The Substitution of soybean meal by fermented tofu dregs in the milkfish (Chanos chanos) diet

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Abstract. This study aims to investigate the effect of fermented tofu dregs as feed raw material to substitute soybean flour in the milkfish (Chanos chanos) formulated feed. The research was conducted from April to May 2019 at the Ujung Batee Brackish Water Aquaculture Center, Mesjid Raya District, Aceh Besar Indonesia. A total of 200 milkfish (Chanos chanos) fry from 1-2 cm total length were randomly put in a volume of 25 liters per container. Fish were fed at 3% body weight daily to apparent satiation twice a day (9:00 and 16:00) for 40 days. A completely randomized design (CRD) with 5 treatments and 4 replications was used with different fermented dregs tofu substitution rate ie, 0% (A), 5% (B), 10% (C), 15% (D) and 20% (E). ANOVA test results showed that the substitution of soybean flour with different doses of fermented dregs flour showed significant different (P<0.05) in absolute weight gain, daily growth rate, specific growth rate, feed conversion rate and feed utilization rate. However there was no significant difference (P>0.05) in the survival rate of the fish in all the treatments. It is concluded that the recommended concentration of fermented dregs tofu added into milkfish (Chanos chanos) diet was 10%.

1. Introduction
Milkfish (Chanos chanos) is one of the potential culture fish in Indonesia due to high market demand. Milkfish is a fish that has high nutritional value, relatively cheap price and delicious meat taste. According to [1], the national production of milkfish has increased from 518,939 tons in 2012 to 667,116 tons in 2013. However, the availability of milkfish in the market is still not fulfilled. This is due to the amount of production that has not been maximized.

Milkfish production has not been maximized due to several factors, like low availability of fish seeds and expensive feed prices. Feed is a very important aspect in milkfish culture because it absorbs 60-70% of the total operational costs. The availability of nutritious ingredient is very important for fish [2, 3]. One of the important nutrients needed by fish is protein [4, 25]. Protein in feed can be obtained from animal and plant based protein [5].

Tofu dreg is one of the organic wastes that can be recycled. However, if not utilized, the tofu waste will become waste and pollute the environment. Utilization of tofu dregs can be used as raw material for feed. However, it has a low protein content, high moisture content and crude fiber so that its use is limited and has not yet given maximum results.

To improve the quality of tofu dreg, fermentation using microbe Aspergillus niger method is commonly done [6]. Utilization of fermented tofu dregs by 10% in catfish feed significantly improved specific growth rate and increased fish weight from 11.43% / gram to 27.4% / gram during 45 days of
rearing [6]. However, there is no study of using fermented tofu dreg in milkfish (Chanos chanos) diet. Therefore, this study aims to investigate the effect of fermented tofu dregs as feed raw material to substitute soybean flour in the milkfish (Chanos chanos) formulated feed.

2. Materials and Methods
2.1. Experimental design
This study used a completely randomized design (CRD) with 5 treatments and 4 replications each. 
- Treatment A = addition of 0% tofu dregs fermentation
- Treatment B = addition of 5% tofu dregs fermentation
- Treatment C = addition of 10% tofu dregs fermentation
- Treatment D = addition of 15% tofu pulp fermentation
- Treatment E = addition of 20% tofu dregs fermentation

2.2. Research procedure
Fermented tofu dregs
Tofu dregs fermentation was done using previous method [6]. A 100 g of Tofu dreg and tapioca flour were mixed with a ratio of 75% tofu dregs and 25% tapioca flour, mixed with 70% water. After steaming for 30 minutes, then it cooled and incubated using Aspergillus niger as much as 9 mL / 100 grams of the material then incubated at room temperature aerobically for 4 days and anaerobically for 3 days.

Test fish
The fish used in this study were 1-2 cm in size. Milkfish bought from the Brackish Water Cultivation Fishery Center (BPBAP) Ujung Batee, Mesjid Raya District, Aceh Besar. The container used was a tank with a volume of 25 liters with a density of milkfish that spread in one container, namely 10 fish / tank. Feeding was given 2 times a day in the morning and evening. Fish rearing was carried out for 40 days.

Feed Formulation Test
The feed formulation test as a treatment was the percentage substitution of fermented tofu dregs flour to soybean flour with details of fermented tofu dregs feed rations are feed A 0%, feed B 5%, feed C 10%, feed D 15% and feed E 20%.

| Feed Materials | Feed A (%) | Feed B (%) | Feed C (%) | Feed D (%) | Feed E (%) |
|----------------|------------|------------|------------|------------|------------|
| Fish meal      | 20         | 20         | 20         | 20         | 20         |
| Soybean meal   | 38         | 33         | 28         | 23         | 19         |
| Fermented tofu dregs | 0         | 5          | 10         | 15         | 20         |
| Bran           | 13         | 13         | 14         | 13         | 13         |
| Tapioca        | 11         | 11         | 11         | 8          | 7,5        |
| Cornstarch     | 10         | 10         | 9          | 13         | 12,5       |
| Fish oil       | 5          | 5          | 5          | 5          | 5          |
| Premix         | 3          | 3          | 3          | 3          | 3          |
| **total**      | **100**    | **100**    | **100**    | **100**    | **100**    |

2.3 Research Parameters
Weight Gain
We calculated the weight gain using the formula [5, 7] as follows:

\[ \Delta G = W_t - W_0 \]
Information:
\[ \Delta G = \text{weight gain (g)} \]
\[ Wt = \text{Weight of fish at end of the experiment (g)} \]
\[ Wo = \text{fish weight at the beginning of the experiment (g)} \]

**Specific Growth Rate (SGR)**

The specific growth rate is the percentage of the difference in final weight and initial weight divided by the length of maintenance time. The specific growth rate was calculated \[3, 5, 8\]:

\[ SGR = \frac{\ln Wt - \ln Wo}{T} \times 100\% \]

**Daily Growth Rate**

The formula used to determine the daily length growth rate was calculated based \[10\]:

\[ DGR = \frac{Wt - Wo}{t} \]

**Survival (SR)**

Survival rate was calculated using the formula \[8, 9, 11, 27\]:

\[ SR = \frac{N_t}{N_0} \times 100\% \]

**Feed Conversion Rate (FCR)**

Calculation of the feed conversion ratio using the formula \[9; 10, 19\]

\[ FCR = \frac{F}{Wt - Wo} \]

2.4 **Water quality**

Water quality parameters measured during the study were temperature, pH, DO, and water quality measurements were carried out every 10 days. One way ANOVA was used for data analysis. The significance of the difference among the means was analyzed by Duncan’s multiple ranges \[12\]. The statistics software of SPSS version 22 was utilized. Tabular form was used to display data.

3. **Results and Discussions**

ANOVA test results showed that the different dosage of fermented tofu dregs significantly affect the growth of milkfish (Chanos chanos) \(P < 0.05\). Fish yield was obtained in treatment C (10% tofu dreg fermentation) (Table 2). The feed conversion ratio (FCR) for milkfish (Chanos chanos) ranged from
1.52 to 3.97, the feed utilization efficiency ranged from 25.3% to 65.6% and the survival rate ranged from 82.5% to 95.0%. Based on Table 2, there was no significant effect on survival rate (P > 0.05).

**Table 2.** The growth status of milkfish fed 5 experimental diets. Means in a column with different letters were significantly different (P < 0.05).

| Concentration% | Absolute weight gain (g) | Daily Growth Rate (g / day) | Specific Growth Rate (% / day) | Feed conversion ratio | Feed utilization efficiency (%) | Survival rate (SR) (%) |
|----------------|--------------------------|-----------------------------|--------------------------------|-----------------------|---------------------------------|----------------------|
| 0              | 0.12 ± 0.005<sup>c</sup> | 0.003 ± 0.00009<sup>c</sup> | 2.86 ± 0.06<sup>c</sup>       | 1.94 ± 0.07<sup>b</sup> | 51.6 ± 2.00<sup>d</sup>         | 82.5 ± 9.5           |
| 5              | 0.09 ± 0.009<sup>b</sup> | 0.002 ± 0.0002<sup>b</sup>  | 2.29 ± 0.16<sup>b</sup>       | 2.77 ± 0.30<sup>c</sup> | 36.3 ± 3.98<sup>e</sup>         | 85.0 ± 12.9          |
| 10             | 0.16 ± 0.005<sup>d</sup> | 0.004 ± 0.0001<sup>d</sup>  | 3.28 ± 0.05<sup>d</sup>       | 1.52 ± 0.04<sup>a</sup> | 65.6 ± 1.99<sup>e</sup>         | 92.5 ± 9.5           |
| 15             | 0.06 ± 0.006<sup>e</sup> | 0.001 ± 0.0001<sup>e</sup>  | 1.79 ± 0.12<sup>a</sup>       | 3.97 ± 0.38<sup>e</sup> | 25.3 ± 2.43<sup>a</sup>         | 95.0 ± 5.7           |
| 20             | 0.07 ± 0.006<sup>ab</sup> | 0.001 ± 0.0001<sup>ab</sup>| 2.04 ± 0.13<sup>b</sup>       | 3.28 ± 0.32<sup>d</sup> | 30.6 ± 2.88<sup>ab</sup>        | 90.0 ± 8.1           |

Based on the proximate test, it was found that the protein content of tofu dregs fermentation ranged from 18.42 to 22.32% (table 3). The best protein was obtained in treatment C with 10% tofu dregs fermentation. While the lowest protein was obtained in treatment E with 20% fermented tofu dreg (table 3).

**Table 3.** Proximate test feed test used in the study

| No. | Treatment% | Protein% |
|-----|------------|----------|
| 1   | A (tofu dregs 0%) | 22.17 %  |
| 2   | B (tofu dregs 5%)  | 20.72 %  |
| 3   | C (tofu dregs 10%) | 22.32 %  |
| 4   | D (15% tofu dregs) | 19.32 %  |
| 5   | E (tofu dregs 20%) | 18.42 %  |

Based on the results, fermented tofu dregs in fish feed had a significant effect on the absolute weight gain of milkfish, it can be seen that the growth of milkfish has increased during experiment (table 2). The highest absolute weight growth of milkfish was found in the 10% treatment of tofu dregs fermentation with an absolute weight gain of 0.16 grams, while 15% treatment of tofu dregs fermentation was the lowest weight gain of 0.06 grams. Fish weight gain is largely determined by the protein content in the feed. It can be seen that the highest weight growth was obtained in treatment C. The results of the proximate test showed that treatment C had the highest protein content (22.32%) compared to other treatments.

Fermented tofu dregs have better nutritional value than unfermented tofu dregs. The fermentation process has caused a decrease in water content, this is due to changes in complex compounds into simple compounds. The dry matter of the media was broken down by *Aspergillus niger* into energy for growth and some of it is released into CO<sub>2</sub> and H<sub>2</sub>O [6]. Daily and specific growth rates in milkfish also increased over time (table 2). The highest daily growth rate in milkfish was in treatment C with 10% tofu dregs fermentation with a daily growth rate of 0.004 grams / day and the lowest growth rate in treatment D with 15% tofu dregs fermentation with a daily growth rate of 0.001 gram / day. The specific growth rate in the best milkfish fish was in the C treatment with 10% tofu dregs fermentation with a specific growth rate of 3.28% / day and the lowest in the D treatment with 15% tofu dregs fermentation with a weight of 1.79% / day, the growth at milkfish has increased every week. This showed that the addition of 10% tofu dregs fermentation in milkfish feed is more optimal than the feed without the addition of tofu dreg and feed with the addition of 5%, 15% and 20% tofu dregs.

The feed efficiency in this study showed the best feed efficiency, namely the treatment of 10% tofu dregs fermentation, namely 65.6% and the lowest was 15% tofu dregs treatment, namely 25.3%. Provision of feed with the addition of fermented tofu dreg has a significant effect on feed efficiency (P <0.05). This study showed that feed efficiency was better than the research by [13] where the addition
of coconut dregs flour to milkfish feed obtained the best feed efficiency in 0% coconut dregs treatment, namely 41.76% and the lowest in commercial pellet treatment with the addition of 30% coconut dregs (35.83%). Feed efficiency showed that the feed value can change in increasing fish weight, feed efficiency can be seen from several factors, one of which is the feed conversion ratio [14].

The best feed conversion ratio was in treatment C which was 1.52. Feeding with the addition of tofu dregs fermentation had a significant effect on feed conversion ratio (P <0.05). Research by [15] the addition of phytase enzymes in milkmfish-made feed showed a better feed conversion ratio with the lowest feed conversion ratio in treatment C (enzyme phytase 1000 mg / kg feed) of 0.69 ± 0.02 and the highest was in treatment A (without the addition of phytase enzymes) of 1.11 ± 0.08. In addition to the application of fermented tofu in milkfish, other studies on efforts to increase growth performance and immunity in aquaculture organisms have been carried out. Among them are the application of astaxanthin to improve the color quality of Green Swordtail fish, Xiphophorus helleri [16], the application of Sargassum oligocystum extract to the immunity of vaname shrimp [17], application of probiotics of yeast and lactic acid bacteria to tiger prawns [18], as well as the use of herbal plants against viral disease of grouper and its detection method [19, 20, 21].

Water quality parameters in milkfish culture were still in the tolerance range for milkfish. The parameters observed were temperature, pH and DO. The temperature at the time of the study ranged between 28-30°C, and the pH at the time of the study was 7.7-2. The optimal temperature for milkfish cultivation is 27-30°C [22, 23]. The DO ranged from 3.9 to 4 mg/l, according to [24] several types of fish were able to survive in waters with a concentration of 3 mg/l, but dissolved oxygen which is good for fish life is 5 mg/l. The pH at the time of the study was 7.7-2, according to [15, 26] the standard pH for milkfish ranged from 7.0 to 8.5.

**Conclusion**
The use of fermented tofu dregs in milkfish feed showed a significant effect on absolute weight growth, specific growth rate, daily growth rate, feed efficiency, and feed conversion ratio. However, it has no significant effect on survival rate. The optimal addition of tofu dregs in the feed in this study was 10%.

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