

# THE EFFECT OF STORAGE TIME ON THE QUALITY OF ARTIFICIAL FEED FOR FEMALE COBIA FISH (*Rachycentron canadum*)

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## ABSTRACT

The purpose of the study was to determine the quality of artificial feed after the storage process. There are two types of artificial feed: P1 feed (n-3 HUFA content 2.82% dry weight) and P2 feed (n-3 HUFA content 2.76% dry weight). The formula feed was made of isoprotein (43%), isolipid (16%), and isoenergy (417 kcal/100 g). Parameters observed were physical changes (color, odor, texture, broken pieces, fungal infestation, and overall quality); results of proximate and fatty acid analysis after two months of closed storage in plastic bags at 17 °C. Observation of physical parameters showed that the physical condition of both types of feed was good, but P1 feed is more crumbly than P2 feed. The results of proximate analysis of the P1 feed showed no significant difference during storage. The proximate analysis of P2 feed showed no significant difference in ash content, fat content, energy from fat, and total energy during storage; that experienced significant changes were water, protein, and carbohydrate content. The water and carbohydrate content increased on the 1<sup>st</sup> and 2<sup>nd</sup> months, while the protein content decreased in the 1<sup>st</sup> and 2<sup>nd</sup> months. The analysis results of fatty acids DHA, EPA, AA, and n-3 HUFA did not show significant change during the storage of the two types of feed. The conclusion was that P1 feed had more stable chemical properties than P2 feed after two months of storage. However, the P1 feed was physically more crumbly than the P2 feed.

**Keywords:** Artificial Feed; Quality; Storage; Time

## INTRODUCTION

Cobia (*Rachycentron canadum*) is a pelagic fish in the sea, living in tropical and sub-tropical waters and warm waters (Shaffer and Nakamura, 1989). Cobia is a type of predatory fish, the preferred kind of feed is fish and crab. Besides that, it can also consume shellfish, zoobenthos, and nekton (Coriolano and Coelho, 2012). Cobia fish has good white meat quality, so that it has economic value as a consumption fish and can be used as a candidate species in aquaculture.

Cobia fish farming activities have been carried out in several countries, including Texas (Faulk and Holt, 2007), India (Gopakumar *et al.*, 2011), Vietnam (Nhu *et al.*, 2011), and Australia (Lee *et al.*, 2015). In hatchery and rearing activities in offshore cages, Cobia fish weighing 100-600 gram in 1-1.5 years can reach 6-8 kg (Liao *et al.*, 2004).

In Cobia fish farming activities, the first activity carried out is the maturation of the parent gonads to produce eggs of good quality, so that other activities (larvae rearing, nursery, rounding, and rearing) can be carried out. One of the factors that influence the success of parental gonad maturation is nutrition. Izquierdo *et al.*, (2001) stated that improving feed and nutrition in broodstock can improve not only egg and sperm quality but also seed production. Essential nutrients influence gonad development and fecundity in the feed.

So far, the feed used to stimulate the gonadal maturation process is fresh feed, in the form of fresh fish and squid (Liao *et al.*, 2004; Benetti *et al.*, 2008; Nhu *et al.*, 2011; Nguyen *et al.*, 2012; Lee *et al.*, 2015). Fresh feed has varying quality throughout the spawning season, thus affecting the biochemical

composition of Cobia eggs (Faulk and Holt, 2008; Nguyen *et al.*, 2010, 2012).

Information on formula feed for gonadal maturation and spawning of Cobia broodstock is still limited (Fraser and Davies, 2009; Estrada *et al.*, 2016). Nguyen *et al.* (2010) suggested that formula feed for Cobia broodstock should contain n-3 HUFA higher than 1.86% dry weight, and AA content should be below 0.42% dry weight. Asmanik (2020) reported no significant difference in the percentage of mature stage oocytes between the artificial feed treatment (n-3 HUFA content 2.82% dry weight) and the fresh Kurisi fish feed on the gonadal maturation activity of female Cobia fish. The use of fresh Kurisi fish feed can be replaced by artificial feed with a production cost of Rp. 30.004.00/kg of feed. This cost is cheaper ( $\pm$  60%) than the price of commercial feed for broodstock.

Fish farmers are usually less concerned about the importance of proper feed storage (De la Cruz *et al.*, (1989); Adaga (2014)). They are more concerned with seasonal fluctuations in feed availability and prices and make bulk purchases for long-term storage. Improper storage and for a long time, as well as the influence of physical conditions (moisture, heat, light) and microorganisms (fungi, bacteria, yeast), can cause deterioration of feed quality. It can reduce the palatability and nutritional value of the resulting product, including the breakdown of amino acids, vitamins, and fats (Chow, 1980). It can lead to economic losses. The rate of fat oxidation is affected by storage temperature, and at an increase of 10 °C, it doubles (Kulikov, 1978). Fat oxidation causes foodstuffs to have lower biological energy values (Rumsey,

1980), which can cause a decrease in animal growth (Stuart *et al.*, 1985).

In this article, we will describe the quality of artificial feed after undergoing the storage process. This study is helpful as consideration for determining the length of time for feed storage.

## RESEARCH METHODS



(a)



(b)

**Figure 1.** Artificial feed (a) P1 Feed (b) P2 Feed

**Table 1.** Feed Formula for Female Cobia

Ingredients (%)	Feed Treatment	
	P1	P2
PSDF	36.15	9.86
SCLP	14.16	11.33
SLP	22.80	53.80
FSP	0.00	9.69
CSFO	9.00	5.67
Tapioca	15.28	7.03
Vitamin premix (VP)	0.20	0.20
Vitamin C	0.02	0.02
Mineral mix	0.40	0.40
Phosporus	1.00	1.00
CMC	1.00	1.00
Total	100	100
K-dry	95	95
Protein	43	43
Lipid	16	16
Energy (kcal/100 g)	417	417
n-3 HUFA	2.82	2.76

After the feed formula was made, the feed was made with the size of the feed (2 cm diameter and 1.5 cm thick), which was adjusted to the size of the mouth opening of the Cobia. The feed was made at the Feed Laboratory, BBPBL Lampung. Feeding is done manually (feed production is done per 2 kg of feed for each type of artificial feed. All feed ingredients are mixed and stirred evenly in dry conditions, then

## Feed Formulation

The formula feed was made of isoprotein (43%), isolipid (16%), and isoenergy (417 kcal/100 g). There are two types of artificial feed made, namely: P1 feed (n-3 HUFA content 2.82% dry weight) and P2 feed (n-3 HUFA content 2.76% dry weight) (Figure 1). The feed formulations are presented in Table 1.

600 ml/1 kg of water is added, then stirred until evenly distributed. The feed is printed Manually). The feed was dried in an oven at 50 °C for 24 hours for P1 feed, while P2 feed was for 48 hours. The feed finished in the dryer is then dried and packed in a closed plastic container and stored in a room with a temperature of 17 °C. Furthermore, observations of physical parameters (change in color, odor, texture, infestation, fraction, and overall quality) and proximate and fatty acid analysis of the test feed were carried out to ensure feed content.

## Analysis of Feed Ingredients

The analysis was carried out in the laboratory of PT Saraswanti Indogenetech Bogor. The test feed's proximate and fatty acid test was carried out three times, namely at 0 month, 1<sup>st</sup> month, and 2<sup>nd</sup> month, with three replications each. It is to see whether or not there is a change in the chemical composition of the test feed. Evaluation of the chemical composition of feed ingredients consisting of: peruvian steam dried fish meal (PSDF), scallop liver powder (SCLP), squid liver powder (SLP), fish soluble powder (FSP), crude salmon fish oil (CSFO), which includes proximate analysis and fatty acids. The method used in the proximate analysis is as follows: carbohydrate measurement refers to 18-8-9/MU/SMM-SIG, GC., water content refers to SNI 01-2891-1992, point 5.1., ash content refers to SNI 01 -2891-1992, point 6.1., protein content refers to 18-8-3/MU/SMM-SIG, Kjeltex, and total fat content refers to 18-8-5/MU/SMM-SIG, Weilbull., while fatty acid analysis refers to 18-6-1/MU/SMM-SIG.

### Statistical Analysis

Proximate test data and fatty acid content in the test feed were analyzed using one-way ANOVA and continued with the Tukey test if the ANOVA test found  $F_{count} > F_{table}$  ( $H_0$  rejected and  $H_1$  accepted).

### RESULT AND DISCUSSION

The results of the observation of physical parameters are presented in Table 2, while the results of the proximate analysis are shown in Table 3, and the results of the analysis of fatty acids are presented in Table 4.

**Table 2.** Feed Formula

Fisic	Feeds	Months		
		0	1st	2nd
Color	P1	Dark brown	Dark brown	Dark brown
	P2	Dark brown	Dark brown	Dark brown
Odor	P1	Typical	Typical	Typical
	P2	Typical	Typical	Typical
Texturs	P1	Normal	Normal	Normal
	P2	Normal	Normal	Normal
Yeast	P1	Clean	Clean	Clean
	P2	Clean	Clean	Clean
Spillikins	P1	little	little	little
	P2	Clean	Clean	Clean
Total Quality	P1	Acceptable	Acceptable	Acceptable
	P2	Acceptable	Acceptable	Acceptable

Observation of physical parameters during two months of storage showed that the overall feed quality was good. The P1 feed showed the nature of breaking more easily than the P2 feed. This is influenced by the essential ingredients of feed ingredients. The P1 feed mainly consisted of fish meal (PSDF), while the P2 feed consisted mainly of squid liver meal (SLP). SLP is more sticky than PSDF. It causes the P1 feed to break more easily (crumbs) than the P2 feed (denser).

The drying process on P2 feed takes a longer time (2 x 24 hours) than P1 feed (1 x 24 hours) at a temperature of 50 °C to reach a moisture content of  $\pm 5\%$ . Buckle *et al.*, (1987) stated that several factors that affect the drying speed are the

product's physical properties, the temperature of the drying equipment, humidity, and air velocity. P2 feed contains more SLP ingredients than P1 feed. SLP is more sticky and slightly wet than PSDF (crumb and dry).

The results of the proximate analysis of P1 feed and P2 feed at zero month, the results were isoprotein (43%), isolipid (16%) and isoenergy (417 kcal/100 g). Based on the results of the ANOVA test, the results of the proximate analysis at 0, 1st, and 2nd months on P1 feed showed no significant differences in water content, ash, fat, protein, energy from fat, total energy, and carbohydrates for two months. The storage is at room temperature at 17 °C. The results of the ANOVA test on the results of the proximate analysis of P2 feed showed no significant differences in the content of ash, fat, energy from fat, and total energy for two months of storage at room temperature 17 °C; the changes are water content, protein, and carbohydrates. The water and carbohydrate content showed an increase in the 1st and 2nd months, while the protein content decreased in the 1st and 2nd months. The increase in water content is thought to be influenced by the temperature and relative humidity of the storage environment. The high level of relative humidity causes water vapor to enter during packaging and storage. Moisture promotes the growth of mold and bacteria, so feed manufacturers target moisture content below 12%. Feeds exposed to high humidity tend to increase the moisture content. Moist feed is relatively soft and easy to compress (Cruz, 1996).

The increase in carbohydrate levels in P2 feed during storage can be explained from the results of research by Lee *et al.*, (2015); in closed storage indicates that storage time has a positive effect on net calorific value (NCV) (an increase of 1% to 2%). The increase in potential energy content, such as aldehydes and ketones produced during storage, increased NCV.

The decrease in protein content is due to differences in feed ingredients (Solomon *et al.*, 2016) and their susceptibility to protein aging (Shyong *et al.*, 1998). Hossain *et al.*, (2011) reported that changes in the chemical composition and nutritional value of feed could occur during storage. Sun and Leopold (1997) stated that increasing seed moisture content and humidity increased protein breakdown. Protein damage is indicated by a decrease in its levels and changes in its profile.

**Table 3.** Proximat Analysis.

Parameters	Unit	P1			P2		
		Months			Months		
		0	1st	2nd	0	1st	2nd
Water	%	5.43 $\pm$ 0.01 <sup>a</sup>	5.27 $\pm$ 0.09 <sup>a</sup>	5.44 $\pm$ 0.04 <sup>a</sup>	5.17 $\pm$ 0.04 <sup>a</sup>	5.24 $\pm$ 0.09 <sup>ab</sup>	5.47 $\pm$ 0.01 <sup>b</sup>
Ash	%	10.41 $\pm$ 0.33 <sup>a</sup>	10.38 $\pm$ 0.13 <sup>a</sup>	10.09 $\pm$ 0.16 <sup>a</sup>	11.38 $\pm$ 0.1 <sup>a</sup>	11.43 $\pm$ 0.32 <sup>a</sup>	11.27 $\pm$ 0.04 <sup>a</sup>
Lipid	%	16.11 $\pm$ 0.28 <sup>a</sup>	16.29 $\pm$ 0.21 <sup>a</sup>	15.58 $\pm$ 0.23 <sup>a</sup>	16.62 $\pm$ 0.36 <sup>a</sup>	15.79 $\pm$ 0.13 <sup>a</sup>	16.23 $\pm$ 0.18 <sup>a</sup>
Protein	kkal/100 g	43.28 $\pm$ 0.57 <sup>a</sup>	41.97 $\pm$ 0.11 <sup>a</sup>	41.95 $\pm$ 0.37 <sup>a</sup>	43.50 $\pm$ 0.51 <sup>b</sup>	42.67 $\pm$ 0.21 <sup>ab</sup>	41.42 $\pm$ 0.52 <sup>a</sup>
Energy from Lipid	kkal/100 g	144.99 $\pm$ 2.55 <sup>a</sup>	146.61 $\pm$ 1.91 <sup>a</sup>	140.18 $\pm$ 2.10 <sup>a</sup>	149.54 $\pm$ 3.25 <sup>a</sup>	142.11 $\pm$ 1.15 <sup>a</sup>	146.07 $\pm$ 1.65 <sup>a</sup>
Total energy	%	417.23 $\pm$ 0.17 <sup>a</sup>	418.89 $\pm$ 1.23 <sup>a</sup>	415.76 $\pm$ 1.96 <sup>a</sup>	416.90 $\pm$ 0.06 <sup>a</sup>	412.31 $\pm$ 0.27 <sup>a</sup>	414.23 $\pm$ 0.81 <sup>a</sup>
Carbohydrate	%	24.78 $\pm$ 0.13 <sup>a</sup>	26.11 $\pm$ 0.28 <sup>a</sup>	26.95 $\pm$ 0.40 <sup>a</sup>	23.33 $\pm$ 0.01 <sup>a</sup>	24.89 $\pm$ 0.15 <sup>ab</sup>	25.62 $\pm$ 0.74 <sup>b</sup>

Note: the same notation means that there is no significant difference.

**Table 4.** Summary of the ANOVA Test Results On The Fatty Acid Content of The Test Feed

No	Lipid Acid		P1			P2		
			Month			Month		
			0	1st	2nd	0	1st	2nd
1	Butyrate	C 4:0	0.002 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.001 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>
2	Caproate	C 6:0	0.002 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.001 ± 0.000 <sup>b</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>
3	Caprylate	C 8:0	0.001 ± 0.000 <sup>b</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.001 ± 0.000 <sup>b</sup>	0.000 ± 0.000 <sup>a</sup>	0.003 ± 0.000 <sup>c</sup>
4	Caprate	C 10:0	0.001 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.001 ± 0.000 <sup>b</sup>	0.000 ± 0.000 <sup>a</sup>	0.003 ± 0.000 <sup>c</sup>
5	Undecanoate	C 11:0	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>
6	Laurate	C 12:0	0.010 ± 0.000 <sup>a</sup>	0.011 ± 0.000 <sup>a</sup>	0.016 ± 0.000 <sup>b</sup>	0.011 ± 0.000 <sup>a</sup>	0.012 ± 0.000 <sup>a</sup>	0.024 ± 0.001 <sup>a</sup>
7	Tridecanoate	C 13:0	0.004 ± 0.000 <sup>a</sup>	0.005 ± 0.000 <sup>b</sup>	0.005 ± 0.000 <sup>c</sup>	0.003 ± 0.000 <sup>a</sup>	0.004 ± 0.000 <sup>a</sup>	0.004 ± 0.000 <sup>a</sup>
8	Myristate	C 14:0	0.470 ± 0.012 <sup>a</sup>	0.569 ± 0.019 <sup>a</sup>	0.563 ± 0.002 <sup>a</sup>	0.509 ± 0.013 <sup>a</sup>	0.591 ± 0.002 <sup>a</sup>	0.576 ± 0.007 <sup>a</sup>
9	Myristoleate	C 14:1	0.006 ± 0.001 <sup>a</sup>	0.010 ± 0.000 <sup>b</sup>	0.015 ± 0.000 <sup>c</sup>	0.005 ± 0.000 <sup>a</sup>	0.007 ± 0.000 <sup>c</sup>	0.006 ± 0.000 <sup>b</sup>
10	Pentadecanoate	C 15:0	0.077 ± 0.002 <sup>a</sup>	0.088 ± 0.002 <sup>a</sup>	0.087 ± 0.001 <sup>a</sup>	0.057 ± 0.002 <sup>a</sup>	0.061 ± 0.001 <sup>a</sup>	0.063 ± 0.000 <sup>a</sup>
11	Pentadecenoate	C 15:1	0.012 ± 0.000 <sup>a</sup>	0.014 ± 0.000 <sup>a</sup>	0.011 ± 0.000 <sup>a</sup>	0.009 ± 0.000 <sup>a</sup>	0.011 ± 0.000 <sup>a</sup>	0.010 ± 0.000 <sup>a</sup>
12	Palmitate	C 16:0	2.606 ± 0.051 <sup>a</sup>	2.980 ± 0.057 <sup>a</sup>	2.812 ± 0.002 <sup>a</sup>	2.477 ± 0.057 <sup>a</sup>	2.680 ± 0.007 <sup>a</sup>	2.687 ± 0.012 <sup>a</sup>
13	Palmitoleate	C 16:1	0.633 ± 0.011 <sup>a</sup>	0.736 ± 0.014 <sup>a</sup>	0.705 ± 0.000 <sup>a</sup>	0.673 ± 0.019 <sup>a</sup>	0.756 ± 0.002 <sup>a</sup>	0.725 ± 0.008 <sup>a</sup>
14	Heptadecanoate	C 17:0	0.159 ± 0.002 <sup>a</sup>	0.181 ± 0.003 <sup>a</sup>	0.176 ± 0.001 <sup>a</sup>	0.148 ± 0.004 <sup>a</sup>	0.162 ± 0.001 <sup>b</sup>	0.137 ± 0.002 <sup>a</sup>
15	Heptadecenoate	C 17:1	0.089 ± 0.001 <sup>a</sup>	0.104 ± 0.002 <sup>a</sup>	0.099 ± 0.001 <sup>a</sup>	0.105 ± 0.003 <sup>a</sup>	0.118 ± 0.000 <sup>a</sup>	0.113 ± 0.001 <sup>a</sup>
16	Stearate	C 18:0	0.794 ± 0.014 <sup>a</sup>	0.816 ± 0.011 <sup>a</sup>	0.793 ± 0.019 <sup>a</sup>	0.737 ± 0.014 <sup>a</sup>	0.703 ± 0.007 <sup>a</sup>	0.744 ± 0.007 <sup>a</sup>
17	Oleate n-9t	C 18:1	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>
18	Oleate n-9c	C 18:1 n-9T n-9C	4.341 ± 0.075 <sup>a</sup>	4.434 ± 0.041 <sup>a</sup>	4.193 ± 0.063 <sup>a</sup>	5.199 ± 0.116 <sup>b</sup>	4.825 ± 0.056 <sup>c</sup>	5.069 ± 0.047 <sup>a</sup>
19	Linoleate n-6t	C 18:2 n-6T	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>
20	Linoleate n-6c	C 18:2 n-6C	1.957 ± 0.033 <sup>a</sup>	2.013 ± 0.023 <sup>a</sup>	1.909 ± 0.037 <sup>a</sup>	2.090 ± 0.045 <sup>a</sup>	1.956 ± 0.015 <sup>a</sup>	2.027 ± 0.016 <sup>a</sup>
21	Arachidate	C 20:0	0.072 ± 0.001 <sup>a</sup>	0.072 ± 0.001 <sup>a</sup>	0.072 ± 0.003 <sup>a</sup>	0.059 ± 0.001 <sup>a</sup>	0.054 ± 0.001 <sup>a</sup>	0.056 ± 0.002 <sup>a</sup>
22	Linolenate n-6	C 18:3 n-6	0.020 ± 0.000 <sup>a</sup>	0.023 ± 0.000 <sup>a</sup>	0.023 ± 0.000 <sup>a</sup>	0.030 ± 0.001 <sup>a</sup>	0.028 ± 0.000 <sup>a</sup>	0.029 ± 0.000 <sup>a</sup>
23	Eicosenoate	C 20:1	0.628 ± 0.009 <sup>a</sup>	0.241 ± 0.002 <sup>a</sup>	0.314 ± 0.005 <sup>a</sup>	0.490 ± 0.010 <sup>a</sup>	0.231 ± 0.002 <sup>a</sup>	0.301 ± 0.007 <sup>a</sup>
24	Linolenate n-3	C 18:3 n-3	0.754 ± 0.013 <sup>a</sup>	0.773 ± 0.010 <sup>a</sup>	0.744 ± 0.016 <sup>a</sup>	0.722 ± 0.015 <sup>a</sup>	0.679 ± 0.006 <sup>a</sup>	0.689 ± 0.007 <sup>a</sup>
25	Heneicosanoate	C 21:0	0.010 ± 0.000 <sup>a</sup>	0.017 ± 0.000 <sup>b</sup>	0.018 ± 0.000 <sup>b</sup>	0.008 ± 0.000 <sup>a</sup>	0.010 ± 0.000 <sup>b</sup>	0.013 ± 0.000 <sup>c</sup>
26	Eicosadienoate	C 20:2	0.093 ± 0.001 <sup>a</sup>	0.088 ± 0.001 <sup>a</sup>	0.088 ± 0.000 <sup>a</sup>	0.127 ± 0.001 <sup>c</sup>	0.107 ± 0.001 <sup>a</sup>	0.119 ± 0.002 <sup>b</sup>
27	Behenate	C 22:0	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.042 ± 0.001 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.022 ± 0.000 <sup>b</sup>
28	Eicosatrienoate	C 20: n-6	0.034 ± 0.000 <sup>a</sup>	0.033 ± 0.000 <sup>a</sup>	0.032 ± 0.001 <sup>a</sup>	0.049 ± 0.001 <sup>a</sup>	0.042 ± 0.000 <sup>a</sup>	0.044 ± 0.001 <sup>a</sup>
29	Erucate	C 22:1 n-9	0.322 ± 0.005 <sup>b</sup>	0.033 ± 0.000 <sup>a</sup>	0.032 ± 0.001 <sup>a</sup>	0.195 ± 0.004 <sup>b</sup>	0.028 ± 0.000 <sup>a</sup>	0.032 ± 0.001 <sup>a</sup>
30	Eicosatrienoate	C 20:3 n-3	0.032 ± 0.000 <sup>a</sup>	0.033 ± 0.000 <sup>a</sup>	0.034 ± 0.001 <sup>a</sup>	0.040 ± 0.001 <sup>a</sup>	0.036 ± 0.001 <sup>a</sup>	0.041 ± 0.001 <sup>a</sup>
31	Tricosanoate	C 23:0	0.006 ± 0.000 <sup>a</sup>	0.011 ± 0.000 <sup>ab</sup>	0.012 ± 0.000 <sup>b</sup>	0.000 ± 0.000 <sup>a</sup>	0.006 ± 0.000 <sup>ab</sup>	0.008 ± 0.000 <sup>b</sup>
32	Arachidonate	C 20:4 n-6	0.149 ± 0.002 <sup>a</sup>	0.147 ± 0.001 <sup>a</sup>	0.140 ± 0.005 <sup>a</sup>	0.117 ± 0.002 <sup>a</sup>	0.108 ± 0.003 <sup>a</sup>	0.113 ± 0.002 <sup>a</sup>
33	Docosadienoate	C 22:2	0.008 ± 0.000 <sup>a</sup>	0.008 ± 0.000 <sup>a</sup>	0.007 ± 0.000 <sup>a</sup>	0.011 ± 0.000 <sup>a</sup>	0.009 ± 0.000 <sup>a</sup>	0.009 ± 0.000 <sup>a</sup>
34	Lignoserate	C 24:0	0.023 ± 0.000 <sup>a</sup>	0.030 ± 0.000 <sup>a</sup>	0.031 ± 0.000 <sup>a</sup>	0.017 ± 0.000 <sup>a</sup>	0.021 ± 0.000 <sup>b</sup>	0.026 ± 0.000 <sup>c</sup>
35	EPA	C 20 : 3 n-3	0.968 ± 0.016 <sup>a</sup>	1.002 ± 0.011 <sup>a</sup>	0.916 ± 0.033 <sup>a</sup>	1.194 ± 0.025 <sup>a</sup>	1.109 ± 0.012 <sup>a</sup>	1.115 ± 0.019 <sup>a</sup>
36	Nervonate	C 24:1	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>
37	DHA	C 22:6 n-3	1.825 ± 0.030 <sup>a</sup>	1.821 ± 0.014 <sup>a</sup>	1.682 ± 0.058 <sup>a</sup>	1.530 ± 0.029 <sup>a</sup>	1.437 ± 0.018 <sup>a</sup>	1.421 ± 0.042 <sup>a</sup>
38	n-3 HUFA		2.825 ± 0.045 <sup>a</sup>	2.856 ± 0.025 <sup>a</sup>	2.631 ± 0.092 <sup>a</sup>	2.763 ± 0.055 <sup>a</sup>	2.690 ± 0.034 <sup>a</sup>	2.576 ± 0.061 <sup>a</sup>
39	DHA/EPA		1.886 ± 0.000 <sup>a</sup>	1.818 ± 0.006 <sup>a</sup>	1.837 ± 0.003 <sup>a</sup>	1.282 ± 0.002 <sup>a</sup>	1.296 ± 0.002 <sup>a</sup>	1.275 ± 0.016 <sup>a</sup>
40	EPA/AA		6.507 ± 0.002 <sup>a</sup>	6.838 ± 0.029 <sup>a</sup>	6.535 ± 0.003 <sup>a</sup>	10.175 ± 0.03 <sup>b</sup>	10.229 ± 0.14 <sup>b</sup>	9.872 ± 0.007 <sup>b</sup>
41	DHA/AA		12.275 ± 0.001 <sup>a</sup>	12.42 ± 0.01 <sup>a</sup>	12.01 ± 0.02 <sup>a</sup>	13.042 ± 0.01 <sup>a</sup>	13.25 ± 0.158 <sup>b</sup>	12.53 ± 0.149 <sup>a</sup>
42	n-3 / n-6		1.656 ± 0.001 <sup>b</sup>	1.638 ± 0.002 <sup>b</sup>	1.605 ± 0.018 <sup>b</sup>	1.525 ± 0.002 <sup>a</sup>	1.528 ± 0.005 <sup>a</sup>	1.475 ± 0.018 <sup>a</sup>

Note: the same notation means that there is no significant difference

The results of fatty acid analysis (Table 4) showed that the n-3 HUFA fatty acid content in the P1 feed was  $2.825 \pm 0.045$  % dry weight, while in P2 feed was  $2.763 \pm 0.055$  % dry weight. Based on the ANOVA results from the DHA, EPA, AA, and n-3 HUFA fatty acids analysis, there was no change in value during two months of storage in both P1 and P2 feeds.

The difference in the content of n-3 HUFA between P1 feed and P2 feed was not too big, is 0.062 % (n-3 HUFA content of P1 feed = 2.825% dry weight while in P2 feed = 2.763% dry weight). However, because the composition of the constituent materials is different, there are differences, among others, in the value of the ratio of DHA/EPA, EPA/AA, DHA/AA, and n-3/n-6. From the proximate and fatty acid analysis of feed for two months of storage, it was seen that the P1 feed was more stable than the P2 feed.

## CONCLUSION

The conclusions were 1) The physical condition of the feed after two months of storage in plastic bags at 17 °C was good. The P1 feed is more crumbly than the P2 feed; 2) The results of proximate analysis on the P1 feed showed no significant difference during storage. The proximate analysis of P2 feed showed no significant difference in ash content, fat content, energy from fat, and total energy during storage; that experienced significant changes were water, protein, and carbohydrate content. The water and carbohydrate content showed an increase in the 1st and 2nd months, while the protein content decreased in the 1st and 2nd months; 3) The results of the analysis of fatty acids DHA, EPA, AA, and n-3 HUFA did not show significant changes during storage of the two types of feed.

The suggestion is that further research is needed on adhesive materials, significantly to improve the structure of the P1 feed so that it is more compact and not crumbly.

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