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Growth, enzymatic glutathione peroxidase activity and biochemical status of juvenile barramundi (*Lates calcarifer*) fed dietary fermented lupin meal supplemented with organic selenium

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Abstract

To investigate the effects of high level of lupin meal (LM) supplemented with organic selenium (OS) on the growth and blood biochemistry of barramundi (*Lates calcarifer*), four isocaloric and isonitrogenous diets were prepared, containing either non-fermented or fermented LM, and either supplemented with 2 mg OS/kg (LM, LM_{OS}, FLM and FLM_{OS}), or not. A fishmeal (FM)-based diet formulated for juvenile barramundi was used as a control diet. Fish (initial mean weight of 5.88 g) were triplicated and fed the test diets for 75 days. The findings demonstrated that growth performance of fish fed with the FLM and FLM_{OS} diets were similar to fish fed with the FM diet ($p > .05$). The antioxidant glutathione peroxidase (GPx) activity, and haemoglobin (Hb) of fish fed with the FLM_{OS} diet were significantly higher than that of FM-fed fish ($p < .05$). Plasma alanine aminotransferase (ALT) activity was significantly increased in fish fed with non-fermented diets (LM and LM_{OS}) than in those fed with fermented LM diets (FLM and FLM_{OS}) ($p < .05$). However, there were no significant differences in ALT activity among LM_{OS}, FLM, FLM_{OS} and FM diets. There was an interaction between the LM and OS on plasma CK activity; the CK of fish fed with diets supplemented with OS was higher in non-fermented LM diets but lower in fermented LM diets ($p < .05$). This study suggests that fermented LM have an obvious potential to substantially replace 75% FM protein in the diets of barramundi.

KEYWORDS
fermentation, *Lates calcarifer*, lupin meal, organic selenium

1 | INTRODUCTION

Barramundi (*Lates calcarifer*) is a commercial aquaculture species in Asia Pacific region and Australia. According to Food and Agriculture Organisation (2016), global aquaculture production of barramundi has, due to intensive farming systems, grown considerably over the last half-decade to 75,374 tonnes in 2013. Thailand, Indonesia, Malaysia and China were the major producers (FAO 2016); however, as with the farming of other carnivorous marine finfish species, potential damage to marine resources through a high reliance on fishmeal (FM) as the major input of protein may further threaten the sustainability of barramundi aquaculture (Tacon & Metian, 2008). Therefore, many scientific investigations representing a variety of ingredients, diet formulation and experimental layouts have been conducted to study the incorporation of a non-FM dietary ingredient derived from plant protein (PP) sources, which are considered to be economically and ecologically sustainable.

Lupin meal (LM) has gained significant attention as a potential substitute to FM, owing to its balanced nutritional properties, desirable palatability, high digestibility, inexpensive price and reliable

supply. When fed to barramundi, digestibility of LM was high (Tabrett, Blyth, Bourne & Glencross, 2012) and 45% LM protein inclusion in their diets was possible without impairing growth performance and protein metabolism (Katersky & Carter, 2009). Lactobacillus-fermented LM replacing 60% FM protein improved the performance of barramundi, though the protein retention in FM was higher than in LM diets (Van Vo, Phan, Nguyen & Fotedar, 2015). These findings suggest that barramundi appear to have a high tolerance for PP sources, particularly LM, which agrees with the previous work of Glencross, Rutherford and Jones (2011) that proposed a threshold level of 15% FM in their diets.

However, a major hindrance of using PP ingredients as component of aquaculture feeds is the presence of antinutritional factors (ANF) such as phytate, which may induce morphological and physiological problems, thus affecting overall fish growth. Phytate, also known as myo-inositol hexakisphosphate or IP6, is the principal storage form of both phosphorus (P) and inositol in plant tissues (Salimur, Awan, Anjum & Randhawa, 2014). Because fish lack the digestive enzyme needed to disintegrate P from the phytate molecule, the digestibility of phytate by fish is generally poor (Kumar, Sinha, Makkar, De Boeck & Becker, 2012). In addition, phytate can interfere with protein utilization by forming phytate-protein complexes that may diminish the function of digestive enzymes (Selle, Cowieson, Cowieson & Ravindran, 2012). Furthermore, phytate has also been demonstrated to complex with minerals such as zinc (Zn), nickel (Ni), manganese (Mn), iron (Fe), magnesium (Mg), calcium (Ca) and selenium (Se) (Connelly, 2011; Reddy, Pierson, Sathe & Salunkhe, 1989), reducing the bioavailability of these minerals for absorption. As reviewed by Kumar et al. (2012) and Prabhu, Schrama and Kaushik (2014), it is generally agreed that trace mineral is poorly bioavailable in diets containing high phytate levels. For this reason, attempts to formulate nutritionally improved PP-based diets with decreased phytate levels and thus, enhance trace mineral bioavailability remains seminal.

One possible technique is the exogenous supplementation of trace minerals; of those, selenium (Se) is gaining a great deal of interest in animal feed either at research-level or at commercial-scale application. Se is an important trace element required for normal life processes (Watanabe, Kiron & Satoh, 1997); it is responsible for skeletal development (Lemly, 2002), antioxidant enzymatic function (Ashouri, Keyvanshokooh, Salati, Johari & Pasha-Zanoosi, 2015; Zhou, Wang, Gu & Li, 2009) and a proper immune system (Le, Fotedar & Partridge, 2014; Wang et al., 2013). Se's underlying role is an element of the antioxidant enzyme glutathione peroxidase (GPx), which protects cell membranes at both cellular and subcellular levels against oxidative damage by eliminating strong prooxidants such as hydroperoxides (Rotruck et al., 1973). Fish may obtain Se from food and also from surrounding water; however, dietary exposure to Se compounds is the predominant source of Se for fish (Janz, 2011). Therefore, the activity of GPx can be modified by dietary Se level. Furthermore, for fish, organic Se (OS) has been reported to be more bioavailable than inorganic Se (Bell & Cowey, 1989; Le & Fotedar, 2014; Wang & Lovell, 1997). Selenoamino

acids are the major form of OS, with seleno-methionine (Se-Met) representing about half of the total Se (Lyons, Papazyan & Surai, 2007). Trace element amino acids chelates have been widely applied in terrestrial and aquatic animal nutrition, yet very limited information is available on the supplementation effect of OS in PP-based diets.

Another possible method to improve the quality of PP sources is by fermentation. A solid-state fermentation process was reported to destroy phytate and tannins present in LM (Van Vo et al., 2015). The degradation of ANF components such as phytic acid, trypsin inhibitor and lectin from soybean meal (SBM) was reported after 12 days of incubation with *Candida utilis* (Zhou et al., 2011). In addition, the fermentation of LM and SBM with lactic acid bacteria was reported to increase nutrient digestibility (Bartkiene, Krungleviciute, Juodeikiene, Vidmantiene & Maknickiene, 2015), in grouper *Epinephelus coioides* (Zhuo, Liu & Lin, 2016). Through fermentation with *Bacillus subtilis*, phytate and free gossypol from cottonseed meal (CSM) were reduced by 88% and 60% respectively (Sun et al., 2016). The nutritive value of PP products could also be enhanced by fermentation with baker's yeast *Saccharomyces cerevisiae*, as reported by Hassaan, Soltan and Abdel-Moez (2015).

However, based on the above highlight, an integrated approach that combines trace mineral supplementation and fermentation seems to be the best available strategy to enhance the content and bioavailability of trace elements in PP-based diets for sustainable aquaculture settings. Our recent study suggests that LM-derived phytate might have constrained Se bioavailability and thus promotes adverse performance in juvenile barramundi (Ilham, Fotedar & Munilkumar, 2016). It is thus expected that juvenile barramundi fed with LM should perform as well as fish fed with FM diets, as the sole protein source provided trace mineral bioavailability of the diets are simultaneously enhanced. Therefore, an attempt was designed to assess the efficacy of dietary OS supplementation in diets containing fermented LM replacing 75% FM protein on growth performance, enzymatic activity of GPx, and biochemical status of juvenile barramundi *L. calcarifer*.

2 | MATERIALS AND METHODS

The feeding experiment was performed at the Curtin Aquatic Research Laboratory (CARL), Curtin University, WA, Australia. All the procedures were conducted in accordance with the Australian Code of Practice for the care and use of animals for scientific purposes. The methodology of this experiment was approved by the Animal Ethics Committee of Curtin University (Approval Number: AEC-2013-07).

2.1 | Preparation of fermented LM

Lupin meal were powdered and sieved through a 0.5-mm sieve and used as the raw material for fermentation. The condition of

fermentation follows a technique as describe by Hassaan et al. (2015), with some modifications. Briefly, 2 kg LM and 66 mg of baker's yeast, *Saccharomyces cerevisiae* were added with 1.6 L of distilled water and homogenized in a food mixer for 15 min. The mixture was placed in a rectangular glass container covered with aluminium foil and incubated at 30°C for 5 days. Then, the product of fermentation was dried at 60°C for 24 hr and used as an ingredient for feed preparation. A representative sample was taken for amino acids (AA) and phytic acid analysis of the ingredients as presented in Table 1.

2.2 | Experimental diet

All feed ingredients were used in this study were commercially obtained from Specialty Feeds (Glen Forrest, WA, Australia). Four isonitrogenous and isocaloric diets were formulated to contain 49% crude protein and 20 MJ/kg gross energy. LM replacing 75% FM protein either fermented or non-fermented (FLM and LM, respectively) and supplemented with 2 g OS/kg (FLM$_{OS}$ and LM$_{OS}$ respectively). A FM-based diet formulated for barramundi according to known nutritional requirements (Catacutan & Coloso, 1995, 1997; Glencross, 2006) was used as the control diet. The composition of the experimental diets is presented in Table 2.

Experimental diets were prepared according to standard CARL methods. All dry ingredients were thoroughly ground and sieved through a 0.5 mm mesh screen, mixed manually and transferred to

a food mixer for 15 min. 5 g/kg chromic oxide (Cr_2O_3, Thermo Fisher Scientific, Scoresby, Vic., Australia), which was used as an inert indicator for digestibility measurement, was dissolved in 100 ml of distilled water and sprayed on the mash during mixing. Fish oil was dispersed and distilled water were added to the premixed ingredients and mixed for another 15 min, yielding stiff dough. The mixture was then pelleted using a laboratory pelleting machine to the desired size (2 mm) and air-dried. 50 g of each diet was sampled for proximate composition and Se analysis of the diets as shown in Table 2. The experimental diets were stored at 4°C until use.

2.3 | Fish and experimental condition

Juvenile barramundi were sourced from the Australian Centre for Applied Aquaculture Research (ACAAR), Fremantle, Western Australia (WA), Australia. Fish were acclimated to laboratory

TABLE 1 AA (g/100 g protein), crude protein (%) and phytate composition (%) of FM, LM and fermented LM

Amino acid	FM	LM	Fermented LM
Essential			
Arginine	4.35	4.67	4.80
Histidine	2.09	1.11	1.24
Isoleucine	3.04	1.80	2.63
Leucine	5.17	2.90	3.85
Lysine	4.77	1.84	2.81
Methionine	1.86	0.31	0.91
Phenylalanine	2.87	1.78	2.66
Threonine	3.19	1.58	2.17
Valine	3.26	1.70	2.42
Non-essential			
Alanine	4.42	1.52	2.12
Aspartic acid	6.20	4.15	4.97
Glutamic acid	8.25	8.84	9.93
Glycine	4.73	1.75	1.98
Proline	3.81	2.12	4.21
Serine	3.05	2.14	2.89
Crude protein	63.97	38.59	50.08
Phytic acid	–	0.61	0.18

TABLE 2 Formulation and proximate composition of experimental diets for juvenile barramundi

Ingredient[a] (g/kg DM basis)	Diets[b]				
	LM	LM$_{OS}$	FLM	FLM$_{OS}$	FM
FM	150	150	180	180	460
LM[c]	510	510	450	450	–
Wheat gluten	100	100	100	100	100
Wheat flour	–	–	–	–	80
Casein	120	120	120	120	120
Fish oil	80	80	90	90	100
Wheat starch	15	13	20	20	65
Cellulose	–	–	15	13	50
Se-free premix[d]	20	20	20	20	20
Organic selenium[e]	–	2	–	2	–
Chromic oxide	5	5	5	5	5
Proximate composition					
Dry matter (%)	90.17	90.78	88.72	88.75	85.17
Ash (%)	5.37	5.33	6.51	6.58	8.25
Crude protein (%)	48.21	48.30	48.67	48.71	49.07
Crude lipid (%)	14.50	14.52	14.65	14.66	15.06
Gross energy (MJ/kg)	20.65	20.57	20.43	20.42	21.33
Se (mg/kg DM basis)	1.53	3.52	1.84	3.81	3.17

[a]Supplied by Specialty Feeds, Perth, WA, except for Sel-Plex and chromic oxide, obtained from AllTech, Lexington, Kentucky, USA and Thermo Fisher Scientific, Scoresby, VIC, Australia respectively.
[b]LM, lupin meal; LM$_{OS}$, LM + Se; FLM, fermented LM; FLM$_{OS}$, fermented LM + Se; FM, fishmeal.
[c]Australian sweet lupin, *Lupinus angustofolius*.
[d]Contains the following (as g/kg of premix): iron, 10; copper, 1.5; iodine, 0.15; manganese, 9.5; zinc, 25; vitamin A retinol, 100 IU; vitamin D3, 100 IU; vitamin E, 6.25; vitamin K, 1.6; vitamin B1, 1; vitamin B2, 2.5; niacin, 20; vitamin B6, 1.5; calcium, 5.5; biotin, 0.1; folic acid, 0.4; inositol, 60; vitamin B12, 0.002; choline, 150; ethoxyquin, 0.125.
[e]Sel-Plex®.

conditions for 1 week prior to the commencement of the trial, and all fish were fed the commercial diet twice daily during this period. Fish were then starved for 24 hr, bulk-weighed, and a total of 300 healthy and homogenous sized juveniles (average individual weight of 5.88 ± 0.18 g) were randomly distributed among fifteen 300-L experimental tanks at stocking density of 20 fish per tank. Each of the experimental diets was randomly assigned to triplicate groups. All fish were hand-fed the respective experimental diets and the control diet to apparent satiation twice daily at 09:00 and 15:00 hours, 7 days a week for 75 days. The fish were fed unhurriedly to ensure no uneaten food. Every 15 days, fish were weighed in bulk to calculate growth. Dead fish were weighed and recorded to adjust the calculation of the feed conversion ratio (FCR) and survival. During the experimental period, the tanks were supplied with recirculated water from an external biofilter (Fluval 406, Hagen, Italy) at a flow rate of 10 L/min. All tanks were supplied with continuous aeration and pure oxygen (compressed oxygen, BOC, Perth, WA, Australia). One-third of the water was exchanged every 2 weeks during the rearing period. Water quality parameters such as temperature, dissolved oxygen and salinity were measured daily and maintained at 28–29°C, >5 mg/L, 32–34 ppt respectively. These values are considered within optimal ranges for juvenile barramundi. The light-dark regime was maintained at 12:12 hr per day.

2.4 | Digestibility assessment

The digestibility of the diets was determined by total collection of faecal matter, for which faecal collections from individuals were pooled by tank, over a 7-day span prior to the end of the feeding experiment. The faecal was manually stripped immediately before the morning feeding by applying pressure the lower abdominal region of the fish. Care to exclude urine, mucus or water from the faecal samples was applied (Austreng, 1978; Glencross et al., 2005). Pooled faecal samples were dried at 55°C and stored at −20°C prior to analysis. Apparent digestibility coefficient (ADC) of dry matter (DM), protein and lipid for experimental and control diets was calculated according to the method of Maynard and Loosli (1969).

2.5 | Sampling and chemical analysis

After the feeding trial, all fish were deprived of food for 24 hr before sampling. The fish were then anaesthetized with tricaine methanesulfonate (MS-222, Sigma-Aldrich, Castle Hill, NSW, Australia) at 100 mg/L and weighed individually to compute the weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival. For the determination of haematological indices, blood was withdrawn from the caudal vein puncture of three randomly selected fish per tank (nine fish per dietary treatment). The extracted blood in a 1 ml plastic syringe was transferred BD Vacutainer® blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Subsamples of whole blood were immediately analysed for haemoglobin (Hb), haematocrit and leucocrit concentrations. Remaining blood samples were then centrifuged for 5 min at 1,500 g at 4°C, and the plasma samples collected were stored at −80°C until subsequent blood chemistry analyses were performed. Enzyme activity of GPx was quantitatively measured using the Randox Laboratories test combination (Ransel, Antrim, UK).

Blood samples were sent to the Animal Health Laboratories (South Perth, WA, Australia) for plasma enzyme and biochemistry analysis. The assays were run on an Olympus AU400 automated chemistry analyser (Olympus Optical, Mt Waverley, VIC, Australia). Each of the assays used was a standard kit developed for the auto-analyser. The tests performed included creatinine kinase (CK) (Olympus kit Cat. No. OSR6179), alanine aminotransferase (ALT) (Olympus kit Cat. No. OSR6107), urea (Olympus kit Cat. No.OSR6134), creatinine (Olympus kit Cat. No. OSR6178), phosphate (Olympus kit Cat. No. OSR6122), cholesterol (Olympus kit Cat. No. OSR6116), total protein (Olympus kit Cat. No. OSR6132) and albumin (Olympus kit Cat. No. OSR6102). Haptoglobin was measured based on method as described by Eckersall et al. (1999).

The proximate composition of the diet and faecal samples were determined in triplicate based on standard procedures (AOAC 1990). Briefly, dry matter was calculated by gravimetric analysis following oven drying to constant weight at 105°C. Crude ash was determined by combustion in a furnace at 550°C. Crude protein content was determined by measuring nitrogen ($N \times 6.25$) using the Kjeldahl digestion method. Lipid content was determined by ether extraction using Soxhlet technique. Gross energy content was measured by an IKA oxygen bomb calorimeter (Heitersheim, Germany). AA content of the ingredients was quantified after samples were hydrolysed in HCl (Barkholt & Jensen, 1989; Rayner, 1985). Analyses were performed on an Agilent 1100 series high-performance liquid chromatography (HPLC, Agilent Technologies, Germany) system using conditions similar to those described by Gratzfeld-Huesgen (1998). Phytate content of the ingredients was determined using automated high-performance liquid chromatography (HPLC) analysis as suggested by Kwanyuen and Burton (2005).

2.6 | Se determination

The total Se in diet and muscle tissue samples was analysed at the Intertek Genalysis Laboratory (Perth, WA, Australia) using inductively coupled plasma-mass spectrometry instrument (ICP-MS, 7500 series, Agilent Technologies, Australia), as previously described in detail (Ilham, Fotedar & Munilkumar, 2016). Se concentration was measured as dry weight.

2.7 | Calculations

Growth, feeding utilization and ADC were measured using the calculated parameters as follows:

$$\text{Specific growth rate (SGR, \% per day)} = 100 \times \left[\frac{\text{ln final weight} - \text{ln initial weight}}{\text{days}}\right] \quad (1)$$

$$\text{Weight gain (\%)} = 100 \times \left[\frac{\text{final weight} - \text{initial weight}}{\text{initial weight}}\right] \quad (2)$$

$$\text{Feed intake (FI, g} \cdot \text{fish}^{-1} \cdot \text{days}^{-1}) = \left[\frac{\text{dry diet given} - \text{dry remaining diet recovered}}{\text{number of fish}}\right] / \text{days} \quad (3)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake}}{\text{weight gain}} \quad (4)$$

$$\text{Survival (S, \%)} = 100 \times \left[\frac{\text{final number of fish}}{\text{initial number of fish}}\right] \quad (5)$$

$$\text{ADC (\%)} = 100 - \left[\left(100 - \frac{\%Cr_2O_3 \text{ in diet}}{\%Cr_2O_3 \text{ in faeces}} \times \frac{\%\text{nutrient in faeces}}{\%\text{nutrient in diet}}\right)\right] \quad (6)$$

2.8 | Statistical analysis

All statistical analyses were performed using SPSS (version 22, IBM, Australia). Data regarding the effects of LM product, Se level and their interactions on growth, feeding, digestibility, enzymatic activity, haematological and biochemical status were subjected to two-way analysis of variance (ANOVA). Assumptions of homogeneity of variances were checked using Levene's equal variance test. One-way ANOVA was performed to compare the control FM diet against each experimental diet. When significant effects were obtained for a factor, the Duncan test was used to compare the reference diet against each test diet (FM versus LM, LM$_{OS}$, FLM, and FLM$_{OS}$). Means were considered to be significantly different when $p < .05$. To meet ANOVA requirements, percentage data such as survival and ADC were computed using arcsine square root transformation.

3 | RESULTS

3.1 | Growth performance

Final survival, growth performance and feed utilization of *L. calcarifer* fed with diets containing fermented lupin meal and OS for 75 days are shown in Table 3. No interaction between fermentation and OS level was found to affect survival, growth, or feeding performances. Instead, at the termination of the feeding experiment, the survival of juvenile barramundi in all dietary treatments was not significantly different, ranging between 95% and 100% ($p > .05$). Meanwhile, FW, SGR, WG, and FCR of the fish were influenced by the type of LM diet ($p < .05$). During the feeding trial, the fish grew from 5.88 g to an average of 39.11 g in non-fermented dietary groups and 43.52 g in fermented dietary groups. SGR varied between 2.72% and 2.92% body weight per day among the dietary groups. Fish fed with fermented LM diets grew significantly faster and weighed substantially

more than fish fed with non-fermented LM diets. Fish fed with diets containing fermented LM diets were observed to gain better FCR compared to fish fed with the non-fermented LM diets ($p < .05$). However, the FW, SGR, and FCR of fish fed FLM and FLM$_{OS}$ diets were similar to those of fish fed with the control FM diets ($p > .05$).

3.2 | Digestibility

As shown in Table 4, fermentation of LM increased the ADC of DM similar to FM diets ($p > .05$). The ADC of protein was affected by both LM type and OS level ($p < .05$). Fish fed with diets containing fermented LM attained higher ADC of protein compared to those fed with non-fermented LM. Likewise, OS supplementation in the diet increased the ADC of protein by around 3%. However, the ADC of lipid was affected neither by LM type and OS level, nor by their interaction ($p > .05$).

3.3 | Muscle Se

At the end of feeding trial, muscle Se concentration was significantly influenced by OS supplementation (Figure 1). The Se level in the muscle of juvenile barramundi fed with OS-supplemented diets was higher than that of fish fed with other diets ($p < .05$). In addition, there was no significant differences in the muscle Se concentration between fish fed with fermented LM-based diets and those fed with LM-based diets without fermentation ($p > .05$). When fish fed with FM-based diets, the muscle Se level was similar to those fed FLM diets, but lower than those fed with OS-supplemented diets.

3.4 | GPx activity and haematology

GPx activity of the experimental fish is shown in Figure 2. Fish fed with diets supplemented with OS attained higher GPx activity than

TABLE 3 Survival, growth performance and feed utilization of juvenile barramundi fed test diets formulated with lupin meal (LM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Analysis of variance (ANOVA)			
	LM	LM_{OS}	FLM	FLM_{OS}	SEM	LM	OS	LM × OS	FM
Survival	95	95	100	97	1.058	ns	ns	ns	100
FW	38.63*	40.19*	43.19	43.86	0.481	0.001	ns	ns	44.25
SGR	2.72*	2.77*	2.88	2.92	0.025	0.003	ns	ns	2.93
WG	667.9*	678.92*	716.8	757.7	0.015	0.002	ns	ns	798.5
FI	0.63	0.61	0.62	0.62	0.011	ns	ns	ns	0.65
FCR	1.39**	1.33**	1.27	1.25	0.045	0.044	ns	ns	1.28

Two-way ANOVA performed involving only the experimental diets.

ns, not significant; SEM, pooled standard error of the mean.

Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $p < .05$).

FW, final weight (g); SGR, specific growth rate (% body weight per day); FI, feed intake (g·fish^{-1}·day^{-1}); WG, weight gain (%); FCR, feed conversion ratio; S, survival (%).

TABLE 4 Apparent digestibility coefficients (ADC) of dry matter (%), protein (%) and lipid (%) of juvenile barramundi fed test diets formulated with lupin meal (LM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Analysis of variance (ANOVA)			
	LM	LM_{OS}	FLM	FLM_{OS}	SEM	LM	OS	LM × OS	FM
Dry matter	83.1*	83.2*	88.4	88.3	0.692	0.006	ns	ns	89.3
Protein	91.3*	93.5	95.1**	96.2**	0.513	0.001	0.036	ns	93.5
Lipid	94.7	94.5	96.2	95.5	0.664	ns	ns	ns	96.1

Two-way ANOVA performed involving only the experimental diets.

*ns*, not significant; SEM, pooled standard error of the mean.

Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $p < .05$).

those fed with diets without OS supplementation. In the same way, fish fed with diets containing fermented LM had significantly higher GPx activity than fish fed with non-fermented LM diets. The GPx activity of fish fed with the control diets was similar to that of fish fed with the LM, FLM, and LM_{OS} diets, but lower than FLM_{OS} diets.

As displayed in Table 5, the highest levels of Hb were in fish fed with fermented LM diets ($p < .05$). However, these levels were not significantly different from that of fish fed with the control diets ($p > .05$). No significant differences in haematocrit were shown among dietary groups ($p > .05$). The leucocrit levels of fish fed with the fermented LM and the control FM diets were similar ($p > .05$) and significantly higher than that of fish fed with non-fermented LM diets ($p < .05$). Meanwhile, GPx activity was significantly influenced by both fermentation and OS supplementation.

3.5 | Biochemical status

Blood biochemistry of the experimental fish is presented in Table 6. The highest total protein (TP) was found in fish fed with fermented LM diets. The TP plasmatic concentrations between non-fermented LM and the control FM diets were similar ($p > .05$), which was around 40–43 g/L. There was an effect in plasma albumin concentration in the dietary treatments. With fermentation of LM, albumin level was higher than in fish fed with diets containing non-fermented
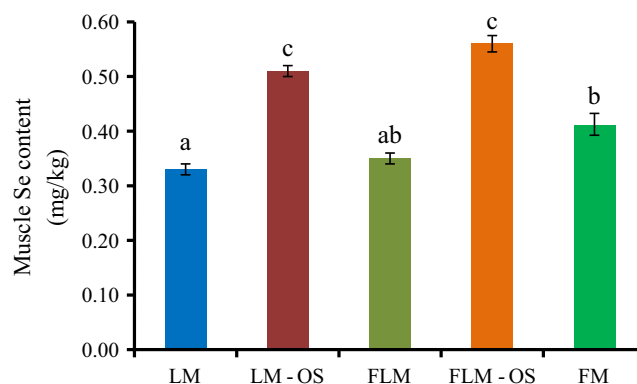
FIGURE 1 Mean muscle Se content of juvenile barramundi fed experimental diets over 75 days. Error bars represent the standard error of the mean. Different letters denote significant differences ($p < .05$)

LM. However, the A/G ratio was not affected either by fermentation or OS supplementation. Furthermore, plasma ALT activity was significantly higher in fish fed with non-fermented diets (LM and LM_{OS}) than in those fed with fermented LM diets (FLM and FLM_{OS}) ($p < .05$). However, there were no significant differences in ALT activity among LM_{OS}, FLM, FLM_{OS} and FM diets. There was an interactive effect between the LM and OS on plasma CK concentration; the CK of fish fed with diets supplemented with OS was higher

in non-fermented LM diets but lower in fermented LM diets ($p < .05$). In addition, both fermentation and OS supplementation level affected the CK plasmatic concentration ($p < .05$). CK
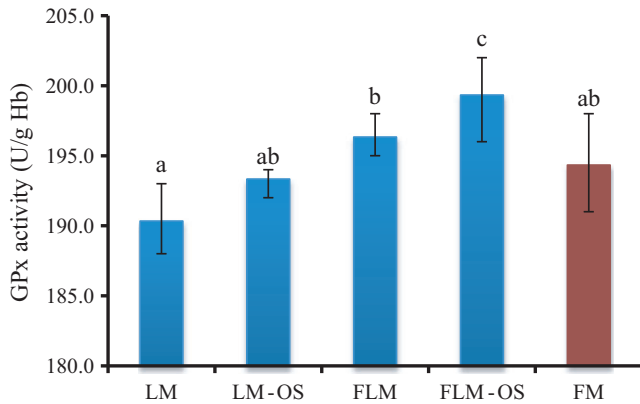

FIGURE 2 Mean GPx activity of juvenile barramundi fed experimental diets over 75 days. Error bars represent the standard error of the mean. Different letters denote significant differences ($p < .05$)

concentration of fish fed with fermented LM diets was lower than that of fish fed with non-fermented diets. Also, OS-supplemented diets were able to reduce the CK levels of fish. No significant differences were observed in haptoglobin, urea, creatinine, phosphate, and cholesterol among the dietary treatments ($p > .05$).

4 | DISCUSSION

Our previous study suggested that 75% of FM protein can be replaced by LM with OS supplementation in the diets of juvenile barramundi (Ilham, Fotedar & Munilkumar, 2016). Thus, this study was designed to investigate the effects of fermentation of LM simultaneously supplemented with OS on growth, feeding, enzymatic activity, and the biochemical status of the fish. Fermentation of LM (FLM and FLM$_{OS}$) significantly improved growth performance and FCR over the 75-day period. SGR observed in barramundi in this study is comparable to data from our previous study (Ilham, Fotedar & Munilkumar, 2016). The works of Van Vo et al. (2015) on similar species and Molina-Poveda, Lucas, and Jover (2013) on juvenile

TABLE 5 Haematological indicator and GPx of juvenile barramundi fed test diets formulated with lupin meal (LM) and simultaneous supplementation with OS for 75 days

	Diet					Analysis of variance (ANOVA)			
Parameter	LM	LM$_{OS}$	FLM	FLM$_{OS}$	SEM	LM	OS	LM × OS	FM
Hb	72	73	75**	75**	0.553	0.001	ns	ns	73
Haematocrit	32.3	34.7	35.7	35.4	0.648	ns	ns	ns	34.8
Leucocrit	1.08*	1.23*	1.37	1.44	0.076	0.011	ns	ns	1.47

Two-way ANOVA performed involving only the experimental diets.
ns, not significant; SEM, pooled standard error of the mean.
Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $p < .05$).
Hb, haemoglobin (g/dl); haematocrit (%); leucocrit (%).

TABLE 6 Plasma biochemistry contents of juvenile barramundi fed test diets formulated with lupin meal (LM) and simultaneous supplementation with OS for 75 days

	Diet					Analysis of variance (ANOVA)			
Parameter	LM	LM$_{OS}$	FLM	FLM$_{OS}$	SEM	LM	OS	LM × OS	FM
Total protein	40	43	45**	45**	1.130	0.029	ns	ns	41
Albumin	13*	15	16	16	0.726	0.018	ns	ns	16
A/G ratio	0.6	0.6	0.7	0.7	0.024	ns	ns	ns	0.6
Haptoglobin	0.18	0.17	0.17	0.16	0.009	ns	ns	ns	0.15
Urea	9	9	10	9	0.687	ns	ns	ns	8
Creatinine	20	21	18	19	1.130	ns	ns	ns	20
Phosphate	3.3	3.6	3.5	4.1	0.421	ns	ns	ns	3.5
Cholesterol	7.1	6.3	6.4	6.5	0.232	ns	ns	ns	7.4
ALT	18**	15	14	14	1.167	0.046	ns	ns	13
CK	3,280**	2,892	2,723	2,767	47.237	0.001	0.033	0.012	2,788

Two-way ANOVA performed involving only the experimental diets.
ns, not significant; SEM, pooled standard error of the mean.
Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $p < .05$).
TP, total protein (g/L); albumin (g/L); A/G (albumin to globulin); urea (mmol/L); creatinine (μmol/L); phosphate (mmol/L); cholesterol (mmol/L); haptoglobin (mg/L); ALT, alanine aminotransferase (U/L); CK, creatinine kinase (U/L).

whiteleg shrimp *Litopenaeus vannamei* led to similar results. PP products resulting from microbial fermentation have been shown to successfully replace high levels of FM in the diets of grouper *E. coioides* (Shiu, Hsieh et al., 2015) and whiteleg shrimp *L. vannamei* (Shiu, Wong, Guei, Shin & Liu, 2015). Hassaan et al. (2015) found improved growth performance of Nile tilapia *Oreochromis niloticus* when 50% FM protein was replaced by yeast-fermented SBM. In this study, the replacement of 75% FM protein with fermented LM resulted in favourable growth performance, similar to that in fish fed with the control FM diet.

Fermentation allows a higher substitution level of FM with plant-derived proteins through deactivation of ANFs, formation of low molecular weight protein and improvement in protein digestibility (Hotz & Gibson, 2007). The commercial strain of baker's yeast *S. cerevisiae* were used for the production of microbial phytase, an enzyme that plays a well-characterized role in the hydrolysis of antinutritional phytate to phosphate and inositol via penta-to monophosphates (Caputo, Visconti & De Angelis, 2015; Kumar et al., 2012). In addition, microbial phytase play an important role in disintegrating the plant cell wall matrix and thus accelerating the flavonoids extraction, which provides physiological and dietary antioxidants, thereby enhancing the animal's natural resistance to oxidative damage (Đorđević, Šiler-Marinković & Dimitrijević-Branković, 2010). In this study, a significant reduction in phytate content (70.52%) in yeast-fermented LM was found, indicating that the phytase activity of the baker's yeast was boosted during the fermentation process. This agrees with earlier findings that fermentation of PP sources with *S. cerevisiae* can degrade phytic acid to below unfavourable levels (Tudor, Jones, Hughes, Holt & Wiegand, 2013), which may offer nutritional advantages to monogastric animals such as fish. Sharawy, Goda and Hassaan (2016) reported that solid-state fermentation of PP feedstuffs with *S. cerevisiaei* is able to reduce phytate and trypsin inhibitor concentrations and thus increase the inclusion level of plant-derived protein by up to 50% in the diets of Indian white shrimp *Fenneropenaeus indicus*. In addition, Belewu and Sam (2010) examined the effect of solid-state fermentation of *Jatropha curcas* kernel cake by various fungi on ANFs and reported a dramatic decrease in phytate, lectin, trypsin inhibitors, and saponin concentrations in fermented *J. curcas* kernel cake. In the present work, fermented LM-fed fish outperformed those fed with the non-fermented LM diets, suggesting that the superior growth promoting capacity of the fermented LM diets may have been partly due to decreased antinutritional content.

The major alterations during the fermentation process include the breakdown of proteins into peptides, AA, and low molecular weight compounds (Bartkiene et al., 2015; Zhuo et al., 2016). Increased AA content could be indicative of the refinement of LM product, as achieved in this study; overall, after fermentation, essential and non-essential AA content increased by 43.55% and 22.67% respectively. The AAs predominantly credited for this increase were methionine, proline, and lysine followed by phenylalanine, isoleucine, valine, alanine, threonine, serine, leucine, aspartic acid, glycine, glutamic acid, histidine, and arginine. It is essential that the AA content

of fermented LM echoes a balanced dynamic equilibrium, which might have been induced by proteolytic activities during fermentation. Increased AA content in solid-state fermentation of various PP ingredients was also reported in earlier studies with LM (Van Vo et al., 2015), SBM (Gao, Wang, Zhu & Qian, 2013; Hassaan et al., 2015; Shimeno, Mima, Yamamoto & Ando, 1993), and CSM (Lim & Lee, 2011; Sun et al., 2015). Increased crude protein (29.78%) observed in this study was attributable to increases in overall AA content, as demonstrated by Shiu, Wong et al. (2015). Accordingly, the improvement in crude protein might lead to reduced amounts of fermented LM being incorporated in fish diets.

This study indicates that, although barramundi seem to tolerate a high inclusion level of LM in their diets, based on ADC observed in our previous study (Ilham, Fotedar & Munilkumar, 2016), high amounts of non-fermented LM in the diet deleteriously affects FCR, more so than fermented LM and FM. Poor FCR have been recorded in Nile tilapia *O. niloticus* fed with non-fermented PP sources than in those fed fermented feeds (Lim & Lee, 2011). In contrast, the inclusion of 400 g/kg fermented SBM in the diet of pompano *Trachinotus ovatus* resulted in higher FCR and poor growth performance (Lin et al., 2012). In fish, unsatisfactory nutrient intake can affect overall metabolism, thus it might be that the energy required to maintain metabolism would have reduced the energy available for growth and decreased FCR.

The results of this study revealed that a high inclusion level of LM decreased the ADC of DM, which seemed to be associated with phytate level, as found in previous studies (Baruah et al., 2007; Plaipetch & Yakupitiyage, 2014; Zhou et al., 2011). Therefore, fermentation appears to confer important benefits to the improvement of ADC of DM, through the degradation of phytate contained in PP sources. Zhuo et al. (2016) reported ADC of DM and protein of fermented SBM in excess of 83% and 93%, respectively, for grouper *E. coioides*. In comparison, Ng, Lim, Lim and Ibrahim (2002) showed that, without fermentation, poor nutrient digestibility values were obtained from palm kernel meal by red hybrid tilapia *Oreochromis* sp. Furthermore, it was observed that juvenile barramundi efficiently utilize protein from the fermented LM diets (FLM and FLM$_{OS}$). The ADCs of protein were substantially higher for the fermented LM diet than for the non-fermented LM and FM control diet. The findings of this study agree with an earlier investigation by Zhou and Yue (2012) who found that fermented PP products improved the ADC of protein more than that of FM. Apparently, high protein digestibility of fermentation-treated PP-based diets can be related to increases in indispensable AA content, as previously explained, and the reduction of phytate content. The progressive increase in protein digestibility with decreased levels of phytate was recorded in FM replacement studies on various fish species (Baruah et al., 2007; Hussain et al., 2017; Mwachireya, Beames, Higgs & Dosanjh, 1999; Storebakken, Shearer & Roem, 1998). Interestingly, although the digestibility of LM is low on account of its high fibre content (Glencross, 2009), the ADC of DM and protein in this study were higher than the findings reported by previous studies (Borquez, Serrano, Dantagnan, Carrasco & Hernandez, 2011; Farhangi & Carter, 2007; Ilham, Fotedar & Munilkumar, 2016; Molina-Poveda et al.,

2013), this is likely due to an amelioration effect of the fermentation process.

Regardless of fermentation, the ADC of protein in fish fed with OS-supplemented diets was significantly higher than that of fish fed with diets without OS supplementation and FM control diet. When fed to juvenile barramundi, the FLM$_{OS}$ diet gave the highest ADC value; therefore, these results suggest that both fermentation and OS supplementation play an important role in protein metabolism. However, the effect of OS supplementation on the ADC of protein did not lead to improvement in the growth performance of the fish. Although there was a slight reduction in SGR of juvenile barramundi fed with diets without OS supplementation, the growth performance of the fish was not affected by dietary OS supplementation, as reported for rainbow trout *Oncorhynchus mykiss* (Hilton, Hodson & Slinger, 1980). Previously, PP-based diets deficient in Se were acceptable for growth although supplemental Se was required to maintain optimal GPx activity in rainbow trout *O. mykiss* (Fontagne-Dicharry et al., 2015). In addition, in a FM-based study, supplemental Se was not required to enhance growth although it seemed to be necessary for GPx activity maintenance in common carp *Cyprinus carpio* (Elia, Prearo, Pacini, Dörr & Abete, 2011).

The OS used in this study was in the form of selenized yeast (Sel-Plex®, Alltech, Lexington, Kentucky, USA) that contains a mixture of selenoamino acids with Se-Met representing more than half of the total Se (Lyons et al., 2007). Due to protection of the structural alignment of chelates, it seems that, when ingested as trace element amino acid chelates, less chance of the complexing action of phytate on mineral cations occurring (Apines-Amar et al., 2004). This may describe, indirectly, the increased ADC of protein observed in fish fed the OS-containing diets, as shown in the current trial. As OS was added in the diets, high absorption of amino acid-chelated Se into the mucosal tissues may stimulate an augmented source of important trace element that was later used as a co-factor in the production of hydrolytic enzymes, such as gastro-intestinal (GI) GPx, in the mucosal tissues. Moreover, GI-GPx is the most effective selenoprotein antioxidant in protecting the intestinal mucosal integrity (Lindh, 2013). A considerable quantity of digestive enzymes in the gut would presumably prompt the improvement of feed digestion.

In contrast, a FM-based diet lacking Se supplementation resulted in depressed growth and reduced GPx activity when fed to channel catfish *Ictalurus punctatus* (Gatlin & Wilson, 1984). Similar results were found for grouper *E. coioides* (Lin & Shiau, 2005), cobia *Rachycentron canadum* (Liu, Wang, Ai, Mai & Zhang, 2010), gibel carp *Carassius auratus gibelio* (Han et al., 2011), largemouth bass *Micropterus salmoide* (Zhu et al., 2012), and yellowtail kingfish *S. lalandi* (Ilham & Fotedar, 2016; Le & Fotedar, 2013). Moreover, our previous studies also suggested that an appropriate level of dietary Se was needed to support growth and antioxidant GPx activity of barramundi when fed with diets containing a high inclusion level of PP sources such as LM and SBM (Ilham, Siddik & Fotedar, 2016; Ilham, Fotedar & Munilkumar, 2016). However, Prabhu et al. (2014) conducted a systematic review on the mineral requirements of fish and concluded that when WG is used as the response criterion, dietary

Se affects GPx activity rather than growth, thus GPx activity comprises a more robust marker of the bioavailability and utilization of dietary Se in fish. In this study, the FLM$_{OS}$ diet (3.81 mg Se/kg) generated the highest GPx activity level in juvenile barramundi, indicating that the combined strategy of Se supplementation and fermentation promotes beneficial effects in enhancing the antioxidant capacity of fish.

In this study, the muscle tissue Se levels of fish fed with the LMOS and FLMOS diets were higher than that of fish fed with the other diets, indicating that trace mineral (Se) utilization was improved by OS supplementation. Similarly, Se contents in juvenile yellowtail kingfish *S. lalandi* were markedly increased with the supplementation of dietary OS (Ilham, Fotedar & Suyasa, 2016). As some minerals such as P, Zn, and Se are known to be less bioavailable in plant-derived protein sources (Prabhu et al., 2014), the decline in FM use in aquaculture feeds might result in the adjustment of those minerals' supply to fish. The presence of ANF in PP-based diets can be of nutritional significance, particularly with the aforementioned minerals; for instance, phytate in plant feedstuffs interferes with the absorption of trace minerals and diminishes their bioavailability (Francis, Makkar & Becker, 2001). Storebakken et al. (1998) demonstrated that the inclusion of soy concentrate with high phytate concentration (18 g/kg) in the diets of Atlantic salmon *Salmo salar* led to the reduction of bioavailable P, Ca, Mg, and Zn. Sajjadi and Carter (2004) reported that P digestibility and retention efficiency were reduced when Atlantic salmon *S. salar* were fed with a phytate-containing canola meal-based diet. SGR of mrigal *Cirrhinus mrigala* was significantly reduced when the phytate level exceeded 1% of their total diet (Usmani & Jafri, 2002). In addition, declining WG, FCR, and Zn content in the vertebrae of channel catfish *I. punctatus* was related to the elevation of phytate levels (2.2%) in their diet (Satoh, Poe & Wilson, 1989). Therefore, the exogenous supplementation of Se to meet the requirements of fish has been a major point of concern in recent years.

Apart from the abovementioned interaction of dietary sources with Se, particular nutrients that exist in the diet may also interact with Se. Prabhu et al. (2014) described that the interaction with a specific trace nutrient may influence the minimum dietary incorporation level of Se. The synergistic interaction between Se and vitamin E has been documented (Watanabe et al., 1997). Although Se and vitamin E have particular roles, both nutrients are involved in the enhancement of the cellular antioxidant protection system. While Se functions as component of enzymatic GPx, which devastates hydrogen peroxide and lipid hydroperoxides, vitamin E is a cellular-related antioxidant and free radical scavenger, which protects biological membranes against lipid peroxidation (Combs & Combs, 1986). Accordingly, the mutual sparing effects of Se and vitamin E allow practical implication in aquaculture feed formulation (Lin & Shiau, 2009). Thus, the synergism between Se and vitamin E when fish are fed with high levels of plant-derived protein sources merits further investigation.

Blood haematological indices of fish are known to be influenced by a variety of both internal and external factors including species, age, size, physiological circumstance, environmental conditions, and

nutritional status (Soltanzadeh, Esmaeili, Ouraji & Khalili, 2015). Little information is available regarding the potential haematological effects of dietary PP intake in carnivorous marine finfish, particularly in barramundi. A well-defined linkage between FM substitution and haematological indicators has not been established in fish; nonetheless, the inclusion of PP ingredients in the diet significantly modifies both qualitative and quantitative descriptions of Hb in great sturgeon *Huso huso* (Jahanbakhshi, Imanpoor, Taghizadeh & Shabani, 2013). In the present experiment, although OS supplementation did not affect the haematological status of the fish, as demonstrated in our previous study (Ilham, Fotedar & Munilkumar, 2016), dietary PP did. Fish fed with diets containing fermented LM (FLM and FLM$_{OS}$) had higher Hb than those fed with any other diet, suggesting a propitious utilization of dietary fermented LM by juvenile barramundi, particularly in the maintenance of red blood cell function. No published data were available with which to contrast the differences of Hb and leucocrit values in barramundi *L. calcarifer* reared on a fermented PP diet achieved in this study. However, in beluga *H. huso*, a decreasing trend was observed in the amounts of Hb when FM was replaced with PP sources (Jahanbakhshi et al., 2013). In addition, the Hb concentration obtained in this study (75 g/dl) was comparable to that reported in healthy juvenile yellowtail kingfish *S. lalandi* (Le, Dao, Fotedar & Partrigde, 2014). Furthermore, leucocrit concentrations can indicate the health condition of fish (Wedemeyer, Gould & Yasutake, 1983), since malnutrition and decreased resistance to disease are associated with low levels of leucocrit (El-Asely, Abbass & Austin, 2014). In this study, significantly higher levels of leucocrit were observed in barramundi fed with fermented LM diets, indicating that fermentation may induce specific compounds that promote leucocrit production. Adams, Brown and Goede (1993) suggested that the leucocrit assay is a quick and efficient tool for assessing fish health status.

Plasma TP was used as a fundamental indicator of physiological condition and health status of juvenile barramundi, in accordance with data reported for other species (Al-Dohail, Hashim & Aliyu-Paiko, 2009; Han et al., 2015; Katya, Yun, Yun, Lee & Bai, 2016; López, Flores-Ibarra, Bañuelos-Vargas, Galaviz & True, 2015; Sardar, Randhawa, Abid & Prabhakar, 2007). The TP level for fermented LM-fed fish obtained in this study (43 g/L) was slightly higher than the reported TP concentration for barramundi when fed with narrowleaf lupin *L. angustofolius* kernel meal (43 g/L). TP is a key indicator of the diet's metabolism, and its deterioration might be affected by deprived nutrient digestion and metabolism (Soltanzadeh et al., 2015). Plasma proteins such as albumin are an essential fraction in the delivery of substances and in the enhancement of vigorous immunological competence (Andreeva, 2012). Decreased plasmatic albumin was associated with inflammation processes as demonstrated in juvenile totoaba *Totoaba macdonaldi* (López et al., 2015). In this study, it was observed that fish fed with non-fermented, OS-unsupplemented diet (LM) had the lowest albumin concentration, suggesting that the fish would have undergone inflammatory processes in response to antinutrients' harmful effects. Interestingly, the concentrations of TP and albumin in fish fed with LM$_{OS}$ diets were similar to that in fish fed with the FM diet. Further research with longer duration is needed to evaluate whether the form of Se (i.e. organic versus inorganic) has the observable effect in barramundi with regard to plasmatic TP and albumin concentrations.

In fish, the elevation of plasma enzymes is indicative of liver and muscle damage. While ALT activity plays a significant role as a biochemical indicator for liver health, CK activity is used as a biomarker enzyme for muscle injuries (Glencross et al., 2011). The elevation of ALT and CK activities was reported in the plasma of barramundi when lupin kernel meal or wheat gluten was included in the diet (Glencross et al., 2011). Similarly, increased ALT in fish fed with diets containing non-fermented LM was observed in this study. However, there was no significant difference in ALT activity of fish fed with the non-fermented LM diet supplemented with OS (LM$_{OS}$) relative to FM control diet, indicating that a diet without fermentation and Se supplementation treatment (LM) might induce liver-associated syndromes. If this would be the case, the results of this study corroborate the findings reported in our previous research, in which lipid droplet congregation in hepatocytes was found when fish were fed with diets containing a high inclusion of LM (Ilham, Fotedar & Munilkumar, 2016). On the other hand, decreased ALT activity with increased inclusion of faba bean *Vicia faba* was reported in beluga *H. huso* (Soltanzadeh et al., 2015). Lin and Luo (2011) also observed that there were no negative influences on liver enzymes when 50% or more SBM replaced FM in hybrid tilapia *O. niloticus* × *O. aureus* diets. Variations in CK activity, as demonstrated in these studies, may reflect a lack of recognition in accordance to the biochemical roles of ALT in fish (Soltanzadeh et al., 2015). However, as evident from the literature cited hereby, and the findings of the current experiment, the refinement of any PP ingredient is a promising way to prevent lipotoxic damage to the liver in fish. For instance, in starry flounders *Platichthys stellatus*, the inclusion of soy protein hydrolisates to replace 50% FM protein resulted in reduced ALT activity (Song et al., 2014). In line with this, in terrestrial animals, the solid-state fermentation of rapeseed meal has been reported to maintain ALT activity at levels appropriate for pigs (Shi et al., 2016). Moreover, dietary PP ingredient refinement through fermentation with *S. cerevisae* might improve liver function performance in juvenile barramundi, as demonstrated in this study.

Furthermore, plasma CK activity appeared to be influenced by fermentation, OS supplementation, and their interaction. Fermentation of LM was an important element responsible for reduced plasma CK activity in juvenile barramundi. Likely, for Se, fermentation enhanced functionality. As LM was fermented, the fish fed with diets supplemented with OS were able to maintain their CK activity, similar to those fed with the FM diet. Reduced phytate concentration due to fermentation might have decreased the potential for phytate-Se complex formation, thus allowing better utilization of LM to support overall growth and physiological properties. Further research examining the synergism between PP-based diet and trace mineral supplementation in fish is required. However, irrespective of OS supplementation, feeding fish with fermented LM diets (FLM and FLM$_{OS}$) resulted in reduced amounts of plasma CK activity. These results indicated that fermentation with *S. cerevisae* provided protection from the degeneration of

muscle fibres, thus structurally improving the integrity of the muscle cells. Previously, Glencross et al. (2011) observed that the inclusion of LM *L. angustofolius* in the diet of barramundi negatively affected their CK activity, achieving a level of 8,515 U/L. The authors also suggested that healthier fish maintained their CK activity at a level around 3,000 U/L, which was similar to the results in this study. In comparison, the reported normal values of CK activity for sturgeon *Acipenser stellatus* were 5,958–7,233 U/L (Shahsavani, Mohri & Gholipour, 2008). The effect of variation in species, nutritional status, and environmental conditions in the quantification of CK activity should be taken into account (Shi, Li, Zhuang, Nie & Long, 2006).

5 | CONCLUSION

The findings of the present investigation reinforce an obvious potential to substitute FM with 75% fermented LM protein in the diet of barramundi (*L. calcarifer*), without triggering adverse impacts on growth and health performance. When compared with FM, fish fed with diets containing fermented LM gained similar growth and FCR, but better protein ADC, Hb, and TP, which are attributable to the degradation of antinutritional phytate during the *S. cerevisae* fermentation process. In addition, the superiority of fish fed with the FLM$_{OS}$ diets, as shown in this study, regarding the enzymatic antioxidant GPx activity and TP status might be due to proper utilization of dietary nutrients in the presence of supplemental OS.

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