The utilization of sludge of dairy wastewater as an additive to increase protein and iron levels in fish

[1]Widya Pangestika, [2]Sugili Putra

[1] The Department of Fisheries Product Processing, Politeknik Kelautan dan Perikanan Pangandaran, 46396 Pangandaran, Indonesia; [2] The Department of Nuclear Chemical Engineering, Sekolah Tinggi Teknologi Nuklir, 55281 Yogyakarta, Indonesia. Corresponding author: W. Pangestika, widya.pangestika@kkp.go.id

Abstract. Sludge of dairy wastewater could be used as an additive in fish feed formulas for containing high iron. This iron was obtained from the coagulant of ferric chloride ($FeCl_3$) which was used to precipitate liquid waste from dairy plant. There were two feed formulas studied in this research, namely: feed containing 0.085% iron (feed B), and feed containing 1.703% iron (feed C), while commercial feed (feed A) was used as a comparison. After 4 weeks feeding trial, the biggest increase in fish weight was found in Nile tilapia (*Oreochromis niloticus*) that was fed by feed A. In addition, the specific growth rate SGR) of Nile tilapia fed with feed C increased more rapidly than other feeds, which was 1.219% d^{-1} from 0.466 % d^{-1} at the previous week. The iron-richest feed (feed C) was able to boost the iron content in Nile tilapia, which was 45.12 mg Fe kg of $fish^{-1}$. On the other hand, the protein in the feed did not really affect the protein content in Nile tilapia. The results have been proved that sludge of dairy wastewater could be used as fish feed.

Key Words: fish feed, fish weight, iron, protein, SGR.

Introduction. The high cost of fish feed makes many fish farmers complain and reduce the benefit of their businesses. Many researchers have tried to solve their problems by studying how to utilize wastes to make fish feed. Hartami & Rusydi (2016) studied the utilization of tofu by product to make fish feed. The usage of organic waste to make fish pellets was also studied by Kusumanto & Hidayat (2018), while Nazullawaty (2013) had done research about the utilization of dairy wastewater to make fish feed.

Dairy wastewater as a side product of dairy plant could be utilized into something useful for living things, such as fish feed (Nazullawaty 2013). It is a new breakthrough that is very beneficial to the industry. Dairy wastewater has to be precipitated before added to fish feed formulation. Chemical precipitation has been used these days by many wastewater treatment plants. Coagulants that used to process the wastewater could be Poly Aluminium Chloride (PAC), aluminium salts, or ferric salts (Nurhasni et al 2013; Loloei et al 2014).

We used ferric chloride as the coagulant since it has been specified by the dairy plant. According to Dabhi (2013), by using ferric salts in wastewater treatment could reduce the energy consumption of biology proccesses at the final step and remove pollutants in the wastewater more efficiently. Ferric chloride is used because it will ionize into a cation. This cation will react with hydroxide ions and settle into insoluble metal hydroxide solids. Then, it will attract colloids in the wastewater to settle (Nurhasni et al 2013).

The way to measure specific growth rate (SGR) of Nile tilapia (*Oreochromis niloticus*) while fed by artificial fed has been done by Lawal et al (2013) and Abd El-Maksoud et al (1999). The rise in protein content occurred in Nile tilapia after being fed from fermented sludge of dairy wastewater had been proved by Nazullawaty (2013). From the results of her studies, it was found that the protein content of Nile tilapia increased after fed with the addition of their feed. The highest increase in protein content in Nile tilapia was 18.51%.

Fish feed that made from dairy wastewater has a long term goal if it consumed by human. Fish proteins are considered as high nutritional food for human because of some excellent amino acid and easily digestible proteins in healthy diet (Tilami & Sampels 2017). Iron in fish feed could increase the haemoglobin, ferritin, transferrin, and plasma iron. It was also said that high level of iron supplement could enhance more adaptive immunity for fish (Hosseini et al 2019). In addition, fish that has high value of iron would prevent anemia in human. Therefore in this study, our focus will be narrowed to sludge of dairy wastewater that could be utilized into fish feed.

Material and Method. This study was held in Laboratory of Chemical Engineering Operation at Sekolah Tinggi Teknologi Nuklir during 4 months, started on March 2015 until July 2015. The processes comprised fish feed production, feeding and measurement of Nile tilapia body weight, testing of protein levels in Nile tilapia, and testing of iron levels in Nile tilapia.

Fish feed production. Sludge of dairy wastewater, rebon flour, starch, and fermented wheat bran were mixed according to the results of the feed formulation in Table 1 and then stirred using the mixer evenly. Then, vitamin C and the pith of cassava stem were added into the B and C feed mixture. The dough then formed into small pellet which matched the size of the fish's mouth. After being formed into pellets, the feed was dried in an oven at 60^oC.

Feeding and measurement of tilapia body weight. Nile tilapias were available in 3 different ponds. Each pond was consisted of 17 fish with an average mass of 94-100 g. At the beginning of the trial, fish in each pond were starved for 24 h and weighed. Three types of pellets used in this feeding trial, namely: commercial pellets (feed A), pellets containing iron 0.085% (B feed) and pellets enriched by iron 1.703% (feed C) were prepared. Each feed was given to fish as much as 3% of total weight of fish. Pond A was prepared to Nile tilapia that fed by feed A while pond B was intended to Nile tilapia that fed by feed B. Nile tilapia in pond C was fed by combination of feed C and feed A as informed below. Feed A and feed B were given to Nile tilapia four times a day for four weeks. The way of feeding fish with feed C was quite different to other variations. Feed C was given once in two days during feeding trial, besides that time, Nile tilapia was also fed by commercial feed (feed A). We could say that feed given in pond C was a combination among feed C and feed A. Then, fish were weighed every week during feeding trial period. Besides the weight, we also measured the specific growth (SGR) of Nile tilapia by using the formula below (Schram et al 2009):

$$SGR = \frac{(\ln W_t - \ln W_o)}{t} \times 100$$

where: SGR = specific growth rate;
W_t = the final average weight of tilapia (g);
W_o = the initial average weight of tilapia (g);
t = the feeding trial time (d).

Testing of protein levels in Nile tilapia. One Nile tilapia was taken randomly from each pond once in a week. Before being tested for protein level, it was weighed, then we hit the fish's head with a wooden until it died. The dead fish was cleaned up on water, and after that it was filletted with a special knife. About 0.51 g of fish fillet sample was put into a 100 mL Kjeldahl flask, then 2 g of selen mixture catalyst was added to the flask. Sulphate acid solution was also added as much as 25 mL. The mixture was heated above the electric heater until the color of the solution became clear greenish. The solution was allowed to cool, then diluted with distilled water to 100 mL. After that, as much as 5 mL of the solution, 30% NaOH, and a few drops of the PP indicator were added to the distiller. The mixture was distilled for approximately 10 minutes. We used 2% boric acid solution which had been mixed with indicator to catch the distillate. The mixture solution was titrated with 0.1 N HCl solution. The blank titration was also done. The protein levels were obtained by using the formula below (Sinaga et al 2016):

$$\text{Protein level} = \frac{(V_1 - V_2) \times N \times 0.014 \times f_k \times f_p}{B} \times 100$$

where: B = the weight of the sample before it was ignited (g);
V_1 = the volume of 0.01 N HCl used for sample titration (mL);
V_2 = the volume of 0.01 N HCl which is used for the blank titration (mL);
N = the normality of HCl;
fk = protein conversion factor from food in general (6.25);
fp = dilution factor.

Testing of iron levels in tilapia. As much as 1 g (dry weight) of fish samples were put into the furnace and the temperature was increased slowly to 500^oC. The ignition was carried out for 10 hours. Ten (10) mL of 1 N HCl was added to the ash. The dissolving ash was carried out by heating it on a hot plate. The sample was cooled and filtered. Then, it was diluted to 100 mL by using distilled water. The 1000 ppm iron standard solution was diluted gradually to 5 different concentrations, namely: 0 ppm, 2 ppm, 5 ppm, 8 ppm and 10 ppm. The iron addition solution was made by diluting 100 ppm Fe to 5 ppm Fe. After that, 1 mL sample was added to iron addition solution and was diluted with distilled water. The Atomic Absorption Spectrophometer (AAS) unit was activated. Measurement of blank absorbance was carried out through three times suction. Absorbance measurements were carried out for 5 variations of standard solutions, until the calibration curve was obtained. In addition, the measurements of sample solution absorbance were carried out through the same step as the blank.

Results and Discussions

Proximate analysis results in fish feeds. Nutrients, such as protein and fat must be fulfilled for fish growth. Table 1 indicates that feed B and feed C had a low-fat content. The fat content in feed B was 2.108% while the fat content in feed C was 3.509%. The level of fat in these feeds did not match to standard stated by the government (SNI 01-7242-2006). According to the standard, the minimum level of fat content in fish feed for fish enlargement should be 8% (SNI 2006).

Based on the measurement results by using AAS, iron content in commercial fish feed was 320 mg kg $feed^{-1}$, which was equal to 0.032%. It resembles the statement of Lall (2000) that commercial fish feed usually contains iron amount to 150-800 mg kg $feed^{-1}$.

Table 1

Proximate analysis results

Parameter	*Feed A*	*Feed B*	*Feed C*
Crude protein (%)	26-28	30.043	32.057
Crude fat (%)	3-5	2.108	3.509
Iron level (%)	0.032	0.085	1.703

Nile tilapia's weight. Pond system was good for semi-intensive aquaculture because the toxicity of the pond could be maintained and reduced (Dauda et al 2019). We used pond system in feeding trial to prevent unexpected variables from occurring. In this study, the amount of feed given was as much as 3% of the total weight of the fish in every day. According to Chowdhury (2011), at a weight of 80-115 g, the effective feeding of tilapia is as much as 3% of the total fish weight in a day. We can see from Table 1 that feed B has a protein about 30.043%, while feed C had protein as much as 32.057%. This is suitable to Bolorunduro (2002) statement that the amount of protein needed by juvenile or adult tilapia should be between 30 and 35%. Jishun et al (1998) added that the protein needed for fish that weighs more than 40 g was 35%. The weight of tilapia after being fed by feed A, feed B, and feed C could be seen in Table 2.

Table 2

The average of Nile tilapia's weight during feeding trial period

Week	*The average of Nile tilapia's weight (g)*		
	A	*B*	*C*
0	94.65±13.84	97.18±13.71	100.29±15.19
1	100.53±16.19	95.44±14.43	110.71±22.62
2	105.94±17.86	96.73±15.48	110.06±18.49
3	109.64±20.26	98.77±15.97	113.71±21.81
4	116.23±23.07	100.58±17.45	123.85±24.22

To make it easier to be understood, we put the data in Table 2 to a graph. Figure 1 compares Nile tilapia's weight when was fed by 3 different feeds. It shows an upward trend for all types of feed.

We can see that the weight of Nile tilapia that was fed by feed A had risen steadily. Meanwhile, it climbed sharply when fed by feed C and Nile tilapia fed by this kind of feed had the highest weight increase of all feed types. Feed A was still looked better in increasing fish weight than feed B. In comparison with feed A and feed C, fish which was fed by feed B had weight decrease in the first week due to the fish's adaptation to a new feed.

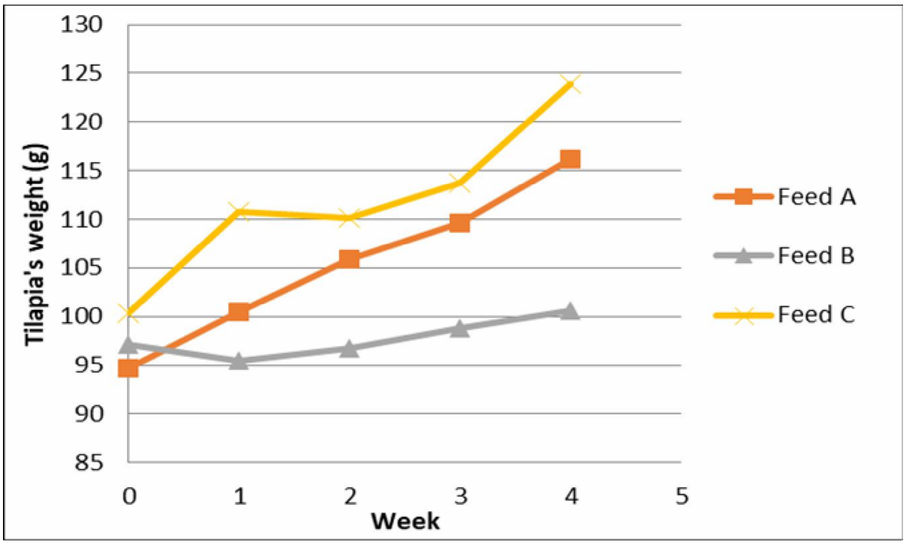

Figure 1. Nile tilapia's weight during feeding trial period.

Specific growth rate (SGR) of tilapia. From Figure 2, we know that the line graphs of SGR which was affected from 3 types of feed are quite fluctuated. Fish fed by feed A gave a stable rise of SGR. This feed was proven could increase SGR from 0.491 to 0.834 % d^{-1} in the last week of feeding trial. Meanwhile, fish fed by feed B was not growing better than when fed by feed A. It increased significantly at the initial week, but dropped at the final week. Feed C had the biggest effect for fish growth. At the fourth week, SGR of Nile tilapia at feed C had reached the highest value of all. It was almost two times bigger than feed A growth effect. However, in the second week, there was no rapid change in fish growth when fed by feed C. It was appeared because fish were still adapting to the new feed that they have consumed.

If we see the data in Table 3 accurately, we could find that there is an increase of SGR in each pond but, there were also declines of SGR in weeks 2 for Nile tilapia fed by feed C and feed A. These might be occurred because the fish used their energy to spawn.

Suyanto (2010) stated that fish would spawn at a weight of 100-150 g. Fish would grow fast if they had excess energy besides the energy that was spent for spawning and other activitites. When fish were spawning, they preferred to be at the bottom of the pond, so they would not eat the feed that floated on the surface. It made the amount of fish intake fell, and the growth rates declined. In addition, when Nile tilapia was prepared to lay eggs, it began to stop to eat. The spawning process took place in Nile tilapia's mouth because Nile tilapia is a mouth breeder so, they would keep the eggs in their mouths until they hatch.

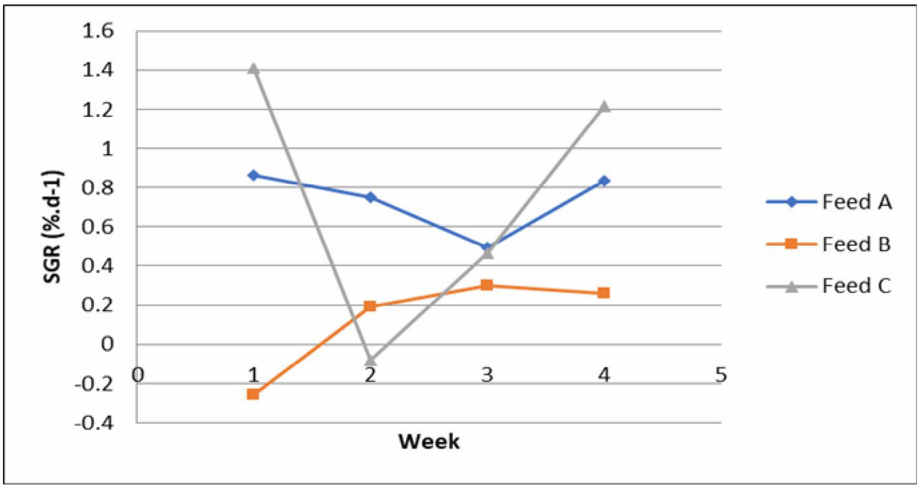

Figure 2. Specific growth rate of Nile tilapia (SGR) during feeding trial period.

Table 3

The specific growth rate of Nile tilapia

Week	*The specific growth rate of Nile tilapia (% d^{-1})*		
	A	*B*	*C*
0	-	-	-
1	0.861	-0.258	1.411
2	0.749	0.193	-0.083
3	0.491	0.298	0.466
4	0.834	0.260	1.219

Protein levels in Nile tilapia. From Figure 3, we can see that the protein levels are so fluctuative. These might be appeared because not all digested nutrients were used by fish to sustain their lives. This was in accordance to the opinion of Dani et al (2005) that not all proteins were converted to meat. In addition, the formation of protein in Nile tilapia also relied on the physiological ability of the fish.

This slight change of protein content inside Nile tilapia was also might be apppeared because the fat level in feed B and feed C did not meet the quality standards for Nile tilapia's intensive cultivation. We know that fat act as an energy source for fish. If the feed lacked fat, protein would be used to substitute its function as nutrients for fish growth and energy source. It made the protein that should be stored in the body declined sharply. This fact was consistent to the opinion of Fujaya (2004), that fish converted digested feed into a protein to fulfill its needs. So, if the energy requirements were not fulfilled, the digestive system would break down the protein from digested feed to become an energy source. Nwanna et al (2014) also had the same opinion that 25-35% dietary protein in fish feed retarded the protein efficiency ratio in fish.

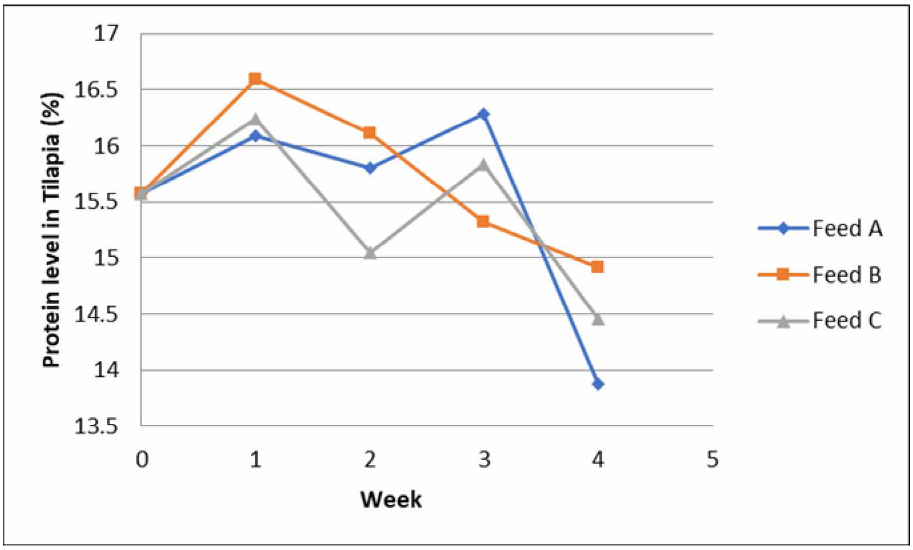

Figure 3. Protein level in Nile tilapia during feeding trial period.

Iron levels in Nile tilapia. The line graph of Nile tilapia fed by feed C in Figure 4 expresses an upward trend, while the graph of feed A and feed B rise considerably during feeding trial time.

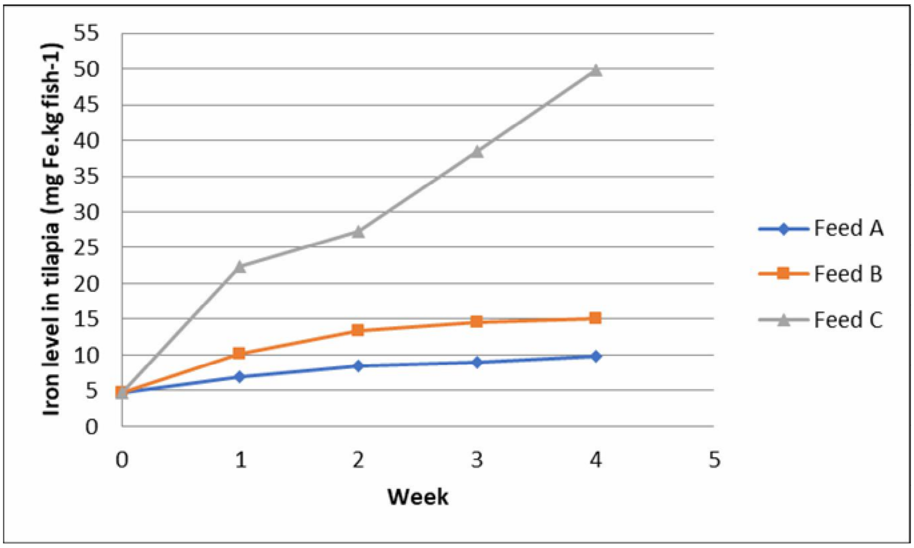

Figure 4. Iron level in Nile tilapia during feeding trial period.

Table 4 gives us information that feed B, which was consisted of 0.085% iron, did not give a rapid increase of iron level inside the Nile tilapia. Actually, the iron level of fish that was fed by feed B was higher than feed A, but it was smaller than feed C.

Table 4

Iron level and survival rate of Nile tilapia

Week	*Iron level in Nile tilapia (mg Fe kg$_{-1}$ fish)*			*Survival rate of tilapia (%)*
	Feed A	*Feed B*	*Feed C*	
0	4.657	4.657	4.657	100
1	6.898	10.089	22.420	100
2	8.486	13.457	27.298	100
3	9.050	14.607	38.424	100
4	9.798	15.031	49.777	100

Another feed that was made in this research was feed C. It was enriched with iron as much as 17030 mg kg feed^{-1}. Feed C were given like a supplement to Nile tilapia, with a frequency of feeding once in 2 days. It was proved that feeding Nile tilapia with feed C could increase iron level in Nile tilapia dramatically. The increased iron level in Nile tilapia could reach 45.12 mg Fe kg fish^{-1} from the first week to the last week of feeding trial.

Although the iron level in feed C was very high, the data in Table 4 illustrate that by giving the feed once in two days could provide a 100% survival rate of Nile tilapia. This fact was occurred because not all iron in the digestive feed were stored completely in the fish. The frequency of feeding with feed C gave time for the fish's body to release the excess iron in the body obviously. It caused the accumulation of iron inside Nile tilapia not be too large, so the fish was not poisoned with iron because they could regulate essential metals (Darmono 1995). Another theory stated that the amount of heavy metal concentration possessed by aquatic biota was influenced by the ability of aquatic biota to absorb and excrete metals in the waters (Supriyanto et al 2001).

Nile tilapia could be dead if the water, which is fish's habitat, contained iron in an excessive amount. Fish could reach LC_{95} if the water contained 128.5 mg L^{-1} total iron and the pH was 6.5; 92.8 mg L^{-1} of total iron at pH 7.0; and 136.8 mg L^{-1} of total iron with a pH that reached 8.5 (Mukhopadhyay & Konar 1984). Toxicity of fish due to exposure to excessive amounts of iron in the water was caused directly by iron deposition which could clog the respiratory system in fish gills (Phippen et al 2008). Fish fed by feed C had iron content as much as 49.777 mg kg fish^{-1} inside. It still had not outcome the iron content in broiler liver and domestic chicken liver, which reached to 71.6 mg kg^{-1} and 249.4 mg kg^{-1} respectively (Simbolon et al 2012).

Conclusions. Sludge of dairy wastewater consisted of high iron level. It could be utilized as fish feed rather than being thrown away. We tested the fish feed that we had made to Nile tilapia in 3 different ponds. After 4 weeks feeding trial, we knew that protein levels in fish feed did not affect protein levels inside Nile tilapia significantly. On the other hand, iron levels in fish feed could increase iron levels in Nile tilapia. Feed enriched with 1.703% iron (feed C) experienced the highest increase in iron content inside Nile tilapia, which was amount to 45.12 mg Fe kg fish^{-1}. Meanwhile, fish fed by feed containing 0.085% iron (feed B) and commercial feed (feed A) had the iron levels uplifted to 10.374 mg Fe kg fish^{-1} and 5.141 mg Fe kg fish^{-1}, respectively.

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Authors:
Widya Pangestika, The Department of Fisheries Product Processing, Politeknik Kelautan dan Perikanan Pangandaran, 46396 Pangandaran, Indonesia, e-mail: widya.pangestika@kkp.go.id
Sugili Putra, The Department of Nuclear Chemical Engineering, Sekolah Tinggi Teknologi Nuklir, 55281 Yogyakarta, Indonesia, e-mail: sugili@batan.go.id

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