protein digestibility of tofu pulp flour in the feed at a dose of 5% was different from that of 10, 15, 20%. In addition, the results of the Variety Analysis of fermented tofu pulp flour at a dose of 15% showed the influence of proteolytic bacteria on the fermentation process so that it gave the best treatment by increasing the crude protein content. This treatment shows a significant difference compared to the 5% dose. This is because in mixed microorganisms there are *Bacillus cereus* bacteria which are proteolytic bacteria which have the ability to produce protease enzymes, where the protease enzyme is simpler to hydrolyze protein and increase protein digestibility.

Bacteria and fungi contained in mixed microorganisms will produce protease, lipase and α - amylase enzymes. Bacteria contained in mixed microorganisms include *Bacillus* sp. which will produce the three enzymes, *Rhizopus* sp and *Streptococcus* sp produce amylase and protease enzymes. *Aspergillus niger* mushrooms found in mixed microorganisms will also produce protease and amylase enzymes, while *Aspergillus oryzae* will produce lipase enzymes[15].

The W-Tuckey test showed that the digestibility of crude fiber in the provision of tofu pulp flour in the feed at a dose of 5% was different from that of 10, 15, 20%, but the doses of 15 and 20% showed the same results. Crude fiber contained in tofu dregs flour is thought to be degraded due to the fermentation process. Fermentation carried out by bacteria and fungi results in chemical changes from a complex compound to a simple one, so that it can have a positive effect [16]. explained that crude fiber is not a nutrient for fish or shrimp because it cannot be digested by fish and shrimp. Crude fiber tolerance is only four percent. Meanwhile, according to [17], the limit of fiber contained in fish feed is eight percent.

The W-Tuckey test shows that the digestibility of carbohydrates in the provision of tofu pulp flour in feed with doses 5 and 10 is the same but different from the 15 and 20% doses, but the doses of 15 and 20% show the same results. The difference in the digestibility of carbohydrates in the feed containing mixed microorganisms with various doses of tofu dregs flour is due to the mixed microorganisms that are supplemented into the vaname shrimp feed containing cellulase, protease and amylase enzymes. Where the cellulase enzyme is an enzyme breaking down fiber and amylase enzyme as a carbohydrate breaker. The high digestibility of carbohydrates and fiber of vaname shrimp at 15 and 20% doses of tofu dregs occurs because at that level the activity of the cellulase enzyme in the digestive tract of vaname shrimp also increases due to the fermentation of mixed microorganisms which are rich in fiber-degrading enzymes so that they can increase the activity of cellulase enzymes in the digestive tract of vaname shrimp.

The enzymes derived from the mixed microorganisms give influence or contribute to the activity of the cellulase enzymes in the digestive tract of vaname shrimp in the process of overhauling complex compounds in feed into simpler compounds and causing carbohydrate components, especially fiber in feed, to be degraded optimally so that the digestion process could be easier, a [18]which states that the hydrolysis of macro nutrients in digestion occurs due to the presence of digestive enzymes. In addition, [19]also explain that the difference in the value of dry matter that can be digested is probably due to differences in the properties of processed food, including its suitability for enzyme hydrolysis and the activity of substances contained in feed.

The W-Tuckey test showed that the fat digestibility of tofu pulp flour in artificial feed at a dose of 5% was different from that of 10, 15, 20% of tofu doses, while 15 and 20% were the same but different. with 5 and 10%. There is a difference in the digestibility of fat in the dosage of tofu dregs at 5% with other doses of tofu dregs, presumably because the treated feed contains high crude fiber and ash so that crude fat nutrients are difficult to digest. Suprpto *et al.* (2013) stated that too high crude fiber levels can interfere with the digestion of other substances. In addition, the higher the ash content of a feed ingredient causes feed nutrients to be undigested, consequently decreasing the digestibility value.

The difference in dosage treatment between fermented tofu dregs and nutrient digestibility above is thought to have a large effect on the substitution of soybean cake flour with tofu dregs flour, wherein feed with 5% tofu pulp and 20% soybean meal produced the lowest digestibility and the content of soybean cake dregs 15 and 15. 20% with 10 and 5% soybean meal content resulted in the highest digestibility, this is presumably because the higher the soybean meal flour, the lower the digestibility was because the soybean meal flour did not undergo a process of predijes / fermentation so that the resulting digestibility was very low.

3.3. Feed Conversion Rate (FCR)

Table 2. Average feed conversion ratio (FCR) of vaname shrimp during maintenance.

Feed (%tofu waste)	Parameter±std
	FCR (%)
A (5%)	4,141±0,64
B (10%)	3,237±0,35
C (15%)	3,723±0,31
D (20%)	3,563±0,18

Description: Different superscript letters in the same column indicate significant differences between treatments at the 95% confidence level ($P < 0.05$)

The results of the analysis of variety showed that the treatment of feeding with various doses of tofu pulp resulted in no significant effect on the feed conversion ratio ($p > 0.05$). The feed conversion ratio is the ratio between the amount of feed consumed and the resulting weight gain. The feed conversion value shows how much shrimp can use the feed given to form 1 kg of meat. The value of the feed conversion ratio that is getting smaller shows the better quality of the feed which is the higher the digestibility of the feed [12]. This means that when a feed has a low FCR, it takes less feed to produce one kilogram of fish then it would if the FCR were higher. A low FCR is a good indication of a high quality feed.

The feed conversion ratio in this study was not statistically significant. The higher the feed conversion ratio, the less effective and efficient the treatment was given. The feed containing fermented tofu pulp flour produced the same feed conversion ratio in each treatment. The value of the feed conversion ratio obtained in this study is slightly higher than the need for the feed conversion ratio for shrimp and fish, where a feed conversion value above 3 is considered bad.

The high value of the feed conversion ratio is thought to be because the feed given cannot be utilized properly by vaname shrimp. Besides, the feed given is wasted filtered recirculation so that the shrimp cannot use the feed given. Feed with a dose of 5% tofu pulp has the highest feed conversion ratio, this is presumably because the resulting nutrient digestibility value in feed with a dose of 5% tofu pulp gives the lowest result, this is the reason why vaname shrimp does not utilize. This is the same with [20] findings that the test feed containing 5% fermented tofu pulp flour showed lower yields. According to [21], the size of the feed conversion ratio is influenced by several factors, namely the quality and quantity of feed, species, size and water quality. The size of the feed conversion ratio determines the effectiveness of the feed. In addition, the microbes that fight in feed fermentation are thought to be able to increase metabolism in crustacean bodies.

4. Conclusions

Based on the research, it can be concluded that a dose of 15% fermented tofu dregs using mixed microorganisms can be used in the formulation of vaname shrimp feed.

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