OPEN ACCESS

Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.

Research Article
Palm Kernel Meal as a Fish-feed Ingredient for Milkfish (*Chanos chanos*, Forskall 1755): Effect on Growth and Gut Health

[1]Ilham Zulfahmi, [2]M. Herjayanto, [3]Agung S. Batubara and [4]Ridwan Affandi

[1]Department of Biology, Faculty of Science and Technology, Ar-Raniry State Islamic University, Banda Aceh 23111, Indonesia
[1]Department of Fisheries, Faculty of Agriculture, Sultan Ageng Tirtayasa University, Serang, Banten 42124, Indonesia
[3]Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia
[4]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, West Java 16680, Indonesia

Abstract

Background and Objective: Alternative feed ingredients is needed to reduce and replace fish meal use in milkfish production. One of the alternative feed ingredients that has potential to be milkfish feed is palm kernel meal (PKM). The ability of milkfish to utilize PKM formulated in feed can be analized through performance of growth rate and gult health (biometric condition and histological structure of gut). This study aimed to evaluate the effect of PKM inclusion as a fish feed ingredient for milkfish (*Chanos chanos*) on growth performance, biometric condition and histological structure of gut. **Materials and Methods:** A total of 200 fish sample were divided into four treatments followed by five replications. Each of experimental treatment had a different inclusion level of PKM, namely treatment control: 0% PKM (commercial diet), treatment A: 16.36% PKM, treatment B: 45.08% PKM and treatment C: 61.14% PKM. The feeding trial last for 45 days with the feeding frequency of twice a day, according to 5% of body weight. **Results:** The result show that treatment A did not adversely affect growth performance and gut health of milkfish. Although, there was no significant difference in the relative gut length and the average length of gut villi among treatments, histologically, it was known that the feed with high iclusion level of PKM may caused damage to the milkfish gut tissue **Conclusion:** The use of PKM in milkfish feed formulations high as 16.36% did not shown adverse effect on growth performance and gut health. Nevertheless, if the inclusion level of PKM was too high, it may damage the gut tissue structure. Consequently, the growth rate and feed efficiency of fish will reduced.

Key words: Feed efficacy, fish meal, growth performance, gut histology, gut villi, milkfish, palm kernel meal

Received: Accepted: Published:

Citation: Ilham Zulfahmi, M. Herjayanto, Agung S. Batubara and Ridwan Affandi, 2019. Palm kernel meal as a fish-feed ingredient for milkfish (*Chanos chanos*, forskall 1755): Effect on growth and gut health. Pak. J. Nutr., CC: CC-CC.

Corresponding Author: Ilham Zulfahmi, Department of Biology, Faculty of Science and Technology, Ar-Raniry State Islamic University, Banda Aceh 23111, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Milkfish, (*Chanos chanos*, Forskall 1755) is one of the major consumption fish for people of Indonesia, Philippines and Taiwan[1].Previous research reported that yearly production of milkfish in Indonesia, Philippines and Taiwan reach 300,000t with a commercial value approaching US$ 380 million[2,3]. In addition, milkfish also contains essential nutrient content for human in form of Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA), omega-3, omega-6 and omega-9[4].

Fish feed is the substantial component in fish farming, constitutes to 80% of the total operational cost[5]. Currently, the major source of dietary protein in fish feed is still being dominated by fish meal. Up to 2006, the fish farming sector spent 3,724,000 t of fish meal which potentially generates a scarcity of stock and increased price[6,7]. According to Chandrapal[8], the use of fish meal as the main source of protein for milkfish feed was no longer relevant, due to not economical and environmentally friendly, thus various efforts of finding alternative feed ingredient in accordance with milkfish nutrition requirement were continually improved. Several alternative feed ingredients have been tested in milkfish feed formulations, for instance rice bran[9], peas (*Pisum sativum*)[10], copra meal[11], earthworm meal[12] and soybean meal[13].

Palm Kernel Meal (PKM) is one of the alternative feed ingredients that have potential to be milkfish feed. This ingredient is a by-product of palm oil processing, available in abundant quantity particularly in tropical countries and does not compete with human[14-16]. According to Sundu *et al.*[17], PKM contained approximately 14-21% crude protein, 8-17% crude lipid, 3-6% ash and 4,998 kcal kg^{-1} gross energy. PKM has been used as an established ingredient for processed feed of ruminant and poultry[18,19]. Until now, information underlying the use of PKM in the aqua culture field is still very limited. Some studies have reported that PKM had been formulated in feed of hybrid catfish (*Clarias macrocephalus*)[14], hybrid red tilapia[15] and nile tilapia *Oreochromis niloticus*[20,21]. In addition, Lim *et al.*[21] proved that tilapia fed by inclusion of 30% PKM showed the same growth rate as the control feed.

Gut performance has linked to the growth and the health of fish. According to Raskovic *et al.*[22], the monitoring of gut performance was indispensable due to the important role of gut in the digestive process and nutrient absorption from the feed. Analysis of digestive organs with histological approaches is considered a good indicator to assess the nutritional status of fish (malnutrition), starvation and negative effect of feed[23,24]. To date, the suitability of the inclusion of PKM as an alternative feed ingredient and its relation to the biometric and histological structure of milkfish gut is still not widely studied. Thus, this research aimed to evaluate the effect of inclusion of PKM as fish feed ingredient for milkfish (*Chanos chanos*) on growth performance, biometric condition and the histological structure of gut.

MATERIALS AND METHODS

Total of 250 milkfish with a range of weight from 4-5 g and total length from 6-7 cm were obtained from Fish Breeding Center of Batee Iliek, Bireuen District, Aceh Province, Indonesia. Fish were transported to Aquaculture Laboratory, Faculty of Agriculture, Al Muslim University by using a closed system equipped with an aeration system. The fish acclimatisation was performed for seven days in $30\times40\times50$ cm^3 fiber glass tank, containing 30 L brackish water (salinity ranged from 13-15 parts per thousand). During acclimatisation, fish were fed by commercial diet (35% protein, 10% moisture content, 13% ash, 5% crude lipid and 7% crude fiber) with a frequency of three times a day.

PKM was obtained from oil palm processing mill located in Gandapura Sub-district, Bireuen District, Aceh Province, Indonesia. Before use, PKM was dried and pounded into homogeneous meal by using a flour mill machine. The milkfish maintenance was in $30\times40\times50$ cm^3 fiber glas tank, containing 30 L brackish water (salinity ranged from 13-15 parts per thousand) and equipped with aeration. The number of tested fish was 10 for each fiber glass tank. The feeding trial last for 45 days with the feeding frequency of twice a day, according to 5% of body weight. Fish faeces were stripped every day using siphon method and the water was completely changed every 10 days.

The fish were divided into four treatments followed by five replications. Each of experimental treatment had a different inclusion level of PKM, namely treatment control: 0% PKM (commercial diet), treatment A: 16.36% PKM, treatment B: 45.08% PKM and treatment C: 61.14% PKM. The diet was formed in pellet shape by using manual pellet press machine. The description of ingredients of the experimental diets is presented in Table 1.

Proximate analysis: The proximate analysis of the experimental diets(crude protein, moisture content, ash content, crude lipid and crude fiber) was conducted at the Laboratory of Nutrition and Feed Technology, Faculty of Agriculture, Syiah Kuala University. The method to determine protein referred to the Kjeldhal method[25], the determination of moisture content was done by using the Karl Fischer method[26], ash content and crude fiber by combustion in a muffle furnace at 600°C[27], while crude lipid was measured by using the Soklet method[26].

Table 1: Ingredient composition of the experimental diets

	Treatments			
Ingredient (g/1000 g diet)	Control	A	B	C
Fish meal	Commercial diet	601.0	313.8	153.2
Palm kernel meal (PKM)	Commercial diet	163.6	450.8	611.4
Bran meal	Commercial diet	106.9	106.9	106.9
Wheat flour	Commercial diet	108.5	108.5	108.5
Mineral premix	Commercial diet	10	10	10
Vitamin premix	Commercial diet	10	10	10
Total		1000	1000	1000

Biological and feed efficacy measurements: The main observed parameters in this study consisted of relative gut length, the average length of gut villi and histological structure of gut, while the supporting observed parameters consisted of survival rate, specific growth rate, average length growth and feed efficiency. The relative gut length was measured by using the following formula by Berumen *et al.*[28]:

$$RGL(\%) = \frac{GL(cm)}{TL(cm)} \times 100$$

where, RGL is the relative gut length (%), GL is the gut length (cm) and TL is the total length of fish (cm). The measurement of the average length of gut villi was conducted by using a micrometer (Mitutoyo 293-340-30, error 0.001 mm, Japan) and observed under a microscope (Olympus CX 31, Tokyo, Japan) with 40x magnification. The average length of gut villi was calculated by using formula referring to German and Horn[29], as follows:

$$PRV = \frac{pv_{lg} + pv_{rg} + pv_{ug} + pv_{bg}}{4}$$

where, PRV is the average length of gut villi (µm), pv_{lg} is the length of left gut villi (µm), pv_{rg} is the length of right gut villi (µm), pv_{ug} is the length of upper gut villi (µm) and pv_{bg} is the length of bottom gut villi (µm). Then, survival rate of fish was measured by using following formula by Berkeley *et al.*[30]:

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

where, SR is the survival rate (%), Nt is the number of fish that live at the end of feeding trial period (fish) and No is the number of fish that live at the early stage of maintenance (fish). Specific Growth Rate (SGR) was analyzed by using equation by Hakim *et al.*[31], as shown below:

$$SGR\ (\%/day) = \frac{\ln Wt - \ln Wo}{\Delta t} \times 100$$

where, SGR is daily specific growth rate (% day^{-1}), Wt is the final weight of fish (g), Wo is the initial weight of fish (g) and t is feeding days (days). The average length growth of milkfish was measured by using equation by Acar*et al.*[32], as shown below:

$$Pm = Pt\text{-}Po$$

where, Pm is the average length growth of fish (cm), Pt is the average length of fish at the end of feeding days (cm), Po is the average length of initial fish (cm). Then, feed efficiency was measured using equation by Handeland *et al.*[33] 2008, as shown below:

$$Fe(\%) = \frac{(Wt + D) - Wo}{F} \times 100$$

where, Fe is feed efficiency (%), Wt is final weight of fish (g), Wo is initial weight of fish (g), D is the weight of dead fish during the feeding trial (g) and F is the amount of feed intake (g).The chemical and physical parameters of maintenance media were observed at every change of water including temperature, dissolved oxygen, pH and salinity. The result of measurement showed that the temperature during maintenance period in every treatment ranged from 27-29°C, the dissolved oxygen ranged from 5.7-6.3 mg L^{-1}, pH value ranged from 6.7-7.9 and salinity ranged from 13-15 ppt. The value of chemical and physical parameters of maintenance media remained in the optimum range which supports the growth of milkfish[34].

Histology: The preparation of milkfish gut histological section was conducted at Pathology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University by using a histotechnique method including sampling, fixation, dehydration, purification, infiltration, planting, cutting, deparaffinization and staining. Fish gut were taken by using a scalpel, then were placed in a bowl that has been added with preservative Buffered Neutral Formalin (BNF). The process of removing water was performed by soaking the tissue in graded alcohol solution from 80, 90

Table 2: Proximate composition of experimental diets

	Treatments			
Proximate	Control	A	B	C
Crude protein (%)	35.15	26.56	24.32	22.66
Crude lipid (%)	5.21	13.00	12.19	11.65
Crude fibre (%)	7.00	30.27	34.61	37.06
Ash (%)	13.10	7.28	10.84	11.45
Moisture (%)	10.03	9.05	8.11	8.61

and 95% to absolute alcohol[35]. The sectioning of paraffin blocks was done by using microtome with a thickness of five microns. Gut section was floated in warm water and then was put on a hotplate for 10-15 min until all the water evaporated. The staining process was done by soaking preparation with hematoxylin for seven minutes followed by eosin for three minutes. Micrographs were generated using a digital camera-equipped microscope. Structure of gut in each treatment was analyzed descriptively.

Statistical analysis: Data analysis was performed using analysis of variance (one-way ANOVA) on relative gut length, the average length of gut villi, survival rate, daily specific growth rate, average length growth and feed efficiency. The confidence interval used in the Duncan test was 95% ($p<0.05$). The statistical software used in this document was SPSS version 22 program (SPSS Inc., Michigan Avenue, Chicago, IL, USA) for Macintosh.

RESULT

Proximate content: The highest crude protein content in diet with inclusion of PKM was found in treatment A (26.56%). However, crude protein content in treatment A remained lower than that of the control treatment (commercial diet). In general, experimental diet had lower ash content than commercial diet (Table 2). The lowest percentage of ash content was found in treatment A (7.28%) followed by treatment C and B which were 11.45 and 10.84% respectively. In addition, the feed with PKM inclusion had higher crude lipid and crude fiber content than commercial feed. The highest crude lipid content was found in treatment A (13%), while the highest crude fiber content was found in treatment C (37.06%).

Relative gut length and average length of gut villi: Relative gut length of milkfish did not differ significantly between treatment ($p>0.05$). Quantitatively, the highest gut length value was found in the control treatment (255.66%), while the lowest gut length value was found in treatment B (241.20%)

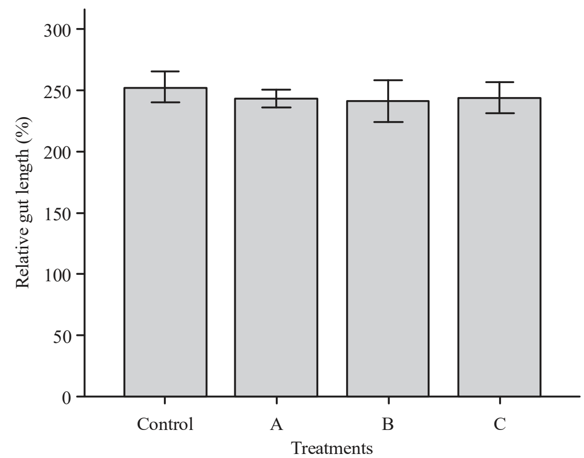

Fig. 1: Relative gut length of milkfish (*Chanos chanos*) fed experimental diets for 45 days

Treatment control: 0% PKM (commercial diet), treatment A: 16.36% PKM, treatment B: 45.08% PKM and treatment C: 61.14% PKM

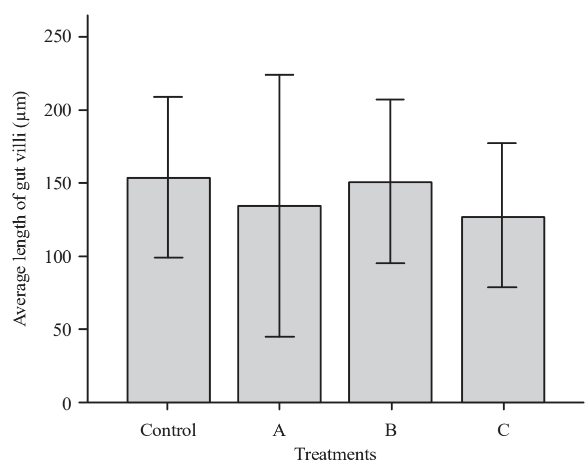

Fig. 2: Average length of gut villi of milkfish (*Chanos chanos*) fed experimental diets for 45 days

Treatment control: 0% PKM (commercial diet), treatment A: 16.36% PKM, treatment B: 45.08% PKM and treatment C: 61.14% PKM

(Fig. 1). Similar results were also seen from the average length of gut villi, where did not present significant differences among treatments ($p>0.05$). The highest average length of gut villi (153.75 µm) was found in control treatment, while the lowest value (128 µm) was found in treatment C (Fig. 2).

Table 3: Growth performance and feed utilization of milkfish (*Chanos chanos*) fed experimental diets for 45 days

	Treatments			
Parameters	Control	A	B	C
SR (%)	86.66±2.86[a]	88.33±2.88[a]	88.33±2.88[a]	78.33±2.88[b]
SGR (% day^{-1})	1.10±0.03[a]	1.33±0.03[a]	0.92±0.051[b]	0.70±0.055[b]
Pm (cm)	4.10±0.10[a]	4.70±0.10[a]	3.30±0.15[b]	2.60±0.15[b]
Fe (%)	21.51±0.50[a]	25.32±0.06[a]	17.33±0.21[a]	13.96±0.29[b]

SR: Survival rate, SGR: Specific growth rate, Pm: Average length growth, Fe: Feed efficiency, Values shown as Mean±STD. The different letters indicate significant differences (p<0.05)

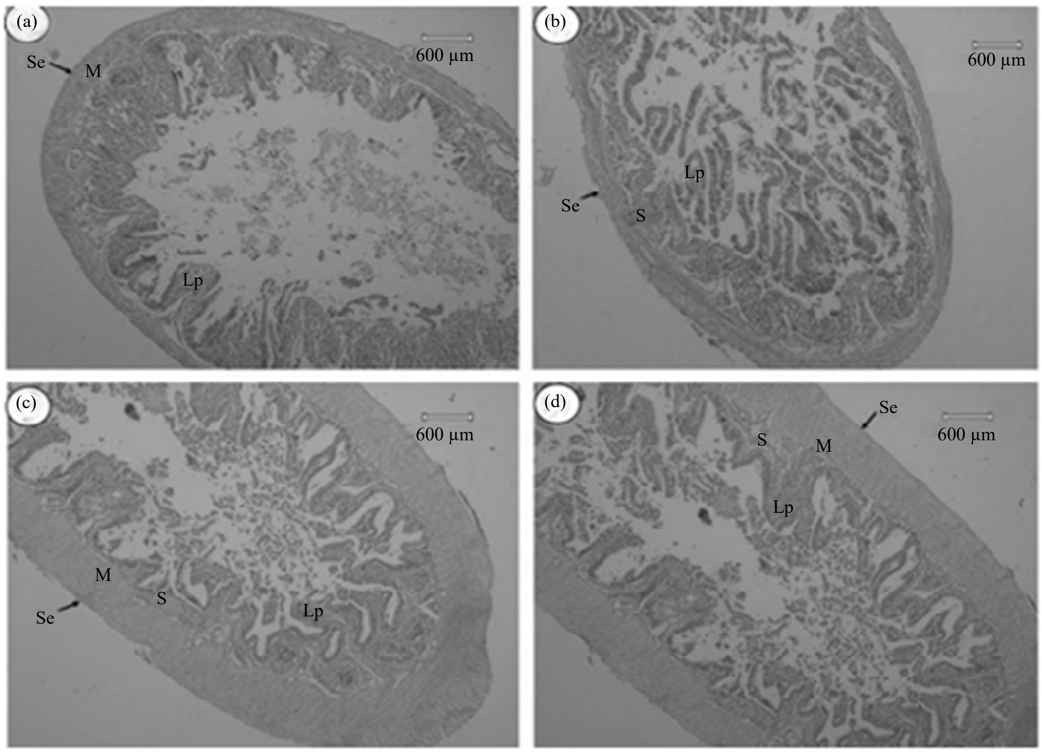

Fig. 3: Histological structure of gut of milkfish (*Chanos chanos*) in each treatment (a) Treatment control, (b) Treatment A, (c) Treatment B and (d) Treatment C. Se: Serosa layer, M: tunica muskularis, S: Submucosa layer, Lp: Lamina Propia. Scale Bar. 600 µm. Magnification 100×

Growth and feed efficiency: The highest survival rate of milkfish was obtained in treatment A and B (88.33±2.89%) while the lowest survival rate was found in treatment C (78.33±2.88%). There was no significant difference between the milkfish survival rate in the control treatment, treatment A and treatment B (p>0.05). Nevertheless, the survival rate of milkfish decreased significantly in treatment C (p<0.05) (Table 3). The use of PKM in treatment A was able to produce specific growth rate that was not different from control treatment with a value of 1.33±0.03% day^{-1} (p>0.05). However, the value of specific growth rate decreased significantly in treatments B and C (p<0.05). Similar result was also found in average length growth and feed efficiency value, where significant difference was not observed between treatment A and control treatment (p>0.05). Nevertheless, the value of average length growth and feed efficiency were slightly reduced in treatments B and C (p<0.05).

Histology of gut: Histologically, milkfish gut comprised of several major parts namely serous layer, tunica muscularis, submucosa and lamina propia. The result showed that control treatment had normal histological structure of gut and it was seen from the gut fragments that tend to be round in shape with a regular arrangement of lamina propia. Change in histological structure of gut was very blatant in treatment B and C. In this treatment, the lamina propia showed an irregular shape. (opposite with control treatment and treatment A) accompanied by thickening of tunica muscularis layer (Fig. 3).

DISCUSSION

The gut performance has a close correlation with the digestive process and nutrient absorption of fish. According to Grosell *et al*.[36], the gastrointestinal tract (including gut) had various functions such as nutrient absorption and ionic and osmotic regulation. Furthermore, according to Dolomat ov *et al*.[37], the gut also played an important role in regulating water balance, electrolyte and immunity. Some researchers began to use parameters of gut health to evaluate the effectivity of using alternative feed source and condition of fish health due to pollutants exposure, for instance, in *Lateolabrax japonicus*[38], *Solea solea*[39], *Oncorhynchus* mykiss[40] and *Dicentrarchus labrax*[41].

The result of this study showed that the relative gut length of milkfish in each of treatment reached twice of a total length of the fish body. The milkfish gut had longer size than carnivorous fish and was shorter than herbivorous fish, so it tended to be classified into omnivorous fish. The length of fish gut was affected by phylogeny factors, the age of fish, the type of food consumed and fish physiological conditions. Paujiah *et al*.[42] revealed that insectivorous carnivorous fish such as *Rhyacichthys aspro* had a shorter gut, which was 0.69 time of their body length. Conversely, herbivorous fish-eating plants such as *Sicyopterus ouwensi* had a longer gut, which was 5.54 times of their body length. According to Starck[43], the presence of stomach and the structure of animal feed that was easily digested were causes of rapid digestion of food in carnivorous fish.

There are no significant differences in the relative gut length among treatments. It was assumed to be caused by a relatively short maintenance period. Hoseinifar *et al* .[44] have stated that the change in gut length rarely occurred except in fish exposed to the same type of food over a long period. Similar results were also seen in the average length of gut villi where there was no significant difference among treatments. This showed that feed containing PKM did not have a negative effect caused length abnormality of gut villi length. Some similar results had also been revealed by several other feed formulation such as the addition of clove oil in the feed of *Pangasianodon hypophthalmus*[45] and *Cyprinus carpio*[46]. Commonly, the average length of gut villi of milkfish is lower than carnivorous fish. Omnivorous fish tend to have gut structures with shorter villi than carnivorous fish. Hence, it requires longer digestive time and tract. The length value of gut villi in this research was ranging from 128-153.75 µm which was lower than the length of gut villi of *Pangasius hypophthalmus* which was ranging from 500-600 µm[45].

Although, there was no significant difference in the relative gut length and the average length of gut villi among treatments, histologically, it was known that the feed with high inclusion level of PKM may caused damage to the milkfish gut tissue. The observed damage encompassed the irregular shape of gut structure, thickening of the tunica muscularis layer accompanied with the occurrence of lysis in gut lamina propia. This was assumed to be caused by high crude fiber content in the feed which was led to performance disturbances and damage to gut tissue. One similar research by Poleksic *et al*.[47] revealed that substitution of excessive soybean meal could have a negative impact on gut performance which can be signaled by an increasing in leukocyte infiltration in epithelium, increasing in mucus production and occurrence of gut inflammation.

Fish are required to adapt to the presence of food sources in their environment even though these types of food are not suitable with their nutritional needs. This has the potential to reduce food nutrient uptake capacity, energy transfer ability, growth and physiology performance of gut[48]. Histological damage of gut can also occur if feed containing pathological compound. These pathological feeds can cause disturbance of palatability, low nutrient digestibilities, changes in nutrient balance, intestinal dysfunction, gut dysfunction, change in gut microflora, goitrogenesis, pancreas hypertrophy and hypoglycemia[48]. The damage structure of gut in treatment B and C was presumably the cause of declining growth rate and fish feed efficiency.

Based on the measurement result of growth parameter, it was known that the high inclusion level of PKM (45.08 and 61.14%) could reduce the survival rate, specific growth rate, average length growth and feed efficiency in milkfish. Besides pertinent to histological damage of gut, this was also assumed to be related to the high crude fiber in experimental diet. The crude fiber content in the experimental diet particularly in treatment B and C had a high value (30.27-37.06%). This is the common problem with feed ingredients derived from plants because it can reduce digestibility, thus, the fish growth was not optimal.

CONCLUSION

The use of PKM in milkfish feed formulation as high as 16.36% did not shown adverse effect on growth performance and gut health. Nevertheless, if the inclusion level of PKM was too high, it may damage the gut tissue structure. Consequently, the growth rate and feed efficiency of fish will reduced. Further research involving efforts to enhance digestibility and nutritive value of PKM-based feed as well as analysis of their physiological effects on other digestive organs is still necessary.

ACKNOWLEDGMENT

The research was funded by Center of Research and Community Service, State Islamic University Ar-Raniry under the "Dosen Pemula" Scheme (Reg No. 171010007446). The authors thank Yusrizal Akmal, Muliari and Rindhira Humairani for their technical assistance.

REFERENCES

1. Martinez, F.S., M.C. Tseng and S.P. Yeh, 2006. Milkfish (*Chanos chanos*) culture: Situations and trends. J. Fish. Soc. Taiwan, 33: 229-244.
2. Yap, W.G., A.C. Villaluz, M.G.G. Soriano and M.N. Santos, 2007. Milkfish Production and Processing Technologies in the Philippines. Milkfish Project Publication Series, WorldFish and SEAFDEC., Philippines.
3. Holmer, M., N. Marba, J. Terrados, C.M. Duarte and M.D. Fortes, 2002. Impacts of milkfish (*Chanos chanos*) aquaculture on carbon and nutrient fluxes in the Bolinao area, Philippines. Mar. Pollut. Bull., 44: 685-696.
4. Agustini, T.W., I. Susilowati, S. Subagyo, W.A. Setyati and B.A. Wibowo, 2011. Will soft-boned milkfish-A traditional food product from Semarang city, Indonesia-breakthrough the global market. J. Coastal Dev., 14: 81-90.
5. Silva, C.R., L.C. Gomes and F.R. Brandao, 2007. Effect of feeding rate and frequency on tambaqui (*Colossoma macropomum*) growth, production and feeding costs during the first growth phase in cages. Aquaculture, 264: 135-139.
6. Tacon, A.G.J. and M. Metian, 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture, 285: 146-158.
7. Hardy, R.W., 2010. Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. Aquacult. Res., 41: 770-776.
8. Chandrapal, G.D., 2005. Status of trash fish utilization and fish feed requirements in aquaculture. Proceedings of the Regional Workshop on Low Value and Trash Fish in the Asia-Pacific Region, June 7-9, 2005, Hanoi, Viet Nam, pp: 1-7.
9. Sumagaysay, N.S., F.E. Marquez and Y.N. Chiu-Chern, 1991. Evaluation of different supplemental feeds for milkfish (*Chanos chanos*) reared in brackishwater ponds. Aquaculture, 93: 177-189.
10. Borlongan, I.G., P. Eusebia and T. Welsh, 2003. Potential of feed pea (*Pisum sativum*) meal as a protein source in practical diets for milkfish (*Chanos chanos* Forsskal). Aquaculture, 225: 89-98.
11. Apines-Amar, M.J.S., R.M. Coloso, C.J. Jaspe, J.M. Salvilla, M.N.G. Amar-Murillo and C.A. Saclauso, 2015. Partial replacement of soybean meal with fermented copra meal in milkfish (*Chanos chanos*, Forsskal) diet. Aquacult. Aquar. Conserv. Legisl., 8: 1019-1026.
12. Spikadhara, E.D., T.S. Subekti and M.A. Alamsjah, 2012. Pengaruh pemberian pakan tambahan (suplement feed) dari kombinasi tepung cacing tanah (*Lumbricus rubellus*) dan tepung *Spirulina platensis* terhadap pertumbuhan dan retensi protein benih ikan bandeng (*Chanos chanos*). J. Mar. Coastal Sci., 1: 81-90, (In Indonesian).
13. Hassan, S., K. Altaff and T. Satyanarayana, 2009. Use of soybean meal supplemented with cell bound phytase for replacement of fish meal in the diet of Juvenile milkfish, *Chanos chanos*. Pak. J. Nutr., 8: 341-344.
14. Ng, W.K. and M.L. Chen, 2002. Replacement of soybean meal with palm kernel meal in practical diets for hybrid Asian-African catfish, *Clarias macrocephalus* × *C. Gariepinus*. J. Applied Aquacult., 12: 67-76.
15. Ng, W.K. and K.K. Chong, 2002. The nutritive value of palm kernel meal and the effect of enzyme supplementation in practical diets for red hybrid tilapia (*Oreochromis* sp.). Asian Fish. Sci., 15: 167-176.
16. Ng, W.K., 2003. The potential use of palm kernel meal in aquaculture feeds. Aquac. Asia, 8: 38-39.
17. Sundu, B., A. Kumar and J. Dingle, 2006. Palm kernel meal in broiler diets: Effect on chicken performance and health. World's Poult. Sci. J., 62: 316-325.
18. Devendra, C., 1978. The utilization of feeding stuffs from the oil palm plant. Proceedings of the Symposium on Feeding Stuffs for Livestock in South East Asia, October 17-19, 1977, Kuala Lumpur, Malaysia, 116-131.
19. Swick, R.A. and P.H. Tan, 1995. Considerations in using common Asian protein meals. ASA Technical Bulletin No. 083/12/94, Vol. P025.
20. Adjanke, A., K. Tona, C.M. Ble, I.I. Toko and M. Gbeassor, 2016. Effect of dietary inclusion of palm kernel meal on feed intake, growth and body composition of Nile tilapia, *Oreochromis niloticus* reared in concrete tanks in Togo. Int. J. Fish. Aquat. Stud., 4: 642-646.
21. Lim, H.A., W.K. Ng, S.L. Lim and C.O. Ibrahim, 2001. Contamination of palm kernel meal with *Aspergillus flavus* affects its nutritive value in pelleted feed for tilapia, *Oreochromis mossambicus*. Aquac. Res., 32: 895-905.
22. Raskovic, B., M. Stankovic, Z. Dulic, Z. Markovic, N. Lakic and V. Poleksic, 2009. Effects of different source and level of protein in feed mixtures on liver and intestine histology of the common carp (*Cyprinus carpio*, Linnaeus, 1758). Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., 153: S112-S112.
23. Caballero, M.J., M.S. Izquierdo, E. Kjorsvik, D. Montero, J. Socorro, A.J. Fernandez and G. Rosenlund, 2003. Morphological aspects of intestinal cells from gilthead seabream (*Sparus aurata*) fed diets containing different lipid sources. Aquaculture, 225: 325-340.

24. Raskovic, B.S., M.B. Stankovic, Z.Z. Markovic and V.D. Poleksic, 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. J. Agric. Sci., 56: 87-100.
25. Persson, J. and M. Wennerholm, 2008. Handbook for Kjeldahl Digestion, A Recent Review of the Classical Method with Improvements Developed by FOSS. 4th Edn., FOSS., Hilleroed, Denmark.
26. Christie, W.W., 2003. Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids. 3rd Edn., The Oily Press, Bridgwater, UK., ISBN-13: 9780953194957, Pages: 416.
27. AOAC., 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA., Pages: 684.
28. Berumen, M.L., M.S. Pratchett and B.A. Goodman, 2011. Relative gut lengths of coral reef butterflyfishes (Pisces: Chaetodontidae). Coral Reefs, Vol. 30. 10.1007/s00338-011-0791-x
29. German, D.P. and M.H. Horn, 2006. Gut length and mass in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): Ontogenetic, dietary and phylogenetic effects. Mar. Biol., 148: 1123-1134.
30. Berkeley, S.A., C. Chapman and S.M. Sogard, 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology, 85: 1258-1264.
31. Hakim, Y., Z. Uni, G. Hulata and S. Harpaz, 2006. Relationship between intestinal brush border enzymatic activity and growth rate in tilapias fed diets containing 30 or 48% protein. Aquaculture, 257: 420-428.
32. Acar, U., O.S. Kesbic, S. Yilmaz, N. Gultepe and A. Turker, 2015. Evaluation of the effects of essential oil extracted from sweet orange peel (*Citrus sinensis*) on growth rate of tilapia (*Oreochromis mossambicus*) and possible disease resistance against *Streptococcus iniae*. Aquaculture, 437: 282-286.
33. Handeland, S.O., A.K. Imsland and S.O. Stefansson, 2008. The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. Aquaculture, 283: 36-42.
34. Capkin, E., I. Altinok and S. Karahan, 2006. Water quality and fish size affect toxicity of endosulfan, an organochlorine pesticide, to rainbow trout. Chemosphere, 64: 1793-1800.
35. Zulfahmi, I., A. Ridwan and T.F.L. Djamar, 2014. Perubahan struktur histologis insang dan hati ikan nila (*Oreochromis niloticus* Linnaeus 1758) yang Terpapar Merkuri. J. Edukasi Sains Biol., 4: 25-31, (In Indonesian).
36. Grosell, M., A.P. Farrell and C.J. Brauner, 2010. Fish Physiology: The Multifunctional Gut of Fish. Vol. 30, Academic Press, Jamestown Road, London, UK.
37. Dolomatov, S., W. Zukow, M. Dzierzanowski, J. Mieszkowski, R. Muszkieta and M. Klimczyk, 2016. Role of nitrates in the adaptation of fish to hypoxic conditions. Water Resour., 43: 177-183.
38. Zhang, C., S. Rahimnejad, Y.R. Wang, K. Lu, K. Song, L. Wang and K. Mai, 2018. Substituting fish meal with soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*): Effects on growth, digestive enzymes activity, gut histology and expression of gut inflammatory and transporter genes. Aquaculture, 483: 173-182.
39. Bonvini, E., L. Parma, L. Mandrioli, R. Sirri and C. Brachelente *et al*., 2015. Feeding common sole (*Solea solea*) juveniles with increasing dietary lipid levels affects growth, feed utilization and gut health. Aquaculture, 449: 87-93.
40. Bruce, T.J., R.D. Neiger and M.L. Brown, 2018. Gut histology, immunology and the intestinal microbiota of rainbow trout, *Oncorhynchus mykiss* (Walbaum), fed process variants of soybean meal. Aquacult. Res., 49: 492-504.
41. Bonvini, E., A. Bonaldo, L. Parma, L. Mandrioli and R. Sirri *et al*., 2018. Feeding European sea bass with increasing dietary fibre levels: Impact on growth, blood biochemistry, gut histology, gut evacuation. Aquaculture, 494: 1-9.
42. Paujiah, E., D.D. Solihin and R. Affandi, 2013. Struktur trofik komunitas ikan di Sungai Cisadea Kabupaten Cianjur, Jawa Barat [Trophic structure of fish community in Cisadea river, Cianjur, Jawa Barat]. J. Iktiol. Indonesia, 13: 133-143.
43. Starck, J.M., 2005. Structural Flexibility of the Digestive System of Tetrapods: Patterns and Processes at the Cellular and Tissue Level. In: Physiological and Ecological Adaptations to Feeding in Vertebrates, Starck, J.M. and T. Wang (Eds.)., Science Publishers Inc., USA., pp: 175-200.
44. Hoseinifar, S.H., A. Mirvaghefi and D.L. Merrifield, 2011. The effects of dietary inactive brewer's yeast *Saccharomyces cerevisiae* var. *ellipsoideus* on the growth, physiological responses and gut microbiota of juvenile beluga (*Huso huso*). Aquaculture, 318: 90-94.
45. Pratiwi, N., D. Jusadi and S. Nuryati, 2016. Pemanfaatan minyak cengkeh *Syzigium aromaticum* untuk meningkatkan efisiensi pakan pada ikan patin *Pangasianodon hypophthalmus* (Sauvage, 1876). J. Iktiol. Indonesia, 16: 233-242.
46. Silvianti, T., D. Jusadi and S. Nuryati, 2016. Penambahan minyak cengkeh *Syzygium aromaticum* dalam pakan untuk memperbaiki kinerja pertumbuhan ikan mas *Cyprinus carpio* Linnaeus 1758 [The supplementation of clove oil *Syzygium aromaticum* in the diet to improve the growth performance of common carp *Cyprinus carpio* Linnaeus 1758]. J. Iktiol. Indonesia, 16: 211-225.
47. Poleksic, V., B. Raskovic, Z. Markovic, Z. Dulic, M. Stankovic, I. Zivic and N. Lakic, 2007. Effects of different dietary protein sources on intestine and liver morphology of carp yearlings. Proceedings of the 3rd Serbian Congress for Microscopy, September 25-28, 2007, Serbian Microscopy Society, Belgrade, Serbia, pp: 237-238.
48. Krogdahl, A., M. Penn, J. Thorsen, S. Refstie and A.M. Bakke, 2010. Important antinutrients in plant feedstuffs for aquaculture: An update on recent findings regarding responses in salmonids. Aquacult. Res., 41: 333-344.