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# Prebiotics$^{BAS}$ (*Bacillus sp.*, *Aspergillus n.*, and *Sacharomyces c.*) as Feed Supplement on Nutrients and its Effects on Digestibility Value of Fish Feed

Kiki HAETAMI[1], Abun[2], Yuniar MULYANI[1]

[1]Faculty of Fisheries and Marine Science, Padjadjaran University, Jalan Raya Bandung-Sumedang KM. 21, 45363, Indonesia, (phone) +6222 7798241, (fax) +6222 7798212.
[2]Faculty of Animal Husbandry, Padjadjaran University, Jalan Raya Bandung-Sumedang KM. 21, 45363, Sumedang-West Java, Indonesia, (phone) +6222 7798241, (fax) +6222 7798212.
Email: kiki.haetami@gmail.com

***Abstract***— *Feed quality shown from level of digestibility can affect fish growth.Some of omnivorous fish have complete digestive organs as a place to live abiotic and biotic ecosystems in the form of living microflora. Growth performance can be improved through the addition of exogenous microflora as feed supplements to help produce simpler components of food substances (amino acids, fatty acids, simple sugars, organic vitamins and minerals). The microflora tested consisted of bacteria Bacillus sp. and fungi (Aspergillus niger and Saccharomycescereviseae) with optimization of its prebiotic bioprocess conditions (bioprocess temperature, inoculum dose, and bioprocess time). Furthermore, to see the quality and value of benefits of feed supplement products, measurements were taken of their digestibility.The experiment was carried out experimentally in a laboratory in two stages. The first stage, using a nested design (3×3) which was repeated three times. The second stage used a completely randomized design, consisting of six ration treatments and repeated four times. The variables observed in the first stage: nutrient content (crude protein, crude fibre, extract ether, calcium and phosphorus) of prebiotics$^{BAS}$; second stage: digestibility of dry matter and crude protein.The data were subjected to analysis of variance, and the differences between treatments were tested by Duncan's multiple range test.Conclussion: The following results were obtained the best bioprocess conditions for making Prebiotics$^{BAS}$ from Bacillus sp. was a dose of 2% with temperature of 45°C, and fermentation time 2 days, while Aspergillus niger 2% at a temperature of 35°Calong 2 days, and Saccharomyces cereviseae 2% with a temperature of 35°C, and fermentation time 2 days. The use of a mixture of three types of microbial each a much 1.5% in the ration, resulting in the best digestibility value in fish. The dry matter and crude protein digestibility valueof Prebiotics$^{BAS}$ were respectively 76.07%, and 75.28%.*

***Keywords— BAS (Bacillus sp. Aspergillus niger, Saccharomyces cereviseae), digestibility of fish feed,optimization of bioprocess, Prebiotics.***

## I. INTRODUCTION

Fish growth is influenced by feed quality, because it has a simple digestive tract which is a small tube that extends. The performance of the digestive tract can be increased through the addition of exogenous microflora as feed supplements (prebiotics) to help increase digestibility and feed efficiency. The mechanism of prebiotics that is quite beneficial is that it can stimulate add enzymes related to the digestive process of complex substances or enzymes that are not present in the digestive tract; and synthesize essential substances that are not enough in quantity from food [3,6]. The prebiotics tested consisted product of bacteria (*Bacillus sp.*), and fungi (*Aspergillus niger*and *Saccharomyces cereviseae*), and their mixtures. The combination of these cultures is expected to be able to support each other (synergism) in excellence and cover up each other's shortcomings, so as to improve the performance of microflora that live in the intestines of fish, which in turn can increase the digestibility of food substances [7, 9]. To get quality feed additives,optimization of Prebiotic bioprocess conditions (inoculum, duration, and bioprocess temperature) was carried out. Furthermore, to see the quality and value of benefits of feed additives products, measurements were taken of their digestibility.

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## II. MATERIALS AND METHODS

**Producing Prebiotics$^{BAS}$(Optimization of Bioprocess Conditions)**

The first stage of the experiment was to obtain product optimization, namely: Inoculum doses of *Bacillus sp*., *Aspergillus niger* and *Saccharomycescereviseae*, long and bioprocess temperatures that produce the best nutritional content.Fermented media (shrimp skin, rice flour and molasses), *Bacillus sp*. bacteria, molds of *Aspergillus niger*, yeast *Saccharomyces cerevisiae*, nutrient agar and standard mineral solutions. Other ingredients used were distilled water, glucose, yeast extract, technical glucose, tryptone, NaCl, NaOH, azo-casein reagent, borate buffer, phosphate buffer, citrate buffer, bicarbonate buffer, TCA, oxygen gas and Bovine Serum Albumin. The tools used are stainless jars (reactors), water heaters, autoshakerbath, autoclaves, goblets, Bunsen burners, petri dishes, porcelain cups, centrifuges, funnels, pH-meter Knick, spectrophotometer, test tubes, furnaces, HPLC, and grinding machines.

**Stages of Making the Prebiotics**

Make a starter inoculum by culturing microbes in 125 ml Erlenmeyer containing 50 ml of sterile Luria broth, pH 7, incubated in an incubator (2 days at 30-35$^{o}$C), and the number of colonies is calculated using the Total Plate Count (TPC) method (minimum number of colonies is 109 per ml or per g).Bioprocess media (shrimp skin, rice flour, and molasses, 0.5% (b/v) yeast extract; 0.5% (b/v) $KH_2PO_4$; 0.1%(b/v) $CaCl_2$; 0,5% (b/v) NaCl, and 0.05% (b/v) $MgSO_4$ was inoculated by *Bacillus sp*., *Aspergillus niger*, and *Saccharomyces cereviseae*, and a standard mineral solution was added.Bioprocess in auto-shakerbath (temperature 25$^{o}$C; 35$^{o}$C; 45$^{o}$C, dose 1%; 2%; 3%, time 1 day, 2 days, 3 days for each treatment.

**Experimental Design**

Experiments used a completely randomized design (7×3) for each process condition for each microbe used. From the combination of treatments, the variables he observed were; nutritional content of the product (crude protein, extract ether, crude fiber,calcium, and phosphorus. The selected treatment was used for the second phase of the study.

*Table.1: Composition of Ration and Nutrient Content (%)*

| Treatments of Ration | CP | EE | CF | Ca | P |
|---|---|---|---|---|---|
| | ……..……..……..…….. % | | | | |
| R0 (Basal of ration) | 30,02 | 6,90 | 7,56 | 1,51 | 0,87 |
| R1 (97% R0 + 3% FSPB) | 30,06 | 6,83 | 7,59 | 1,59 | 0,91 |
| R2 (97% R0 + 3% FSPA) | 30,00 | 6,84 | 7,56 | 1,58 | 0,90 |
| R3 (97% R0 + 3% FSPS) | 29,98 | 6,85 | 7,56 | 1,58 | 0,90 |
| R4 (97% R0 + 1,5% FSPB+1,5 FSPA) | 30,03 | 6,83 | 7,57 | 1,59 | 0,91 |
| R5 (97% R0 + 1,5% FSPB+1,5 FSPS) | 30,02 | 6,84 | 7,58 | 1,58 | 0,90 |
| R6 (97% R0 + 1,5% FSPA+1,5 FSPS) | 29,99 | 6,84 | 7,56 | 1,58 | 0,90 |
| R7 (97% R0 + 1% FSPA+1 FSPS+ 1% FSPS) | 30,02 | 6,84 | 7,57 | 1,58 | 0,90 |

FSP = feed supplement of prebiotics (B : *Bacillus sp*.; A : *Aspergillus niger*; S : *Saccharomyces cerevisiae*).

**Second Phase Experiment (Determination of Digestibility Value)**

**Materials and Tools**

Tested fish: 240 red tilapia fish with 200 ± 10 g body weight.

The tools used in this study are: 1m$^3$ volume of fiber-blower tubes, aerator,thermometer analytical scales O-thirst scales, pH meters and spectrophotometers "Milton Roy Spectron, gloves, wipes, tweezers, threads, and scalpels, ovens and aluminum foil, pellet printing machine, and installation of lignin testers and protein testing installations the Kjehdahl method.

**The experiment was carried out in three stages**

Adaptation stage to familiarize fish with the test feed and estimate the length of feed in the digestive tract which is indicated by the initial discharge of feces, and determine the frequency of feeding.

collection of feces (2 weeks): Feed is given ad libitum, on the last day of the study fish were dissected and feces were taken.Stool analysis phase, which includes: fresh weight, dry weight, and oven dryness, protein analysis and feed lignin content.

**The variables observed**

Consumption of dry ingredients and ration lignin (grams); drymatter and faecal lignin (gram) and calculated [10, 13] formula as follows:

$$\text{Digestion coefficient} = 100\% - 100\left(\frac{\%\ ligninfeed}{\%\ ligninfeces} \times \frac{\%nutrientsfeces}{\%nutrientsfeed}\right)$$

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The experiments were performed in the laboratory using acomplete randomized design, consisted of 8 treatments of Prebiotic each of which was repeated four times. The data obtained were analyse byVariance (Test F) and differences between treatments were tested by Duncan Test.

## III. RESULTS AND DISCUSSION

### Nutritional Contents of Prebiotics$^{BAS}$

Observations on the bioprocess temperature conditions were carried out at the specified time and dose, i.e. for 2 days at a dose of 2%. While the observation of the dose and time is carried out at the selected temperature. The results of the Analysis Variance showed that various levels of temperature, dose, and time had significant (P <0.05) content of crude protein, crude fat, crude fibre, calcium and phosphorus both in *Bacillus sp*., *Aspergillus niger*and *Saccharomyces cerevisiae* products. To find out how much difference in effect between treatments, Duncan's multiple distance test was carried out which results can be seen in Table 2, Table 3, and Table 4.

*Table.2: Effect of Bioprocess Temperature on Product Nutritional Content*

| Microbes | Doses | Nutrients | | | | |
|---|---|---|---|---|---|---|
| | | CP | EE | CF | Ca | P |
| | | ……..(%)…….. | | | | |
| *Bacillus sp.* | S1 | 27.59 A | 5.04 B | 9.33 A | 3.88 A | 1.69 A |
| | S2 | 28.09 A | 4.76 AB | 9.00 B | 4.05 AB | 1.83 A |
| | S3 | 30.91 B | 4.63 A | 8.74 B | 4.16 B | 2.09 B |
| *Aspergilus niger* | S1 | 27.89 A | 5.07 B | 9.07 A | 3.70 A | 1.56 A |
| | S2 | 29.19 B | 4.85 AB | 8.14 B | 4.04 B | 1.91 B |
| | S3 | 29.23 B | 4.70 A | 7.93 B | 4.08 B | 1.97 B |
| *Sacharomyces cerevisiae* | S1 | 27.76 A | 5.20 B | 8.22 A | 3.67 A | 1.70 A |
| | S2 | 28.48 A | 5.06 AB | 8.16 A | 3.74 A | 1.86 B |
| | S3 | 28.52 A | 4.89 A | 7.99 A | 3.83 A | 1.88 B |

S1 = 25ºC; S2= 35ºC ; S3 = 45ºC

Table 2 shows that the increasing treatment temperature tends to increase protein, calcium and phosphorus products, both in bioprocess *Bacillus sp*., *Aspergillus niger* and *Saccharomyces cerevisiae*. This is supported by the opinion of [15], that protein and mineral content of bioprocess Prebiotics microbiologically will experience an increase in line with the increase in temperature to some extent. The use of 45 $^0$C (S3) temperature in the Prebiotic bioprocess of *Bacillus sp*. significantly affected the highest crude protein (30.91%) compared to the temperature (25$^0$C) S1 and 35$^0$C (S2). This shows that *Bacillus sp*. is more effective at working on substrate at a temperature of 45 $^0$C. The results of this study are in line with opinion [8], that *Bacillus sp*. is thermophilic and has a maximum growth temperature of 50-55$^0$C. The Effect of Temperature on Bioprocess *Bacillus sp*. and *Aspergillus niger* on changes in other nutritional composition, are: a decrease in crude fibre content, an increase in fat content, and an increase in calcium levels which produce at a temperature of 45 $^0$C produce the greatest changes. Bioprocess of *Aspergillus niger* can be done at 35ºC. Whereas in the Prebiotic S. cerevisiae bioprocess, all three temperature treatments did not show significant differences in protein, crude fibre and calcium content. According [5], *Aspergillus niger* fungi grow well in the temperature range of 32-33ºC, with a pH of 2.8-8.8 and humidity of 80-90%. Whereas *Bacillus sp*. has a maximum growth temperature of 50-55ºC, and *Saccharomyces cereviseae* can grow at room temperature [8]. However, these three temperature treatments can change the substrate into a product whose nutritional content is better. Observation of the 2condition of bioprocess dosage was carried out at the selected temperature, namely *Bacillus sp*. 45ºC, *Aspergillus niger* 35ºC and*S. cereviseae* 25ºC; with bioprocess for 2 days. The number of microbes planted determines bioprocess products. The dosage level of the inoculum and time is related to the size of the microbial population that has the opportunity to determine the speed of microbial development in producing enzymes to remodel the substrate, which in turn affects the final product. In this study, D1 turned out to produce the lowest nutritional content, meaning that the inoculated microbial population was not enough to be used to remodel the substrate to its full potential. From the results of this study almost in total doses of 2% inoculum (D2) produced a nutritional content that was not significantly different (P <0.05) with a dose of 3% inoculum (D3), although the crude fibre content of the product feeds on Prebiotic supplements at lower D3. D2 is an effective inoculum dose to produce optimal crude protein content of Prebiotic$^{BAS}$ products. In accordance with the opinion [17], that the number of

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microbes that are too much can cause sporulation that is too fast so that some of the energy is not used to multiply cells, and vice versa, the number of microbes that are too few causes optimal growth.

*Table.3: Effect of Bioprocess Dose on Nutritional Content Product*

| Microbes | Doses | Nutrients | | | | |
|---|---|---|---|---|---|---|
| | | CP | EE | CF | Ca | P |
| | | ……..……..……..……..……..(%)……..…. | | | | |
| *Bacillus sp.* | D1 | 27.74 A | 5.00 B | 9.33 A | 3.88 A | 1.54 A |
| | D2 | 31.19 B | 4.27 AB | 8.84 AB | 4.23 B | 1.91 B |
| | D3 | 30.98 B | 4.13 A | 8.66 B | 4.22 B | 2.11 B |
| *Aspergilus niger* | D1 | 27.70 A | 5.08 B | 8.77 A | 3.61 A | 1.57 A |
| | D2 | 29.36 B | 4.63 AB | 7.12 B | 4.17 B | 1.86 B |
| | D3 | 29.54 B | 4.51 A | 6.90 B | 4.22 B | 1.97 B |
| *Sacharomyces cerevisiae* | D1 | 26.72 A | 5.26 B | 8.25 A | 3.66 A | 1.62 A |
| | D2 | 28.65 B | 5.14 AB | 7.13 B | 3.89 B | 1.79 AB |
| | D3 | 28.39 B | 4.96 A | 7.14 B | 3.99 C | 1.96 B |

D1 = 1%; D2= 2% ; D3 = 3%

Observation of the condition of the time when bioprocess was carried out at the specified temperature and dosage, i.e. for each *Bacillus sp*. bacterium 45ºC,*A.niger* 35ºC and *S. cerevisiae* 25ºC; with a dose of 2%. Table 4 shows that time has a significant effect on increasing the nutritional content of the prebiotic products of the three types of microbes (bacteria and fungi). Bioprocess time 1 day (W1) produces the lowest protein, calcium and phosphorus. The size of the three nutrients can show the quality of nutrients in terms of chemistry. Similarly, W1has the highest crude fibre content, which means that the crude fibre component in the substrate has not been optimally converted into simple sugars. Whereas bioprocess time 2 and 3 days did not show a significant difference in nutrient content. As with other bioprocess conditions (inoculum dose level), the length of time the microbiological fermentation process is related to the size of the microbial population that has the opportunity to determine the speed of microbial development in producing enzymes to remodel the substrate so that in turn affects the nutritional content of the final product. Table 4. Duncan's Multiple Distance Test Effect of Bioprocess Time on Product Nutritional Content.

*Table.4: Duncan's Multiple Distance Test Effect of Bioprocess Time on Product Nutritional Content*

| Microbes | Time | Nutrients | | | | |
|---|---|---|---|---|---|---|
| | | CP | EE | CF | Ca | P |
| | | ……..……..……..……..(%)……..…. | | | | |
| *Bacillus sp.* | W1 | 26.77 A | 4.72 B | 9.22 A | 3.81 A | 1.64 A |
| | W2 | 31.59 C | 4.24 A | 8.35 B | 4.29 B | 2.14 B |
| | W3 | 28.18 B | 4.17 A | 8.26 B | 4.11 B | 1.92 AB |
| *Aspergilus niger* | W1 | 26.97 A | 5.13 B | 8.74 A | 3.74 A | 1.65 A |
| | W2 | 29.47 B | 4.53 A | 6.93 B | 4.10 B | 2.08 B |
| | W3 | 30.44 B | 4.47 A | 6.80 B | 4.31 B | 2.05 B |
| *Sacharomyces cerevisiae* | W1 | 27.08 A | 5.38 A | 8.74 A | 3.59 A | 1.62 A |
| | W2 | 29.64 B | 5.22 A | 7.43 B | 4.13 B | 1.95 B |
| | W3 | 29.17 B | 5.05 A | 7.20 B | 3.90 AB | 2.01 B |

W1 = 25 ºC; W2= 35 ºC ; W3 = 45 ºC

Sum of colonies andNutrient content, Before andAfter Bioprocess.

*Table.5: Nutrients content of Substrate and PrebioticsBAS Product of Bioprocess.*

| No | | Sum of colonies | CP | EE | CF | Ca | P |
|---|---|---|---|---|---|---|---|
| | | × $10^9$CFU | ……..……..(%)……..…. | | | | |
| 1 | Initial Bioprocess | 4,01-4,42 | 22,19 | 5,91 | 12,82 | 3,41 | 1,44 |
| 2 | Product *Bacillus sp.* | 15,22 | 31,23 | 4,38 | 8,64 | 4,22 | 2,05 |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3 | Product *A.niger* | 11,92 | 29,34 | 4,67 | 7,40 | 4,10 | 1,95 |
| 4 | Product *S.cerevisiae* | 10,66 | 28,68 | 5,18 | 7,59 | 3,90 | 1,77 |

In Table 5 it appears that there is a change in the composition of the bioprocess substrate in the manufacture of Prebiotic products. This is in line with the opinion [14], which states that in bioprocess there will be changes in complex molecules or organic compounds such as proteins, carbohydrates and fats into simpler molecules. The protein content of Prebiotic B products contains the highest protein because *Bacillus sp*. is a species of bacteria that is able to produce relatively high amounts of protease [8], and multiply rapidly so that it becomes the microbial protein (from 12.82% to 7.4%). According [12], *Aspergillusniger* is one of the fungi that is reported to be capable of producing cellulase enzymes. Cellulase derived from *Aspergillus niger* is in the form of a cellulase complex and is capable of being produced in sufficient quantities. Fish use protein as an energy source compared to other types of livestock, and tend to be less able to utilize carbohydrate sources, especially those with high crudefibres[2]. The use of microbes has changed the composition of the substrate to be more qualified as a process of digesting "outside the body" and a source of microbial enzymes. *Aspergillus niger* produces cellulase enzymes which can degrade cellulose (a component of crude fibre) into glucose (a source of energy for fish) as well asS. cerevisiae can work to break down starch to be simpler.

**Digestibility of Feed Supplements**

Supplements on Digestion Bioprocess results were selected in stage 1, used as supplement feed (supplementary feed) containing Prebiotics.Results of biological tested for effectiveness through measurement of digestibility value at tilapia fish, can be seen in Table 6.

*Table.6: The Effect of Feed Supplement Prebiotics BAS on Digestibility Value of Dry Matter and Protein.*

| RationTreatments | Digestibility Value | |
|---|---|---|
| | DM Dig. | Protein Dig. |
| | ..... (%)..... | |
| R0 (Basal Ration; without prebiotic) | 65,83 E | 64,17 F |
| R1 (97% R0 + 3% Prebiotic$^{B}$) | 70,11 D | 69,10 D |
| R2 (97% R0 + 3% Prebiotic$^{A}$) | 70,16 D | 68,82 DE |
| R3 (97% R0 + 3% Prebiotic$^{S}$) | 69,11 D | 67,68 E |
| R4 (97% R0 + 1,5%Prebiotic$^{B}$ + 1,5% Prebiotic$^{A}$) | 74,52B | 73,64 B |
| R5 (97% R0 + 1,5%Prebiotic$^{B}$ + 1,5% Prebiotic$^{S}$) | 74,07 B | 72,34 C |
| R6 (97% R0 + 1,5%Prebiotic$^{A}$ + 1,5% Prebiotic$^{S}$) | 72,35 C | 71,21 C |
| R7 (97% R0 + 1% Prebiotic$^{B}$+ Prebiotic$^{A}$ +1% Prebiotic$^{S}$ | 76,10 A | 75,39 A |

Table 6 shows that the value of digestibility of dry matter and crude protein in treatment R0 was lower ($p<0.05$) compared to the treatment of rations containing feeds of Prebiotic supplements. The low digestibility value in the R0 treatment was caused by the fact that rations without using supplement feeds were not sufficiently supportive to improve the performance of the digestive tract, even though the ration contained protein that was in accordance with the nutritional needs of red tilapia. Especially in R0 as well as other treatment rations containing crude fibre that exceeds the 4% tolerance limit according [16], so that in the absence of Prebiotics as a source of exogenous enzymes and intestinal microflora balancer, it does not support the effectiveness of the digestive tract.

High digestibility with R3, while R2 shows no significant difference with R3 and R1 treatments. *Bacillus sp*. is proteolytic so it helps digest protein [11], so that it can help protein digestibility more than other microbes. Protein digestibility which contains a combination of 1.5% Prebiotics and 1.5% Prebiotics (R4) was significantly higher than the combination of two other types of Prebiotics. This is because *Bacillus sp*. is a protein remover, *Aspergillus niger* is a rough fibre remover so that both are synergistic. Decrease in crude fibre content will have an impact on the digestibility value, which in turn will also affect digestibility. In line with the opinion [18], which states that crude fibre is one of the food substances that affect digestibility. The use of feed supplement combination of the three types of bacterial bioprocess, mold and yeast products resulted in the highest dry matter digestibility and protein digestibility compared to a combination of two types of Prebiotics, and differed significantly when compared with the use of one type of prebiotics. This can be understood because *Bacillus sp*. is proteolytic so it helps digest protein [11], *A. niger* is cellulolytic and amylolytic [19], so it helps to degrade carbohydrates; whereas *S. cerevisiae* is amylolytic and stimulates appetite [1, 19].

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The combination of these cultures is expected to be mutually supportive (synergism) in excellence and cover up for each other's deficiencies, because according [4]Prebiotics can improve the performance of living microflora and intestinal ecosystems in fish intestines, which in turn can increase the digestibility of nutrients.

## IV. CONCLUSIONS AND SUGGESTIONS

### Conclusions

1) The temperature of $45^0$C in the Prebiotic bioprocess *Bacillus sp*. is the best bioprocess condition to increase the protein content of the product. While the Prebiotic*Aspergillus niger* can be carried out at temperatures of $35^0$C and $45^0$C. Preparation of *Saccharomyces cerevisiae*Prebiotics can be carried out at a temperature of 25-$45^0$C.
2) The effective dose in making Prebiotics$^{BAS}$is 2%, with bioprocess time for two days. Bioprocess feed supplement The Prebiotics$^{BAS}$product produces an increase in the number of colonies and nutrient content of the substrate. The initial substrate protein content is 22.19%; and the bioprocess results obtained protein content of Prebiotic$^{B}$ products of 31.23% higher thanPrebiotic$^{A}$(29.34%); and Prebiotic$^{S}$(28.68%).
3) The use of a mixture of three types of microbes (bacteria, and yeast) from Prebiotics$^{BAS}$ Products can increase the value of dry matter digestibility and crude protein digestibility of basal rations (without using Prebiotics$^{BAS}$ supplement feed). Value of dry matter digestibility and crude protein basal ration, that is equal to 65.83% and 64.17%; each increased to 76.10% and 75.39%.

### Suggestions

Further research is needed to determine the effectiveness of the use of Prebiotics$^{BAS}$ as feed supplements to growth, feed conversion, composition of intestinal microflora and feed efficiency through experiments on feeding red tilapia fish starting from the seed stage.

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