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Utilization of Lemna Flour (*Lemna minor*) As A Source of Feed Nutrition Substituted with Commercial Feed on The Results of Chemical Tests on Fish Feed

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ABSTRACT

Weeds pose a major challenge to crop productivity, significantly impacting maize (*Zea mays* L.), which is Nepal's second-most important staple crop. Although attainable yield 5.8 t ha^{-1}, the national average is only 3.15 t ha^{-1}, largely due to issues like weed competition that can lead to yield losses of up to 48%. This study, carried out during the winter season at the National Maize Research Program in Rampur, Chitwan, evaluated nine different weed management treatments, weedy check, weed free, green polythene mulching, clear polythene mulching, cowpea co-culture, black polythene mulching, atrazine + one hand weeding @ 30 DAS, silver black polythene mulch and *Lantana camara* as mulch in a randomized complete block design with four replications. The results showed that mulching methods, particularly silver-black polythene mulch, significantly enhanced growth parameters, grain yield (4,537.5 kg ha^{-1}) and stover yield (6,500 kg ha^{-1}). Furthermore, mulching facilitated earlier tasseling (73.19 DAS) and silking (85.31 DAS), while also improving soil moisture retention, and weed suppression. Both clear and black polythene mulches had beneficial effects on growth and yield. Similarly, grain yield in *Lantana camara* as mulch and Atrazine @ 0.75 kg ha^{-1} + one hand weeding also remain same with clear polythene mulch.

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KEYWORDS: Utilization, Lemna minor, Fish feed, Substitution, Chemical test.

INTRODUCTION

Feed is one of the important production factors in fish farming activities, especially in intensive farming systems. In fish farming, feed is always a problem due to the expensive price of feed (Mose et al., 2020). Feed that has good nutritional and physical quality is the key to achieving production and economic goals for fish farmers. Therefore, the nutrients contained in the feed must be truly controlled and meet the needs of the fish (Gunawan and Munawwar, 2015). Raw materials for making fish feed are still 80% imported (Melati et al., 2010). The main source of protein in making fish feed in general that has not been replaced so far is fish meal (Kordi, 2007).

The availability of feed in intensive aquaculture is essential. Fish feed needs must be met from outside the pond, namely in the form of artificial food known as fish feed (Iskandar and Subhan, 2017). The availability of sufficient fish feed, both in quality and quantity, is one of the key factors in the success of fish farming. The problem that often arises in feed procurement is the high cost of feed, considering that fish feed requirements reach 60-70% of total production costs (Mulia et al., 2017). Feed is one of the production costs that contributes 65% of total production costs, so there needs to be an alternative feed ingredient that can reduce feed costs (Pomeroy et al., 2017). Another alternative to overcome the feed problem is in the form of making independent feed using local ingredients at a more affordable price (Mose et al., 2020).

Quality feed helps achieve optimal production goals (Darmawiyanti & Baidhowi, 2015). Therefore, it is necessary to know the knowledge of nutrition, composition, and physical quality (Suryaningsih, 2010). The use of commercial feed can be reduced by making feed with local raw materials so that it can reduce production costs in fish farming; thus, high fish growth will increase fish production (Amin et al., 2020). In increasing feed efficiency and fish growth, feed nutrition must be in accordance with fish needs

(Hidayat & Sasanti, 2013). However, the current problem is that in the formulation of pellet feed, the main raw material used for protein sources is fish meal. Fish meal has a high protein content of around 55-60%. The problem is that pellet feed that relies on imported fish meal tends to be expensive. Therefore, it is necessary to formulate feed by substituting alternative ingredients from other animals (Gunawan and Munawwar, 2015). Some requirements for alternative feed ingredients include easy availability, low price, and high nutritional content (Suprayudi et al., 2011).

One of the materials that has not been utilized is lemna, which is a water weed that has no selling value and does not compete with human needs. Lemna is cosmopolitan and can grow anywhere, such as in swamps, rice fields, puddles, and ponds (Asriyanti et al., 2018). Its availability and development in nature are very good; it has nutritional content, such as vegetable protein, and does not compete with human needs (Asriyanti et al., 2018). The use of Lemna minor as a raw material for fish feed is constrained by the high crude fiber content, which is 20.08% (Zidni et al., 2017). This can cause the level of digestibility of feed for fish to be low. Therefore, to determine the importance of fish feed in the world of fish farming, a study was conducted on the use of Lemna minor as one of the main ingredients of fish feed, where the Lemna minor plant is one of the wild plants whose use has not been widely carried out.

MATERIAL AND METHOD

The method used in this study is an experimental method to determine the effect of ingredients on the nutritional content of fish meal formulated in pellet feed using the main ingredients, namely, lemna minor flour, commercial feed, and feed adhesive in the form of tapioca flour, with the treatment as shown in Table 1. This research design used a Completely Randomized Design with 3 treatments and 1 control, namely commercial feed and 3 replications. This research was conducted for 2 months, namely April to May 2024, at the Laboratory of the Department of Agricultural Technology, Makassar State University, while the proximate test of feed was carried out at the Chemistry and Nutrition Laboratory of the Pangkep State Agricultural Polytechnic.

Table 1. Research treatments

Materials for making fish feed	Treatments (%)			
	K	A	B	C
Lemna minor flour	0	18	20	22
Commercial feed (Brand : HI-PRO-FIT 781)	100	78	76	74
Tapioca flour	0	4	4	4
Amount	100	100	100	100

After obtaining the amount of the required value of the materials needed that have been calculated in making feed, the next step is to make feed. The process of making pellet feed is that each raw material is boiled at a temperature of 80°C for 15 minutes, then dried and ground until smooth, after which it is dried again until it forms like flour. After becoming flour, the next step is mixing until the ingredients are evenly mixed while mixing warm water until it becomes dough. Next, molding and forming of pellet feed is carried out. The last stage of the feed that is made is dried using a drying chamber. After the feed is dry, a proximate test is carried out consisting of a protein content test, water content test, ash content test, fat content test, and carbohydrate content test.

Protein content

A total of 20 g of feed was ground and dissolved using water, then stirred until evenly distributed; if it is still in solid form, then it is centrifuged at a speed of 3000 RPM for 10 minutes. Before centrifuging, each treatment had 1 ml of 10% TCA added so that the protein was denatured and precipitated. The centrifuge results in the form of supernatants were discarded, and denatured protein sediment was taken. After that, 2 ml of ethyl ether was added to the protein sediment and then centrifuged again. After that, it was dried again at a temperature of 28°C for 10 minutes. Then 4 ml of water was added to the sediment and mixed evenly, and 6 ml of biuret reaction was added to each treatment or test tube. After that it was stored again at a temperature of 30°C for 10 minutes until the formation of a perfect purple color then the absorbance was measured in a spectrometer at a speed of 520 nm (Gunawan and Munawwar, 2015).

Water content

Water content test refers to (Gunawan and Munawwar, 2015). Weigh 1 gram of feed, then grind it. After that, it is put into a porcelain cup. The porcelain cup is heated in an open oven for 1 hour at a temperature of 110°C. Then it is cooled in a desiccator for 15 minutes. After that, the cup is weighed and the initial weight is recorded. Next, the cup was reheated for 30 minutes 3 times to get the middle value. After that, the feed that had been ground for 3 g was opened for 2 hours at a temperature of 110°C. The cup was cooled in a desiccator for 15 minutes and weighed. The weight of the cup containing the sample was reheated for 30 minutes 3 times, and then the water content was calculated using the following formula:

$$\text{Water content (\%)} = \frac{(A+B)-C}{B} X100\%$$

Where :
A= Weight of porcelain cup
B= Weight of sample
C= (cup weight + sample weight) after heating

Ash content

The ash content test refers to (Gunawan and Munawwar, 2015); namely, the sample is ground and weighed as much as 1 g, then put into a porcelain course after that, put into the furnace. Before being put into the furnace, the furnace is first heated for 1 hour and cooled in a desiccator for 15 minutes; after that, it is reheated for 30 minutes and then cooled in a desiccator for 15 minutes. This is done for 3 repetitions. After that, the furnace containing the sample was heated in an oven at a temperature of 550°C for 2 hours until the sample was whitish; after that, it was cooled in a desiccator for 15 minutes and then weighed. The cup was reheated for 30 minutes, repeated 3 times. Then calculate the ash content using the formula:

$$\text{Ash content (\%)} = \frac{(W_1+W_2)-C}{W} X100\%$$

Where:
W_1= Weight of cup + sample
W_2= Final ashing container weight
W = Initial cup weight

Fat content

The fat content test refers to (Gunawan and Munawwar, 2015); namely, the feed is weighed as much as 5 g and then put into a Soxhlet wrapper. The Soxhlet wrapper is placed in the Soxhlet extraction, after which a condenser is installed on top and equipped with water circulation so that it is not too hot, and the end of the condenser is covered with cotton. At the bottom of the Soxhlet, a fat flask that has been weighed is installed as the initial weight of the flask, then 100 ml of ethyl ether is added to the fat flask and heated using an electric heater. The function of ethyl ether is to wash all the fat content in the feed system by refluxing. Refluxing is the event of the rise and fall of ethyl ether in an effort to filter fat in feed. Reflux was done 33 times, or approximately 5 hours. After 5 hours, the flask was taken, and distillation was carried out for 30 minutes until the ethyl ether moved to another fat flask after distillation. The fat flask containing fat was heated in an oven at a temperature of 100°C. After drying, the fat flask was weighed again and then calculated using the formula:

$$\text{Fat content (\%)} = \frac{(C-B)}{A} X100\%$$

A= Weight of Feed sample
B= initial weight of the flask container
C= final weight of flask container

Carbohydrate content

The carbohydrate content test refers to (Gunawan and Munawwar, 2015). Carbohydrate analysis is carried out using the anthrone method. The feed is weighed and ground until smooth, then put into filter paper and washed using 80% alcohol with a ratio of 1:2. The filtered results are collected in an Erlenmeyer flask, 200 ml of water and 2 grams of $CaCO_3$ are added, and then boiled at a temperature of 100°C for 30 minutes. After that, it is cooled and transferred to a 500 ml measuring cylinder, then saturated Pb acetate is added slowly until the solution is clear. Pb acetate was added as much as 5 ml, then mixed evenly and filtered again with Whatman paper. Then sodium acetic acid was added again, as much as 1 g, to precipitate all Pb mixed evenly and filtered again. The filtered results were put into an Erlenmeyer. The filtration results above are ready to be used for the determination of carbohydrates, where all treatments are put into a test tube, then anthrone solution is made with the following steps: Weigh 5 mg of anthrone, then put it into a measuring flask with a flask size of 50 ml. Then, mixed with concentrated sulfuric acid, after that a standard glucose solution of 0.2 ml was taken and diluted to 100 ml in a 100 ml measuring flask. After that, a standard glucose solution was taken and also put into 5 test tubes that had been filled with blanks of 0.2, 0.4, 0.6, 0.8, and 1 ml. Then added water to each test tube. 1 ml to the blank; after that, each test tube, both in the treatment and blank, was added 5 ml of anthrone reaction; after that, the test tube was closed using cotton and heated at a temperature of 100°C for 12 minutes (soaked in boiling water). After that cooled quickly using water, and then all the solutions in the clear test tube were put into the spectrometer cuvette, and the absorbance was read at a speed of 630 nm. The data analysis used in this study was descriptive analysis.

RESULTS AND DISCUSSION

Protein content

Figure 1 shows that the highest protein content of feed in the study was in treatment K and the lowest in treatment B. This shows that the addition of Lemna minor can reduce the protein content of feed in feed K, although it is known that Lemna minor also contains vegetable protein. Protein is the main source of energy in fish; if the protein requirement is not met in its food, there will be a drastic decrease or cessation of growth or loss of body weight because the fish will pull back protein from several tissues to maintain the function of more vital tissues (Iskandar and Subhan, 2017). Rostika (1997) also stated that the goodness of the protein content of the feed is not seen from the protein content of the feed but also from the completeness of its amino acids. In general, fish need protein around 20-60%, with an optimum of 30-36% (Masyamsir, 2001). Protein content of around 60% is generally found in fish meal (Handajani & Widodo, 2010). The results of the ANOVA test showed a sig. value of 0.000, which means P < 0.005, which means that the treatment has an effect on the protein content of the feed.

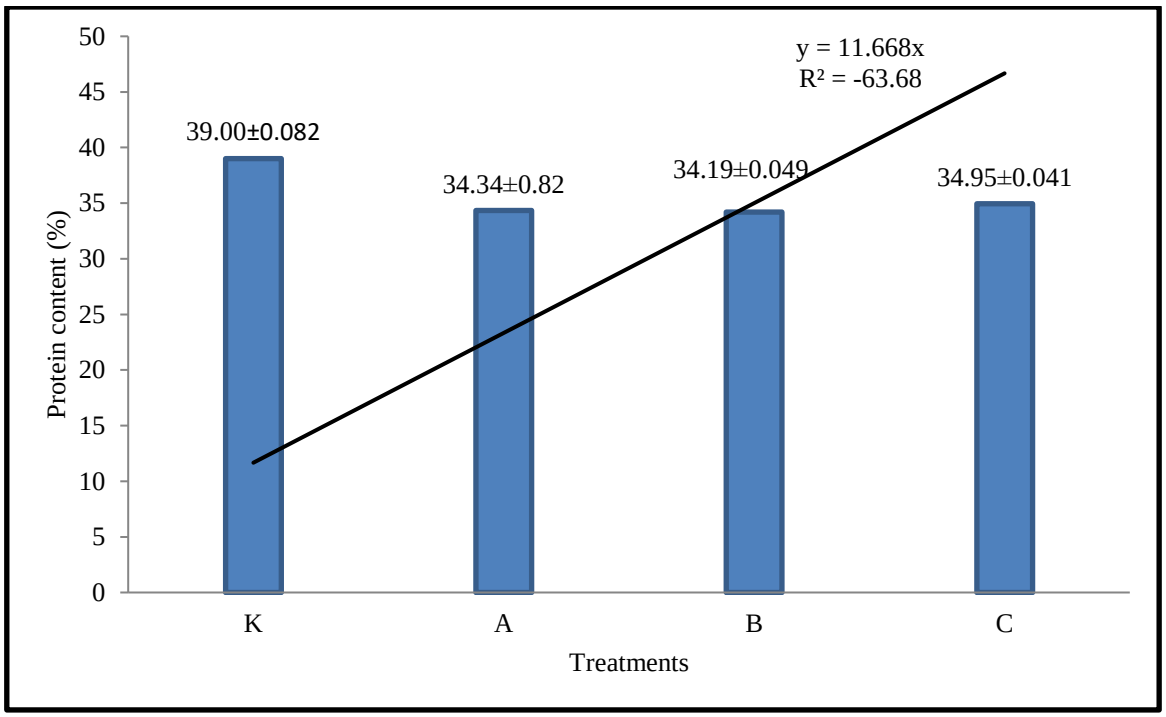

Figure 1 Protein content of fish feed

Water content

Figure 2 shows the highest water content in the control treatment, while the lowest water content is in treatment A. Thus it can be stated that the use of Lemna minor as one of the ingredients for making fish feed can be used because it has a low water content. The results of the ANOVA test showed a sig. value of 0.000, which means P < 0.005, which means that the treatment has an effect on the water content of the feed.

Water in feed plays a very important role because the nature of water in feed is to unite all ingredients in the feed. The level of dryness of this feed greatly determines the durability of the feed because if the artificial feed contains a lot of water, it will become damp. In this condition, if the feed is stored for too long, mold will grow (Gunawan and Munawwar, 2015). Excess water in feed can cause the feed to spoil easily (Mulia et al., 2017). According to Winarno (2004), the water content in food ingredients can affect the appearance, texture, and taste of food. Water content is the main parameter involved in most food spoilage reactions.

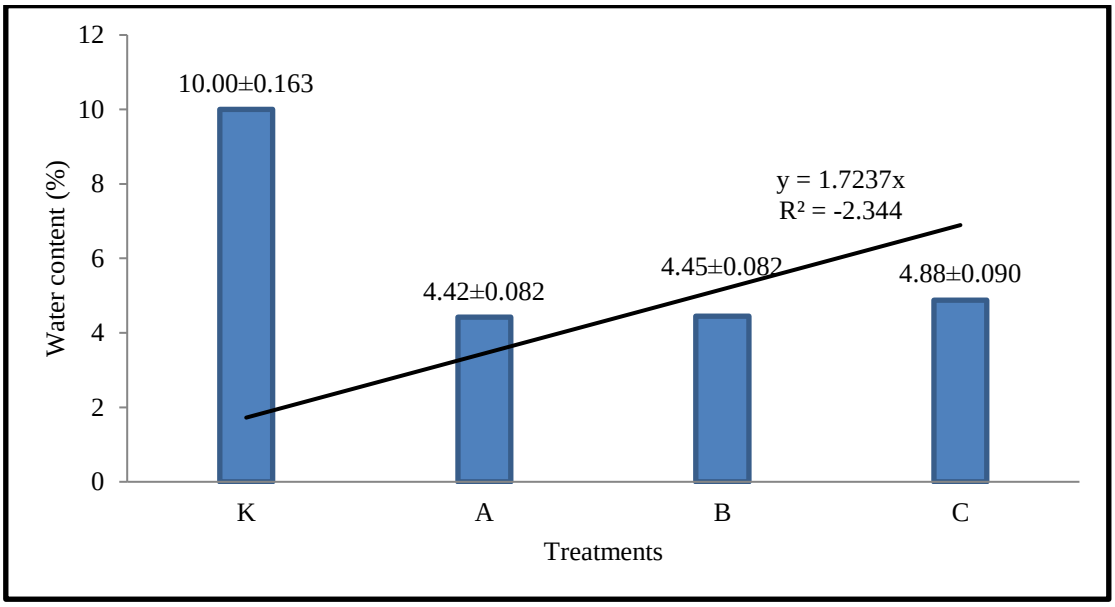

Figure 2 Water content of fish feed

Ash content

Figure 3 shows that the ash content in the feed is lower in the treatment of giving Lemna minor in the feed compared to the control treatment. The results of the ANOVA test showed a sig. value of 0.000, which means P < 0.005, which means that the treatment has an effect on the ash content of the feed. The ash content of fish feed in this study was in the range of 11.48 ± 0.082 to 12.00 ± 0.163%.

The standard ash content for good fish feed, according to the Indonesian National Standard (SNI), is below 13% (Gunawan., and Munawwar, 2015). This means that the ash content in the study is still relatively high because, according to (Winarno, 1997), the ideal ash content of fish feed is around 3–7%, where this ash content represents the mineral content of fish feed. Total ash is defined as the residue produced in the combustion process of organic materials in the form of inorganic compounds in the form of oxides, salts, and minerals. The total ash contained in a product is limited in amount. Ash is the residue produced by the combustion of organic materials in the form of inorganic materials in the form of oxides, salts, and minerals (Gunawan., and Munawwar, 2015).

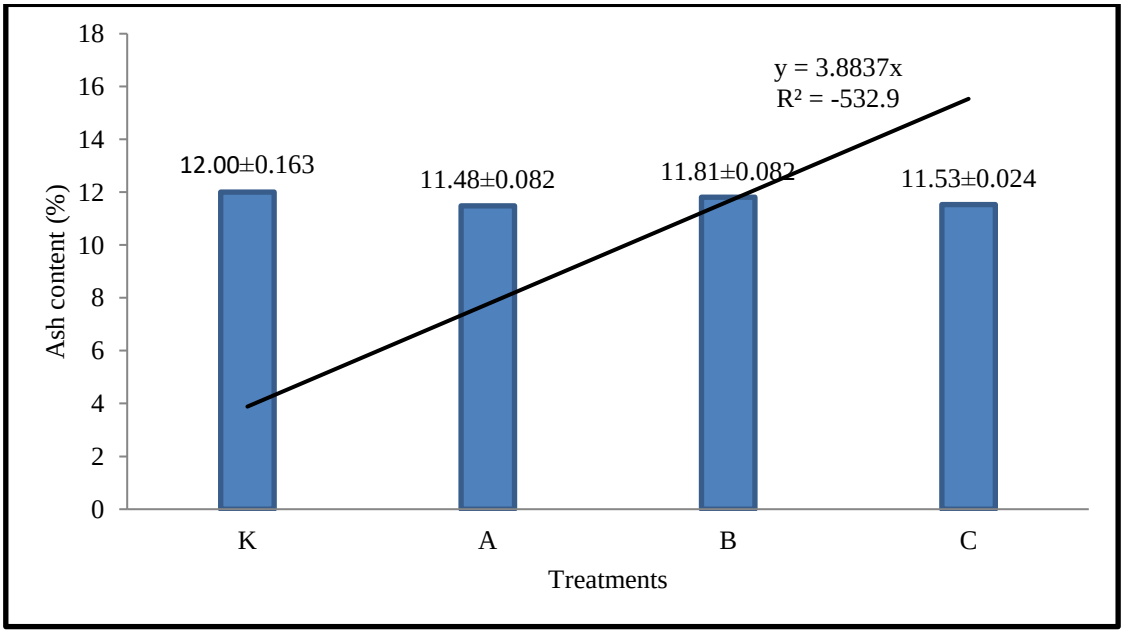

Figure 3 Ash content of fish feed

Fat content

Figure 4 shows that the fat content of fish feed is in the range of 5.00±0.41 to 7.56±0.049, with the lowest fat content in treatment K. The results of the ANOVA test show a sig. value of 0.000, which means P < 0.005, which means that the treatment has an effect

on the fat content of the feed. The role of fat as an energy source is second only to protein (Afrianto and Liviawaty, 2005). Fat and carbohydrates have a sparing effect on the use or utilization of protein. High levels of fat in feed will cause fat storage in the body, decreased feed consumption and growth, and liver degeneration (Huisman, 1976).

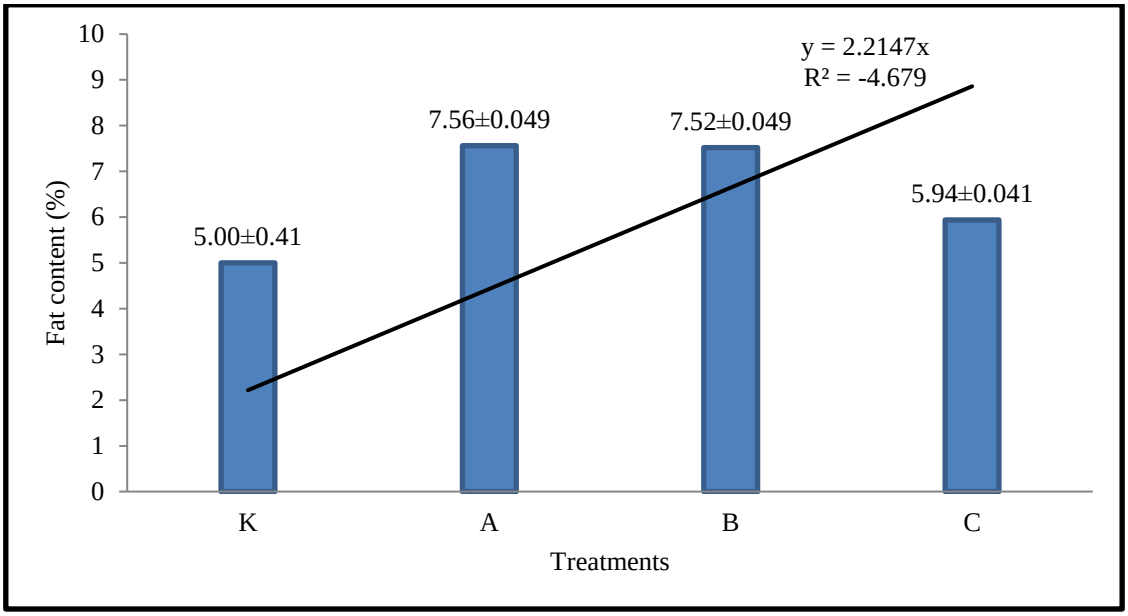

Figure 4 Fat content of fish feed

Carbohydrate content

Figure 5 shows that the carbohydrate content of fish feed is in the range of 30-42.7, with the highest carbohydrate content in treatment C. The results of the ANOVA test showed a sig. value of 0.000, which means P < 0.005, which means that the treatment has an effect on the carbohydrate content of the feed. If there is too much crude fiber (> 10%), it will decrease digestibility, decrease absorption, increase metabolic waste, and decrease the quality of culture water (Watanabe, 1988). While increasing the carbohydrate content of feed followed by decreasing the fat content results in higher fat retention. Brauge et al. (1994) stated that high levels of digestible carbohydrates stimulate the lipogenesis process and increase fat storage. Crude fiber is a part of carbohydrates that cannot be digested and is not an essential nutrient for marine fish. Crude fiber will cause dirt in the culture container but is still needed to facilitate the excretion of feces.

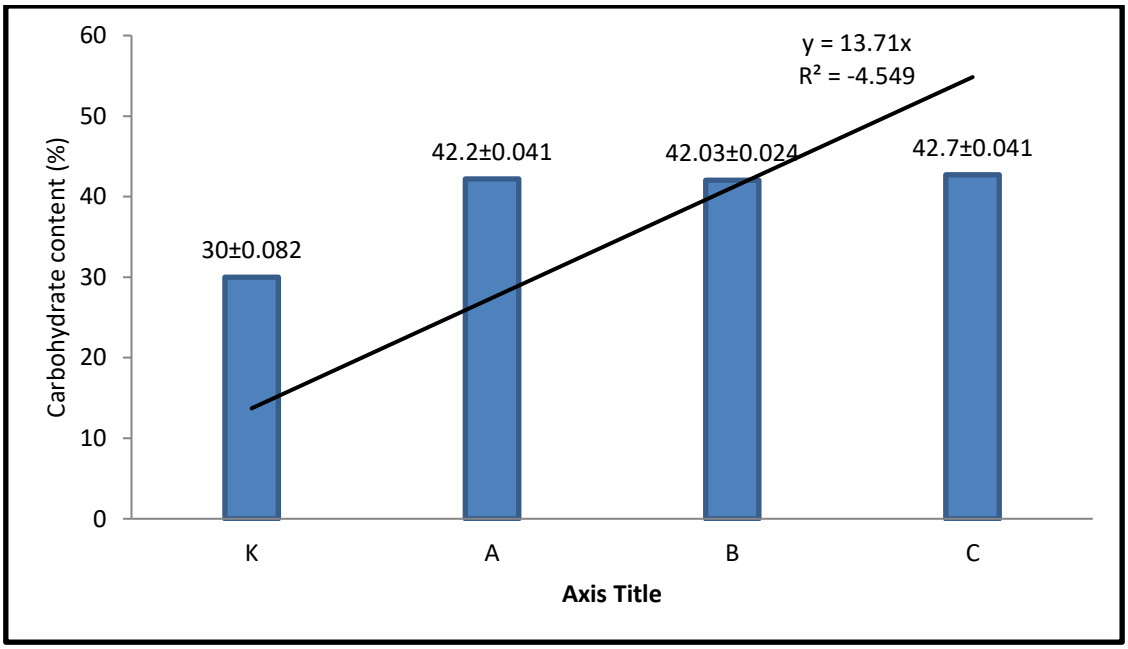

Figure 5 Carbohydrate content of fish feed

CONCLUSION

The results of the study showed that the chemical content of fish feed had met the nutritional value requirements of fish feed; namely, the highest feed protein in the study was in treatment K and the lowest in treatment B, with a protein content of 34.19±0.049%. The protein content is still considered high for several types of fish. The highest water content was in treatment K, while the lowest water content was in treatment A, which means that the feed treatment is still better than the K treatment from commercial feed. Likewise, the ash content is lower than the K treatment. While the fat content of fish feed is still higher than the K treatment. Thus it can be stated that the use of Lemna minor as one of the ingredients for making fish feed can be used because it has a low water content. The results of the ANOVA test also showed a P value <0.005 for all treatments, which means that the treatment has an effect on the protein content, water content, ash content, fat content, and carbohydrate content of fish feed.

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