Measuring the Substitution of Vegetable Waste Fermented Rumen Fluid with Tofu Waste in *Vannamei* Shrimp Feed

Murni^{#1}, Haryati^{*}, Herry Sonjaya^{*}, Siti Aslamyah⁺

[#] University of Muhammadiyah Makassar of South Sulawesi Province, Indonesia E-mail:¹murni@unismuh.ac.id

^{*}Aquaculture, Faculty of Marine Science and Fishery, Hasanuddin University, Makassar, South Sulawesi, Indonesia

⁺Faculty of Animal Husbandry, Hasanuddin University, Makassar, South Sulawesi, Indonesia

Abstract— *Vannamei* shrimp cultivation in Indonesia deals with the complication at high feed fees as a result of raw material feed protein sources. Rumen fluid fermented vegetable waste has the potential to substitute tofu in feed *Litopenaues vannamei*. This study aims to determine the substitution of vegetable waste with the rumen fluid fermented tofu is best to improve the performance of *vannamei* shrimp growth. Research carried out for 60 days with evaluation substitution levels of rumen fluid fermented vegetable waste with tofu in *vannamei* shrimp feed. designed using a completely randomized design (CRD) with 4 treatments each with 3 replications, thus totaling 12 experimental units. Feed treatment tested is A) 0 (control), B) 33.33, C) 66.67, D) 100% rumen fluid fermented vegetable waste. The results showed that the substitution of tofu with fermented vegetable waste rumen fluid in *vannamei* shrimp feed a significant effect (P <0.05) on digestive enzyme activity, total and nutrient digestibility, growth, and survival rate of *vannamei* shrimp. The research shows that the tofu substitution more than sixty percent of fermented vegetable waste in feed could increase the activity of digestive enzymes, total digestibility, nutrient digestibility, growth, and survival of *juvenile vannamei* shrimp.

Keywords- bioactivate enzyme; survival rate; feed; fermented vegetable waste; hydrolyze fiber.

I. INTRODUCTION

Vannamei shrimp cultivation in Indonesia faced obstacles at high feed prices due to raw material feed protein sources such as fish meal, and soy are imported. It is, therefore, necessary raw materials other sources of protein that have a quality and quantity such as tofu. Tofu is the result of processing know who has a protein content of 17.63%, 8.11% crude lipid, crude fiber 17.93%, ash content of 3.35% and 39.80% BETN. To substitute tofu should have relatively the same protein, abundant and cheaper prices as well as vegetable waste has a high nutrient content, low-cost, and sustainable. Therefore, vegetable waste can be used to substitute tofu in shrimp feed.

Vegetable waste is an alternative raw material that can be utilized. However, the main obstacle in the use of vegetable waste is a high cellulose content (30.71%). To cope with high cellulose content can be made through fermentation. Fermentation is the process of decomposition of complex molecules into simpler molecules. One fermenter can be used to lower the cellulose content of vegetable waste is rumen fluid. Andriani [1] states that microorganism cow

rumen contains cellulase and amylase enzyme sufficient to hydrolyze the fish feed.

Murni and Darmawati [2] reported that the vegetable waste fermentation using rumen fluid at a dose of 10-15 mL/kg of vegetable waste and long incubation time 7 days increases the activity of the enzyme amylase (0.250 U/mL/min), proteases (0.49 u/ml/min), cellulase (0.124 u/ml/min). Murni et al. [3] reported that fermentation of vegetable waste with a dose of 15 mL/kg and long incubation time 4 days can lower crude fiber, vegetable waste 29.35% to 14.83%, and the nutritional content of 18.45% to 19.19%; vegetable waste fermentation with rumen fluid dosage of 3% and incubation period of 4 days can reduce levels of waste vegetable fiber from 30.73 to 11, 79% [4]. Jusadi et. al. [5] reported that the addition of enzymes sheep rumen fluid 125 ml/kg of material with a 24hour long incubation time may lower crude fiber, coconut cake, the highest of 67.8% from 13.76% to 6.98%; Fitriliani [6] reported that administration Lamtoro gung leaf starch content as much as 0, 10, and 15% increase specific growth rate, feed efficiency and tends to decrease with increased

levels *Lamtoro gung* leaf in the feed. Jusadi et.al. [5] says that the addition of sheep rumen fluid enzymes can improve skin cocoa fruit digestibility on tilapia fish feed. Listiowati and Pramono [7] said that the use of 25% fermented liquid cassava leaf flour in feed provided the best growth performance for tilapia (*Oreochromis sp*) compared to the increase in the percentage increase in fermented cassava leaf flour.

Fermented vegetable waste in feed can reduce fiber and increased nutrients. Thus, the fermented vegetable waste can be used as a raw material for feed without lowering the quality of feed. However, the concentration of fermented vegetable waste in *vannamei* shrimp feed is not yet known, so it is necessary to find the percentage that can be substituted by the fermented vegetable waste pulp in the feed can improve feed efficiency, nutrient digestibility, and ultimately improve the growth and survival of shrimp *vannamei*. Tujuan this study was to evaluate the rumen fluid fermented vegetable waste as substitute tofu in artificial feed can improve feed efficiency and growth of *vannamei* shrimp.

II. MATERIALS AND METHOD

This research was conducted at the Mini FIKP Unhas Hatchery, from April to August 2017. Chemical analysis was carried out at the Integrated Laboratory of the Faculty of Animal Husbandry, Hasanuddin University, the Research Institute for Brackish Water Research in Maros regency and the Water Quality Laboratory of FIKP Unhas, South Sulawesi, Indonesia

A. Materials

The cow's rumen liquid is taken from the Slaughterhouse. Cattle rumen is taken from the contents of the cattle rumen by filtration (filtering with cotton cloth) under 40C. The enzyme extract of cattle rumen fluid was obtained following the method of digestibility [8]. The waste vegetable used in the study is mustard greens, cabbage, kale, and carrots 25%, respectively. Feed samples used in this study were pelleted feed formulated with fermented vegetable waste rumen fluid.

B. Method

The study begins with a rough cut and milled vegetable waste. Vegetable waste inserted into a plastic clip, rumen fluid was added at a dose of 1, 2, 3% sealed and incubated with a period of 4, 6, 8, and 10 days in anaerobic, then stored in boxes for the same room temperature. The feed manufacturing process begins with the preparation of raw materials, mixing of feed raw materials, and feed printing, drying of feed and feed packaging. The waste vegetable used is the result of fermentation using rumen fluid with a dose of 3%- and 4-day long incubation time.

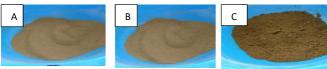


Fig. 1 Sample of feed-in experimental diets with fermented vegetable waste rumen fluid.

Results of the proximate analysis of vegetable waste before rumen fluid fermented flour and the proximate analysis pulp used as raw material for feed formulation testing (Table 1).

 TABLE I

 Results of the proximate analysis of vegetable waste without fermentation rumen fluid and tofu

Raw material (%)	Crude protein	fat rough	crude fiber	ash content	NFE
Vegetable waste (kg)	22.63	3.87	30.73	29.88	35.77
Tofu (kg)	16.73	8,11	17.93	3.35	39.80

Description: Lab analysis results, UNHAS, 2017.

The crude protein content of feed testing substituted tofu with rumen fluid fermented vegetable waste and energy content of the feed trials (Table 2).

 TABLE II

 The content of crude protein and energy feed test substituted tofu with rumen fluid fermented vegetable waste

Composition	feed A	feed B	feed C	feed D
Crude protein	28.41	29.43	30.21	29.88
Feed energy (kcal/kg)	4197.22	4160.22	4110.95	3996.67

Description: The results of lab analysis. Nutrition BPPBAP Maros, 2017.

1) Growth trail: At the end of the study period was measured against total digestibility, nutrient digestibility, digestive enzyme activity, the efficiency of feed utilization, growth, and survival rate. All treatments are determined using the digestibility total and digestibility of nutrients [9]. Vannamei shrimp digestive enzyme activity measured in some measurements. Measurement of the activity of digestive enzymes protease estimated by the method enzymatic analysis [10]. Measurement of the enzyme amylase and cellulase activity was estimated by the method of Bernfeld [11]. The growth trail also considers the feed efficiency [9], protein retention [9], retention fat [9], SGR [12], and survival [12].

2) Water quality parameters: Water quality parameters like temperature, pH, salinity, dissolved oxygen were measured every day, and the ammonia was measured three times during the study with standard methods [13]. The parameters analyzed are presented in the table.

3) Biochemical analysis: Ingredients, experimental feeds and were analyzed for proximate using a method of AOAC [14]. Amino acids were analyzed using 18-5-17/MU/SMM-SIG, UPLC.

4) Statistical analysis. Nutrient digestibility, digestive enzyme activity, feed efficiency, growth and survival rate of one-way variance analysis (ANOVA). If there are differences among the treatments, then continued using the Duncan test at a 95% confidence interval (p < 0.05) using SPSS version 20.

III. RESULTS AND DISCUSSION

Total digestibility and nutrient *juvenile vannamei* shrimp tofu substituted with a different vegetable waste in feed during the study are presented in Table 3. TABLE III

AVERAGE TOTAL DIGESTIBILITY AND NUTRIENT SUBSTITUTED JUVENILE VANNAMEI SHRIMP TOFU WITH FERMENTED VEGETABLE WASTE IN FEED IN ALL TREATMENTS DURING THE STUDY.

The concentration of vegetable	Total and nutrient digestibility (%)							
waste (%)	Digestibility	Digestibility	Digestibility	Digestibility	Digestibility			
waste (70)	Total	protein	Fat	Fiber	Carbohydrate			
Feed A	$60.98 \pm 1.14a$	$60.26\pm0.41a$	$69.27\pm0.73a$	$78.83 \pm 0.49a$	$88.04\pm0.62a$			
Feed B	$64.71 \pm 1.01b$	$74.70\pm0.33c$	$84.22\pm0.52c$	$83.44 \pm 1.04c$	$92.21\pm0.17c$			
Feed C	$68.13\pm0.66c$	$79.51 \pm 0.88 d$	$88.33 \pm 0.41 d$	$86.70 \pm 0.24 d$	$93.75\pm0.43d$			
Feed D	$63.17\pm0.34b$	$71.58 \pm 0.61 b$	$81.87\pm0.59b$	$81.15\pm0.61b$	89.40 0.10b			

Note: (1) Mean bearing superscript the same in a row significant (p <0.05). (2) Feed A: 0% fermented vegetable waste. Feed B: 33.33% fermented vegetable waste. Feed C; 66.67% fermented vegetable waste. Feed D: 100% fermented vegetable waste

Substitution of tofu with vegetable waste is fermented in the rumen fluid artificial feed of *juvenile vannamei* shrimp showed significant effect (p<0.05) on total digestibility, protein, fiber, carbohydrates, and fats (Table 4, 5, 6, 7 and 8).

 TABLE IV

 The results of variance analysis (Anova) of total digestibility of JUVENILE VANNAMEI SHRIMP

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	81.47	3	27.16	37.38	.000
Within Groups	5.81	8	.73		
Groups Total	87.28	11			

 TABLE V

 Results of analysis of variance (Anova) digestibility protein of Juvenile Vannamei shrimp

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	602.39	3	200.80	556.79	.000
Within Groups	2.89	8	.361		
Total	605.28	11			

 TABLE VI

 RESULTS OF ANALYSIS OF VARIANCE (ANOVA) DIGESTIBILITY FIBER OF

 JUVENILE VANNAMEI SHRIMP

	Sum of		Mean		
	Squares	df	Square	F	Sig.
Between Groups	101.23	3	33.74	76.28	.000
Within Groups	3.54	8	.44		
Total	104.77	11			

TABLE VII Results of analysis of variance (Anova) digestibility of carbohydrate *juvenile vannamei* shrimp

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	60.72	3	20.24	130.74	.000
Within Groups	1.24	8	.16		
Total	61.96	11			

 TABLE VIII

 Results of analysis of variance (Anova) digestibility of fat

 JUVENILE VANNAMEI SHRIMP

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	608.39	3	202.80	605.45	.000
Within Groups	2.68	8	.34		
Total	611.07	11			

Duncan advanced test substitution treatment tofu with fermented vegetable waste in feed *juvenile vannamei* shrimp showed that the total digestibility of *juvenile vannamei* shrimp fed substitute tofu with fermented vegetable waste 66.67% (P <0.05) (Table 9). It is higher than the tofu substitution with 33.33% fermented vegetable waste, vegetable waste tofu with fermented 100%, and tofu with fermented vegetable waste 0%.

 TABLE IX

 DUNCAN'S FURTHER TEST RESULTS IN TOTAL DIGESTION OF JUVENILE

 VANNAME! SHRIMP

Duncan ^a						
		Subset for $alpha = 0.05$				
Treatment	Ν	1	2	3		
0% vegetable waste	3	60.98				
100% vegetable waste	3		63.17			
33.33% vegetable waste	3		64.71			
66.67% vegetable waste	3			68.13		
Sig.		1.00	.07	1.00		

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Duncan's further test on the substitution of tofu pulp with fermented vegetable waste in feed of *juvenile vannamei* shrimp showed that digestibility of protein, fat, fiber and carbohydrates (Table 10, 11, 12, and 13) of *juvenile vannamei* shrimp fed with tofu pulp substitution with fermented vegetable waste 66.67% was significantly higher than test feed others.

TABLE X DUNCAN'S FURTHER TEST RESULTS DIGESTIBILITY OF PROTEIN VANNAMEI SHRIMP JUVENILE Duncan^a

Duilean						
		Subset for $alpha = 0.05$				
treatment	Ν	1	2	3	4	
0% vegetable waste	3	60.26				
100% vegetable waste	3		71.58			
33.33% vegetable waste	3			74.70		
66.67% vegetable waste	3				79.51	
Sig.		1.00	1.00	1.00	1.00	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TABLE XI
DUNCAN'S FURTHER TEST RESULTS DIGESTIBILITY OF FAT JUVENILE
VANNAMEI SHRIMP

Duncan^a

VANNAMEI SHRIMP

		Subset for alpha = 0.05				
treatment	Ν	1	2	3	4	
0% vegetable waste	3	69.25				
100% vegetable waste	3		81.87			
33.33% vegetable waste	3			84.22		
66.67% vegetable waste	3				88.33	
Sig.		1.00	1.00	1.00	1.00	

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000.

TABLE XII
DUNCAN'S FURTHER TEST RESULTS DIGESTIBILITY OF FIBER JUVENILE
VANNAMEI SHRIMP

Duncan^a

		Subset for $alpha = 0.05$					
treatment	N	1	2	3	4		
0% vegetable waste	3	78.83					
100% vegetable waste	3		81.15				
33.33% vegetable waste	3			83.44			
66.67% vegetable waste	3				86.70		
Sig.		1.00	1.00	1.00	1.00		

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

 TABLE XIII

 DUNCAN'S FURTHER TEST RESULTS IN DIGESTIBILITY OF CARBOHYDRATE

 JUVENILE VANNAMEI

 SHRIMP

Duncan ^a									
		Subset for $alpha = 0.05$							
treatment	Ν	1	2	3	4				
0% vegetable waste	3	88.04							
100% vegetable waste	3		89.40						
33.33% vegetable waste	3			92.21					
66.67% vegetable waste	3				93.75				
Sig.		1.00	1.00	1.00	1.00				

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Digestive enzyme activity of *juvenile vannamei* shrimp substituted tofu with fermented vegetable waste rumen fluid in artificial feed is presented in Table 14.

TABLE XIV Average digestive enzyme activity of *Juvenile vannamei* shrimp substituted tofu with fermented vegetable waste in feed in all treatments during the study

Treatment (% fermented	Enzyme a	ne activity (U / mL / min)			
vegetable waste $+$ tofu)			cellulas		
(egetable waste volu)	protease	amylase	e		
	0.18 ±	$0.08 \pm$	$0.04 \pm$		
Feed A (0% waste vegetable)	0.00a	0.03a	0.00a		
	0.33 ±	0.28 ±	0.25 ±		
Feed B (33.33 vegetable waste)	0.00c	0.03c	0.00c		
	0.34 ±	0.29 ±	0.27 ±		
Feed C (66.67 vegetable waste)	0.13c	0.02d	0.00d		
	0.31 ±	0.27 ±	0.23 ±		
Feed D (100 vegetable waste)	0.00b	0.03b	0.00b		

Note: Mean bearing superscript same in a row significant (p <0.05)

Analysis of variance showed that substitution of tofu with vegetable waste is fermented in the rumen fluid artificial feed on *juvenile vannamei* shrimp significantly (p < 0.05) influence the activity of the enzyme's protease, amylase, and cellulase (Table 15, 16 and 17) *vannamie* digestive tract of *juvenile* shrimp.

TABLE XV
RESULTS OF VARIANCE ANALYSIS (ANOVA) ACTIVITY OF DIGESTIVE
PROTEASE ENZYME JUVENILE VANNAMEI SHRIMP

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.14	3	.05	594.62	.000
Within Groups	.001	8	.000		
Total	.14	11			

 TABLE XVI

 Results of analysis of variance (Anova) activity of digestive amylase enzyme *Juvenile vannamei* shrimp

	Sum of		Mean		
	Squares	df	Square	F	Sig.
Between Groups	.09	3	.03	3383.63	.000
Within Groups	.000	8	.000		
Total	.09	11			

 TABLE XVII

 Results of analysis of variance (Anova) activity of digestive cellulase enzyme *juvenile vannamei* shrimp

	Sum of		Mean		
	Squares	df	Square	F	Sig.
Between Groups	.10	3	.034	6923.90	.000
Within Groups	.000	8	.000		
Total	.102	11			

Duncan Test Results further showed that the activity of the protease enzyme digestive tract *juvenile vannamei* shrimp fed substitute tofu with fermented vegetable waste 66.67% identical to the substitution of tofu with fermented vegetable waste 33.33%, and significantly higher than the substitution of tofu the fermented vegetable waste 0%, and tofu with vegetable waste fermented 100% (Table 18).

TABLE XVIII
DUNCAN'S FURTHER TEST RESULTS ON DIGESTIVE PROTEASE ENZYME
JUVENILE VANNAMEI SHRIMP

Duncan"							
		Subset for $alpha = 0.05$					
Treatment	Ν	1	2	3			
0% vegetable waste	3	.09					
100% vegetable waste	3		.31				
33.33% vegetable waste	3			.33			
66.67% vegetable waste	3			.34			
Sig.		1.00	1.00	.14			

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Duncan advanced test showed that the enzyme amylase and cellulase activity (Table19 and 20) of the digestive tract of *juvenile vannamei* shrimp fed by substituting tofu and fermented vegetable waste 66.67% were significantly higher than the other treatments. Substitution of tofu with fermented vegetable waste generated 0% real protease enzyme activity is lower than with other treatments.

TABLE XIX
DUNCAN'S FURTHER TEST RESULTS ON THE ACTIVITY OF THE ENZYME
AMYLASE DIGESTIVE JUVENILE VANNAMEI SHRIMP
Duncan ^a

		Subset for $alpha = 0.05$			
Treatment	Ν	1	2	3	4
0% vegetable waste	3	.08			
100% vegetable waste	3		.27		
33.33% vegetable waste	3			.28	
66.67% vegetable waste	3				.29
Sig.		1.00	1.00	1.00	1.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

TABLE XX
DUNCAN'S FURTHER TEST RESULTS ON CELLULASE ENZYME DIGESTION
IIIVENILE VANNAMEL SHRIMP

Duncan^a

		Subset for $alpha = 0.05$			
treatment	Ν	1	2	3	4
0% vegetable waste	3	.04			
100% vegetable waste	3		.25		
33.33% vegetable waste	3			.25	
66.67% vegetable waste	3				.27
Sig.		1.00	1.00	1.00	1.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Substitution of tofu with vegetable waste fermented feed rumen fluid in *juvenile vannamei* shrimp significant effect (p <0.05) (Table 21) on feed efficiency.

TABLE XXI THE RESULTS OF VARIANCE ANALYSIS (ANOVA) FEED EFFICIENCY OF JUVENILE VANNAMEI SHRIMP

	Sum of		Mean		
	Squares	df	Square	F	Sig.
Between Groups	74.621	3	24.874	17.716	.001
Within Groups	11.232	8	1.404		
Total	85.853	11			

Duncan substitution further test tofu with vegetable waste fermented feed rumen fluid in *juvenile vannamei* shrimp showed that juvenile shrimp feed efficiency *vanname* fed substitute tofu with fermented vegetable waste 66.67% were significantly higher than other test feed.

TABLE XXII DUNCAN'S FURTHER TEST RESULTS ON FEED EFFICIENCY OF JUVENILE VANNAMEI SHRIMP

Duncan^a

		Subset for alpha $= 0.05$			
Treatment	Ν	1	2	3	
0% vegetable waste	3	19.94			
100% vegetable waste	3	21.02			
33.33% vegetable waste	3		23.75		
66.67% vegetable waste	3			26.35	
Sig.		.30	1.00	1.00	

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Analysis of variance showed that the administration of substitution tofu with vegetable waste fermented in the rumen fluid different *juvenile vannamei* shrimp feed significant effect (p < 0.05) (Table 23) on the rate of growth.

TABLE XXIII THE RESULTS OF VARIANCE ANALYSIS (ANOVA) OF THE GROWTH RATE OF VANNAMEI SHRIMP

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.08	3	11.70	110.36	.000
Within Groups	.85	8	.11		
Total	35.93	11			

Duncan advanced test showed that the growth rate in the substitution of tofu with vegetable waste 66.67 fermented significantly higher than those with other than test feed (Table 24).

TABLE XXIV DUNCAN'S FURTHER TEST RESULTS IN THE GROWTH RATE OF JUVENILE VANNAMEI SHRIMP Duncan^a

		Subset for $alpha = 0.05$			
Treatment	Ν	1	2	3	4
0% vegetable waste	3	9.88			
100% vegetable waste	3		11.41		
33.33% vegetable waste	3			13.51	
66.67% vegetable waste	3				14.19
Sig.		1.00	1.000	1.00	1.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Analysis of variance showed that substitution of tofu with vegetable waste fermented in the rumen fluid artificial feed on *juvenile vannamei* shrimp has a significant effect (p <0.05) (Table 25) on the survival of *juvenile vannamei* shrimp.

TABLE XXV THE RESULTS OF THE ANALYSIS OF VARIANCE (ANOVA) SURVIVAL OF JUVENILE VANNAMEI SHRIMP

results					
	Sum of		Mean		
	Squares	df	Square	F	Sig.
Between Groups	656.25	3	218.75	26.25	.000
Within Groups	66.67	8	8.33		
Total	722.92	11			

Duncan substitution further test tofu with fermented vegetable waste in *juvenile vannamei* shrimp feed showed that the survival rate of juvenile shrimp fed substitution *vannamei* tofu with fermented vegetable waste 66.67% significantly higher than those with another test feed (Table 26).

 TABLE XXVI

 DUNCAN'S FURTHER TEST RESULTS OF THE JUVENILE VANNAMEI SHRIMP

 Duncan^a

		Subset for $alpha = 0.05$		
Treatment	Ν	1	2	3
0% vegetable waste	3	78.33		
100% vegetable waste	3		91.67	
33.33% vegetable waste	3		93.33	
66.66% vegetable waste	3			98.33
Sig.		1.00	.50	.07

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

The high activity of the enzyme protease, amylase, and cellulase on vannamei shrimp fed substituted tofu with fermented vegetable waste 66.67% influenced by the concentration of the enzyme and substrate concentrations. Lokapirnasari, et. al. [15] reported that the bacterium Enterobacter cloacae WPL 214 isolated from rumen fluid cellulose produces an enzyme with cellulolytic enzyme activity endo-(1,4) - β -D-glucanase, exo-(1,4)- β -D-glukanase and β - glucosidase can reduce the fiber content of feed. Andriani [1] states that microorganism cow rumen contains cellulose and amylase enzyme sufficient to hydrolyze fish feed and Fitriliani [6] get bigger cellulose enzyme activity $(1.66 \pm 0.19 \text{ IU/mL/minute});$ amylase $(1.32 \pm 0.02 \text{ IU/mL})$ /minute); phytase (0.27 ± 0.13 IU/mL/minute); protease $(0.26 \pm 0.07 \text{ IU/mL/minute}); \ lipase \ (0.01 \pm 0.00$ IU/mL/minute) in the sheep rumen fluid. Protease enzyme capable of hydrolyzing proteins into *peptides*, further into amino acids necessary for amylase enzymes hydrolyze starch metabolism and helps digestion in organisms, and lipase hydrolyze function in fat [16]. Values of 0.1247-0.2885U protease enzyme activity/g/minute, and amylase 0,0638-0,1104U/g/minute with the treatment of juvenile vannamei shrimp cake tofu fermented using microorganisms Mix [17], this value is lower than the *protease enzyme* activity 0.183 to 0.340 U/mL/minute, *amylase* 0.077-0.291 U/mL/minute, and *cellulose* 0.043-0.265 U/mL/minute produced in this study, and higher in the results of studies reported by Masria et al. [18], that administration of cow rumen fluid to various *carbohydrates* in milkfish feed produces *protease enzymes* ranging from 0.071-0.113 U/mL/minute, amylase 0.172-0.214 U/mL/minute, and *cellulose* 0.262-1.584 U/mL/minute.

The concentration of the nutrient content of feed substrates such as proteins, fats, and carbohydrates. Results of the proximate analysis test feed showed that the higher the substitution of vegetable waste fermented pulp, and then the protein content, and content is relatively high its Nitrogen-Free Extract (NFE). The high concentration of the substrate is not followed by the high concentration of the enzyme that affects the activity of the enzyme [17]. Research results Rajkumar et al. [19] shows that the activity of digestive enzymes amylase and protease increased because of 25% fish meal replacement by *T. ornata* and *G. corticata*.

Value digestibility of carbohydrates, protein, crude fat obtained at the highest substituted *vannamei* shrimp feed tofu with fermented vegetable waste 66.67% respectively 93.75, 79.51, and 88.33%, while the value of the digestibility of carbohydrates, protein, and the suggested fat is 73.5% digestible carbohydrates, protein digestibility of 91.6%, 70.4% fat digestibility [20]. Furthermore, the value of protein and lipid digestibility in fermented plant products reported by Yang [21], respectively ranged from 87.89 to 93.18% and from 91.57 to 95.28%, digestibility value is higher than the value of digestibility proteins and lipids obtained in this study amounted to 60.26 to 79.51 and from 69.27 to 88.33%.

The high value of total digestibility, protein, fat, carbohydrates and fiber digestibility of feed produced on test substituted tofu with 66.67% of fermented vegetable waste in feed due to the activity of digestive enzymes in the treatment of vannamei shrimp is higher than other treatments. According to Lee and Lawrence [22] that the digestibility of crude protein shrimp is quite high if more than 80%. The highest protein digestibility value in the study of79.51%, this value is lower than 84% protein digestibility in juvenile vannamei shrimp produced [23]; and different in feed ingredients [24]. The value of the resulting total digestibility is 79.50%, the lowest in the digestibility total, protein, fat, carbohydrates and fiber digestibility in *juvenile* vannamei shrimp fed with 100% substitution of tofu and fermented vegetable waste 0%. It is influenced by the substitution of tofu. The higher the substitution of pulp in the feed, the lower the nutrient digestibility because tofu is not fermented so that the resulting low digestibility. This can be seen in the activity of digestive enzymes in the treatment vannamei shrimp is lower than other treatments. These results are consistent with those reported Zuliyan, et al, [25] that the energy digestibility of protein and relatively low in the treatment of substituted 40% and 0% fermented soy flour substitution of Lamtoro leaves compared with 10% and 30% soybean flour fermented Lamtoro leaves.

Feed digestibility value shows the ability of *vannamei* shrimp in digesting food, the quality of feed consumed and is supported by the activity of digestive enzymes. The

influence of feed substituted tofu with fermented vegetable waste against total digestibility, protein, crude fiber, carbohydrates and fat *vannamei* shrimp showed that with an increase in the composition of fermented vegetable waste in feed up to 66.67% can be digested well by the *vannamei* shrimp with the proviso that the feed is fermented using rumen fluid with a dose of 3%. Shiu, et al [26] stated that the maximum level of soybean flour replacement in shrimp feed was 37.42% while *Bacillus subtilis* fermented soy flour with a dose of 5 mL was 61.67%.

The highest feed efficiency value obtained in the test feed substituted tofu with fermented vegetable waste 66.67% amounting to 26.35%. Higher feed efficiency *vannamei* shrimp because it is influenced by the level of feed intake and digestibility of nutrients, it can be seen on the digestibility of nutrients was higher in the feed is compared to other treatments and is followed by the activity of digestive enzymes is also high, so the digestibility of feed is high, will increase feed efficiency. Feed substituted tofu with fermented vegetable waste 0% produced the lowest feed efficiency, due to the feed consumed cannot be digested properly due to the level of feed intake and digestibility of nutrients *vannamei* shrimp produced in such treatment.

IV. CONCLUSION

The rate of growth and the highest survival rate in feed substituted tofu with fermented vegetable waste 66.67%, this was due to feed quality, the ability of *juvenile vannamei* shrimp to consume and digest the feed. Feed quality evaluation and proximate result of feed amino acid test and the results of proximate and amino acid substitution test feed tofu with fermented vegetable waste 66.67% rumen fluid in accordance with the needs of *vannamei* shrimp. It can be seen from the total digestibility and nutrient *juvenile vannamei* shrimp in the treatment higher. This is in line with Jiang et al [27] states that the substitution of soybean meal with the fermented soybean residues in feed can improve the growth of juvenile largemouth bass, weight gain, specific growth rate.

Feeding on *juvenile vannamei* shrimp substituted tofu with waste vegetable fermented 0% is the lowest rise due to the pulp out not through a fermentation process, so it is difficult to digest, it can be seen from the results of the digestibility of nutrients in the treatment was lower than other treatments. The supplementation of dry fermentation biomass 100g/kg in the diet to lose weight (WG) *vannamei* shrimp 340.0% to 311.3% and increase the feed conversion ratio (FCR) of 1.79% to 1.61% due to palatability or nutritional imbalances feed [21]. Thus, the substitution of tofu with 66.67% rumen fluid fermented vegetable waste in feed can increase the activity of digestive enzymes, total digestibility, nutrient digestibility, growth and survival of *juvenile vannamei* shrimp.

ACKNOWLEDGMENTS

Acknowledgments to the Ministry of Research, Technology, and Higher Education for providing research funding for Fiscal Year 2018, so that this research can be completed, and thanks to the 2014 BPDN for providing educational scholarships, the University of Muhammadiyah Makassar that have provided opportunities to continue doctoral education.

REFERENCES

- Y. Andriani, "Assessment on Cow Rumen Fluid Celluloseamylase Enzyme Activity As An Alternative Source of Crude Fiber Degrading Enzyme in Fish Feed Materials," *Lucr. Stiințifice-Universitatea Științe Agric. şi Med. Vet. Ser. Zooteh.*, vol. 63, pp. 242–245, 2015.
- [2] Murni and Darmawati, "Optimize the use of Liquid Rumen in Fermentation Process on Increased the Nutrients Waste Vegetables For Tilapia'S Feed," *Int. J. Ocean. Oceanogr.*, vol. 10, no. 1, pp. 19– 28, 2016.
- [3] Murni, Darmawati, and M. I. Amri, "Optimasi Lama Waktu Fermentasi Limbah Sayur dengan Cairan Rumen terhadap Peningkatan Kandungan Nutrisi Pakan Ikan Nila (Oreochromis Niloticus)," *J. Ilmu Perikan. OCTOPUS*, vol. 6, pp. 541–545, 2017.
 [4] Murni, Haryati, S. Alamsyah, and H. Sonjaya, "The Nutrition Waste
- [4] Murni, Haryati, S. Alamsyah, and H. Sonjaya, "The Nutrition Waste Vegetables with Invitro Using Rumen Liquids for Feed," J. Food Nutr. Sci., vol. 6, no. 2, p. 58, 2018.
- [5] D. Jusadi, J. Ekasari, and A. Kurniansyah, "Improvement of cocoapod husk using sheep rumen liquor for tilapia diet," J. Akuakultur Indones., vol. 12, no. 1, pp. 40–47, 2014.
- [6] I. Fitriliyani, "Evaluation of the nutritional value of Leucaena leucophala leaf meal hydrolyzed by sheep rumen liquor enzyme extract on the growth performance of Nile tilapia (Oreochromis niloticus)," J. Akuakultur Indones., vol. 9, no. 1, pp. 30–37, 2010.
- [7] E. Listiowati and T. B. Pramono, "Potensi pemanfaatan daun singkong (Manihot utillisima) terfermentasi sebagai bahan pakan ikan nila (Oreochromis sp)," *Berk. Perikan. Terubuk*, vol. 42, no. 2, 2014.
- [8] L. R. D'Abramo, D. E. Conklin, D. M. Akiyama, I. W. G. on C. Nutrition, and W. A. Society, *Crustacean Nutrition*. World Aquaculture Society, 1997.
- [9] M. Takeuchi *et al.*, "Comparative study of the asparagine-linked sugar chains of human erythropoietins purified from urine and the culture medium of recombinant Chinese hamster ovary cells," *J. Biol. Chem.*, vol. 263, no. 8, pp. 3657–3663, 1988.
- [10] H.-Ui. Bergmeyer, Methods of Enzymatic Analysis, vol. 2. Elsevier Science, 2012.
- [11] P. Bernfeld, "[17] Amylases, α and β," 1955, pp. 149–158.
- [12] P. G. Dehaghani, M. J. Baboli, A. T. Moghadam, S. Ziaei-Nejad, and M. Pourfarhadi, "Effect of synbiotic dietary supplementation on survival, growth performance, and digestive enzyme activities of common carp (Cyprinus carpio) fingerlings," *Czech J. Anim. Sci.*, vol. 60, no. 5, pp. 224–232, 2015.
- [13] A. D. Eaton et al., Standard Methods for the Examination of Water & Wastewater, no. v. 21. American Public Health Association, 2005.
- [14] AOAC International, Official methods of analysis of AOAC International, 20th Editi. Rockville, Maryland, USA: AOAC International, 2016.
- [15] W. P. Lokapirnasari, D. S. Nazar, T. Nurhajati, K. Supranianondo, and A. B. Yulianto, "Production and assay of cellulolytic enzyme activity of Enterobacter cloacae WPL 214 isolated from bovine rumen fluid waste of Surabaya abbatoir, Indonesia.," *Vet. world*, vol. 8, no. 3, pp. 367–71, Mar. 2015.
- [16] Hamsah, "Growth Performance, Immune Response and Resistance shrimp larvae," Institut Pertanian Bogor, 2017.
- [17] S. Haryati, Aslamyah, and Surianti, "Influence of Tofu Dregs cake by using microorganisms Fermentation Mix on Digestive Enzyme Activity Juvenil Vanname shrimp," in *Symposium on Marine and Fisheries*, 2017.
- [18] A. Masria, S. Aslamyah, and Zainuddin, "Effect of Rumen Fluid Cows at Various Levels Carbohydrates in Feed Enzyme Activity Gastrointestinal against milkfish Chanos Chanos-Forsskal," in Symposium on Marine and Fisheries All 4.
- [19] G. Rajkumar, P. S. Bhavan, V. Srinivasan, R. Udayasuriyan, M. Karthik, and T. Satgurunathan, "Partial Replacement of Fishmeal with Marine Algae Turbinaria ornata and Gracilaria corticata for Sustainable Culture of the Freshwater Prawn Macrobrachium rosenbergii," *Int. J. Res. Stud. Zool*, vol. 3, no. 2, pp. 32–44, 2017.
- [20] D. M. Akiyama, "Penaeid shrimp nutrition for the commercial feed industry: Revised," in *Proceeding of the aquaculture feed processing* and nutrition workshop, 1991, pp. 80–98.

- [21] Q. Yang, X. Zhou, Q. Zhou, B. Tan, S. Chi, and X. Dong, "Apparent digestibility of selected feed ingredients for white shrimp Litopenaeus vannamei, Boone," *Aquac. Res.*, vol. 41, no. 1, pp. 78– 86, Dec. 2009.
- [22] P. G. Lee and A. L. Lawrence, "Digestibility," *Crustac. Nutr.*, vol. 6, pp. 194–260, 1997.
- [23] M. Terrazas-Fierro, R. Civera-Cerecedo, L. Ibarra-Martínez, E. Goytortúa-Bores, M. Herrera-Andrade, and A. Reyes-Becerra, "Apparent digestibility of dry matter, protein, and essential amino acid in marine feedstuffs for juvenile whiteleg shrimp Litopenaeus vannamei," *Aquaculture*, vol. 308, no. 3–4, pp. 166–173, Oct. 2010.
- [24] D. A. Villarreal-Cavazos, "Apparent digestibility of dry matter, crude protein, and amino acids of six rendered by-products in juvenile Litopenaeus vannamei," *Ciencias Mar.*, vol. 40, no. 3, pp. 163–172, Sep. 2014.
- [25] B. Zuliyan, A. Agustono, and W. H. Satyantini, "Pengaruh Subtitusi Kedelai dengan Fermentasi Tepung Daun Lamtoro pada Pakan Udang Vaname (Litopenaeus Vannamei) terhadap Nilai Kecernaan Protein dan Kecernaan Energi," J. Aquac. Fish Heal., vol. 6, no. 3, p. 129, Jan. 2019.
- [26] Y.-L. Shiu, S.-L. Wong, W.-C. Guei, Y.-C. Shin, and C.-H. Liu, "Increase in the plant protein ratio in the diet of white shrimp, Litopenaeus vannamei (Boone), using Bacillus subtilis E20fermented soybean meal as a replacement," *Aquac. Res.*, vol. 46, no. 2, pp. 382–394, Feb. 2015.
 [27] Y. Jiang, P.-F. Zhao, S.-M. Lin, R.-J. Tang, Y.-J. Chen, and L. Luo,
- [27] Y. Jiang, P.-F. Zhao, S.-M. Lin, R.-J. Tang, Y.-J. Chen, and L. Luo, "Partial substitution of soybean meal with fermented soybean residue in diets for juvenile largemouth bass, Micropterus salmoides," *Aquac. Nutr.*, vol. 24, no. 4, pp. 1213–1222, Aug. 2018.