Red Tilapia (Oreochromis Niloticus) Farming in Thailand Using a Split-plot Experiment Design to Improve Feeding Frequency and Feed Intake: Weight Gain and Profit Return

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Abstract

There is still relatively little research examining the feeding frequency and feed intake optimization for culturing Red Tilapia (oreochromis niloticus), especially in Thailand. Meanwhile, Red Tilapia (Oreochromis niloticus or pomegranate fish) is a high-quality meat fish that is crucial to the economy's ability to generate sustained income. Thus, by using a split plot experiment design, this study examined the adequacy of food quantities and feeding frequency for rearing red tilapia for 4- to 5-month-old fish in terms of fish weight and profit. It used a case study of the Red Tilapia farming in the Ping River, Tak Province, involving the use of floating baskets (Oreochromis niloticus). In normal operation, feeding occurs three times daily, and each 20 fish consumes an average of 25 grams of food per feeding. In the experimental design, feeding volume levels were established at 15, 25, and 35 grams each meal, while feeding frequency levels were set at 2, 3, and 4 times per day. The data were examined between 0 and 30 days. It was discovered that Weight increase in Red Tilapia was significantly influenced by feeding frequency and food intake. However, there was no difference in the weight gain of Red Tilapia under the effect of offshore and nearshore trails. In addition, feeding Red Tilapia four times a day with a feed quantity of 35 grams each meal led to the greatest weight increase, but not the greatest profit. With a feed intake of 15 grams each portion and a feeding frequency of four times a day, the optimal conditions for maximizing profit were attained and applied by entrepreneurs. Future research would benefit from an experiment involving a broader range of ages.

Keywords: Oreochromis niloticus, Culturing red tilapia, Feed intake, Feeding frequency, Split plot experiment design

Introduction

Red Tilapia (Oreochromis niloticus or pomegranate fish), produced by Thai-Chitralada strains of Nile Tilapia under the initiative of Charoen Pokphand Group Company Limited, is a high-quality meat fish that is crucial to the economy's ability to generate profits in a sustainable manner. The purpose of Red Tilapia cultivation is to raise the fish's size and weight in accordance with their intended use as food. By feeding methods, the varieties of culture may be categorized as extensive culture, semiextensive culture, and intense culture. It is further subdivided based on the features of surrounding environment, the notably earthen pond culture, cage culture, concrete pond culture, and plastic pond culture. The cultivation of Red Tilapia in cages is intense or semi-extensive, with a focus on accelerating production; hence, it is dependent on food intake and feeding frequency (Department of Livestock Development, n.d.).

The Red Tilapia cage culture on the Ping River in the Muang District of the Tak Province serves as a case study for the present investigation. It holds a valid license from the Fisheries Office of the province of Tak. Since its inception in 2006, the case study farm has been in operation for 16 years. Presently, the case study farm is a Red Tilapia cage culture maintained by an intense or semi-extensive culture; it is a small business with five to ten staff, seventy-two fish cages, and roughly seventy-two thousand Red Tilapia.

Aquaculture of Red Tilapia often involves the use of floating feed pellets. The feeding frequency is three times per day, and the average food intake per 20 fish (only 4– 6-month-old fish) is 25 grams. Throughout the years of fish culture, farmers sometimes feed twice, three times, or even four times, resulting in a fluctuating feed intake at each feeding. Farmers are dubious about the appropriate feed intake per unit of time and feeding frequency per day. A large food intake results in increased prices, but a small food intake would have no effect on fish

weight. Moreover, researchers and entrepreneurs in this field have significant concerns that, under the experimental settings, the biggest weight growth may not provide the most profit, thus this hypothesis will be tested and refuted by the present study. By employing a split-plot experiment design, this study aims to determine the best point of food intake and feeding frequency for the Red Tilapia aquaculture business in terms of fish weight, thereby affecting which establishment will provide the most profit.

Literature Review

Pouomogne and Ombredane (2001) explored Nile Tilapia culture in soil ponds. The experimental conditions consisted of 30.9 g of Nile Tilapia given a 25 percent plant protein diet until the fish were full, fed by hand, for 2, 3, and 6 times per day, from 8:00 a.m. to 3:00 p.m., over the course of 15 weeks. The findings indicated that feeding Nile Tilapia six times each day promoted optimal development. The daily growth rate was 1.3 g/day, while the Feed Conversion Rate (FCR) decreased from 1.6 to 1.3, resulting in an average yearly output of 8.7 kg/year of Nile Tilapia.

Tran-Duy et al. (2008) investigated the effect of water oxygen concentration on food intake and the growth of Red Tilapia fish by studying all male fish. The fish were divided into two age groups with different weights and placed in different oxygen concentration ponds. The experiment was conducted using a Randomized Complete Block Design (RCBD) with four conditions. experimental Two factors

consisted of two types of fish weight: small fish (21 g) and big fish (147 g) and two levels of oxygen concentration in the water: low oxygen concentration (3 mg/l) and high oxygen concentration (5.6 mg/l). The experiment was fed twice daily, and 20 fish were randomly assigned per experimental condition to record Specific Growth Rates (SGR), Feed Conversion Rate (FCR), and Growth rates per metabolic weight unit (GRMBW). The results indicated that (1) feed intake and the growth of fish in high oxygen concentration water were better than in low oxygen concentration water; (2) the relationship between feed intake and the growth of small fish was significantly greater than that of big fish; and (3) fish in low oxygen concentration water had no hematological changes.

Holm et al. (1990) explored the frequency of feed intake and stocking density of rainbow trout (Oncorhynchus mykiss) fish culture. The experiment was conducted for 129 days, and the parameters of the fish's body length, increasing each day, were recorded. Specific Growth Rates (SPR) and death rates were recorded for comparison. Experimental results concluded that for the average growth rate regardless of feeding pattern, fish at low stocking density were able to grow better than fish at high stocking density. The increased growth rate was showing a positive correlation with the increased feed intake frequency. Therefore, fish fed at high stocking density require high feed intake frequency.

Sriyasak et al. (2017) researched the cost and returns of Nile Tilapia cage culture in the Songkhram River. Questionnaires were used to collect data from 142 farmers. It was found that the cost of Nile Tilapia cage culture was divided into fixed costs of 86.49 THB/m³ and variable costs of 1,443 THB/m³. The total cost was 1,530 THB/m3, which included 73.11 percent of the food cost. The cost of Nile Tilapia cage culture in the Songkhram River was 61.56 THB/kg, whereas the average farm-gate price was 47 THB/kg. Farmers had an average loss of 14.56 THB/kg.

Hatachote et al. (2021) measured Nile Tilapia culture returns in Sakon Nakhon Province, comparing soil pond culture with cage culture. Questionnaires were used to gather data. The constant and variable expenses in the cost and return analysis were breeding costs, feeding costs, vitamin supplement costs, medical costs, gasoline costs, fish catching costs, and labor costs. According to the findings, farmers made an average profit of 1,779.18 THB/cage/cycle and 9,917.28 THB/rai/cycle on Nile Tilapia cultures in cages and earthen ponds.

Yuangyai and Lin (2013) outlined how the fractional factorial experiment design is commonly used in industrial and agricultural research. Using random factor level change, the researcher sought to assess the significance of the factor. However, not all trials were able to completely adjust the number of random factors owing to financial restrictions or the difficulty of modifying the level of certain factors. The split-plot experiment design proved to be an intriguing experiment.

The split-plot experiment design mirrored the factorial experiment; hence, it was chosen for the following reasons (Cochran and Cox, 1948): 1) Some experimental factors were naturally limited, or the methods using those factors made it necessary to employ large experimental units, differing from other factors, which were able to use small experimental units.

2) In the case that there were previous experiments to study some factors, but the experimenter required further studies for other factors, the original experimental unit should be divided into smaller experimental units. As a result, the original experimental unit was the main plot, whereas the sub-unit in the original experimental unit was a subplot.

3) In some cases, factors were not equally prioritized by experimenters. The factor that included sufficient guidelines or possessed previous trials was given less weight and classified into the main plot, while the factor that required further studies was assigned more importance and classified as a sub-plot.

4) It is convenient in practice because, in some experiments, when using factorial experiments, the practice method on experimental units was switched randomly, which was inconvenient to practice and caused errors while designing the split-plot experiment. The randomization of the first factor was performed over a large area, making it convenient in practice (Cochran and Cox, 1948).

Duangklang et al. (2018) studied the factors affecting the flatness of fiber-cement flat sheets in the case study factory. The related factors were feeding speed, roll gap, roll pressure, and relative humidity. A splitplot design was selected to be used. The results demonstrated that the feeding speed, roll gap, pressure, and relative humidity, were set to 60 m/s, 7 mm, 90 bar, and 27.98 %, respectively, to satisfy the flatness criteria of the customers and reduce the costs caused by the whole flatness issues of fiber-cement flat sheets for the case study factory.

Chutteang et al. (2021) studied the effects of salt stress conditions on the growth and physiological responses of 4 sugarcane varieties by using the split-plot design method. The main plot was the level of salinity, and the sub-plot was 4 sugarcane varieties, namely wild sugarcane (Saccharum spontaneum), forage-cane Biotech2 (BT2), and cultivated-cane Khonkaen3 (KK3), and Suphanburi72 (SP72). It was found that the growth and the change of physiological characteristics and biomass of sugarcane depended on the level of salt concentration. The 200 mM NaCl salinity affected all four sugarcane varieties on dry leaves and damaged cell membranes.

Hargreaves and Kucuk (2001) and Mac Intyre et al. (2008) showed that feeding rate decreased when body weight increased. On farm condition feeding rate was a negative relationship to total ammonia nitrogen (TAN) level in the pond water and whenever TAN level was higher than 0.5 ppm, the feeding rate decreased.

From the literature review, few studies investigated the effects of feed intake and feeding frequency for culturing Red Tilapia on fish weight. Most of them mainly focused on Nile Tilapia culture. In addition, in most fish research studies, fish were often fed until full, such as in the work of Pouomogne and Ombredane (2001), which did not imply the highest profit return. It was noted that the research was not widely conducted in Thailand, especially in the Ping River, Tak Province. Therefore, this current research was interested in studying the feed intake and feeding frequency factors for Red Tilapia cage-culture in the Ping River, Tak Province, in terms of fish weight gains and profit returns.

Materials and Methods

The Red Tilapia cage culture in the case study farm was divided into 5 periods:

1) The Red Tilapia fingerlings aged between 0 and 2 months were transported from a fish hatchery. This period had a higher mortality rate than other periods.

2) They were then fed in a cage the size of $3x3 \text{ m}^2$ from 2- to 4-month-olds. At this age, the Red Tilapia had a lower mortality rate than in the first period although they were still susceptible to death at this age.

3) From 4- to 5-month-old, the fish had a low mortality rate, or were less likely to die.

4) From 5- to 6-months-old during this period, the fish were almost ready for sale. Farmers modified a new floating pellet formula to accelerate the processing of fish meat before sale.

5) During the sale period, farmers brought 800 g of Red Tilapia from the cage to sell to wholesalers and retailers, as shown in Figure 1.

In this study, the researchers were interested in studying and experimenting with 4-month-old Red Tilapia for 1 month by collecting data and conducting the experiment from December 2021 to January 2022 in the analysis of variance (ANOVA). The criterion for decision-making was used at a significance level of 0.05.



Figure 1. Red Tilapia cage culture in the Ping River

For the scope of this study, food intake and feeding frequency of Red Tilapia aged 4 to 5 months old were studied for a 1-month experimental period. A total of 360 fish were fed in the two large cages. A cage with a size of 3×3 m² was divided into the nine sub-cages, each with a size of 1×1 m², so the two large cages received a total of 18 subcages, culturing 20 Red Tilapia per subcage.

In addition, the two large cages were used as the two experimental blocks, called nearshore and offshore. The two large cages were divided into a total of 18 sub-cages, namely 9 sub-cages for the offshore trial (Block 1) and the 9 sub-cages for the nearshore trial (Block 2), as shown in Figure 2.

This experiment studied a 4-month-old Red Tilapia by weighing and selecting Red Tilapia with a weight range of 0.100 to 0.300 kg. The dependent variables (y) were fish weight gain compared to the initial average weight and a profit increase.



Figure 2. Fish cage frame construction divided into sub-cages in the water were put under an indicating factor level sign.

The two independent variables (x) were as follows:

1) Feeding frequency (x_1) was the main plot, consisting of 3-level factors (2, 3, and 4 times/day).

2) Feed intake (x_2) was the sub-plot, consisting of 3-level factors (15, 25, and 35 g/time).

Preparation of food intake was based on the experimental factor level using clear plastic cups for feeding. Therefore, the food in the plastic cup must be weighed to the factor levels of 15, 25, and 35 g, and based on the factor level, the plastic cup must be marked with lines at weighed food levels, as shown in Figure 3.

The Red Tilapia weighing method for experiments was done following these steps:

1) Preparing four-month-old fish for weighing by bringing 20 Red Tilapia to the water's surface.

2) Preparing a bucket with the appropriate amount of water and set it on a

digital scale to subtract the weight of the bucket and water.

3) Placing the fish into a bucket filled with water one by one to put them on the digital weighing scale, as shown in Figure 4.

4) Recording the weight of each fish (kg) in a logbook.

5) Placing the fish that have been weighed into a plastic pail with the appropriate amount of water.

6) Repeating steps 2 through 5 twenty times.

7) Transferring 20 fish from a plastic bucket to a sub-cage for the experiment.

8) Transferring information on the weight of the fish to the logbook.



Figure 3. Feed intake based on factor levels



Figure 4. Weighing Red Tilapia on a digital scale

For business owners, the water's quality was unquestionable even though it affected how much feed the fish consume. According to McGinty and Rakocy (2007), the water in the river was circulated through the cages to bring clean water in and waste water out. It was comparable to always having to change the water to maintain its quality. This river fish breeding enterprise has been operating for at least 16 years and has yielded acceptable results. There has never been an issue with the water's quality that prevented the fish from being raised. Information about the water quality of the Ping River has been provided here. Surface water samples were collected in the area above Bhumibol Dam, which is referred to

the upper Ping River and can be used to determine the WQI value in Table 1.

Table 1 showed that the upper Ping River's surface water quality index (WQI) from 2013 to 2021 was not poor.

It was noted that the water quality index (WQI) was computed by the following 5-parameter quality index: dissolved oxygen (DO), biological oxygen demand (BOD), total coliform bacteria (TCB), fecal coliform bacteria (FCB) and ammonia (NH₃).

The closest surface water sample station was located at Ban Kong Hin, Hang Dong Subdistrict, Hot District, Chiang Mai Province (Station PI10). The other fundamental water quality criteria for the year 2021 were given in Table 2.

Table 1. Average annual WQI of the upper Ping River

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Year	2013	2014	2015	2016	2017	2018	2019	2020	2021
WQI	64	67	66	66	61	61	70	66	64

[Environmental and Pollution Control Office 1 (Chiang Mai), 2021]

	Table 2. Average surface	water quality	measurements a	at Station	PI10	for 2021
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Index	DO	BOD	ТСВ	FCB	TAN NH ₃	WQI
	(mg/l)	(mg/l)	(MPN/100ml)	(MPN/100ml)	(mg/l)	
Station PI10	6.89	1.22	7,300	155	0.21	69

[Environmental and Pollution Control Office 1 (Chiang Mai), 2021]

Total ammonia nitrogen (TAN) level for fish farming should not be more than 0.5 milligrams per liter, and the optimal dissolved oxygen concentration should not be less than 3.0 milligrams per liter. (Department of Fisheries, Ministry of Agriculture and Cooperatives, Thailand, 2012)

Hence, Table 2 showed that both the DO and TAN NH_3 of the Ping River were acceptable for fish farming.

Results

Following the experiment, data were gathered as shown in Table 3 for the offshore trial (Block 1) and Table 4 for the nearshore trial (Block 2) by measuring the weight of Red Tilapia on day 0 and day 30 as well as the weight growth of the fish under each experimental condition. Each condition used twenty Red Tilapia. As indicated in Table 5, the researcher analyzed the feeding frequency and feed intake to assess the impact on weight increase of Red Tilapia using the SPSS software.

At a significance level of 0.05, Table 5 reveals that feeding frequency and feed intake had an influence on Red Tilapia weight increase. The interaction between food intake and feeding frequency was not statistically significant, nor was the impact of experimental area (blocks).

The condition that gave the highest weight gain in both the offshore trial (Block 1) and the nearshore trial (Block 2) was the 8^{th} condition, which fed at a frequency of 4 times per day with a food intake of 35 g/time. Thus, the weight gain of the Red Tilapia was greater than the other conditions, as shown in Figure 5.

Block 1 (Offshore trial)						
Condition	Fish Weight/2	20 fish	Weight gain			
Conultion	Day 0	Day 30	weight gam			
1	3.257	4.095	0.838			
2	3.241	4.245	1.004			
3	3.374	3.850	0.476			
4	3.231	4.225	0.994			
5	4.711	6.012	1.301			
6	3.052	4.025	0.973			
7	3.235	4.510	1.275			
8	3.060	4.855	1.795			
9	3.339	4.415	1.076			

 Table 3. Experimental data from the offshore trial

Table 4. Experimental data from the nearshore trial

Block 2 (Nearshore trial)							
Canditian	Fish Weight/	'20 fish	Weight goin				
Condition	Day 0	Day 30	weight gam				
1	3.986	4.515	0.529				
2	2.786	3.970	1.184				
3	3.145	3.784	0.639				
4	2.857	4.330	1.473				
5	3.045	4.380	1.335				
6	3.307	3.935	0.628				
7	3.456	4.930	1.474				
8	3.596	5.205	1.609				
9	3.017	4.270	1.253				

Dependent Variable : Weight								
Source		Type Sum Squares	III of df	Mean Square	F	Sig.		
Intercent	Hypothesis	21.903	1	21.903	2565.733	0.013		
mercept	Error	0.009	1	0.009 ^a				
main * block	Hypothesis	0.002	2	0.001	0.024	0.976		
	Error	0.294	6	0.049 ^b		<u> </u>		
main	Hypothesis	1.213	2	0.606	510.328	0.002		
IIIaIII	Error	0.002	2	0.001 ^c				
aub	Hypothesis	0.845	2	0.422	8.622	0.017		
suo	Error	0.294	6	0.049^{b}		<u> </u>		
block	Hypothesis	0.009	1	0.009	7.185	0.116		
	Error	0.002	2	0.001 ^c		$\overline{\mathbf{i}}$		
main * sub	Hypothesis	0.072	4	0.018	0.368	0.824		
	Error	0.294	6	0.049^{b}				

Table 5. Factors affecting fish weight gain

a. MS(block), b. MS(Error), c. MS(main * block)

Note: 'Main' is feeding frequency, 'Sub' is feed intake, 'Block' is the experimental area



Researchers and entrepreneurs are uncertain as to whether the circumstances with the biggest weight also provide the most profit. Using the Excel program, the outcomes of the tests were examined to discover the most profitable factors for the business. As shown in Table 6, the cost of food was only counted as a fixed expense. The cost of fish food was 34.5 THB/kg and the selling price of Red Tilapia was 85 THB/kg. The monthly cost of food, which was 40 Red Tilapia, was also looked at to figure out the company's profit.

Condition	Food Quantity per Time (For 40 Fish)	Frequency per Day	Fish weight gain (kg)	Monthly Feed Rate (kg/40 fish)	Food Cost per Month (THB)	Income	Profit (THB/ month)
1	0.050	2	1.367	3.000	103.5	116.195	12.695
2	0.070	2	2.188	4.200	144.9	185.98	41.08
3	0.030	2	1.115	1.800	62.1	94.775	32.675
4*	0.050	3	2.467	4.500	155.25	209.695	54.445
5	0.070	3	2.636	6.300	217.35	224.06	6.71
6	0.030	3	1.601	2.700	93.15	136.085	42.935
7	0.050	4	2.749	6.000	207	233.665	26.665
8	0.070	4	3.404	8.400	289.8	289.34	-0.46
9	0.030	4	2.329	3.600	124.2	197.965	73.765

Table 6. Profit analysis

* Current State

According to the analysis, it was found that the 9^{th} condition gave the most profit at 73.765 THB/month. The frequency in this condition was 4 times per day, with a feeding intake of 15 g/time.

However, the 8^{th} condition gave the maximum weight gain of Red Tilapia with 35 g of feeding intake and a frequency of 4 times per day. From the highest profit analysis, the 8^{th} condition gave a profit of -0.46 THB/month, indicating a financial loss.

Therefore, the 8th condition, which gave the maximum weight gain, when analyzing the profit, was not profitable. If entrepreneurs wish to obtain the most profit, they should select the factor level in the 9th condition, which has a frequency of 4 times per day with a feeding intake of 15 g/time. Fish physiology and behavior were outside the range of this study, because wholesalers and retailers focused on the main weight of the fish. In the beginning of the trial, it was acknowledged that the fish might be under stress and eat less or not at all. Nonetheless, the fish resumed eating normally after 3-5 days.

Summary and Discussion

Entrepreneurs questioned the adequacy of the food intake and feeding frequency for Red Tilapia farming in the cages of the case study farm. Using the split plot experiment method, this study explored and assessed the optimum point of food intake and feeding frequency.

The experimental study demonstrated that feeding frequency and food intake had a

statistically significant impact on Red Tilapia weight increase.

The main results were summarized as follows:

1) The influence of place for culturing Red Tilapia, offshore trail (Block 1) and nearshore trail (Block 2), had no difference in weight gain of Red Tilapia.

2) A frequency of 4 times per day, with a feeding intake of 35 g/time, resulted in Red Tilapia in the offshore trail having the maximum weight gain, but not making the most profit.

3) The condition that gave the most profit has a frequency of 4 times per day with a feeding intake of 15 g/time.

Researchers and entrepreneurs expected that Red Tilapia would not increase weight if given more than 25 g/time and more than three times per day; nevertheless, the experiment shown that Red Tilapia can gain weight when fed up to 35 g/time and four times per day. This study also demonstrated that Red Tilapia with the greatest weight growth may not be the most profitable due to rising feeding costs and market-determined selling prices.

This study used 4- to 5-month-old Red Tilapia raised in cages in the Ping River, Tak Province, for feeding the case study farm, which may be advantageous to other businesses.

This study did not address the recommended food intake and feeding frequency for Red Tilapia aged 5 to 6 months, which was the age prior to sale. Therefore, it was advised to perform research on 5- to 6-month-old Red Tilapia as a guideline for the case study farm and for the future benefit of other establishments. In addition, the water's quality was unquestionable for the business owners, even though it affected how much feed the fish consumed. Hence, further long-term research on the effects of river water quality (such as total ammonia nitrogen levels) on fish growth and feeding is encouraged.

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