



Olive oil by-products in aquafeeds: Opportunities and challenges

Mohd Khalid Hazreen-Nita^{a,*}, Zulhisyam Abdul Kari^a, Khairiyah Mat^{a,b}, Nor Dini Rusli^{a,b}, Suniza Anis Mohamad Sukri^a, Hasnita Che Harun^{a,b}, Seong Wei Lee^a, Mohammad Mijanur Rahman^{a,b}, N.H. Norazmi-Lokman^{c,d}, Mansor Nur-Nazifah^e, Mohd Firdaus-Nawi^{e,f,**}, Mahmoud A.O. Dawood^{g,h}

^a Department of Agricultural Science, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia

^b Institute of Food Security and Sustainable Agriculture, Universiti Malaysia Kelantan, Jeli Campus, Jeli 17600, Kelantan, Malaysia

^c Fisheries and Aquaculture Centre, Institute for Marine and Antarctic Studies, University of Tasmania, Taroona, Tasmania, Australia

^d Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia

^e Kulliyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia

^f Institute of Oceanography and Maritime Studies (INOCEM), International Islamic University Malaysia, Cheroh Paloh, 26060 Kuantan, Pahang, Malaysia

^g Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt

^h The Center for Applied Research on the Environment and Sustainability, The American University in Cairo, 11835 Cairo, Egypt

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ABSTRACT

The increment in world population has led to the increasing demands for fish supply as principal source of animal protein that represents about 17% of animal protein consumption globally. The world aquaculture industry has expanding remarkably but limited source of fishmeal and fish oil which known as important ingredients in fish feed has led to seeking of alternative sources for their replacement in aquafeed formulation. Many potential and cheaper ingredients derived from vegetable oils or agricultural by-products have been investigated to replace fish meal and fish oil which shown promising results in fish productivity and fish health status including olive oil by-products. Varies depending on its type, olive oil composes of antioxidants, healthy type of fats such as MUFA and PUFA, and high in vitamins that provide human health benefits for heart and liver, help in many diseases and reducing the risk of cancer. In agriculture industry, olive and olive oil production processes have resulted to by-products such as olive mill wastewater (OMW), olive leaf and olive pomace. The inclusion of these by-products in animal feeds is the solution for agricultural waste management. The first report of utilization of olive oil by-products in fish feed was documented in 2004 and since then, many researches has been conducted to investigate fish health benefits that showed improvement in fish carcass composition and fish immunity depending on type of by-products used and fish species. Additionally, antioxidant contents in olive oil extract were reported could provide antimicrobial, antifungal and antioxygenic characteristics which potentially improve the fish health. This review discussed the opportunities and challenges in application of olive oil by-products in aquafeed which provides significant prospects in fish growth performance that could boost aquaculture industry development.

Abbreviations: MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; SFA, Saturated fatty acids; OMW, Olive mill wastewater; FAO, Food and Agriculture Organization; IFFO, The Marine Ingredients Organization; EVOO, Extra virgin olive oil; VOO, Virgin olive oil; ROO, Refined olive oil; POO, Pure olive oil; OPO, Olive pomace oil; OLP, Olive leaf powder; ANFs, Antinutritional factors; SSF, Solid state fermentation; FCR, Feed conversion ratio; SGR, Specific growth rate; BWI, Body Weight Index; OBE, Olive oil bioactive extract; PER, Protein efficiency ratio; GAE, Gallic acid equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; COC, Crude olive cake; OLE, Olive leaf extract.

* Corresponding author.

** Corresponding author at: Institute of Oceanography and Maritime Studies (INOCEM), International Islamic University Malaysia, Cheroh Paloh, 26060 Kuantan, Pahang, Malaysia.

E-mail addresses: hazreen.mk@umk.edu.my (M.K. Hazreen-Nita), zulhisyam.a@umk.edu.my (Z. Abdul Kari), khairiyah@umk.edu.my (K. Mat), nordini@umk.edu.my (N.D. Rusli), suniza@umk.edu.my (S.A. Mohamad Sukri), hasnita@umk.edu.my (H. Che Harun), leeseong@umk.edu.my (S.W. Lee), mijanur.r@umk.edu.my (M.M. Rahman), Lokman.Norazmi@utas.edu.au, lokhakim@umt.edu.my (N.H. Norazmi-Lokman), nurnazifah@iium.edu.my (M. Nur-Nazifah), firdausn@iium.edu.my (M. Firdaus-Nawi), mahmoud.dawood@agr.kfs.edu.eg (M.A.O. Dawood).

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1. Introduction

Aquaculture is defined as the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants with some forms of intervention such as regular stocking, feeding and protection from predators in the rearing process to enhance production (FAO Fishery Information Data and Statistics Unit, 2003). In regard to feeding and production system, aquaculture can be categorized into two general classifications: 1) non-fed aquaculture production and 2) fed aquaculture production. The former involved aquatic farming systems that does not require feeding such as those in seaweed, mollusk and some freshwater finfish while the latter is the farming of aquatic animals which involves feeding intervention (IFFO, 2020). In 2018, fed aquaculture production has outgrown the non-fed aquaculture sub-sector where it contributes to 69.5% of the total world aquaculture production (FAO, 2020). This figure gives an indication of the high aquafeed usage and demands in the industry where it is projected that by 2030, the supply of aquafeed ingredients needed will be close to 30 million tons (IFFO, 2020).

Over the past decades, the aquaculture industry has transformed its fish feeding practice by using high quality compounded pelleted feeds compared to the traditional feeding using trash fish and rice bran/oil cake mixture (Salin et al., 2018). Conventionally, the aquafeed industry uses two main ingredients in fish feed namely fishmeal and fish oil which is mainly sourced from wild captured fish and a small portion comes from the processing of fish by-products such as fish offal and trimmings (Hamid, 2021; Mitra, 2021; Tacon et al., 2011). This practice has now raised a conundrum mainly due to the reliance on wild fish stock to produce both ingredients. Despite both ingredients being considered the most nutritious and digestible ingredients for farmed fish as well as the major source of omega-3 fatty acids, currently there is a clear decreasing trend of their inclusion in aquafeed (FAO, 2020; Mitra, 2021). This is mainly due to its high demands and limited supply which resulted in price increment besides the dependent on wild fish stock to produce both ingredients (FAO, 2020; Salin et al., 2018).

Globally, the search for an alternative ingredient to replace fishmeal and fish oil in total or partially has begun. The past decade has seen the aquafeed industry starting to evaluate and increasingly incorporates plant-based/agriculture-based ingredients in fish feed such as soy, cotton seed, coconut, palm kernel and olive oil to support aquaculture growth (Kari et al., 2022; Napier et al., 2020; Salin et al., 2018). They are considered much cheaper, environmentally sustainable and have better nutritional content such as protein and fatty acids compare to some animal sources (Hamid, 2021; Dawood et al., 2021; Malcorps et al., 2019; Mandal et al., 2010). Nevertheless, the increasing trend on using terrestrial plant-based ingredients comes with a price too where it has social, ecological, and environmental implications (Blanchard et al., 2017; Fry et al., 2016; Troell et al., 2014). This issue can be address by looking into plant protein sources from agriculture by-products. In the past two decades, health benefits of olive oil have steadily boosted its consumption and production globally where the Mediterranean countries has become its main producer (Khemakhem et al., 2018; Manzanares et al., 2020; Nunes et al., 2016). As in other agriculture industry, the olive and olive oil industry produce large number of by-products. For example, the production of olive oil produces 35 kg of solid waste and 440 L of liquid waste per 100 kg of olives used (Molina Alcaide and Nefzaoui, 1996; Nasopoulou and Zabetakis, 2013). One of the solutions to fully utilized olive oil by-products is by using it as an ingredient in animal feed including fish (Alves et al., 2018; Nasopoulou and Zabetakis, 2012, 2013; Peri and Proietti, 2014). The inclusion of olive oil by-products in fish feed was first reported in 2004 where they were incorporated in African catfish feed (Yilmaz et al., 2004), since then the number of literature on the incorporation of olive oil by-products have increased especially by researchers from Mediterranean countries. Therefore, this review was done to look into the opportunities and challenges of using olive oil by-products in the aquafeed industry. The

focus of this review includes the type and source of olive oil by-products and the impacts of its inclusion in aquafeed on fish carcass composition, growth development, feed utilization, intestinal health and microbial diversity, immune response, antioxidative capacity and disease resistance. The future perspectives of this ingredient will also be discussed.

2. Olive oil sources

Olive (*Olea europaea L.*) plants can be divided into two types: 1) cultivated olive trees (var. *europaea*) and 2) wild olive trees (var. *sylvestris*) (Hannachi et al., 2013). In order to get olive oil, oil extraction was carried out by an extraction process conditions using an oleodorsor system. By using the extraction system, products obtained are aqueous liquid (olive oil), solid waste (olive pomace), semisolid waste (olive cake), semisolid destoned waste (paté olive cake), fragments of olive stone and decanters (Benincasa et al., 2021; Veneziani et al., 2017). Conventionally, the method of extraction involves three major steps; crushing, malaxation and centrifugation (Clodoveo, 2013). Malaxation process involved wide range of temperature from cold to high temperature. There are five types of olive oil produced from different phase of olive fruits and temperature which namely extra virgin olive oil (EVOO), virgin olive oil (VOO), refined olive oil (ROO), pure olive oil (POO) and olive pomace oil (OPO) (Yan, 2020). Different types of olive oil have different content of composition (Table 1) (Gimeno et al., 2007).

3. Effects on the carcass composition

'We are what we eat' – a famous phrase by the German philosopher Ludwig Feuerbach can be applied in both human and fish context literally. For humans, fish is an important, cheap and a much healthier choice of protein and lipid source in our daily diets (Jobling, 2007; Tacon et al., 2020). In 2017, the FAO reported that fish and seafood products was the third major source of human dietary protein after cereal and milk (FAO, 2021; Tacon et al., 2020) thus making it important for us to know and understand the carcass/body composition of the fish we consumed and factors that can influence it. In animal science and food industry such as aquaculture, carcass composition is an important aspect that not only helps to understand the ecological, nutritional and physiological aspect of animals but also helps determine suitable

Table 1

Content composition of different type of olive oil (Gimeno et al., 2007).

Quality Parameters	Refined	Common	Virgin
Free acidity (% oleic acid)	0.12	0.17	0.11
Peroxide value (meq O ₂ /kg oil)	1.80	2.93	6.48
K ₂₇₀	0.48	0.20	0.10
C14:0	0.02	0.02	0.01
C16:0	11.25	11.78	13.18
C16:1	0.86	1.05	1.11
C17:0	0.08	0.06	0.10
C17:1	0.15	0.13	0.22
C18:0	2.52	2.59	1.76
C18:1	73.46	75.65	73.37
C18:2	9.97	7.17	9.02
C20:0	0.45	0.39	0.33
C18:3	0.73	0.70	0.48
C20:1	0.34	0.29	0.28
C22:0	0.12	0.10	0.10
C24:0	0.05	0.04	0.04
MUFA (%)	74.83	77.14	74.98
PUFA (%)	10.68	7.86	9.50
SFA (%)	14.49	15.00	15.52
Phenolic Acid (μmol/kg CAE)	0	370	825.00
A-Tocopherol (mol/kg)	65.88	48.22	47.98

CAE, Caffeic acid equivalents; K₂₇₀, UV Spectrophotometric index

MUFA, monounsaturated fatty acids

PUFA, polyunsaturated fatty acids,

SFA, saturated fatty acids

marketing strategy for farmed animal based on carcass value (Breck, 2014; Silva and Cadavez, 2012).

In fish, besides geographic location, age, sex and maturity, the feed it consumed is regarded as one of the primary factors that influence its carcass composition (Aryani et al., 2017; Jobling, 2007). The carcass composition of farmed fish is much stable and easier to be manipulated compared to wild fish since the former received constant and stable food supply throughout the year compared to the later who are subjected to unstable changes of prey abundance (Jobling, 2007). By formulating and establishing feed with certain ingredients and nutrients, farmers can produce fish with desirable and better carcass composition to fulfil human consumption needs. Till date, most literature reports fish carcass composition by analyzing the proximate composition of the body or meat fillet mainly crude protein, lipid, ash, and moisture (Barlaya, Ananda Kumar et al., 2021; Barlaya et al., 2021; Ghosi Mobaraki et al., 2020).

Since 2004, researcher especially those at Mediterranean countries have studied the potential of olive oil by products inclusion in fish feed on both freshwater and marine fish species. Amongst all, effects of olive oil by products on carcass composition were reported in African catfish (*Clarias gariepinus*) (Yilmaz et al., 2004), rainbow trout (*Oncorhynchus mykiss*) (Sicuro et al., 2010a), red sea bream (*Pagrus major*) (Arsyad et al., 2018) and gilthead sea bream (*Sparus aurata*) (Nasopoulou et al., 2011; Sicuro et al., 2010c; Sioriki et al., 2016). Each of the reports uses different types and amount of olive oil by-products in their studies and assess its effects on the proximate composition and fatty acid (FA)

content of the body or meat fillet. The proximate analysis and fatty acid content of body/meat fillet observed in each study based on treatments with significant results are compiled in Table 2.

Overall, olive oil by-products have different effects on fish carcass composition depending on the type of by-products and fish species. Most of the study reported on fatty acid composition compared to carcass/muscle proximate analysis. In terms of proximate analysis, it was found that the muscle of juvenile African catfish (Yilmaz et al., 2004) fed with feed containing 9% and 3% olive pomace oil with and without L-carnitine has higher lipid content compared to controls. However, there were no significant differences found in the fillet of rainbow trout (Sicuro et al., 2010a) and gilthead seabream (Sicuro et al., 2010c) fed with feed enriched with OMW. Mixed results were also seen in the FA analysis of fish fed with olive oil by-products. Feed enriched with OMW does not have significant impact in the muscle/fillet FA in rainbow trout and gilthead seabream (Sicuro et al., 2010a, 2010c). Among all olive oil by-products, olive pomace has shown to have impacted the FA amount in the fish species tested. Report by Nasopoulou et al. (2011) on gilthead seabream showed that all FA classes in muscles of fish fed with OP incorporated feed decreases compared to control. In contrast, Sioriki et al. (2016) who worked on the same species reported that the incorporation of the same amount of OP (8%) in feed enriched the fish lipid profile where most FA amount was higher compared to control.

For olive pomace oil (OPO), it was found that it decreases most FA in the muscle/fillet of African catfish (Yilmaz et al., 2004) compared to control though it must be noted that it was unclear whether the

Table 2
Analysis on carcass/muscle fillet composition of several fish species fed with olive oil by-products.

	Fish Species						
	African catfish (<i>Clarias gariepinus</i>)	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Red sea bream (<i>Pagrus major</i>)	Gilthead sea bream (<i>Sparus aurata</i>)			
Olive oil by-product	Olive Pomace oil ^a	Olive mill wastewater (OMW) ^b	Olive leaf powder (OLP)	Olive mill wastewater (OMW) ^c	Olive Pomace ^d	Olive Pomace oil ^d	Olive Pomace ^e
Treatment/Diet Composition	3% OP oil + 2 g LC kg ⁻¹ feed (%)	5% kg ⁻¹ diet(%)	8% OLP kg ⁻¹ diet	1% and 5% kg ⁻¹ diet	8% replacement of total fish oil in diet (ppm)	8% replacement of total fish oil in diet (ppm)	8% OP mixed in diet prior to extrusion (%)
Proximate Composition							
Crude Protein	16.25	89.47 ± 1.39	Not Examined	No differences between treatments and control.	Not Examined	Not Examined	Not Examined
Lipid	7.85 *	5.93 ± 0.83		Value not reported			
Ash	3.42	6.53 ± 0.16					
Moisture	NA	–	75.17 ± 0.45a				
Fatty Acids							
14:0	1.24	4.14 ± 0.6	Not Examined	No differences between treatments and control.	43.87 ± 2.61	46.89 ± 0.99	2.63 ± 0.35
16:0	27.87	23.94 ± 1.2		Value not reported	150.1 ± 2.81 *	546.7 ± 1.12 *	16.0 ± 1.28
16:1	1.88	5.81 ± 0.7			57.19 ± 4.88	50.91 ± 4.12	3.53 ± 0.21
18:1 ω – 9	29.58	29.85 ± 1.6			162.7 ± 3.48(cis)*	262.9 ± 11.42(cis)*	17.0 ± 1.04 (cis)
					15.09 ± 1.009 (trans)	42.16 ± 4.82(trans)	2.20 ± 0.28 (trans)
18:2 ω – 6	18.63	9.68 ± 1.7			53.14 ± 0.978 *	139.5 ± 3.002 *	8.71 ± 0.65
18:3 ω – 3	–	0.33 ± 0.1			–	–	1.20 ± 0.08
20:5 ω – 3	0.51	3.61 ± 0.2			3.484 ± 0.011 *	40.19 ± 3.982 *	7.51 ± 1.03
22:6 ω – 3	2.68	17.78 ± 2.6			2.298 ± 0.098 *	61.18 ± 1.990 *	30.09 ± 1.97
Saturated	41.32	31.35 ± 1.3			198.1 ± 4.168 *	649.5 ± 5.139 *	23.0 ± 1.89
MUFA	32.62	36.21 ± 2			–	–	22.7 ± 2.01
PUFA	26.55	32.53 ± 2.6			–	–	–
Monoenes	–	–			235.0 ± 1.582 *	356.0 ± 3.012 *	–
ω – 3/ ω – 6	0.27	22.52 ± 2.7			0.11 ± 0.01 *	0.73 ± 0.05 *	3.34 ± 0.03
Protein Content							Not Examined
Sarcoplasmic protein(mg/g muscle)	Not Examined	Not Examined	46.64 ± 10.50	Not Examined	Not Examined	Not Examined	
Myofibril protein (mg/g muscle)			200.4 ± 32.4 *				
Acid-soluble collagen(mg/g muscle)			1.66 ± 0.30 *				
Hydroxyproline (mg/g muscle)			0.45 ± 0.062				

differences are significant since it was not clear whether statistical analysis was conducted on the data. In gilthead seabream (Nasopoulou et al., 2011), OPO seems to only significantly increase only one FA (saturated FA (16:0)) and no significant difference was found in other FA amount compared to control fish. Compared to other olive oil by-products, olive leaf powder (OLP) is the least tested product in terms of studying the impacts on fish carcass/muscle. In a study done by Arsyad et al. (2018), OLP did not have any significant impact on the moisture content of red sea bream muscle but it increases the collagen and myofibril protein content. The authors did not report its effect on other proximate parameters or FA profile since the focus of the study was on the effects of OLP on red seabream muscle protein.

3.1. Effects on the growth performance

Plant proteins' wide availability and reasonable cost have made them the most viable choice as an alternative. As such, the identification and development of plant ingredients as an alternative to fishmeal and its high cost has received sustained interest in the flourishing world aquaculture sector (Azarm and Lee, 2014; Cheng et al., 2013; Goda et al., 2014; Kari et al., 2022, 2021). Several previous studies reported that the antioxidant content on olive oil extract can affect the growth performances of sea bream (*Sparus aurata*) (Sadek et al., 2004; Sanchez-Muros et al., 2003). The growth rate performances of fish normally related with improvement in humoral, mucosal immune parameters and antioxidant enzymes activities (Hoseinifar et al., 2020). The study by Baba et al. (2018) on *Oncorhynchus mykiss* indicated that olive oil leaf improves survival rate and the serum biochemical parameters in the species. In his study, 0.1% of olive leaf in fish feed had a lowest mortality rate compared with others treatment and recommended for aquaculture industry. Improvement in growth performance parameters is recited to be attributed of immune-nutritional constituent such as polysaccharides as complex sugar (Zahran et al., 2014).

Determining the optimum inclusion level of plant protein is very crucial in the fish feed formulation. In general, the high level of inclusion rate can give a negative effect on the growth and health performances of fish (Gabriel, 2019; Hosseinnia et al., 2021; Zulhisyam et al., 2020b, 2020a) and the recommended inclusion level of olive oil plant part were showed in the Table 3. According to Sokooti et al. (2021), the various level of olive leaf extract give a different result on growth and the low

rate of inclusion was recommended. In contrast, the study by Rajabiesterabadi et al. (2020) revealed that there were no significant difference in growth parameters of *C. carpio* and *Oncorhynchus mykiss* fed with various levels of olive leaf extract. The different results obtained from various study on fish growth performance may be related to fish species, different concentrations of olive leaf extract, the fish feed preparation, the diverse intestinal microbiota and morphology and experimental condition.

Limitations related to plant protein use in fish feed are well known. Anti-nutritional factors (ANFs) present in plant-based feed are the primary constraint an example being phytate, which is phosphate (P) in its main storage form (NRC, 2011). Since an olive product is categorized as herbal plant, the higher herbal extract inclusion level has been reported to give a negative effect on the fish growth performances because of the higher concentration of anti-nutritional factors (ANFs) (Irkin et al., 2014; Yilmaz and Ergün, 2012; Yilmaz et al., 2018; Zulhisyam et al., 2020b). Thus, to make the olive product more acceptable in aqua feed, more research was needed to be highlight on ANFs compound to reduce the negative effect on growth and health performances of fish. With the new technology and development, the idea of research into solid state fermentation (SSF) has focused on developing bio-processes for bio-remediation and biological detoxification of agro-industrial wastes to eliminate or reduce these wastes by the process of fermentation which can be applied to the olive plant too (Rusli et al., 2021).

3.2. Effects on the feed utilization

When oil is extracted from olives, a waste product called waste olive cake is generated. It is composed of olive pulp, skin, stones, and water (Berbel and Posadillo, 2018). In addition, the olive pomace also has a high concentration of lipids, water-soluble carbohydrate, polyalcohol, polyphenols, and polyalcohol metabolites. Their moisture level and oil content are highly varied, and this is due to the extraction process that was used to obtain the oil and pomace. As compared to other oil cakes, raw olive pomace contains a low concentration of crude protein, with a large proportion of these proteins (80–90%) attached to the lignocellulose fraction. Crude fibre is plentiful in olive pomace, and its lipids contain a high concentration of unsaturated C16 and C18 fatty acids, which contribute to 96% of the total fatty acid content. However, despite its nutritional value, the olive cake contains a substantial

Table 3
Recommended inclusion level of olive plant part in different species of fish.

Type of olive plant part	Recommended inclusion level of Olive oil	Duration	Species	Effects	References
Olive oil	5%	2 months	Persian sturgeon (<i>Acipenser persicus</i>)	Lowest FCR and the highest SGR, BWI, and GR	Hosseinnia et al. (2021)
Olive oil bioactive extract	0.17% and 0.42%	90 days	Gilthead sea bream (<i>Sparus aurata</i>)	OBE enhanced fish growth primarily by improving the condition and defensive role of the intestine.	Gisbert et al. (2017)
Olive leaf extract	200 mg/kg of olive leaf	75 days	Common carp (<i>Cyprinus carpio</i>)	WG, SGR and PER values were significantly higher in the groups fed the diet containing 200 mg/kg of olive leaf extract compared with the control group	Sokooti et al. (2021)
Olive leaf	1%	2 months	Nile tilapia (<i>Oreochromis niloticus</i>)	olive leaves extract can be used to enhance the immunity, growth and health of fish	Fazio et al. (2021)
Olive leaf	1 g/kg olive leaf extract	60 days	common carp (<i>Cyprinus carpio</i>)	Improve fish immune parameters and survival rate	Zemheri-Navruz et al. (2019)
Olive leaf powder	8%	40 days	Red sea bream (<i>Pagrus major</i>)	Increased the collagen content and myofibril protein content of the dorsal muscles.	Arsyad et al. (2018)
Olive leaf extract	0.1%	60 days	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Enhance the serum biochemical parameters, survival rate and immune gene expression	Baba et al. (2018)
Olive oil extract	0.1–0.5%	8 weeks	Common carp (<i>Cyprinus carpio</i>)	Improve the fish intestinal health	Rajabiesterabadi et al. (2020)
Olive oil	Partial or total dietary replacement	40 days	Young yellowtail (<i>Seriola quinqueradiata</i>)	Prevents discoloration of dark muscle without affecting the growth of young yellowtail	Seno-o et al. (2008)
Olive oil	Low level of olive oil inclusion	10 weeks	Large yellow croaker (<i>Larimichthys crocea</i>)	High level of dietary OO decreased the growth performance and antioxidant capacity	Li et al. (2019)
Olive waste	2.5 g Olive waste kg ⁻¹	6 weeks	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Improved growth rate and feed utilization	Hoseinifar et al. (2020)

amount of low-molecular-weight phenols and high-molecular-weight polyphenols (Clodoveo et al., 2015). These compounds, such as oleuropein and lignin-like polymers, can cause significant damage when disposed of in an open environment. Additionally, the antibacterial activity of phenolics has been demonstrated against a few strains of bacteria that have been connected to intestinal and respiratory disorders (Waterman and Lockwood, 2007).

Olive pomace have been widely utilised in the livestock and aquaculture industries for a variety of purposes, including feeding animals. Because of their high protein content, several of these are also thought to be beneficial for use as feed additives. Furthermore, the olive pomace, may be an effective protein source for feed formulation, especially nowadays with the increased focus on cost reduction and value added of agro-industrial waste. The practise of feeding by-products of agricultural industries to livestock and aquaculture species is widespread around the world (Rahman et al., 2021). The use of agricultural by-products also lessen the reliance of animals on grains, as well as the costs associated with feeding and waste management (Mat et al., 2021). Throughout the year, large volumes of olive oil by-products suitable for livestock feeding are produced in Mediterranean countries. Furthermore, in addition to its high phenolic content, olive pomace also contains vitamins such as tocopherol, hydrocarbons such as squalene, and sterol compounds such as -sitosterol (Tabera et al., 2004), all of which have significant nutritional and physiological benefits for animals. A constituent of olive oil, squalene, a key natural source derived from shark liver oil that has been outlawed, can be detected as a constituent in the hydrocarbon fraction, where it accounts for more than 90% of the total hydrocarbon fraction (Lanzón et al., 1994). Supplementing the feed of animals with squalene has been shown to lower cholesterol and triglyceride levels in the blood. The sterol esters lower serum cholesterol levels by decreasing intestinal cholesterol absorption and blocking the synthesis of LDL apolipoprotein B (Katan et al., 2003). A study conducted by Serra et al. (2017) revealed that inclusion of olive pomace in swine diets had potentially reduced the lipid oxidation in the sausage which also improved the fatty acid composition.

According to Ramachandran et al. (2005), because of the high nutritional value of olive pomace, it may be used as an excellent nutrition source for all types of livestock. Untreated olive pomace for feeding, on the other hand, is restricted due to the high crude fibre content and anti-nutritive chemicals present in the pomace. The olive pomace, when properly processed, has the ability to substitute crops in animal diets, as well as to function as a compound feed and a health-promoting element in animal feed. Equines, goats, and cows showed improved lactation performance when conventional roughage (barley hay and barley straw) was partially replaced with ensiled crude olive pomace silage. The feeding of ensiled olive pomace silage (15% of the total diet) increased the fat percentage of dairy cows' milk in the range of 0.3–0.6 absolute percent units (Hadjipanayiotou, 1999). According to Sadeghi et al. (2009), destoned olive cake is a partial separation of the stone from the olive pulp achieved through screening or ventilation. When fed to sheep, destoned olive cake produced significantly higher body weight gain, growth rate and feed conversions when compared to crude olive pomace and partially destoned exhausted and exhausted olive pomace, all of which contained low digestible content (Sadeghi et al., 2009). In addition, the inclusion of dried stoned olive pomace in the diet of lactating dairy cattle did not affected milk production as well as their body indexes (Zilio et al., 2015). The application of dried stoned olive pomace in lactating buffalos diets also showed an improvement in nutritional characteristic of mozzarella cheese with increment in the mono-unsaturated fatty acids without unfavourable effects on milk production (Taticchi et al., 2017).

Several studies have been carried out by formulating feed using olive pomace and feeding it to tilapia species shows that it can replace wheat meal without having any negative impacts on growth performance or feed evaluation. Al-Asgah et al. (2011) found that utilising olive pomace to replace wheat bran at up to a 25% level in tilapia diets has no adverse

effect and does not compromise the growth performance of fish or efficiency of feed utilisation. Yildirim and Guroy (2015) discovered a similar result when they determined that dietary olive pomace meal may replace wheat meal at a 20% level in *Tilapia zillii* juvenile diets without generating any harmful impacts on growth performance or feed evaluation. As the olive pomace meal is utilised as a feed component in tilapia diets, it has the potential to aid in the development of more cost-effective feedstuffs. Nutritional olive pomace meals are natural by-products that are available at a low cost and are not genetically modified. There was no difference in the acceptability of the meals by the fish when olive pomace meal was added to the diets, and the inclusion of olive pomace meal did not affect the palatability of the feed (Yildirim and Guroy, 2015). In contrast, based on findings from Al-Asgah et al. (2011), it was determined that substituting wheat bran with olive waste at a level of more than 25% in tilapia diets considerably affected their growth performance as well as their efficiency in utilising their feed. Because of the higher crude fibre content of olive waste, the addition of olive waste to tilapia diets resulted in higher overall crude fibre contents of the diets. Tilapia diets include a significant amount of fibre, which may have an inhibiting effect on the digestion of other dietary constituents. Depending on the nutritional composition of individual feed ingredients as well as the fish's capacity to digest and absorb nutrients from various feeds, the nutritional value of mixed diets can vary significantly (Köprücü and Özdemir, 2005). According to Al-Asgah et al. (2011) the poor development performance of tilapia found with increasing amounts of olive pomace in their diets could be attributed to a combination of factors, including high dietary fibre content as well as deficiency in certain amino acids, specifically methionine. A possible limiting amino acid in olive by-products is methionine, which could be the case (García et al., 2003).

A previous study found that utilising olive pomace meal and olive waste oil in sea bass diets instead of fish meal and oil resulted in significantly lower feed conversion ratios (FCR) and lowered specific growth rates (Nasopoulou et al., 2011). The increase in the amount of olive waste in fish diets had an effect on the FCR and the protein efficiency ratio, respectively (Al-Asgah et al., 2011). The low growth performance of sea bass could be linked to the species' limited metabolic capabilities (Yildirim and Guroy, 2015). Fish feed consumption and the amount of olive waste in their diets were shown to be in direct correlation with each other. It was shown that fish fed 100% waste from olive production had the lowest overall feed intake with 40.88 g/fish. Fish given 0% and 25% olive waste, on the other hand, showed no statistically significant variations in feed intake ($P > 0.05$) (Al-Asgah et al., 2011). It has been reported by Yilmaz et al. (2004) the effects of dietary olive pomace oil and L-carnitine on the growth and chemical composition of African catfish (*Clarias gariepinus*). Two different levels of energy diets, including 9% vs 3% olive pomace oil, respectively, were tested in conjunction with the supplementation or not of L-carnitine. The fish fed the low-energy diet (3% olive pomace oil) with supplementary L-carnitine showed greater feed intake and growth than those fed the high-energy diet alone. Total lipids in the muscle were shown to be increased by L-carnitine, while total lipids in the liver were decreased. The inclusion of olive mill effluent in the diet of rainbow trout (*Onchorynchus mykiss*) at two different concentrations (1% and 5%) demonstrated that there were no adverse impacts noticed on the fish's development or feed digestibility when the wastewater was supplemented with the diet. Consequently, olive mill wastewater is being explored as a potential alternative ingredient in trout diet formulation (Sicuro et al., 2010b). Additionally, Nasopoulou et al. (2011) revealed that olive pomace as a partial substitute for fish oil in gilthead sea bream (*Sparus aurata*), reported that feeding olive pomace to the fish leads to an enhancement in the fish's potential to suppress atherogenesis. In addition, Fazio et al. (2021) reported that there were increased haematological and biochemical parameters were observed in the fish fed fortified feed containing 1% olive leaf extract. These improvements may indicate that the fish's immune system is being strengthened, with

positive effects against various diseases and long-term stressful pressures.

3.3. Effects on the intestinal health and microbial diversity

Olive oil by-products have been used as partial replacement of fish oil in aquafeed due to its benefits on intestinal health and microbial diversity. According to, histologically no significant difference was found in intestine villus size, width and height among treatment and control fish group but goblet cell population was found increased in the intestinal epithelium when the fish were given different percentage of 0.17% and 0.42% olive oil bioactive extract in their diets. Comparable to histological findings, intestinal gene expression profile of fish fed with OBE had also demonstrated up-regulation in a goblet specific intestinal differentiation factor which also known as zinc-finger transcription factor (*Klf4*) that signalled a potent immune barrier as protection from potentially pathogenic gut bacteria (Gisbert et al., 2017). Additionally, six out of 16 genes, included occludin, *Cldm3*, claudin 12, junctional adhesion molecule A (*F11r*) and few more genes that related to intestinal architecture and permeability also showed up-regulated results (Gisbert et al., 2017). Occludin and claudin genes are protein families of tight junctions which are crucial as the major component of intestinal barrier function (Liu et al., 2012). This physiological barrier is important for reabsorption of nutrients which play important roles as nutrient transporters that thought to influence digestion and fish growth performance (Centrone et al., 2021). Moreover, olive oil by-products or extra virgin olive oil diet also have shown anti-inflammatory activity where its derivatives such as hydroxytyrosol, tyrosol and oleuropein were mostly found in the lumen of the intestine (Centrone et al., 2021; Corona et al., 2009). In addition, vegetable oil used to replaced fish oil in fish feed particularly are olive oil and rapeseed oil, have demonstrated lower cholesterol content in fish intestinal tissue in comparison with fish oil (Liland et al., 2018).

The olive extract was previously used to produce digestible biofilm which demonstrated antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* (Centrone et al., 2021). The antimicrobial activity of olive oil by-products has been rarely tested against fish pathogens. The ability of olive extract biofilm to inhibit bacterial growth could be tested to investigate its potential in reducing population of pathogenic bacteria in fish gut. In previous study by Leouifoudi et al. (2015), phenol derivative from olive wastewater extract had demonstrated antibacterial activity against pathogenic Gram-positive, *S. aureus* (Leouifoudi et al., 2015; Rebollada-Merino et al., 2019). Branciari et al. (2016) had highlighted the potential of olive mill wastewater polyphenol extract and dehydrated olive cake inclusion in the chicken diet on the shedding of *Campylobacter* spp. in broiler. The inclusion of polyphenols in broiler diets also boosted antioxidant activity in meat but not affecting its (Branciari et al., 2017).

Apart from that, olive oil by-products could stimulate the growth of fish gut microbiota such as *Lactobacillus acidophilus* which has been reported as good candidate as probiotics (Banerjee and Ray, 2016; Gavahian et al., 2019). However, the effects of olive oil by-products on fish gut microbiota have not been studied extensively with most of the available publications were focused on fish growth performance and antioxidant activities. A study conducted by Hidalgo et al. (2014) on the effect of extra virgin olive oil (EVOO) consumption on mice gut bacterial population had observed an increase in microbial diversity in mice group that fed with EVOO diet. Another study by de Wit et al. (2012) also showed better results of gut microbiota composition in mice intestine when the mice fed with olive oil dietary fat compared to palm oil dietary fat. Aside from anti-inflammatory properties, bioactive compounds such as oleuropein and hydroxytyrosol are also known as potential candidates that could balance the gut microbiota diversity (Herrero-Encinas et al., 2019). The gastrointestinal microbiota composition is vital for gut health and nutrient absorption in improving fish growth performance.

3.4. Effects on the immune response

Olive oil by-products is giving good effect on fish immunity and highly potential to be used as an immunomodulators in fish. The fatty acids from refined olive pomace oil such as oleic, linoleic, stearic, palmitic, palmitoleic acids and other bioactive compounds are beneficial for immune modulation and can be used as prophylaxis against infection when added into diet. Study by (Calder, 2001) indicated the fatty acid composition of membrane phospholipids in the phagocytes and monocytes is directly proportional to the total fatty acid intake from food that will affect the physical characteristics of the membrane and the efficiency of the cell to respond toward stimuli. Other than that, bioactive compounds from olive oil by-products was found to enhanced the intestinal mucosal immunity of gilthead seabream (Gisbert et al., 2017). The supplementation of olive oil by-products into the salmon feed was resulted the reduction of inflammation due to diseases, extreme temperature as well as the pathogen load in the body. High composition of antioxidants in the olive oil by-products might contribute to the improvement of immune status of fish by reducing the oxidative stress. Oxidative stress is known to reduce the efficiency of innate immune response in fish that make fish more susceptible to diseases (Billar and Takahashi, 2018). Hajirezaee et al. (2020) suggested that vitamin C supplementation in fish diet has a potential of preventing oxidative stress in common carp and reduce the severity of liver damage when the fish was exposed to titanium oxide nanoparticles.

3.5. Effects on the antioxidative capacity

For a food safety perspective, the use of natural antioxidants in fish feeds is increasing in recent years. To avoid undesirable side effects from the use of synthetic antioxidants, researchers are focusing on several natural compounds. It has been the fruit, leaves and oil of olive trees are significant sources of polyphenols (Salah et al., 2012). For example, Mulinacci et al. (2001) and Balasundram et al. (2006) reported that olive oil by-products (leaves, stems, twigs etc.) contain polyphenols, for instance, oleuropein, hydroxytyrosol, tyrosol, and hydroxycinnamic acids, which also have beneficial antimicrobial, antioxidant, antifungal and antitoxigenic characteristics. The concentration of polyphenol acid in olive oil by-products is significantly more than that found in olive oil products: with the polyphenol acid found in the by-products around 98% and the polyphenol acid in olive oil products around 2% (Alu'datt et al., 2010). Furthermore, a study by Branciari et al. (2020) had demonstrated an increment of tyrosol and hydroxytyrosol sulphate metabolites in ewe's milk and cheese when spray-dried olive mill wastewater was supplemented in the dairy sheep. Olive leaves contain important natural phytonutrients like oleuropein (of approximately 14% dry matter basis) and oleonic acid (of approximately 3% dry matter basis), as reported by (Guinda et al., 2015). Moreover, these natural products can be obtained from various plants such as *Origanum vulgare*, *Melissa officinalis* and *Pistacia vera*, and feeding of these plant derivatives in Nile tilapia can improve the antioxidative capacity and immune-related genes in their body (Mohammadi et al., 2020; Mohammadi et al., 2022)

Many beneficial effects have been noticed due to the presence of antioxidant properties (such as free radicals, antimicrobial, hypoglycemic and hypolipidemic actions) of olives, olive oil and olive mill vegetation water (Yang et al., 2007). The total phenolic and flavonoid contents for the aqueous extracts of the following were: olive pomace: 278.41 mg cat./g and 69.66 mg GAE/g, olive leaves: 195.83 mg cat./g and 40 mg GAE/g, and pomace olive oil: 55 mg cat./g and 20 mg GAE/g, respectively, with the value of phenolic content stated first and flavonoid second (Abdel-Razek et al., 2017). In addition, it was observed that pomace olive extract had an excellent antimicrobial character, which was in consistence with its total phenolic, flavonoid and antioxidant activities. Moreover, its extract consisted highly of bioactive components that reduced toxigenic fungal growth and mycotoxins. Table 4

Table 4

Phenolic components in olive leaves, olive pomace and pomace olive oil (source: Abdel-Razek et al., 2017).

Phenolic compound	Concentration (mg/g)		
	Olive leaves extract	Olive pomace extract	Pomace olive oil
Tyrosol	0.42	0.25	0.025
Luteolin-7-rutinoside	0.02	0.07	Not detected
Rutin	0.14	0.12	0.01
Dihydroquercetin	0.08	0.21	0.02
10-hydroxy-oleuropein	11.48	2.18	2.48
Luteolin-7-glucoside	0.09	0.15	0.00
Verbascoside	0.57	0.00	1.07
Apigenin-7-glucoside	7.4	0.00	Not detected
Chrysoeriol-7-O-glucoside	1.05	0.28	Not detected
Oleuropein glucoside	0.02	0.14	Not detected
Oleuropein	0.46	0.72	0.04
Oleoside	0.07	0.03	18.00
Apigenin	15.08	0.39	20.00

shows that compared to ascorbic acid, olive pomace and leaf extract had a more effective antioxidant capacity.

After estimation, Abdel-Razek et al. (2017) observed that the antioxidant activity values, displayed as DPPH and ABTS inhibition ratios, were: DPPH radical scavenging ability for aqueous extracts of olive pomace, olive leaves and olive oil were 72.24%, 66.49% and 65.7%, respectively, and antioxidant capacity of the aforementioned substances as per ABTS•+ radical assay were 83.33%, 78.24% and 70.5%, respectively (Abdel-Razek et al., 2017). It can be inferred from the above findings that olive by-products possess antioxidant/free-radical scavenging properties, which is probably owing to the presence of high quantity phenolic compounds. Regarding the growing interest in natural antioxidants, especially of those with plant source, to replace synthetic antioxidants because of their potential benefits compared to the former, olive oil by-products should be taken into serious consideration.

3.6. Effects on the disease resistance

Olive oil, *Olea europaea* L. folium, is world oldest cultivated crop was recorded in the literature (Kapellakis et al., 2008). The oil of olive was extracted and widely used in food, beverages as well as traditional medical purposes (Acar-Tek and Ağagündüz, 2020). The usefulness of olive oil attract many investors to venture in extracting oil in olive. However, olive oil extraction activity produce a lot of by products such as olive leaf, crude olive cake (COC) and olive mill wastewater (OMW). In spite of the facts, many studies were conducted to utilise these by products for other uses. There are some reports showed COC was used as ingredient in animal feed formulation as it will contribute oil in the feed (Kamini et al., 2011). On the other hand, Soha et al. (2019) reported that microalgae species *Nostoc muscorum*, *Anabaena oryzae* and *Spirulina platensis* are able to utilise and grow by using OWM. Furthermore, the growth of these microalgal can reduce total phenolic content of wastewater up to 80% (Soha et al., 2019). Application of OMW in microalgal propagation can reduce cost production of microalgal production and minimize OMW impact to environment. Besides COC and OWM, there is other olive by-products such as olive leaf is on-going to be explored its potential uses in fish health management.

The increasing of human population in the world need more protein source to fed people. Fish is one of the cheap and affordable protein source can be derived from natural water bodies such as river, lake and ocean. The activity of harvesting fish from natural water bodies is known as fishery. Recently, fishery activity is over exploited resources from natural water bodies lead to declination of fish resources. Hence aquaculture is carried out to balance the fish stock in natural water bodies and fulfil fish demand in the market. In the year of 2014, aquaculture produced 74 million tons fish or 44% of total world fish production with

the value USD 160 billions (Assefa and Abunna, 2018). As aquaculture industry is heading toward to intensification, the industry development was hindering with fish diseases problem. Assefa and Abunna (2018) reported total loss of aquaculture due to diseases is around USD 6 billions annually. For instance, salmon farming lost about USD 2 billions due to salmon anaemia disease and 20,000 workers lost their job in Chile (Assefa and Abunna, 2018). Furthermore, Leung and Bates (2013) is also claimed that various diseases reduced at least 15% of China fish production through aquaculture (Leung and Bates, 2013). Traditionally, fish farmer will use antibiotic for treatment and fish health management. However, misuse and overuse of antibiotic lead to many antibiotics no longer effective in control diseases. Fish farmer was left with no option and continue to use antibiotic in his fish farm hence there is a need to find alternative antimicrobial agent for aquaculture uses such as vaccine, probiotic, prebiotic, immunostimulant, biological control, antimicrobial compound, medicinal plant and etc (Kumar et al., 2016). The new antimicrobial agent to overcome diseases in aquaculture must cost effective and user friendly. The recent studies and findings revealed olive leaf extract (OLE) have huge potential as immunostimulant agent for aquaculture uses. These findings showed olive by-products extract possess immunostimulant property where can increase immune system of commercial farmed fish to resistant various diseases infection.

Based on literature survey, there are many studies revealed the potential of OLE as immunostimulant for various fish to resistant disease. For instance, Zemheri-Navruz et al. (2019) reported that feeding feed supplemented with 0.1% of OLE for 60 consecutive days can enhance immune parameters of juvenile, common carp, *Cyprinus carpio*, and the fish was found can resistant to *Edwardsiella tarda* infection (Zemheri-Navruz et al., 2019). Similar finding was reported in the study of Baba et al. (2018) showed fish feed supplemented with 0.1% of OLE was found can control *Yersinia ruckeri* infection in rainbow trout, *Oncorhynchus mykiss*, effectively because OLE is able to enhance immune system of rainbow trout hence the fish will resistant to the disease infection (Baba et al., 2018). However, the study revealed OLE did not help in fish growth performance. On the other hand, Fazio et al. (2021) claimed fish feed supplemented with 1% of OLE is not only can enhance immune system of Nile tilapia, *Oreochromis niloticus*, but also can improve its growth rate (Fazio et al., 2021). Other study that revealed OLE is able to enhance fish immune system as well as growth rate in the study of Sokooti et al. (2021) showed feed fish supplemented with 0.02% OLE can enhance the growth, haematological parameters, immune system and carcass composition in common carp, *C. carpio* (Sokooti et al., 2021). Same finding was observed between study of (Sokooti et al., 2021) and Arsyad et al. (2018) where both studies claimed olive leaf can improve quality of flesh texture of fish that received feed supplemented with olive leaf. However, in the study of Arsyad et al. (2018) study olive leaf was fed directly to red sea bream, *Pagrus major*. In the study, dried olive leaf was blended into powder form and mixed with fish feed at 8% of feed weight. The feed was then given to experimental fish for 40 consecutive days. The finding of the study showed olive leaf can enhance flesh texture quality with the present significantly higher content of myofibril and collagen compared to control group of fish. Hence, fish received feed supplemented with 0.1% or less of OLE for a period of 40 days or more has huge benefit to improve fish immune system and allowed them to resistant to various diseases. At the meantime, the quality of fish flesh texture will be enhanced by reducing fat content.

Besides fish, OLE was found can against virus infection in white leg shrimp, *Penaeus vannamei*. The experimental shrimp was given shrimp feed supplemented with OLE for 7 consecutive days before exposed to white spot syndrome virus (WSSV). Experimental shrimp which received feed supplemented with 0.02% OLE showed highest survival rate was recorded (65%). This finding showed OLE can also enhance crustacean immune system. Fish was also reported can survive in poor water quality condition after received OLE. This was supported by Rajabiesterabadi et al. (2020) claimed that OLE is good to improve fish

health and reduce adverse effect of ammonia toxicity. In their study, common carp fish received feed supplemented with 0.1% OLE for 60 consecutive days was found can tolerant high concentration of ammonia at 0.5 ppm (Rajabiesterabadi et al., 2020).

Cho et al. (2020) showed that 90% methanol can perform the best solvent in extracting total phenolic content from olive leaf compared other 2 solvents namely distilled water and ethanol. Total phenol content is bioactive compound in OLE may responsible to immunostimulant activity of OLE. However, further study need to carry to confirm this hypothesis. Olive leaf is olive oil by-products and considered as cost less raw material for medical and nutritional uses (Cho et al., 2020). Hence, methanol solvent can be widely used in preparing OLE for aquaculture uses in the commercial scale. There is huge potential of olive by-products can be used in fish health management. This statement was supported by several recent studies and findings. Further study need to be carried out in order to upscale recent findings for mass production before it can come to a commercial sense.

3.7. Conclusion and future perspectives

The utilisation of agro-industrial biomass provides significant prospects for the creation of novel solutions to existing and future aquaculture difficulties, as well as new phytochemical capable of improving fish health and welfare. It appears that olive by-products are widely utilised in aquaculture to some extent, and researches into their usefulness in the diets of different fish species are ongoing. Each of olive by-products which include olive cake, olive leaves and branches, or vegetative waters, offers a nutritious value that, while minor, should not be overlooked. However, these by-products may have anti-nutritional factors that are not entirely suitable for fish, which may limit the use of olive by feedstuffs in high concentrations in diets. Given this obstacle, there is a huge opportunity for technology advancements to develop high-quality olive oil and plant by-products with improved nutritional profiles on a consistent basis, while economies of scale can increase price competitiveness. Nevertheless, several significant findings have been provided in this review article, allowing for a rather optimistic outlook on the future. These by-products can and should be employed in animal feed to a greater extent. The antioxidant, antibacterial, antimicrobial, antioxidant, antifungal and antioxygenic properties were discovered in the olive by-products, which improved the intestinal health and immune response of fish. The presence of olive by-products in the fish feed also had no deleterious impact on the growth performance. Taking into account the benefits reported here, olive by-products have the potential to be employed in aquafeed, albeit a system for purifying and extracting important polyphenols remains to be developed. More research is required to improve the quality of animal products by incorporating olive by-products into their diets.

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CRediT authorship contribution statement

Hazreen Nita Mohd Khalid: Writing – review & editing, Project administration. **Mohd Firdaus Nawi:** Writing – review & editing, Project administration. **Zulhisyam Abdul Kari:** Writing – review & editing, Writing – original draft. **Khairiyah Mat:** Writing – review & editing, Visualization. **Nor Dini Rusli:** Writing – review & editing, Visualization. **Suniza Anis Mohamad Sukri:** Writing – review & editing, Investigation. **Hasnita Che Harun:** Writing – review & editing. **Lee Seong Wei:** Writing – review & editing, Writing – original draft. **Mohammad Mijanur Rahman:** Writing – review & editing, Investigation. **Norazmi-Lokman N. H:** Writing – review & editing, Writing – original draft. **Nur Nazifah Mansor:** Writing – review & editing.

Mahmoud Abdelhamid Omran Dawood: Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

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