Effect of probiotic duration and dose of coffee peel fermentation (*Coffea* sp.) on crude protein and crude fiber as an alternative fish feed ingredient

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Abstract. The research aimed to find out whether there was interaction between fermentation duration and probiotics dose toward the increase in crude protein and decrease in crude fiber content in coffee peel. This research was experimental using complete randomized design (RAL) factorial, data analysis using duncan. The result of the research showed that there was interaction between duration and probiotics doses in coffee peel fermentation to crude protein in day 5 of fermentation duration with probiotics dose of 5%, it was the highest result of crude protein content. There was no interaction between duration fermentation and probiotics dose in fermentation of coffee peel toward crude fiber content in day 7 duration of fermentation with probiotics dose of 5%, it was the result of the lowest crude fiber. Fermentation in 7 days with dose of 5% was the best result. There was increase of protein from 12.15% to 13.93% and decrease of fiber from 34.27% to 30.8%.

1. Introduction
Feed is one of the important factors in the business of aquaculture because the availability of feed will affect the growth and survival of the cultivated fish, therefore good feed is food that must be given according to the nutritional needs of fish. Food nutrients requirements in fish generally contain 20-60% protein, 4-18% fat, 10-15% carbohydrate, crude fiber no more than 8%, and ash content in feed maximum 15%[1], however, crude fiber in feed that can be tolerated by fish, in general, is 8-12% [2]. Various types of feed ingredients can be used as alternative feed ingredients, that is ingredients which are easily available and do not compare with humans, for example, coffee peel.

Coffee peel (*Coffea* sp.) is a waste from coffee plantations which has a dry weight of 91.77%, crude protein 11.18%, crude fiber 21.74%, crude fat 2.85%, and BETN 50.8%. Moreover, coffee peel also has anti-nutrients in the form of tannin, caffeine, and lignin which can disturb or interfere with digestion [3]. The nutrient content of coffee peel needs to be improved to become good quality feed in a biological way using cellulolytic bacteria [4] by fermentation using probiotics, but the fermentation process is affected by duration and dose [5].

2. Materials and methods
2.1 Materials
The research materials used were coffee peel, probiotics, molasses and aquadest. Robusta coffee peel geted from the farming business group of Dwi Tunggal Pasuruan. Kali Pucang Village, Tutur, Pasuruan, East Java Indonesia.

2.2 Method

2.2.1 Methodology
This research began by taking the coffee peel obtained from farming business group of Dwi Tunggal Pasuruan, East Java. Afterward, the coffee peel were washed and dried it using an oven at temperature $\pm 60^\circ$ on 2 days, then grinding it. The coffee peel was weighed amounted to 100 grams. The next treatment is by adding probiotics and molasses in accordance with the dosage determined in the treatment. The probiotic and molasses are mixed in aquadest ad 30% of the dried ingredients of the sample A mixture of probiotics, molasses and aquadest is sprayed on the coffee peel evenly. All materials that have been mixed evenly in each treatment are put into a plastic bag and tied in anaerobic condition and later the sample is placed into basin for storage. The fermentations process is done for 5 and 7 days, after the fermentation is completed, the plastic knot is opened and aerated to stop the fermentation process, further the proximate analysis of crude protein and crude fiber and after that data analysis is conducted.

2.2.2 Parameter
The main testing parameters of this research were crude protein content and crude fiber content which was fermented with probiotics in different fermentation duration and probiotics doses.

2.2.3 Data analysis
The data obtained were analyzed using factorial ANOVA and if there were significant differences, then Duncan’s Multiple Range test [6]

3. Results and discussion

3.1 Crude protein
Data from the calculation of the proximate analysis of coffee peel that fermented with probiotics at different lengths of duration, based on the average results there were no significant differences $(p>0.05)$ between treatments, both on day 5 of fermentation and on day 7 of fermentation. Based on the average treatment, 5% dose was not significantly different $(p>0.05)$ with a 1% dose and 3% dose, but it is significantly different $(p<0.05)$ with a 0% dose. Data on the average protein content of coffee peel during treatment can be seen in table 1.

| Probiotic Dose | Duration of fermentation |
|---------------|-------------------------|
|               | H₁ (5)                  | H₂ (7)                  |
| P₀ (0%)       | 12.46$±$ 0.07           | 13.3$±$ 0.58            |
| P₁ (1%)       | 13.68$±$ 0.22           | 14.27$±$ 0.27           |
| P₂ (3%)       | 13.66$±$ 0.28           | 13.99$±$ 0.25           |
| P₃ (5%)       | 14.41$±$ 0.68           | 13.93$±$ 0.21           |

Note: Different superscripts in the same column show significant differences $(p<0.05)$. 
Based on the results of Variant Analysis (ANOVA), there was an effect of duration and dosage of crude protein content in the coffee peel, but it showed no interaction. The 7 day with a 5% probiotic dose can be used as the best duration and dosage on the crude protein content of coffee peel, because at this duration and dose, it was not significantly different (p > 0.05) both with 1% and 3% dose treatment at fermentation duration of 5 and 7 days.

3.2 Crude fiber
Data from the calculation of the proximate analysis of coffee peel that fermented with probiotics at different lengths of duration, based on the average results there were significant differences (p < 0.05) between treatments, both on day 5 of fermentation and on day 7 of fermentation. Based on the average treatment 3% and 5% dose, there were no significant differences (p > 0.05), but it is significantly different (p < 0.05) with 0% and 1% dose. Data on the average crude fiber content of coffee peel during treatment can be seen in table 2.

| Probiotic Dose | Duration of fermentation | H₁ (5) | H₂ (7) |
|---------------|-------------------------|--------|--------|
| P₀ (0%)       | 38.99±0.81              | 33.96±0.6 |
| P₁ (1%)       | 37.10±1.02              | 32.39±0.44 |
| P₂ (3%)       | 34.88±1.5               | 31.23±1.15 |
| P₃ (5%)       | 35.44±1.34              | 30.80±0.42 |

Note: Different superscripts in the same column show significant differences (p<0.05).

Based on the results of ANOVA, there was an interaction effect between the length of duration and different doses of crude fiber content, but there was no interaction. The period of 7 days with a dose of 5% can be used as the best duration and dose on the crude fiber content of coffee peel because this duration and dose has the lowest value, although not significantly different (p > 0.05) with a period of 7 days with a dose of 3%.

3.3. Discussion
Feed nutrition needs must be available according to the needs of fish in their growth, such as the protein content in the feed required by fish as the main energy source for fish [7], as well as the content of crude fiber which can inhibit the digestibility of fish, so that by decreasing crude fiber content in feed ingredients, the quality of the ingredients is getting better, because the low value of the content of crude fiber in the feed will increase the digestibility coefficient of feed ingredients [8].

The increasing of protein content in coffee peel fermentation is caused by the large number of microbes in probiotics, in which the microbes are able to break down feed ingredients from complexes to be simpler like amino acids, so the microbes can be used to multiply themselves. Moreover, in the process fermentation, there is the addition of molasses which can also help increase crude protein content because molasses is a source of carbohydrates containing amino acids which are often used as a source of energy for bacteria.

Anaerobic fermentation can increase the crude protein content added with molasses because of anaerobic microbial activity that grows and develops by utilizing energy sources into microbial cells that are rich in protein content [9]. This can be proven from the results of the treatment of 0% for 5 days and 7 days of fermentation which increases even without the provision of probiotics, while the decreasing of crude protein content such as on the 7 day of fermentation with 3% and 5% probiotic doses is caused by the lack of food sources which cause the bacteria die and no longer produces enzymes. Besides, it can also be caused by too much dose, which causes a microbial competition that causes microbes to die and not produce enzymes in carrying out the fermentation process.
The decrease in crude fiber content in this study was due to the role of cellulolytic bacteria found in these probiotics (*Bacillus* sp., *Celullomonas* spp., *Actynomyces* spp., and *Enterobacter* sp.). Cellulolytic bacteria are bacteria that produce cellulose enzymes that are capable of hydrolyzing cellulose as the main constituent of crude fiber in feed into simple forms such as glucose which is used as a carbon source as well as an energy source for bacteria. This will make crude fiber in the feed material decrease [10]. The advantage of fermentation using cellulolytic bacteria is that cellulolytic bacteria have a faster growth rate compared to other microbial groups, so the time needed for enzyme production is faster [11].

4. Conclusion
There is an interaction between the length of duration and the dose of probiotics on coffee peel (*Coffea* sp.) fermentation on crude protein content and on 5 day with a probiotic dose of 5% is the best result on crude protein content. There is no interaction between the length of duration and the probiotic dose in coffee peel (*Coffea* sp.) fermentation on crude fiber content and on 7 day with a probiotic dose of 5% is the best result in crude fiber content.

5. References
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