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Feed Utilisation Efficiency of Lupin Inclusion in Cobia: Role of Dietary Organic Selenium Supplementation

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Abstract

The dietary selenium (Se) requirement has been determined for cobia *Rachycentron canadum* using purified diet; however, its role in the utilisation of plant-derived ingredients has not been evaluated in the species. Therefore, a 3 x 2 factorial experimental design involving three inclusion levels (0, 210 and 315 g/kg) of lupin *Lupinus angustifolius* kernel meal (LKM) and two concentrations (0 and 0.8 mg/kg) of Se was used to evaluate the effects of Se supplementation in conjunction with LKM on the growth, feed utilisation and physiological responses in the cobia. Six isonitrogenous (46.5 % crude protein) and isoenergetic (21 MJ/kg gross energy) diets were formulated and fed to cobia for 7 weeks in a flow-through seawater system. The results showed significant effects of Se supplementation and its interaction with dietary lupin on the growth and feed efficiency of cobia. Se supplementation significantly improved the growth and feed utilisation efficiency in cobia fed lupin-based diets. The nutrient digestibility of fish fed supplemental Se lupin-based diets was significantly higher than that of fish fed diets without Se supplementation at each inclusion level of LKM. There were no significant effects of dietary Se supplementation on the survival, muscle composition or muscle amino acids, whereas regression analysis indicated a positive linear relationship between tissue Se accumulation and dietary Se levels. An enhancement of haematological responses was also observed in cobia fed Se-supplemented diets. In summary, cobia fed lupin-based diets required higher dietary Se supplementation for higher feed utilisation efficiency than previously quantified for the casein-based diet.

Keywords: organic selenium, cobia, lupin, feed utilisation

1. Introduction

Carnivorous marine fish such as cobia *Rachycentron canadum* require relatively high dietary protein to provide adequate amino acids and nitrogen for the synthesis of non-essential amino acids and other essential biological compounds (Fraser & Davies, 2009). Fishmeal contains significantly high protein content, with well-balanced essential amino acids, and high nutrient digestibility to meet the nutritional requirements of fish (Gatlin et al., 2007). However, the rapid development of aquaculture has led to an increased demand and unstable supply of fishmeal (Olsen & Hasan, 2012; Tacon & Metian, 2008). A series of studies have evaluated the use of plant-derived ingredients to reduce the fishmeal dependence in cobia diets (Chou et al., 2004; Luo et al., 2012; Salze, McLean, Battle, Schwarz, & Craig, 2010; Zhou, Mai, Tan, & Liu, 2005). Chou et al. (2004) demonstrated that up to 40 % of fishmeal protein can be replaced with soybean meal without compromising growth and feed utilisation, whereas, the replacement levels are 50 % and 94 % with soy-derived protein and soy protein concentrate, respectively (Salze et al., 2010; Trushenski et al., 2011). The optimal dietary rapeseed meal, corn gluten meal and yeast-based products in cobia diets were 135, 300 and 250 g/kg, respectively (Lunger, Craig, & McLean, 2006; Luo et al., 2012; Luo et al., 2013). Our previous study (unpublished) also demonstrated the beneficial effects of narrow-leafed lupin kernel meal, wherein up to 105 g/kg lupin kernel meal can be incorporated into the cobia diet without impairing specific growth rate and feed efficiency. However, the lysine and methionine limitations and the presence of anti-nutritional factors in lupin products can impair growth and feed utilisation (Carter & Hauler, 2000; Farhangi & Carter, 2001). Fishmeal-substituted diets can also result in histopathological lesions in the stomach, liver and intestine of the host farmed fish (Refstie et al., 2006; Robaina

et al., 1995).

The increased inclusion levels of plant-derived ingredients in aqua-feeds can impact the uptake and digestion of minerals, consequently changing the mineral requirements of the fish (Antony Jesu Prabhu, Schrama, & Kaushik, 2014; Barrows, Gaylord, Sealey, Smith, & Porter, 2010; Read et al., 2014). The anti-nutritional factors presented in plant-derived ingredients can also interact with minerals, making these minerals less available for fish (Antony Jesu Prabhu et al., 2014). The interaction between phytic acid and minerals directly affects the digestion of minerals in fish. Whereas, the metabolised process of minerals can be disrupted due to the presence of other anti-nutrients (Antony Jesu Prabhu et al., 2014), thus, reducing nutrient digestibility (Petterson, 2000).

Selenium (Se) is an essential trace mineral for normal growth and physiological functions in animals (Watanabe, Kiron, & Satoh, 1997). The deficiency of Se can cause negative effects on growth and survival, peroxidative damage to cells and their membranes and reduced host defence function in farmed fish (Liu, Wang, Ai, Mai, & Zhang, 2010) (Lin & Shiau, 2005; Liu et al., 2010). Feed ingredients contain varied amounts of Se, with relatively lower Se contents in plant-derived products than in fishmeal (Antony Jesu Prabhu et al., 2014; Watanabe et al., 1997). Additionally, the low Se level in lupin meal (18 – 240 µg/kg) in Australia due to the low Se concentration in Australian soils (Petterson, 2000) results in inadequate Se in the diets when fishmeal protein is replaced with lupin meal protein. The reduction of growth and feed efficiency caused by deficient dietary Se in lupin-based diets has been reported in barramundi Lates calcarifer (Ilham, Fotedar, & Munilkumar, 2016). Whereas, rainbow trout Oncorhynchus mykiss fry fed soybean-based diets required Se fortification to achieve optimum glutathione peroxidase (GPx) activity (Fontagné-Dicharry et al., 2015). African catfish Clarias gariepinus (Abdel-Tawwab, Mousa, & Abbass, 2007) and barramundi (Ilham et al., 2016) also showed improved growth performance, feed utilization and health after being fed plant-based diets supplemented with organic Se. Although dietary Se required for juvenile cobia fed casein-based diet has been quantified to be 0.788 mg/kg based on the specific growth rate (Liu et al., 2010). The fish fed plant-based diets could require mineral supplementation at higher concentrations than recommended by the National Research Council NRC (1993) (Barrows et al., 2010; Read et al., 2014). Besides, there is no published information on the interactive biological effects of dietary Se and dietary inclusion levels of LKM on cobia. Therefore, the aim of this study was to evaluate the effects of dietary Se supplementation on the nutritional and physiological responses in cobia fed high inclusion levels of narrow-leafed LKM.

2. Materials and Methods

2.1 Experimental Diet Preparation

The fishmeal protein in the control diet (LP0) was replaced with 40% and 60% of narrow-leafed lupin kernel meal (LKM) protein without and with 0.8 mg/kg Se extracted from Se-yeast (Sel-Plex, Alltech, Nicholasville, KY, USA) supplementation to formulate six isonitroenous and isoenergetic test diets, labelled as LP0, LP0Se, LP40, LP40Se, LP60 and LP60Se, respectively. The ingredients were finely grounded, and then homogeneously mixed. Chromic oxide (Cr_2O_3) was added to all test diets as the inner marker. Taurine (Sigma-Aldrich, St. Louis, MO, USA) was added to all diets at a level of 5 g/kg to meet the nutritional requirement of cobia as the recommendation by Watson, Barrows, and Place (2014). Fish oil and distilled water were added and then thoroughly mixed. A laboratory extruder was used to pelletise the diet through 2 mm die. The diets were air-dried, sealed in bags and stored in a freezer until feeding trial commenced. The diet formulation and ingredient composition are presented in Table 1 and Table 2.

2.2 Experimental Fish

Juvenile cobia was obtained from a commercial hatchery in Khanh Hoa Province, Vietnam. Fish was acclimated to the experimental conditions in two weeks prior to the commencement of the feeding trial. During this period, the fish were fed a commercial cobia diet (NRD P16, INVE Ltd, Thailand) containing 53 % protein, 12 % lipid and 21.0 MJ/kg gross energy. Prior to the test feeding, all fish were individually weighed and standard length measured after starving them for 24 hours. The cobia (initial mean weight of 18.56 g/fish) was randomly distributed to eighteen tanks at a density of 12 fish per tank. Each test diet was assigned to three tanks. Fish were hand-fed twice daily at 8:00 and 16:00 until apparent satiation for 7 weeks. Water temperature, dissolved oxygen and pH were measured daily using OxyGuard meters (Handy Polaris 2 and Handy pH, OxyGuard International A/S, Denmark). Total ammonia was monitored with NH_3/NH_4^+ test kit (Mars Fishcare, Chalfont, PA, USA).

2.3 Sample Collection

At the beginning of the feeding trial, 15 fish were randomly selected for the initial whole body composition analysis. At the end of the experiment, three fish per tank were randomly sampled and stored in a freezer for the

chemical analysis. Another three fish were used to collect blood samples by puncturing their caudal vein with a 25-gauge needle attached to a 1-ml syringe and transferred to the BD Vacutainer (with K2E 5.4 mg, UK) for haematological analysis. The muscle tissues from four fish per tank were pooled and used for proximate composition, selenium content and amino acid analysis. Faeces were collected following the method described by Kim, Tibbetts, Milley, and Lall (2007). One hour after the last feeding, air diffusers, and the bottom and walls of the tanks were carefully cleaned to remove any residual feed and waste from the rearing system. Faeces were allowed to settle overnight. The faeces were gently collected into a 200 mL polyethylene bottle in the following morning, and then were centrifuged for 20 min at 10000 rpm, before the supernatant was discarded; the faecal material was stored at -20 ^{0}C until analysis.

Table 1. Ingredients and composition of the experimental diets (g kg^{-1} in dry weight)

Ingredients[a]	Diets					
	LP0	LP0Se	LP40	LP40Se	LP60	LP60Se
Fishmeal	320	320	192	192	128	128
Soybean meal	100	100	100	100	100	100
Lupin kernel meal	0	0	210	210	315	315
Casein	146	146	146	146	146	146
Wheat flour	5	5	5	5	5	5
Wheat gluten	120	120	120	120	120	120
Cellulose	159	157.5	77	75.5	36	34.5
Wheat starch	30	30	30	30	30	30
Fish oil	95	95	95	95	95	95
Taurine[b]	5	5	5	5	5	5
Premix[c]	15	15	15	15	15	15
Sel-Plex[d]	0	1.5	0	1.5	0	1.5
Chromic oxide[e]	5	5	5	5	5	5
Proximate composition						
Moisture (%)	8.82	8.66	10.29	11.79	9.56	10.02
Crude protein (%)	46.51	46.64	46.85	46.24	46.71	46.54
Crude lipid (%)	11.42	11.94	11.35	11.07	10.97	10.12
Ash (%)	8.10	7.09	6.46	5.72	4.53	4.58
Gross energy (MJ/kg)	20.85	20.78	21.38	21.36	22.18	22.21
Se (mg/kg)	1.71	2.42	1.28	2.04	1.03	1.82
Phytic acid (g/kg)	2.51	2.48	3.58	3.61	3.94	4.01
Tannins (g/kg)	0.35	0.33	0.61	0.63	0.91	0.90
Essential amino acids						
Arginine	2.78	2.78	3.17	3.17	3.37	3.36
Histidine	1.45	1.46	1.39	1.38	1.34	1.35
Isoleucine	2.29	2.27	2.31	2.31	2.36	2.37
Leucine	4.31	4.34	4.22	4.21	4.18	4.20
Lysine	3.06	3.06	2.81	2.80	2.61	2.59
Methionine	1.33	1.31	1.14	1.13	1.05	1.06
Phenylalanine	2.41	2.42	2.38	2.40	2.40	2.41
Threonine	2.26	2.26	2.14	2.15	2.13	2.12
Valine	2.60	2.61	2.61	2.62	2.63	2.63
Taurine	0.42	0.41	0.43	0.41	0.39	0.40

Note. Supplied by Specialty Feeds (Glen Forrest, WA 6071, and Australia)

Sigma-Aldrich, St. Louis, MO, USA

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; and ethoxyquin, 0.125

Obtained from Alltech, Nicholasville, KY, USA

Obtained from Thermo Fisher Scientific, Scoresby, Victoria, Australia.

Table 2. Feed ingredient composition (g/100 g or MJ/kg in dry matter) unless specified

Ingredients [a]	Crude protein	Total lipid	Ash	Dry matter	Organic matter [b]	Gross energy	Se (mg/kg)	Phytic acid	Tannins
Fishmeal	64.00	10.70	21.90	91.43	78.10	20.09	4.37	n/d	n/d
Lupin meal	39.00	6.82	2.41	87.61	97.59	20.93	0.35	0.53	0.16

Note. n/d: not detected.

2.4 Chemical Analysis

Crude protein, crude lipid, moisture, ash and gross energy contents of the whole body and muscle tissues of cobia, faecal material and tested diets were determined according to the standard methods of AOAC (1990). The crude protein was determined by Kjeldahl method; crude lipid was analysed gravimetrically following chloroform : methanol extraction of the lipids according to the method of Folch, Lees, and Sloane-Stanley (1957); moisture by drying at 105 ^{0}C in an oven (Thermotec 2000, Contherm Scientific, Hutt, New Zealand) to a constant weight; ash by combustion at 550 ^{0}C for 24 h in an electric furnace (Carbolite, S., UK); and gross energy was determined by bomb calorimeter (C2000, IKA, Staufen, Germany). The tannin was analysed following the method described by Embaby (2011) after extraction in acetone. Phytic acid in phytate salt form was analysed after extraction in hydrochloric acid following the procedure from Haddad, Greiner, and Allaf (2007). The selenium content in the test diets and muscle tissues were analysed following inductively coupled plasma atomic absorption spectrometry. Chromic oxide was determined by the method from Bolin, King, and Klosterman (1952) using a spectrophotometer (UV-1201, Shimadzu, K., Japan). Amino acid profiles were analysed by using high performance liquid chromatography after an acid hydrolysis. Blood constituents were automatically analysed using automated haematology analyser (Sysmex XT-1800i, Kobe, Japan).

2.5 Calculations and Statistical Analysis

The survival rate, specific growth rate (SGR %/day), feed conversion ratio (FCR), protein retention (PR) and energy retention (ER) were calculated using following equations:

$$Survival\ (\%)\ =\ 100\ x\ final\ fish\ number\ /\ initial\ fish\ number \quad (1)$$

$$SGR\ =\ 100\ x\ [(Ln\ FBW\ -\ Ln\ IBW)/feeding\ period\ (days)] \quad (2)$$

Where IBW and FBW are the initial body weight and final body weight, respectively.

$$FCR\ =\ feed\ intake\ in\ dry\ matter\ (g)/body\ weight\ gain\ (g) \quad (3)$$

$$PR\ =\ protein\ gain\ x\ 100/protein\ fed \quad (4)$$

$$ER\ =\ energy\ gain\ x\ 100/energy\ fed \quad (5)$$

Apparent digestibility coefficient (ADC) of tested diets and basal diet were calculated as Equation (6) described by Cho, Slinger, and Bayley (1982):

$$ADCdiet\ =\ 100\ x\ [1\ -\ (Fnut/Dnut\ x\ DCr/FCr)] \quad (6)$$

Where F_{nut} is the % of nutrient or gross energy in faeces, D_{nut} represents % of nutrient or gross energy in the diet. D_{Cr} and F_{Cr} are % of the chromic oxide in diet and faeces, respectively.

All data were statistically analysed using SPSS for Windows version 22 (IBM, New York, USA) unless otherwise specified. The two-way ANOVA was used to analyse the effects of dietary lupin meal inclusions, Se supplementation and their interactions on all of the tested parameters of cobia. When a significant main effect was observed, data were analysed to determine the differences among the dietary groups with or without Se supplementation. When a significant interaction was detected, one-way analysis (ANOVA) with post hoc Turkey's HSD multiple comparison tests were employed to determine differences among dietary treatments, but not for means of main effects. The linear regression analysis was used to determine the relationship between dietary Se level and Se accumulation in the tissues. The statistical significance was evaluated at $P < 0.05$.

3. Results

During the feeding trial, the water temperature ranged from 28 to 30 ^{0}C, total ammonia was less than 0.5 mg/L, and pH was 7.6 - 8.2. The salinity ranged from 28 to 33 ‰.

3.1 Growth Performances

There were significant ($P < 0.05$) effects of dietary LKM, Se supplementation and their interaction on the final weight (FBW), specific growth rate (SGR), feed intake (FI) and feed conversion ratio (FCR) of the cobia after 7

weeks of feeding. In the absence of Se supplementation, cobia fed 315 g/kg LKM resulted in a reduction in FBW, SGR and FI, whereas FCR was increased at an inclusion level of 210 g/kg LKM. Fish fed lupin-based diets supplemented with 0.8 mg/kg Se achieved significantly higher FBW, SGR, FI and FCR than the fish fed lupin-based diets without Se supplementation (Table 3). Reduced protein retention (PR) and energy retention (ER) were observed in the fish fed lupin-based diets without Se supplementation. There was a significant effect of the interaction between dietary LKM and Se supplementation on the PR, but not on ER. High survival rates were attained in all dietary treatments and were not significantly ($P > 0.05$) affected by dietary LKM, Se supplementation or their interaction (Table 3).

Table 3. Growth and feed utilisation of cobia fed the test diets

Diet	FBW (g/fish)	SGR (%/day)	FI (g/fish)	FCR	PR	ER	Survival (%)
LP0	84.46^{cd}	3.11^{cd}	92.08^{c}	1.39^{a}	24.59^{d}	23.42	100
LP40	79.16^{c}	2.96^{c}	89.39^{c}	1.48^{b}	23.36^{c}	21.56	100
LP60	61.55^{a}	2.44^{a}	68.64^{a}	1.60^{c}	20.19^{a}	19.28	97.22
LP0Se	85.28^{d}	3.13^{d}	91.39^{c}	1.37^{a}	25.22^{d}	23.66	97.22
LP40Se	85.71^{d}	3.12^{d}	94.99^{c}	1.42^{a}	24.68^{d}	22.37	100
LP60Se	72.93^{b}	2.79^{b}	80.02^{b}	1.47^{b}	22.23^{b}	20.90	94.44
Pooled SE	2.14	0.06	2.25	0.02	0.43	0.38	0.84
Means of main effects of fishmeal protein replacement level							
0	84.87	3.12	91.74	1.38	24.91	23.54^{C}	98.61
40	82.44	3.04	92.19	1.45	24.02	21.96^{B}	100
60	67.24	2.61	74.33	1.54	21.21	20.09^{A}	95.83
Means of main effects of dietary Se supplementation							
0	75.06	2.84	83.37	1.49	22.72	21.42^{X}	99.07
0.8	81.31	3.01	88.80	1.42	24.04	22.31^{Y}	97.22
Two-way ANOVA: P values							
Lupin	0.000	0.000	0.000	0.000	0.000	0.000	0.139
Se	0.000	0.000	0.000	0.000	0.000	0.002	0.271
Lupin x Se	0.002	0.001	0.003	0.002	0.011	0.084	0.723

Values are displayed as mean of triplicate groups. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significantly differences ($P < 0.05$) among all dietary treatments. Means with different uppercase alphabets (A, B, C or X, Y) within a column indicate the significantly differences ($P < 0.05$) among means of the main effects of fishmeal replacement level and dietary Se supplementation, respectively. FW: Final weight (g), SGR: Specific growth rate, FI: Feed intake, FCR: Feed conversion ratio, PR: Protein retention and ER: Energy retention.

3.2 Digestibility

In the absence of Se supplementation, the cobia fed dietary LKM showed significantly lower nutrient digestibility than the fish fed the control diet. At each inclusion level of LKM, the digestibility of protein, energy and dry matter significantly improved when the diet was supplemented with Se. The interaction between dietary lupin and Se supplementation did not affect the protein, energy or dry matter digestibility of the cobia (Table 4).

3.3 Haematological Parameters

The haematocrit (Ht) was significantly ($P < 0.05$) affected by dietary Se supplementation, but not by dietary LKM levels. There were significant effects of dietary LKM level, Se supplementation and their interaction on the red blood cells (RBCs) and haemoglobin (Hb) of the cobia at the end of the feeding period. In the absence of Se supplementation, the RBC and Hb concentrations were significantly reduced in the cobia fed 315 g/kg LKM. However, the RBCs and Hb concentrations significantly increased in cobia fed 315 g/kg LKM supplemented with Se compared with the fish fed the same diet without Se supplementation (Table 4).

Table 4. Apparent digestibility coefficients (ADC) and haematological parameters of cobia fed test diets

Diets	ADC of protein (%)	ADC of energy (%)	ADC of dry matter (%)	Ht (%)	RBC	Hb
LP0	87.80	75.04	67.17	42.15	4.24^{b}	7.16^{bc}
LP40	85.20	66.32	63.92	42.62	4.18^{b}	7.05^{b}
LP60	82.46	62.80	58.21	41.99	3.97^{a}	6.35^{a}
LP0Se	89.42	76.35	69.63	43.55	4.31^{b}	7.28^{c}
LP40Se	87.96	70.39	64.25	43.99	4.30^{b}	7.24^{bc}
LP60Se	85.11	66.17	60.79	43.64	4.24^{b}	7.13^{bc}
Pooled SE	0.59	1.21	0.95	0.31	0.03	0.33
Means of main effects of fishmeal protein replacement level						
0	88.61^{C}	75.69^{C}	68.40^{C}	42.85	4.28	7.22
40	86.58^{B}	68.36^{B}	64.08^{B}	43.31	4.24	7.15
60	83.79^{A}	64.49^{A}	59.50^{A}	42.82	4.11	6.74
Means of main effects of dietary Se supplementation						
0	85.15^{X}	68.06^{X}	63.10^{X}	42.26^{X}	4.13	6.86
0.8	87.50^{Y}	70.97^{Y}	64.89^{Y}	43.73^{Y}	4.29	7.22
Two-way ANOVA: P values						
Lupin	0.000	0.000	0.000	0.744	0.001	0.000
Se	0.002	0.000	0.008	0.025	0.000	0.000
Lupin x Se	0.990	0.126	0.230	0.977	0.024	0.000

Values are displayed as mean of triplicate groups. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significantly differences ($P < 0.05$) among all dietary treatments. Means with different uppercase alphabets (A, B, C or X, Y) within a column indicate the significantly differences ($P < 0.05$) among means of the main effects of fishmeal replacement level and dietary Se supplementation, respectively. Ht: haematocrit, RBC: Red blood cell, Hb: Haemoglobin.

3.4 Proximate Composition

Neither dietary LKM nor Se supplementation significantly ($P > 0.05$) affected the protein, dry matter and ash contents in the muscle tissues of cobia. However, dietary LKM, but not Se supplementation, significantly affected the lipid content and gross energy in the muscles of the cobia (Table 5). The muscle amino acid profiles did not show any significant differences among treatments (Table 6). There were no significant effects due to the interaction between dietary lupin levels and Se supplementation on the muscle composition or amino acid profiles of the cobia at the end of the feeding period. Dietary LKM level, Se supplementation and their interaction had significant effects on Se deposition in the cobia tissues (Figure. 1). Regression analysis revealed positive linear relationships between dietary Se levels and tissue Se accumulation in cobia ($y = 0.177x + 0.1795$, $R^2 = 0.9585$ with $P < 0.001$ for the muscle Se and $y = 0.5222x + 0.6685$, $R^2 = 0.9516$ with $P < 0.001$ for the liver Se).

4. Discussion

In the absence of Se supplementation, cobia fed high dietary levels of LKM showed reduced FBW, SGR, FI and nutrient digestibility, corresponding to increased FCR. This was consistent when cobia were fed soybean meal (Chou et al., 2004; Zhou et al., 2005) and rapeseed meal (Luo et al., 2012). The reduced growth rates in cobia fed 315 g/kg LKM can be linked to the depression in FI as FI is directly related to weight gain (Espe, El-Mowafi, & Ruohonen, 2012). Therefore, it is essential to maintain an equal feed acceptability in order to evaluate the performance of the fishmeal-substituted diets (Espe et al., 2012).

The suppression of FI has been attributed to the deficiency of essential amino acids in lupin-based diets (Jobling et al., 2007). This is probably due to low concentrations of lysine and methionine in lupin products (Gatlin et al., 2007) as shown by relatively lower growth and feed efficiency in cobia by Zhou, Wu, Tan, Chi, and Yang (2006) and Zhou, Wu, Chi, and Yang (2007). The dietary requirements for lysine and methionine of juvenile cobia are 2.33 % and 1.05 %, respectively (Zhou et al., 2007; Zhou et al., 2006). Although lupin inclusion diets contain lower dietary lysine and methionine concentrations than fishmeal-based diets, however, the higher dietary lupin inclusion levels in the current study are still able to meet the threshold requirement of lysine and methionine for juvenile cobia.

Table 5. The muscle compositions of cobia fed test diets

Diet	Protein (%)	Lipid (%)	Dry matter (%)	Ash (%)	Gross energy (MJ/kg)
LP0	18.22	2.41	23.57	1.51	5.45
LP40	18.08	2.41	22.88	1.55	5.28
LP60	17.98	2.31	22.24	1.58	5.10
LP0Se	18.24	2.40	23.32	1.57	5.44
LP40Se	18.19	2.43	22.96	1.59	5.32
LP60Se	17.97	2.36	22.85	1.58	5.14
Pooled SE	0.05	0.01	0.19	0.01	0.04
Means of main effects of fishmeal protein replacement level					
0	18.23	2.40^{B}	23.44	1.56	5.44^{B}
40	18.14	2.42^{B}	23.08	1.57	5.30^{B}
60	17.98	2.34^{A}	22.54	1.58	5.12^{A}
Means of main effects of dietary Se supplementation					
0	18.09	2.38	23.00	1.56	5.28
0.8	18.13	2.39	23.04	1.58	5.31
Two-way ANOVA: P values					
Lupin	0.152	0.000	0.090	0.567	0.001
Se	0.685	0.078	0.894	0.267	0.548
Lupin x Se	0.870	0.081	0.438	0.551	0.610

Values are displayed as mean of triplicate groups. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significant differences ($P < 0.05$) among all dietary treatments. Means with different uppercase alphabets (A, B, C) within a column indicate the significant differences ($P < 0.05$) among means of the main effects of fishmeal replacement level.

Table 6. Essential amino acid profiles (g/100 g dry weight sample) in muscle of cobia fed test diets

Diets	Arg	His	Iso	Leu	Lys	Met	Phe	Thr	Tyr	Valine
LP0	3.16	2.43	4.10	6.57	9.22	2.01	3.15	2.43	2.94	3.96
LP40	3.14	2.38	4.19	6.45	9.01	1.94	3.17	2.43	3.00	4.02
LP60	3.14	2.37	4.04	6.67	9.25	1.94	3.20	2.41	2.95	3.98
LP0 + Se	3.15	2.39	4.17	6.68	9.01	2.06	3.23	2.44	2.99	3.96
LP40 + Se	3.13	2.42	4.20	6.67	9.26	1.90	3.19	2.42	2.95	3.98
LP60 + Se	2.81	2.36	4.12	6.65	9.23	1.98	3.27	2.41	2.97	3.94
Pool SE	0.06	0.01	0.02	0.01	0.04	0.02	0.02	0.01	0.01	0.02
Two-way ANOVA: P values										
Lupin	0.39	0.25	0.15	0.37	0.43	0.17	0.60	0.13	0.84	0.67
Se	0.34	0.93	0.27	0.09	0.92	0.70	0.22	0.86	0.79	0.48
Lupin x Se	0.43	0.34	0.79	0.10	0.09	0.71	0.80	0.89	0.28	0.87

Values are displayed as mean of triplicate groups. Means with same letters within a column are not significantly different ($P > 0.05$). SEM: pool standard error of the mean.

The high inclusion levels of dietary plant-derived ingredients in aqua-feeds also result in an increased proportion of dietary anti-nutrients (Francis, Makkar, & Becker, 2001; Gatlin et al., 2007). The interactions between anti-nutritional factors presented in protein ingredients and micronutrients can also impact the absorption and metabolism of these micronutrients (Read et al., 2014). The molecules in phytate can attach to minerals such as calcium, zinc, copper, iron, manganese, nickel and Se to form insoluble compounds, reducing the bioavailability of these minerals (Francis et al., 2001). The chelation between phytate and cation groups in protein, amino acids and lipid can also result in a reduction in the digestibility of these nutrients (Francis et al., 2001). Meanwhile, the metabolism of minerals in fish might also be compromised due to the presence of other anti-nutritional factors, such as tannin, saponin, glucosinolates and gossypol (Antony Jesu Prabhu et al., 2014). Thus, the complexity of mineral chelation may depress the growth, feed utilisation and mineral absorption in fish, as observed in Atlantic salmon *Salmon salar* (Storebakken, Shearer, & Roem, 1998) and channel catfish *Ictalurus punctatus* (Satoh, Poe, & Wilson, 1989). Relative to other plant ingredients, narrow-leafed LKM contains insignificant levels of phytic

acid (0.53 %) and tannins (0.16 %), resulting in low dietary phytic acid and tannin concentrations in the test diets (Table 1). Besides, the saponins, trypsin and chymotrypsin inhibitors found in the narrow-leafed LKM at low levels (574, 0.12 and 0.6 mg/kg, respectively) are unlikely to interfere with the uptake and absorption of nutrients in fish (Petterson, 2000).

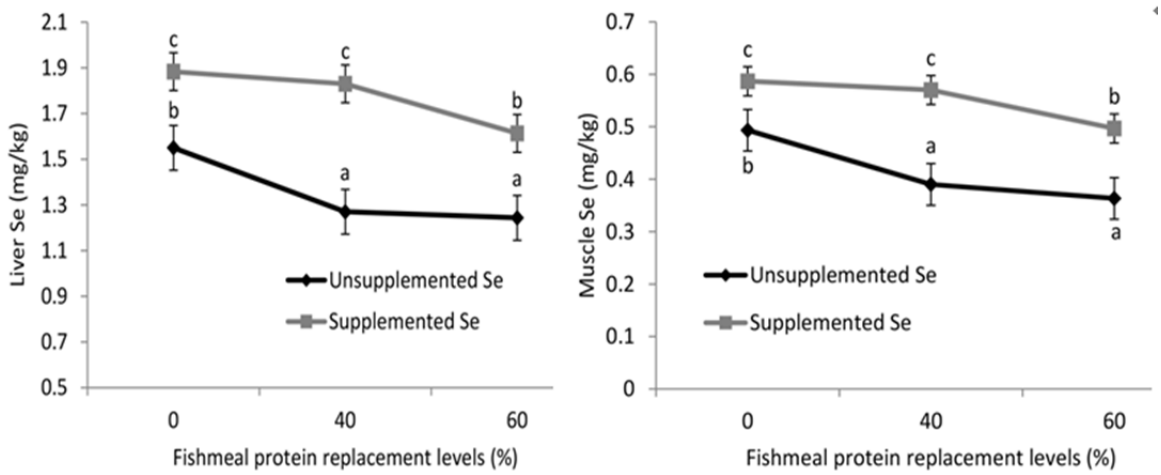

Figure 1. Mean of Se concentrations in the muscle and liver tissues of cobia fed diets containing various inclusion levels of lupin kernel meal with and without Se supplementation, mean with different letters are significantly different ($P < 0.05$)

One possible reason for the reduced nutrient digestibility in cobia fed lupin-based diets could be the presence of oligosaccharides in the diets. The narrow-leafed LKM contains relatively high levels of oligosaccharides in the forms of raffinose, stachyose and verbascose (Glencross, 2001; Petterson, 2000). These anti-nutrients may interact with the digestion of other nutrients (van Barneveld, 1999). They also prevent the activity of digestive enzymes and substrate transportation in the intestine (Francis et al., 2001), thus reducing nutrient digestibility as seen in rainbow trout (Glencross, Boujard, & Kaushik, 2003) and Atlantic salmon (Refstie, Storebakken, & Roem, 1998). Lupin meal also contains a significant amount of carbohydrates (Gatlin et al., 2007). A carnivorous fish such as cobia poorly utilise dietary carbohydrate as an energy source (Ren, Ai, Mai, Ma, & Wang, 2011), resulting in low energy digestibility. In contrast, cobia fed dietary LKM supplemented with organic Se showed improved growth, feed efficiency and nutrient digestibility compared with the fish fed diets lacking Se supplementation. A positive relationship between nutrient digestibility and dietary Se supplementation has been demonstrated in pigs (Chaudhary, Garg, Mittal, & Mudgal, 2010) and sheep (Shi et al., 2011). The reason for this could be due to the increased quantity and activities of digestive enzymes induced by dietary Se (Chaudhary et al., 2010). However, the effects of interaction between dietary Se and plant-derived protein on feed efficiency and nutrient digestibility has been reported in only one study, in which barramundi showed increased protein digestibility after being fed Se-supplemented lupin-based diets (Ilham et al., 2016). In the case of soybean, African catfish fed plant-based diets with supplemented Se demonstrated increased activities of protein metabolism enzymes and FI (Abdel-Tawwab et al., 2007). This could be indirectly associated with the complex mechanism of amino acid-chelated Se (AAC-Se) ingestion, resulting in increased essential trace element sources when high AAC-Se is absorbed into mucosal tissues (Ilham et al., 2016). These elements act as a cofactor in the synthesis of hydrolytic enzymes, such as gastrointestinal GPx, which plays important roles in defending the intestinal mucosal integrity (Lindh, 2013), stimulating nutrient digestion in fish (Ilham et al., 2016).

The dietary Se requirement has been established for juvenile cobia using purified diet (Liu et al., 2010). However, the biological effects of dietary Se on the feed utilisation efficiency of plant-derived ingredients, such as LKM, in this species are still unknown. In the current study, though increased levels of dietary LKM resulted in a corresponding decrease in dietary Se levels, the minimum dietary Se was still higher than threshold requirement for cobia fed casein-based diet (Liu et al., 2010). However, the improved growth and feed utilisation efficiency in cobia fed supplemental Se lupin-based diets indicated that the endogenous dietary Se in the lupin-based diets may not meet the nutritional requirement for juvenile cobia.

Feed ingredients contain a varied amount of Se, with a higher availability of Se from plant-derived ingredients

than from fishmeal (Watanabe et al., 1997). Thus, casein is generally used as the sole protein source in purified or semi-purified diets to overcome the complications of the varied Se availabilities in feed ingredients (Hilton, Hodson, & Slinger, 1980; Lin, 2014; Lin & Shiau, 2005; Liu et al., 2010). However, the use of casein, compared with fishmeal or krill meal, in commercial diets becomes challenging due to its high price and poor feed intake and growth rates (Hertrampf & Piedad-Pascual, 2000). Further, in the current study, the SGR was 74 % higher when fed the control diet containing various protein ingredients than reported by Liu et al. (2010) in the cobia fed a casein-based diet. The higher metabolic rates associated with faster-growing fish require sufficient energy to maximize their growth potential (DeVries & Eastman, 1981), resulting in a need to take up more nutrients, including Se.

The optimum dietary Se concentrations quantified for most fish species fed casein-based diets ranged from 0.15 to 0.98 mg/kg (Hilton et al., 1980; Lin, 2014; Lin & Shiau, 2005; Liu et al., 2010). Meanwhile, fish fed fishmeal or plant meal-based diets resulted in relatively higher dietary Se requirement levels, ranging from 1.62 to 5.35 mg/kg (Abdel-Tawwab et al., 2007; Le & Fotedar, 2013; Zhu et al., 2012). Even though, fishmeal-based diets can provide adequate Se to meet the nutritional requirements of some species (Watanabe et al., 1997). However, the significantly lower Se digestibility from fishmeal (38.48 – 47 %) compared with SeMet or Se-yeast (89.48 – 92 %) (Le & Fotedar, 2014; Watanabe et al., 1997) and relatively lower concentration of Se in lupin kernel meal (0.35 mg/kg) than fishmeal (4.37 mg/kg) may result in Se deficiency when fishmeal protein is replaced with lupin kernel protein, as seen in barramundi (Ilham et al., 2016) and cobia as in the current study. Moreover, Barrows et al. (2010) and Read et al. (2014) also indicated that rainbow trout fed plant-based diets required macro-minerals and inositol and/or dietary copper and zinc supplementation at higher levels to improve growth performance than those previously quantified by the NRC (1993). Similarly, the beneficial effects of Se fortification were also observed in African catfish fed soybean-based diets (Abdel-Tawwab et al., 2007) and barramundi fed plant-based diets (Ilham et al., 2016). This may be, in part, a reason for the enhancement of growth and feed utilisation performances in the current study.

The values of RBC and Hb can provide useful information about oxidative stress and toxicological impacts in fish as their important roles on physiological functions in fish (Kiron, Puangkaew, Ishizaka, Satoh, & Watanabe, 2004). In this study, reduced Hb and RBCs concentrations were shown in cobia fed dietary lupin meal without Se supplementation. This is consistent with a previous report in cobia (Zhou et al., 2005) fed soybean-based diets. The restriction on RBCs production and Hb synthesis imposed by adverse effects of anti-nutrients, as previously reported in cobia (Zhou et al., 2005), could constitute the reason. Conversely, cobia fed dietary lupin meal supplemented with Se did not show any significant differences in haematological performance compared with fish fed the control diet. As RBC and Hb play significant roles in oxygen and carbon dioxide transportation in the blood and haemoglobin synthesis (Olugbemi, Mutayoba, & Lekule, 2010). The increases in the RBC and Hb values in the cobia fed lupin-based diets supplemented with Se can be attributed to the enhancement of fish health stimulated by Se supplementation, as described in other fish species (Abdel-Tawwab et al., 2007; Ilham et al., 2016).

In the current study, dietary LKM did not have any effects on protein, ash and dry matter levels in the muscles of cobia, irrespective of Se supplementation. This is consistent with the study in gilthead sea bream Sparus aurata (Pereira & Oliva-Teles, 2004) and black seabream *Acanthopagrus schlegeli* (Zhang et al., 2012) fed lupin inclusion diets. A deficiency of taurine in plant-based diets has been linked to the reduced lipid deposition in the tissues of cobia (Lunger, McLean, Gaylord, Kuhn, & Craig, 2007). However, in the current study, taurine was added at 5 g/kg to satisfy the nutritional needs of cobia as quantified by Watson et al. (2014). Thus, the reduced muscle lipid contents of cobia fed 315 g/kg LKM diet in the current study might be due to the low energy intake and energy digestibility, similar in cobia fed other plant-derived ingredients (Luo et al., 2012; Luo et al., 2013; Zhou et al., 2005). As the tissue Se depositions of cobia had a strong linear relationship with dietary Se levels, the concentration of Se in these tissues can be used as a biomarker of dietary Se delineation.

In conclusion, cobia fed a lupin-based diet requires Se fortification to meet their nutritional requirements to satisfy potential growth and feed utilisation. Up to 210 g/kg of LKM can be included in the diets of cobia with Se supplementation, without impairing growth, feed efficiency and physiological performances. The dietary Se supplement had no beneficial effects on the muscle composition and amino acid profiles of cobia, but significantly improved their haematological parameters and nutrient digestibility.

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