Current **A**pplied **S**cience and **T**echnology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. 2026, Vol. 26 (No. 1), e0265396

Research article

Flesh Quality of Nile Tilapia (*Oreochromis niloticus*) Cultured in Biofloc System with Different Dietary Protein Levels

Supalug Kattakdad[1], Suriya Udduang[1], Krittima Kasamawut[1], Woranit Muangmala[1], Janejira Phakawan[2] and Nittaya Phungam[1]*

[1]Faculty of Agriculture and Technology, Rajamangala University of Technology Isan, Surin, Thailand
[2]Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Pathum Thani, Thailand

Received: 25 November 2024, Revised: 17 April 2025, Accepted: 18 April 2025, Published: 30 May 2025

Abstract

The effects of dietary protein levels on flesh quality of Nile tilapia cultured in a biofloc system were investigated after an 8-week feeding trial. The experimental design was a completely randomized design (CRD) with 3 treatments and 3 replicates. The fish (initial average weight of 30.70±0.70 g) were fed diets with 32% (32%CP-BFT), 30% (30%CP-BFT) and 28% (28%CP-BFT) crude protein and raised in a biofloc system. The results showed that biofloc particles in the 32%CP-BFT treatment had a higher protein content than those in other treatments, which corresponded with protein accumulation in the whole body of fish ($p \leq 0.05$). However, no differences were found in the protein accumulated in fillets ($p > 0.05$). The fillet color analysis showed that the 32%CP-BFT treatment had the lowest yellowness values, but no significant differences were found between treatments for brightness, redness, and whiteness index ($p > 0.05$). Water holding capacity showed no significant differences in drip loss, thawing loss, and grilling loss ($p > 0.05$). However, the 32%CP-BFT treatment showed the highest boiling loss ($p \leq 0.05$). Texture analysis showed that the 32%CP-BFT treatment had the highest springiness and hardness ($p \leq 0.05$), while the pH values showed no significant differences ($p > 0.05$). The analysis of thiobarbituric acid reactive substances (TBARS) in the fillets stored under chilling conditions showed increasing TBARS values with longer storage duration, with no significant differences among treatments ($p > 0.05$).

Keywords: fish feed; biofloc; fillet; Nile tilapia; protein

1. Introduction

Fish flesh quality is considered an important indicator of consumer acceptance. As a result, many previous research studies have focused on factors that may affect flesh quality (Cheng et al., 2014; Zhuang et al., 2022; Tian et al., 2024). Changes in flesh quality are caused by many factors, such as physical factors, chemical factors, and diverse treatments. Feed ingredients are one of the physical factors that affect flesh quality (Cheng

*Corresponding author: E-mail: Nittaya.ph@rmuti.ac.th
https://doi.org/10.55003/cast.2025.265396

et al., 2014), especially dietary protein intake, which is an important component of muscle tissue. Muscle growth and structure has a profound effect on the flesh texture quality, which depends on the interplay between protein synthesis and degradation (McCarthy & Esser, 2010). Besides, previous studies have reported that a lack of protein is the main cause of inferior flesh quality (Wei et al., 2016). Generally, flesh quality can be measured and evaluated by physical properties such as hardness, flexibility, toughness, toughness, and chewiness, including nutritional value and taste (Choi & Kim, 2009).

A Biofloc system involves raising aquatic animals together with heterotrophic bacteria, which are stimulated to grow by adding carbon organic matter to the farming system and controlling the carbon to nitrogen ratio (Khanjani et al., 2021). Heterotrophic bacteria, preferably aerobic bacteria, assimilate nitrogen waste in the form of ammonia from the water and utilize the carbon source added to the system to produce new cells, forming microbial protein or biofloc particles. These particles provide extra essential nutrients for aquatic animals and maintain nitrogenous waste below toxic levels (Schryver et al., 2008; Avnimelech, 2007; Ekasari et al., 2010; Xu et al., 2012). Biofloc particles can be well used by omnivorous species such as Nile tilapia (Narimbi et al., 2018). Avnimelech and Kochba (2009) demonstrated the ability to use biofloc as a nutritional supplement in terms of protein content in Nile tilapia, which can assimilate 240 mgN· kg^{-1} of biofloc (approximately 25% of protein content in diet). Utilizing protein from biofloc particles allows for the reduction in the amount of diet and/or the protein content in the diet. This is an efficient way to reduce production costs. Therefore, the present study aims to apply the biofloc system to increase microbial protein and decrease the protein composition in the diet of Nile tilapia (*Oreochromis niloticus*) and investigate the effect of dietary protein reduction on the flesh quality of Nile tilapia.

2. Materials and Methods

2.1 Experimental plan and experimental fish

The effects of different dietary protein levels on the flesh quality of Nile tilapia cultured in a biofloc system were studied. The experiment was conducted using a completely randomized design (CRD) with three treatments and three replicates. Fish were fed diets containing 32% (32% CP-BFT; control group), 30% (30% CP-BFT), and 28% (28% CP-BFT) crude protein while being reared in a biofloc technology system. In this context, "CP" denotes the crude protein content of the diets, and "BFT" refers to the biofloc technology system employed in the study.

The biofloc system was prepared in 400 L of water in nine aerated plastic tanks. Biofloc inoculum and expired fish feed (containing 32% protein) were added, with molasses serving as the carbon source. The carbon-to-nitrogen (C:N) ratio was maintained at 20:1 (Samocha et al., 2007), and the system was subjected to aerobic fermentation. Molasses was added daily until the biofloc particle concentration reached 10 mL/L. Subsequently, the fish were stocked in the experimental tanks for rearing. The carbon source was added every week to maintain biofloc particles following the C:N ratio.

Male Nile tilapia sourced from a private aquaculture farm in Surin Province were acclimatized under controlled conditions for two weeks. During the acclimation period, the fish were provided with a diet containing 32% protein. Following the acclimation period, fish of uniform size were carefully selected to commence the experimental procedures. The experimental fish had an initial average weight of 30.70±0.70 g. The fish were randomly distributed into the tanks with a density of 30 fish/tank (density 30 fish/m^3) and were fed

floating pellets according to the experimental treatments, at a quantity of 5% of body weight of the fish, 3 times a day for 8 weeks. After the feeding trial, the biofloc particles were harvested and analyzed for their chemical composition analysis (AOAC, 2000).

Throughout the study, the experimental animal protocol obtained ethical clearance from the Institutional Animal Care and Use Committee, ensuring compliance with the guidelines outlined by the National Research Council of Thailand for the ethical use of animals in scientific research (Certificate of Approval ID: 27/2565).

2.2 Experimental diet preparation

The feed ingredients were analyzed for their chemical composition and subsequently used to formulate the experimental diets. The raw materials were ground, passed through a 320-µm mesh, and thoroughly mixed according to the designated formulations. The feed mixtures were then pelletized using a single-screw extruder at Rajamangala University of Technology Isan, Surin Campus, Thailand. The resulting pellets were dried in a hot air oven at 65°C for 12 h. The nutritional composition of the experimental diets was determined using AOAC methods (2000). The formulation and chemical composition of the experimental diets are presented in Table 1.

Table 1. Formulation and chemical composition of experimental diets (%, based on dry matter)

Ingredient (%)	Treatment		
	32%CP-BFT	30%CP-BFT	28%CP-BFT
Fish meal	20	20	20
Soybean meal	38	34	30
Rice bran	10	10	10
Corn meal	13	15	17
Cassava	12	14	16
Soybean oil	3	3	3
Dicalcium phosphate	1	1	1
Vitamin-mineral premix[a]	1	1	1
Vitamin C	1	1	1
Feed binder	1	1	1
Chemical composition by proximate analysis (% dry weight basis)			
Crude protein	32.03	30.32	28.43
Crude lipid	5.65	5.70	5.76
Crude fiber	4.92	4.78	4.62
Crude ash	9.22	9.25	9.45
Moisture	9.86	9.51	9.54
Nitrogen free extracts[b]	38.32	40.44	42.20

Notes: [a]Vitamin-mineral premix provided per kg of diet: vitamin A 12,000,000 IU; vitamin D 2,000,000 IU; vitamin E 6000 IU; vitamin K 2000 mg; vitamin B1 800 mg; vitamin B2 2500 mg; vitamin B6 800 mg; and vitamin B12 10 mg. Minerals provided per kg of diet: Mn 18 mg; Mg 200 mg; Co 0.1 mg; I 0.25 mg; Fe 140 mg; Cu 2.5 mg; Zn 65 mg; Se 0.2 mg.
[b] Nitrogen-free extract = 100 − (moisture + crude protein + crude lipid + crude fiber + ash)

2.3 Flesh quality measurement

After 8 weeks of experimentation, the fish were immediately asphyxiated by immersion in ice bath (Robb & Kestin, 2002). Fillets were taken from one side of the dorsal muscle in the epaxial myotomes below the dorsal fin of each fish, excluding the ventral section, as a sample for flesh quality (Tian et al., 2024). The five fillet samples per tank were divided for analysis of flesh quality.

2.3.1 Chemical composition analysis

The whole bodies of five fish per tank and fillets samples of five fish per tank were analyze for the chemical composition including crude protein, crude lipid, crude ash, and crude fiber, following the procedures of AOAC (2000).

2.3.2 Color

Color values were evaluated using a colorimeter (model Hunter Lab Ultra Scan VIS, Germany) to record lightness (L*), redness (a*), and yellowness (b*).

2.3.3 Water holding capacity

Water holding capacity was evaluated through drip loss, thawing loss, grilling loss, and boiling loss. The dorsal fillet samples were cut into 5×3×1 cm size for water holding capacity analysis. For drip loss, the samples were initially weighed (D1), and placed in a plastic bag with small drainage and then refrigerated at 4°C for 48 h. After the storage period, the samples were reweighed (D2) (Tian et al., 2024). The thawing loss analysis was evaluated following the method of Zhou and Xie (2021) with modification. The samples were weighed (T1) and placed in a vacuum packaging bag, and then frozen in a refrigerator at -18°C for 72 h. The samples were then thawed in a 4°C constant temperature refrigerator until their central temperature reached 4°C, and were then reweighed (T2). For grilling loss analysis, the samples were weighed (G1) and placed in a convection oven until an internal temperature of 80°C was reached. The samples were then reweighed (G2) (Jaturasitha et al., 2008). The boiling loss was determined by weighing the samples (B1) and placing in a water bath at 100°C for 15 min. Then, the samples were removed from the water bath, the surface water absorbed using paper towels, and then the samples were reweighed (C2) (Tian et al., 2024). The water holding capacity was calculated as follows:

$$\text{drip loss (\%)} = 100 \times (D1-D2) / D1$$
$$\text{thawing loss (\%)} = 100 \times (T1-T2) / T1$$
$$\text{grilling loss (\%)} = 100 \times (G1-G2) / G1$$
$$\text{boiling loss (\%)} = 100 \times (B1-B2) / B1$$

2.3.4 Texture analysis

Texture analysis was investigated on the dorsal fillet samples, which were cut in to 1.5×1.5 ×1.0 cm size for springiness and hardness analysis using texture analyzer (model TA.XT.Plus, Germany), following the method of Zhou and Xie (2021).

2.3.5 pH

The pH value was determined by pH meter (model PONPE 59PH, China). Ten grams of samples were homogenized with 100 mL of distilled water for 1 min, and then the pH of the resulting solution was measured using a pH meter (Sumer & Oz, 2023).

2.3.6 Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) in the fillets were determined after 0, 1, 3, 6, and 9 days of storage under chilling conditions at 4°C, following the method of Sumer and Oz (2023). The samples (2 g) were homogenized with trichloroacetic acid (TCA) solution (12 mL) for 30 s, and the mixture was then filtered through Whatman 1 filter paper. The thiobarbituric acid (TBA) solution (3 mL) was added to the filtrate solution (3 mL). The mixture was incubated in a 100°C water bath for 40 min, then cooled in cold water and centrifuged at 2000 rpm for 5 min. The absorbance values were determined against a blank sample at 530 nm using a spectrophotometer (WTW–photoLab® 7600 UV-VIS; U.S.A). TBARS values were given as mg malondialdehyde· kg^{-1} (mgMDA·kg^{-1}).

2.4 Data analysis

Mean values and standard deviations (SD) were calculated, and the differences among treatments were assessed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at a significance level of $p \leq 0.05$.

3. Results and Discussion

3.1 Chemical composition analysis

The chemical composition analysis of biofloc particles, fillets and whole bodies of the Nile tilapia are shown in Table 1. The results revealed that biofloc particles from the 32%CP-BFT and 30%CP-BFT treatments had a higher protein content than that of the 28%CP-BFT treatment, with values of 25.02±0.17%, 24.16±0.38%, and 20.37±0.71%, respectively. Additionally, crude fat results showed that biofloc particles from the 32%CP-BFT treatment had the highest crude fat content (2.12±0.03%) compared to other treatments ($p \leq 0.05$). The biofloc particles from the 32%CP-BFT treatment showed the lowest crude ash at 10.78±0.26%, with statistically significant difference with other treatments ($p \leq 0.05$). The biofloc particles from the 32%CP-BFT treatment contained the lowest crude fiber (8.63±0.22%) compared to other treatments. This was consistent with the study of Khanjani et al. (2021), which reported protein values in biofloc particles from raising tilapia fed with a 35% crude protein. Their results showed that the protein in the biofloc particles was between 24.39-31.70% and the ash content was as high as 25.17-32.04%. Lopez-Elias et al. (2015) reported that the protein content in biofloc particles ranged from 23.7-25.4%, the fat content was 2.6-3.5% and the ash content was 33-40.4%. Becerril-Cortes et al. (2018) reported protein content in biofloc particles from different carbon sources. Their values were in the range of 30.2-48.0%, the crude fat content was in the range of 2-2.5% and the ash content was in the range of 6.7-16.5% for fish reared in plastic tanks with a biofloc system. Kumari et al. (2021) reported that for red tilapia fed 32% crude protein in a biofloc system under varying salinity levels from 0-20 ppt, the protein content in biofloc particles was between 25.01-29.63%, the fat content was between 2.37-3.30% and the ash

content was between 19.97-27.41%. Biofloc particles are considered a high- quality feed for aquatic animals because they are rich in nutrients such as essential amino acids, fatty acids, vitamins, and minerals (Ekasari et al., 2014; Wang et al., 2015; Bakhshi et al., 2018). However, the chemical composition of biofloc particles also varies based on factors such as the source of carbon added to the biofloc system (Crab et al., 2010; Ekasari et al., 2015; Bakhshi et al., 2018), C:N ratio (Minabi et al., 2020), water salinity (Khanjani et al., 2020) and feeding rate (Khanjani et al., 2016).

The chemical composition analysis of Nile tilapia fillets revealed that crude protein showed no statistically significant differences among the treatments ($p>0.05$), with crude protein values between 82.04-82.14%. The fillet of the 28%CP-BFT treatment had the highest crude fat (3.51±0.07%), with no statistically significant difference from the 32%CP-BFT treatment (3.34±0.12%) ($p>0.05$). In addition, the 28%CP-BFT treatment gave the highest crude ash in the fillets (8.90±0.12%). The analysis did not find crude fiber content in the fillets of any treatments. The results of the present study were similar to those reported by Lima et al. (2018), who showed that fillets of Nile tilapia reared in a biofloc system contained 76.47-79.98% moisture, 17.3-20.28% protein, 0.81-1.24% lipids, 1.18-1.32% ash, and <0.02% fiber. However, the fillet from a biofloc system tended to be higher in crude protein than fillet from normal conditions (rearing with water exchange method), with values of 78.43% moisture, 17.08% protein, 1.99% lipid, and 1.09% ash (Biscalchin-Grÿschek et at., 2003).

The chemical analysis of the whole body of fish revealed that the 32%CP-BFT treatment had the highest crude protein content (60.01±0.92%) ($p\leq0.05$) compared to the 30%CP- BFT and 28% CP-BFT treatments, which had the values of 58.16±0.82% and 56.77±0.49%, respectively. The 32%CP- BFT treatment resulted in the highest crude fat content (2.79±0.05%) ($p\leq0.05$). Additionally, the 28%CP-BFT treatment was found to have the highest crude ash content (6.87±0.11%) compared to other treatments ($p\leq0.05$), and the 32%CP-BFT treatment contained the highest value of crude fiber (3.21±0.03%) ($p\leq0.05$), as shown in Table 2. The chemical composition analysis of the fillets from Nile tilapia fed different protein levels and reared in a biofloc system revealed that the fish fed 32% crude protein exhibited increased protein and fat contents, which could be attributed to the higher amount of protein-rich feed from the biofloc system. Biofloc technology can reduce the level of toxic ammonia-nitrogen (Burford et al., 2004) through the activity of heterotrophic bacteria. Moreover, biofloc can utilize carbon and nitrogen as its energy sources, with the C:N ratio needing to be maintained within an appropriate range. A high level of dietary protein has a positive impact on the level of crude protein in biofloc particles and results in a higher amount of protein accumulation in the fillet and whole body of Nile tilapia. Kayan et al. (2015) reported that the chemical composition of Nile tilapia fillets also depended on slaughter weight and fillet section. Protein accumulation in fillet varies inversely with slaughter weight, while fat accumulation in fillet varies directly with slaughter weight. However, no differences in protein accumulation were found in fillet section (dorsal and ventral), but higher fat accumulation was found in the ventral compared to the dorsal fillet section.

3.2 Color

The color values of Nile tilapia fillets are shown in Table 3. The color values, i.e. brightness (L^*) and redness (a^*) of the fillets showed no significant differences among treatments ($p>0.05$). The brightness value was between 44.13-43.66 and the redness value ranged

Table 2. Chemical composition analysis (%, based on dry matter) of biofloc particles, fillets and whole bodies of Nile tilapia cultured in a biofloc system with different dietary protein levels for 8 weeks (n=3)

Parameter	Treatment			p-value
	32%CP-BFT	30%CP-BFT	28%CP-BFT	
Biofloc particles				
Crude protein	25.02±0.17^{a}	24.16±0.38^{a}	20.37±0.71^{b}	0.000
Crude lipid	2.12±0.03^{a}	1.08±0.04^{c}	1.89±0.03^{b}	0.000
Crude ash	10.78±0.26^{b}	14.00±0.51^{a}	13.60±0.39^{a}	0.000
Crude fiber	8.63±0.22^{c}	12.74±0.80^{b}	17.72±0.50^{a}	0.000
Fillets				
Crude protein	82.14±0.25	82.04±0.62	82.10±0.77	0.978
Crude lipid	3.34±0.12^{a}	2.32±0.12^{b}	3.51±0.07^{a}	0.000
Crude ash	7.99±0.40^{b}	8.54±0.39^{ab}	8.90±0.12^{a}	0.040
Crude fiber	ND	ND	ND	
Whole body				
Crude protein	60.01±0.92^{a}	58.16±0.82^{b}	56.77±0.49^{b}	0.006
Crude lipid	2.62±0.03^{a}	2.43±0.05^{b}	2.26±0.05^{c}	0.000
Crude ash	6.21±0.06^{b}	6.07±0.09^{b}	6.87±0.11^{a}	0.000
Crude fiber	3.21±0.03^{a}	2.48±0.04^{b}	1.75±0.13^{c}	0.000

Notes: mean±SD in each row with different lowercase superscript letters represent significant differences (p≤0.05). ND = Not detected

Table 3. The color values of Nile tilapia fillets cultured in a biofloc system with different dietary protein levels for 8 weeks (n=3)

Parameters	Treatment			p-value
	32%CP-BFT	30%CP-BFT	28%CP-BFT	
L*	43.36±0.44	43.66±0.38	44.13±0.17	0.095
a*	3.54±0.04	3.35±0.12	3.41±0.08	0.072
b*	2.78±0.67^{b}	4.34±0.28^{a}	4.31±0.02^{a}	0.006
Whiteness index	43.18±0.41	43.40±0.36	43.86±0.17	0.108

from 3.35 to 3.54. The yellowness (b*) of the 30%CP-BFT and 28%CP-BFT treatments exhibited higher values compared to that of the 32%CP-BFT treatment, with b* values of 4.34±0.28, 4.31±0.02 and 2.78±0.67, respectively. The whiteness index of the fillets was not significantly different (p>0.05), with values ranging from 43.18 to 43.86. The coloration of fish fillets results from the fat accumulation in the muscles. Fish with high fat content generally accumulate a lot of fat in their muscles, giving fillet its yellow, grey, pink, red, or other colors (Gurr, 1992; NurSyahirah & Rozzamri, 2022). Therefore, protein supplementation in the feed contributes to a lower proportion of fat in the fish, resulting in increased whiteness of the fish fillets and a decrease in the redness and yellowness values. This finding is consistent with the study of Aramli et al. (2013), who investigated the characteristics of Siberian sturgeon (*Acipencer baerii*).

3.3 Water holding capacity

The water holding capacity of Nile tilapia is presented in Table 4. The results of water holding capacity showed that the drip loss, thawing loss, and grilling loss were not significantly different ($p>0.05$). Drip loss ranged from 3.53 to 3.73%, which was higher than that observed in large yellow croaker (*Larimichthys crocea*) (Tian et al., 2024). Thawing loss ranged from 3.50 to 3.58%, and grilling loss ranged from 10.92 to 11.74% (Table 3). The 32% CP-BFT treatment resulted in the highest boiling loss value (9.72±0.14%). The analysis of water holding capacity of the tilapia fillet showed that the 28%CP-BFT treatment resulted in lower boiling loss than other treatments. Protein supplementation in the feed resulted in the growth of the tilapias, with large amounts of protein being deposited in the fish tissue or muscle. Heat during cooking, such as boiling, results in protein denaturation (Kayan et al., 2015). Due to the amino acid polymeric structure of protein, which contains both hydrophilic and hydrophobic R-units, the hydrophilic amino acid molecules can attract and hold water in the structure of the fish fillet (Qing et al., 2022). Even though freezing tilapia fillets generally increases porousness, sponge-like appearance, and water loss during thawing, the high protein content of the fillet samples resulted in lower losses of water holding capacity. However, Biscalchin-Grÿschek et al. (2003) reported an increase in amount of drip loss when there was an increase in the insolubility of the myofibrillar proteins. Protein aggregation can cause insolubility, which results in loss of water retention ability. In addition, stressed conditions may decrease the water holding capacity of fish meat (Goes et al., 2015). Stressed Atlantic cod pre-slaughter was observed to have decreased water holding capacity compared to non-stressed fish (Hultmann et al., 2012).

Table 4. Texture profile of Nile tilapia fillet cultured in a biofloc system with different dietary protein levels for 8 weeks (n=3)

Parameter	Treatment			p-value
	32%CP-BFT	30%CP-BFT	28%CP-BFT	
Water holding capacity (%)				
Drip loss	3.73±0.26	3.53±0.22	3.67±0.22	0.569
Thawing loss	3.50±0.09	3.53±0.13	3.58±0.10	0.689
Grilling loss	10.95±0.45	10.92±0.67	11.74±0.16	0.134
Boiling loss	9.72±0.14^{a}	9.49±0.06^{b}	9.35±0.07^{b}	0.010
Texture analysis (N)				
Springiness	0.54±0.03^{a}	0.34±0.02^{c}	0.48±0.01^{b}	0.000
Hardness	0.64±0.01^{a}	0.44±0.00^{c}	0.57±0.02^{b}	0.000
pH value	6.84±0.10	6.91±0.10	6.96±0.13	0.470

Notes: mean±SD in each row with different lowercase superscript letters represent significant differences ($p\leq0.05$).

3.4 Texture analysis

The analysis of the texture characteristics of the tilapia fillets (Table 4) showed that the 32% CP-BFT treatment had the highest springiness (0.54±0.03 N), which was consistent with its highest value of hardness (0.64±0.01N) ($p \leq 0.05$). The analysis of the textural characteristics indicated that the 32% CP-BFT treatment had higher values than other treatments. This could be because of the supplementation of protein from biofloc particles. The biofloc system allowed appropriate water quality and ecology, which encouraged the fish to fully utilize protein from the feed for growth. Protein is a vital nutrient that contributes to the formation of connecting tissues, which are mainly collagen, as well as elastin and reticulin. These connecting tissues could result in a chewy texture. The amount of connecting tissue relates to the function of the muscle, with very active muscles containing larger amounts of connecting tissues (NurSyahirah & Rozzamri, 2022). In addition, other factors affecting the texture and structure of fish include storage temperature and time, solubility, hydrophobicity, aggregation of protein, and fillet section (Badii & Howell, 2002; Rawdkuen et al., 2010).

3.5 pH

The pH of Nile tilapia is presented in Table 4. The tilapia fillets showed the pH level of weak acid, ranging between 6.84 to 6.96 ($p>0.05$). The analysis of the pH values of the tilapia fillets demonstrated that they were in the condition of weak acid to neutral, which is the normal condition of animal meats that do not experience stresses during slaughter or dissection. After slaughter, the pH in muscle decreases or changes to a more acidic state (Goes et al., 2015). The rate of pH decline depends on multiple factors, such as the type of animal and the tolerance to stress conditions before slaughter. The changes in pH after slaughter also affects the quality of meat. Stress before slaughter could influence the formation of lactic acid, causing a drop in the pH. The meat obtained from animals that are stressed, panicked, and struggled before slaughter contains a large quantity of lactic acid, causing a significantly lower than usual pH (Kayim & Can, 2010). The decrease in pH is an indicator of stress conditions before slaughter in Atlantic salmon (*Salmo salar*) (Einen et al., 2002), eel (Anguilla anguilla L.) (Morzel & De Vis, 2003) and gilthead seabream (*Sparus aurata*) (Matos et al., 2010). In addition, pH changes also depend on the storage temperature, salt composition, physiological state, buffering capacity of protein and enzymatic action (Biscalchin-Grÿschek et al., 2003).

3.6 Thiobarbituric acid reactive substances (TBARS)

The TBARS analysis in the tilapia fillets after 0, 1, 3, 6, and 9 days of storage under chilling conditions (4°C) revealed increasing values of TBARS with longer storage duration (Figure 1). The 28% CP-BFT treatment exhibited a trend toward lower TBARS levels during storage compared to the other treatments; however, the difference was not statistically significant ($p>0.05$). After 9 days of storage, the TBARS of all groups ranged from 2.34 to 2.45 mgMDA·kg^{-1}. The oxidation of fat induces the oxidation of myoglobin, which causes color changes in the fish fillet. The analysis of the fat oxidation progress by measuring TBARS in the fish fillets stored under chilled conditions for 9 days indicated that TBARS values increased with longer storage duration across all treatments. However, without a clear standard, the values of TBARS of all samples during all storage periods

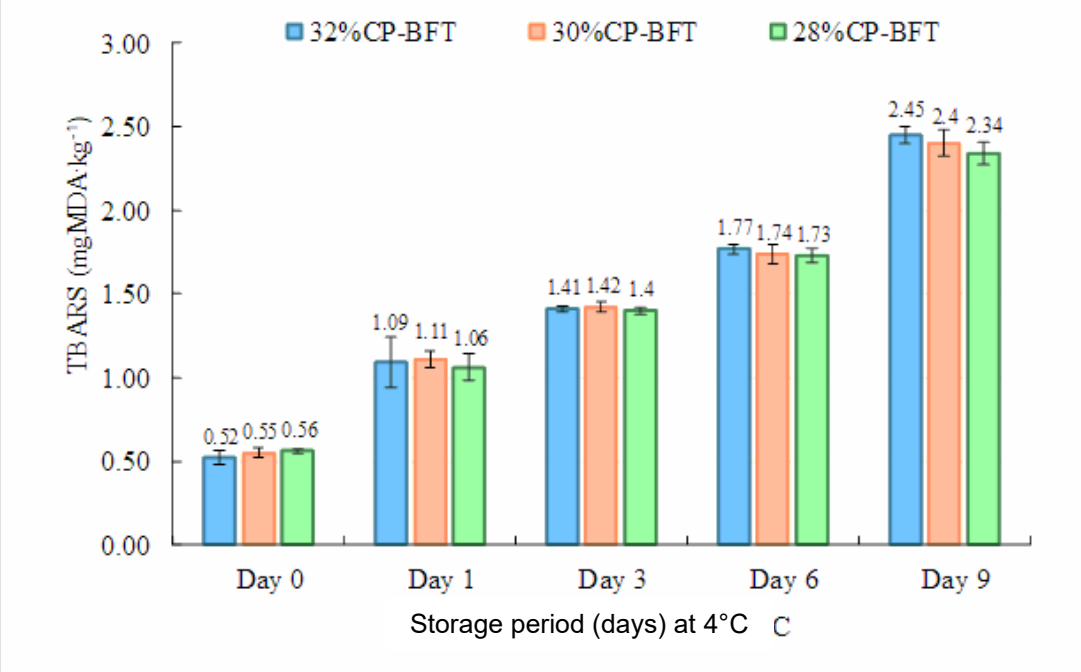

Figure 1. Thiobarbituric acid reactive substances analysis of Nile tilapia fillets cultured in a biofloc system with different dietary protein levels for 8 weeks (n=3)

remained below the recommended limit of 8 mgMDA·kg-1 for fish fillets (Fan et al., 2014). The TBARS test measures malonaldehyde, which is the secondary product from hydroperoxide decomposition, resulting in aldehyde compounds that cause unpleasant odors and flavors in food (Kobayashi & Park, 2017).

4. Conclusions

Different dietary protein levels influenced the flesh quality of Nile tilapia reared in a biofloc system. However, the lowest protein levels in this experiment (28% crude protein) did not affect some important parameters, such as protein accumulation, whiteness index, water holding capacity (in terms of drip loss, thawing loss, and grilling loss), and pH value in the fillets. In addition, it decreased the weight loss of fillets from boiling and tended to reduce TBARS after storage. Therefore, 28% dietary protein can be used to raise Nile tilapia in a biofloc system to save the production costs and decrease the environmental impact of excess dietary protein without significantly affecting the flesh quality.

5. Acknowledgements

This research project was supported by Science Research and Innovation Fund (Contract No. FF66-P2-007).

6. Author Contributions

Supalug Kattakdad: Writing original draft, review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Suriya Udduang: Formal analysis. Krittima Kasamawut: Formal analysis. Woranit Muangmala: Writing –review & editing. Janejira Phakawan: Writing –review & editing. Nittaya Phungam: Methodology, Investigation, Formal analysis, Writing original draft, review & editing.

7. Conflicts of Interest

The authors declare that they have no conflicts of interest.

ORCID

Supalug Kattakdad https://orcid.org/0000-0002-8312-6885

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