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Evaluating dietary supply of microminerals as a premix in a complete plant ingredient-based diet to juvenile rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Two basal diets M0 and V0 were formulated with marine and plant based ingredient composition. Seven experimental diets were prepared from the two basal diets namely M0, M100, V0, V30, V60, V100 and V150 by incorporating different levels of a micro-mineral premix (Cu, Fe, Mn, Se and Zn). Triplicate groups of rainbow trout (initial weight: 20 g) reared at 17°C were fed one of each diet to apparent visual satiation over 12 weeks. Among the V diet fed fish, growth and feed intake exhibited maximal response at V60 level of premix inclusion; Apparent availability coefficient of Fe, Cu and Zn decreased linearly with increasing level of premix whereas apparent availability coefficient of Mn and Se was unaffected. The available dietary concentration in basal V0 diet was for Fe, 20.6; Cu, 2.8; Mn, 6.5; Zn, 17.3 and Se, 0.195 (in mg/kg DM) and in the M0 diet for Fe, 63.3; Cu, 5.2; Mn, 2.9; Zn, 35.2 and Se, 0.87 (in mg/kg DM). In reference to NRC (*Nutrient requirements of fish and shrimp*. Washington, DC: National Research Council, The National Academies Press, 2011) recommendations, the V0 basal diet accounted for 34.3%, 92.9%, 53.9%, 115% and 130.2% and the contribution from M0 diet for 105.5%, 173.3%, 24.2%, 234.7% and 580% of the minimal dietary inclusion levels of Fe, Cu, Mn, Zn and Se to rainbow trout, respectively. However, data on whole body mineral contents showed that normal levels were maintained only for Cu and Mn through supply from basal V0 diet. For Zn and Se, available supply even from the highest supplemented diet (V150) was not sufficient to maintain normal body mineral levels of rainbow trout in the present study. On the whole, optimal dietary inclusion levels of microminerals are altered while using fishmeal-free diets for rainbow trout.

KEYWORDS

fishmeal replacement, micro minerals, premix, rainbow trout, requirement, supplement, trace minerals

1 | INTRODUCTION

Mineral requirements of fish and optimal dietary inclusion level in particular of micro minerals such as Fe, Cu, Mn, Zn and Se in fish feeds are not well understood (Lall, 2008). As regards rainbow trout,

estimates on requirements for all micro minerals, except for iron (Fe), are available (NRC, 2011). The recommendations of National Research Council (NRC) are based on "requirement estimates determined with highly purified ingredients in which the nutrients are highly digestible and therefore the values represent near 100% bioavailability."



Under practical conditions, the dietary inclusion level of micro minerals needed to meet the requirement can vary based on a number of physiological and environmental factors (Hilton, 1989; Sugiura, Dong, & Hardy, 2000). The ingredient composition of practical fish feeds is one such factor (Watanabe, Kiron, & Satoh, 1997). The latter authors found that micro minerals such as Fe, Cu, Mn, Se and Zn are important to be supplemented in fish feeds due to the low levels in practical feed ingredients and interactions with other dietary components. The magnitude of supplementation depends on the intrinsic contribution from the basal ingredients and interactions affecting the availability of the micro minerals. Over the years, the use of marine ingredients such as fishmeal and fish oil in fish feeds has seen a significant reduction, and they are replaced to a large extent by plant-derived protein and lipid sources (Tacon & Metian, 2008). Fishmeal is generally rich in micro minerals (Julshamn, Haugsnes, & Utne, 1978), but appreciable levels of certain micro minerals are also found in plant-derived ingredients, at times even many folds higher than in fishmeal, as seen for the Mn content of white lupin (NRC, 2011). Considering the supply of micro minerals from the different ingredients themselves (Lall, 2008; Sugiura, Dong, Rathbone, & Hardy, 1998), it is necessary to evaluate the need for and level of supplementing one or more micro minerals to plant ingredient-based diets which are completely devoid of fishmeal. Based on the foresaid considerations, a study with juvenile rainbow trout was undertaken to evaluate the NRC (2011) recommendations for micro minerals and determine the optimal range of micro mineral supplementation required to a complete plant ingredient-based diet.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

Two basal diets (M0 and V0) were formulated with the basal ingredient composition provided in Table 1. The diets were made either with fishmeal as the major protein source (M) or entirely of plant-derived ingredients (V) and supplemented with di-calcium phosphate to meet the available phosphorus requirement of rainbow trout (Antony Jesu Prabhu et al., 2014; NRC 2011). A micromineral (Fe, Cu, Mn, Zn and Se) premixture was designed to meet the theoretical requirements of rainbow trout (NRC, 2011) at an inclusion level of 10 g/kg diet contributing Fe, 52.5 mg; Cu, 7.5 mg; Mn, 12 mg; Zn, 14 mg; and Se, 0.15 mg/kg diet, on as fed basis. This premix was included at incremental levels of 0, 3, 6, 10 and 15 g/kg diet to the basal V diet to provide diets V0, V30, V60, V100 and V150, respectively, whereas, in the M diet, the premix was included at 0 and 10 g/kg diet to give M0 and M100, respectively. For the measurement of the apparent availability of minerals, the diets were prepared with the incorporation of 0.1 g/kg yttrium oxide (Y_2O_3) as the inert marker.

2.2 | Fish, feeding and rearing condition

Rainbow trout juveniles (19.8 ± 0.8 g, IBW) were randomly distributed into 21 experimental units, each of 500 L (40 fish/unit). Triplicate groups of fish were hand fed twice a day to apparent visual satiation

for a period of 12 weeks (6 days a week). The fish were reared in flow-through systems at the INRA experimental fish farm at Donzacq (Landes, France). Water temperature was $17 \pm 0.5^\circ\text{C}$, and the flow rate of water was maintained at 50 L/min during the experimental period. The analysed concentrations of minerals in the rearing water (mg/L) were P, <0.2 ; Ca, 41.7 ± 6.3 ; Mg, 19.2 ± 0.8 ; K, 1.8 ± 0.1 ; Fe, <0.02 ; Mn, <0.02 ; Cu, <0.008 , Zn, <0.007 ; and Se, 0.002.

2.3 | Fish, tissue and faecal sampling

Fish samples for initial body composition analysis ($n = 20$) were collected at the start of the experiment. Fish from each experimental unit were bulk weighed at the start, every 3 weeks and at the end of the 12-week growth trial. Feed was withheld for 24 hr before every weighing. At the end of the growth trial and 24 hr after the last meal, six fish were randomly selected from each experimental unit and pooled for final body composition analysis. Out of the remaining fish, 25 fish per tank were used for the determination of apparent availability coefficient (AAC) of minerals. The fish were fed a single meal (86 g/tank), and 9 hr after the meal, faecal samples were collected by the method of stripping (Antony Jesu Prabhu et al., 2014). The samples were collected over ice, frozen immediately and stored at -20°C until mineral analysis. Animal experiments and sampling procedures followed the guidelines of the National Legislation on Animal Care of the French Ministry of Research (Decree no. 2001-464, May 29, 2001) and the animal ethics committee of INRA (INRA 2002-36, April 14, 2002).

2.4 | Analytical methods

Moisture content of the pooled fish samples was determined by drying freshly ground samples at 105°C for 24 hr. The chemical composition of the diets and of fish samples were analysed by the following methods: dry matter after drying at 105°C for 24 hr, ash by combustion at 600°C for four hours in a muffle furnace, crude protein (Nx6.25) by Kjeldahl method in acid digested samples, crude lipid by petroleum ether extraction using Soxhlet method (Soxtherm) and gross energy content in an adiabatic bomb calorimeter (IKA, Heitersheim Griebheimer, Germany). The concentrations of P, Ca, Mg, K, Fe, Cu and Zn in the diets, whole fish and faeces were analysed using inductively coupled plasma-mass spectrometry (ICP-OES) at USRAVE-INRA, Bordeaux, France. The Se and yttrium content in the samples were analysed using ICP-MS at LCABIE-UPPA, Pau, France. The analysed chemical composition and macro-mineral concentration of the basal V0 and M0 diets are presented in Table 1, and the micromineral concentration of the diets are provided in Table 2.

2.5 | Data analysis

Tanks ($n = 3$) were used as experimental units for data on growth, body composition and mineral balance. All data were subjected to one-way ANOVA followed by Tukey's multiple comparison test at a probability of $p < .05$. All the data analysis was performed using SPSS version 20, IBM Statistics Inc., USA.

TABLE 1 Ingredient and chemical composition of the basal diets

Ingredients	V0	M0
Norwegian herring meal, (CP 70; Sopropêche, France)	-	625.7
Corn Gluten meal (CP 60; Inzo, France)	180	-
Wheat Gluten (CP 70; Roquette, France)	200	-
Soybean meal (CP 48; Inzo, France)	50	-
Soyprotein concentrate (Estrilvo; CP 70; Sopropêche, France)	170	-
White lupin meal (Terrena, France)	50	-
Extruded peas (Aquatex, Sotexpro, France)	30	-
Rapeseed meal (Primor 00; SudOuestAliments, France)	40	-
Whole wheat	31.8	245.6
Soylecithin (Louis François, France)	20	-
L-Lysine (Eurolysine)	13.4	-
L-methionine (Evonik, Germany)	3	-
CaHPO ₄ .2H ₂ O (18%P; 22% Ca)	21.7	-
Attractant Mix ^a	15	-
Micro mineral premix ^b	0.00	0.00
Vitamin premix ^c	10	10
Yttrium oxide (Sigma-Aldrich, USA)	0.1	0.1
Fish oil (southern hemisphere, Sopropêche, France)	-	118.6
Rapeseed Oil (Daudruy, France)	66	-
Linseed Oil (Daudruy, France)	66	-
Palm Oil (Daudruy, France)	33	-
Analysed proximate composition		
Dry matter (DM), g/kg	949.4	948
Crude Protein, g/kg DM	499.8	494
Crude Lipid, g/kg DM	227.3	221
Crude Ash, g/kg DM	40.2	82
Energy, kJ/g DM	25.1	23.8
Analysed concentration of major minerals (g/kg DM)		
Phosphorus	9.4	12.9
Calcium	8.0	11.3
Ca/P ratio (no unit)	0.9	0.9
Magnesium	0.95	1.76
Potassium	4.2	12.2
Sodium	1.4	9.4

^aAttractant premix: glucosamine, 5 g; taurine, 3 g; betaine, 3 g; glycine, 2 g and alanine, 2 g.

^bMicro mineral premix (g/kg premix): FeSO₄.7H₂O (21% Fe; 11.5% S; Merck 103965; Fontenay-sous-Bois, France), 25 g; CuSO₄.5H₂O (25.45% Cu; 12.8% S; Sigma C8027, Saint-Quentin-Fallavier, France), 3 g; MnSO₄.H₂O (33% Mn; 19% S; VWR Chemicals 25303.233; Fontenay-sous-Bois, France), 3 g; ZnSO₄.7H₂O (36% Zn; 18% S; VWR Chemicals 29253.236; Fontenay-sous-Bois, France), 4 g; KI (76% I; 24% K; Sigma P4286, Saint-Quentin-Fallavier, France), 0.04 g; Na₂SeO₃ (46% Se; 27% Na; Sigma 71950, Saint-Quentin-Fallavier, France), 0.03 g; α-cellulose (carrier)964.93 g.

^cVitamin premix (IU or mg/kg diet): DL-α tocopherol acetate, 60 IU; sodium menadione bisulphate, mg; retinyl acetate, 15,000 IU; DL-cholecalciferol, 3,000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1,000 mg; biotin, 2.5 mg; calcium pantothenate, 50 mg; choline chloride, 2,000 mg (UPAE, INRA).

3 | RESULTS

3.1 | Feed intake and growth performance

Data on feed intake (FI), weight gain (WG) and other indices of growth performance such as feed efficiency (FE) and daily growth index (DGI) are provided in Table 3. During the 12-week feeding

trial, fish gained more than sixfold their initial weight. Fish fed M diets had significantly higher feed intake, weight gain and feed efficiencies compared with V diet fed fish. Inclusion of the micromineral premix did not have an impact on the growth of the fish, irrespective of the type of basal diet. However, among the five groups fed the V diet, a tendency for higher weight gain was observed in the fish fed with the V60 diet.



Diet code	V0	V30	V60	V100	V150	M0	M100
Iron	153.8	169.1	185.2	205.4	234.8	161.6	212.9
Copper	7.0	8.8	10.0	12.9	14.2	6.9	14.0
Manganese	88.6	92.2	94.1	100.9	102.1	9.4	22.7
Zinc	42.9	44.1	46.8	52.5	58.1	62.8	72.5
Selenium	0.24	0.28	0.33	0.40	0.54	1.09	1.27

TABLE 2 Analysed dietary concentration of microminerals (mg/kg DM)

V0, Basal plant ingredient-based diet; M0, Basal fishmeal-based diet; V30, V60, V100 and V150, plant ingredient-based diets with 3, 6, 10 and 15 g/kg inclusion of micromineral premix; M100, Fishmeal-based diet with 10 g/kg inclusion of micromineral premix.

TABLE 3 Growth performance of rainbow trout fed the experimental diets for 12 weeks

	IBW (g/fish)	FBW (g/fish)	Feed intake (g/fish)	Weight gain (g/fish)	FE	DGI
V0	19.8 ± 0.6	144 ± 12.8 ^a	113.7 ± 5.7 ^a	123.8 ± 12 ^a	1.2 ± 0.04 ^a	3 ± 0.15 ^a
V30	19.8 ± 0.5	145.8 ± 4.5 ^a	116.9 ± 1.3 ^a	126 ± 4.8 ^a	1.1 ± 0.02 ^a	3 ± 0.08 ^a
V60	19.9 ± 0.8	155.1 ± 2.2 ^a	121.6 ± 1.4 ^{ab}	135.2 ± 1.5 ^a	1.2 ± 0.02 ^a	3.2 ± 0.02 ^b
V100	19.7 ± 0.5	145.3 ± 5.7 ^a	115.9 ± 3.5 ^a	125.5 ± 5.4 ^a	1.1 ± 0.03 ^a	3 ± 0.07 ^a
V150	20 ± 0.3	146.8 ± 4.5 ^a	120.4 ± 4.5 ^{ab}	126.8 ± 4.8 ^a	1.1 ± 0.07 ^a	3.1 ± 0.1 ^{ab}
M0	19.7 ± 0.5	187.9 ± 6 ^b	127.6 ± 1.7 ^b	168.3 ± 5.3 ^b	1.4 ± 0.03 ^b	3.6 ± 0.1 ^c
M100	19.7 ± 0.5	190.8 ± 5.5 ^b	131.2 ± 2.4 ^b	171.1 ± 4.1 ^b	1.4 ± 0.03 ^b	3.6 ± 0.04 ^c
<i>p</i> -value	.52	.01	.03	.01	.02	.02

V0, Basal plant ingredient-based diet; M0, basal fishmeal-based diet; V30, V60, V100 and V150, plant ingredient-based diets with 3, 6, 10 and 15 g/kg inclusion of micromineral premix; M100, fishmeal-based diet with 10 g/kg inclusion of micromineral premix; IBW, Initial body weight; FBW, final body weight; FE, feed efficiency; DGI, daily growth index. $DGI = 100 \times (FBW^{1/3} - IBW^{1/3}) / \text{duration (84 days)}$; FE = wet weight gain (g)/dry feed intake (g). Values within the same column with different superscript are statistically significant.

TABLE 4 Apparent availability (%) of minerals in experimental diets fed to rainbow trout

	P	Mg	Ca	K	Fe	Cu	Mn	Zn	Se
V0	58.5 ± 7.9 ^a	68.6 ± 5.2 ^b	7.4 ± 1.1	88.4 ± 1.1 ^a	13.4 ± 1.9 ^d	39.8 ± 5.5 ^c	7.3 ± 0.4 ^a	40.2 ± 3 ^{ba}	81.4 ± 1.4 ^b
V30	60.8 ± 0.8 ^a	69.4 ± 4.5 ^b	8.4 ± 0.9	88.9 ± 0.6 ^a	12.9 ± 0.1 ^d	35.9 ± 3.1 ^c	8.7 ± 1 ^a	38.6 ± 2.2 ^{ba}	80.7 ± 1.8 ^b
V60	60.9 ± 6.2 ^a	68.9 ± 4.8 ^b	9.2 ± 7.1	88.9 ± 1.2 ^a	13.1 ± 1.1 ^d	27.7 ± 2.1 ^b	9.9 ± 2.6 ^a	40.4 ± 2.3 ^{ba}	80.4 ± 2.4 ^b
V100	59.6 ± 5.9 ^a	70 ± 3.9 ^b	11.8 ± 1.9	89.2 ± 0.4 ^a	10.1 ± 1.2 ^{bc}	28.3 ± 4.4 ^b	10 ± 2.6 ^a	39.4 ± 2 ^{ba}	79.8 ± 1.7 ^b
V150	58.7 ± 2.3 ^a	68.1 ± 4.9 ^b	7.9 ± 5.6	88.7 ± 0.4 ^a	7.5 ± 0.5 ^b	19.1 ± 2.3 ^a	6.6 ± 2.1 ^a	34.5 ± 1.8 ^a	80.8 ± 2.1 ^b
M0	70.5 ± 1.8 ^b	65.4 ± 3.3 ^{ab}	13.4 ± 3.1	97.4 ± 0.2 ^b	39.2 ± 3.1 ^e	74.8 ± 0.2 ^e	31 ± 4.8 ^b	56.1 ± 7.4 ^c	80.5 ± 0.7 ^b
M100	67.5 ± 1.2 ^{ab}	58.5 ± 3.8 ^a	8.3 ± 0.1	97.1 ± 0.1 ^b	3.7 ± 0.9 ^a	35.1 ± 3.7 ^d	25.7 ± 6.8 ^b	45.8 ± 3 ^b	74.4 ± 1.5 ^a
<i>p</i> -value	.051	.069	.707	.000	.000	.000	.000	.000	.004

V0, Basal plant ingredient-based diet; M0, Basal fishmeal-based diet; V30, V60, V100 and V150, plant ingredient-based diets with 3, 6, 10 and 15 g/kg inclusion of micromineral premix; M100, Fishmeal-based diet with 10 g/kg inclusion of micromineral premix. Data presented as mean ± SD; values within the same column having different superscripts are statistically different.

3.2 | Apparent availability of minerals

Data on apparent availability coefficient (AAC, %) of minerals in the experimental diets to rainbow trout are presented in Table 4. The AAC of all the analysed macro-minerals and microminerals were higher in fish fed the M diet. Supplementation of micromineral premix to the M0 diet resulted in reduction of AAC of the microminerals, most prominent for Fe. Among the V diet fed fish, the AAC of microminerals such as Fe, Cu and Zn decreased with increasing level of premix and attained the lowest with V150 fed group. Premix supplementation to basal V diet did not have an impact on the AAC of Mn, Se and also on

the AAC of the macro-minerals such as P, Ca, Mg and K, for which the dietary levels were kept constant.

3.3 | Contribution of basal diet in meeting available micromineral requirements

A comparison between the NRC recommendations (2011) and the contribution of available micromineral supply from the basal V0 and M0 diet to the requirements of corresponding microminerals for rainbow trout are presented in Table 5. In reference to NRC (2011) recommendations for rainbow trout, the V0 basal diet covered 34.3%,

92.9%, 53.9%, 115% and 130.2% and the M0 diet for 105.5%, 173.3%, 24.2%, 234.7% and 580% of the minimal dietary inclusion levels of Fe, Cu, Mn, Zn and Se, respectively.

3.4 | Chemical composition of fish

The major chemical composition of the body such as moisture (674 ± 5 g/kg), crude protein (155 ± 2 g/kg), crude fat (166 ± 6 g/kg), ash (17 ± 1 g/kg) and energy (9.2 ± 0.2 kJ/g) were not different between the treatments (data not shown). The initial and final mineral concentrations of fish fed experimental diets are presented in Table 6. Micromineral premix supplementation did not affect the concentration of macro-minerals such as P, Ca, Mg, K and Na. The final body concentration of Cu ($p < .05$), Zn ($p < .01$) and Se ($p < .01$) increased with premix inclusion level, while Fe and Mn were not significantly different between the V diet fed groups. In comparison with M diet fed groups, it was found that Zn and Se levels even in the V150 diet were insufficient to maintain the normal body mineral levels in rainbow trout.

4 | DISCUSSION

Excessive use of inorganic minerals in practical diets can be avoided by utilizing the intrinsic potential of minerals in feed ingredients (Sugiura et al., 1998). Tacon and De Silva (1983) documented exceedingly high concentrations above recommended levels and twofold to 11-fold variations for microminerals such as Fe (80–540 mg/kg), Cu (5–40 mg/kg), Mn (35–100 mg/kg) and Zn (50–260 mg/kg) within similar feed categories of commercial salmonid feeds that were available in Europe. Three decades down the line, the situation has not changed much, according to the recent report based on a survey on different Norwegian fish feeds over a decade from 2000 to 2010 (Sissener et al., 2013). The reported minimal and maximal dietary levels are as follows: Fe (65–493 mg/kg); Cu (2.5–21 mg/kg); Mn (4.4–226 mg/kg); Zn (36–330 mg/

kg); and Se (0.39–4.1 mg/kg). These data suggest that (i) high variations could be related to the differences in micromineral composition of the major feed ingredients; due to a diverse array of source and origin (ii), commercial fish feed formulations are more often negligent to the negative effects of excess micromineral supply. Given the diverse array of alternate feed ingredients of varying mineral concentrations, necessary strategies need to be devised to provide optimal levels, avoiding deficient or excess of microminerals in fish feeds.

The available micromineral levels from the basal diets (both M0 and V0) fulfilled more than 90% of the respective micromineral requirements to rainbow trout except for Mn with reference to NRC (2011) recommendations. However, the final whole body concentrations indicated deviations from the earlier statement. Final body composition is a robust response to quantify macro-mineral requirement (Antony Jesu Prabhu, Schrama, & Kaushik, 2013; Rodehutsord, 1996; Shearer, 1989) and can also be applied for microminerals (Shearer, 1995). Iron is the only essential micromineral for which the requirement is yet to be tested in rainbow trout (NRC, 2011). Shearer (1995) predicted an estimate of 21 mg/kg diet for normal Fe homeostasis in 10 g rainbow trout. Dietary Fe requirement of Atlantic salmon is reported to be 60–100 mg/kg diet (Andersen, Maage, & Julshamn, 1996; Naser, 2000), when fed FeSO_4 supplemented casein-based diets. It can be assumed that total Fe supply of 154 mg/kg DM (from V0 diet) almost satisfied the minimal dietary Fe needs of rainbow trout. The AAC of Fe was constant at 13% until V60 and reduced significantly thereafter to 10% and 7.5% in V100 and V150, suggestive of an excess dietary supply only beyond V60. This is more likely because iron homeostasis is primarily regulated at intestinal absorption stage by body iron stores and dietary Fe levels (Standal, Dehli, Rorvik, & Andersen, 1999). Judging from the AAC and the higher but statistically non-significant values of whole body Fe in V30 and V60, it could be proposed that supplementation of 30 mg Fe/kg (V60, 185 mg Fe/kg) would be required to the basal V0 diet.

The NRC (2011) recommended and the only available report on dietary Cu requirement of rainbow trout (3 mg/kg DM) was based on growth and body Cu concentration, obtained by testing only two dietary

TABLE 5 Comparison between the NRC (2011) recommendation on micromineral requirements to rainbow trout and available supply of microminerals from the basal M0 and V0 diets in the present study

	Requirement of rainbow trout NRC (2011)	Available supply from basal diet (mg/kg DM) ^a		Percentage contribution of M0 diet to NRC (2011) recommendation ^d	Percentage contribution of V0 diet to NRC (2011) recommendation ^d	Mineral balance from V0 diet based on whole body levels in this study ^b
		M0-diet	V0-diet			
Iron	NT (21 ^c)	63.3	20.61	301.4	98.1	–
Copper	3	5.2	2.79	173.3	92.9	++
Manganese	12	2.9	6.47	24.2	53.9	++
Selenium	0.15	0.87	0.19	580.0	130.2	--
Zinc	15	35.2	17.25	234.7	115.0	--

NT, not tested in rainbow trout as per NRC (2011).

Available supply of microminerals from the basal diet was calculated as a product of the analysed dietary mineral concentration (mg/kg) and the AAC (%).

^aCalculated from data presented in Table 2 (dietary micro mineral concentration) and Table 4 (AAC of micro minerals) for M0 and V0 diet.

^bIndicates sufficiency of available supply from V0 diet in maintaining whole body mineral balance.

^cFe requirement provided within parenthesis is based on data from Shearer (1995).

^dCalculated as relative percentage of available supply from basal diet to the respective available requirement estimates.

**TABLE 6** Mineral composition of rainbow trout fed the experimental diets for 12 weeks (fresh weight basis)

	Ash (g/kg)	P (g/kg)	Ca (g/kg)	Mg (g/kg)	K (g/kg)	Na (g/kg)	Fe (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Se (mg/kg)
Initial	23.4	4	4.2	0.3	3.7	1.1	26	0.9	0.9	24.9	0.25
V0	17.9 ± 0.4	3.3 ± 0.1	2.4 ± 0.1	0.3 ± 0.02	3.6 ± 0.02	0.8 ± 0.02	17.9 ± 0.6	1.9 ± 0.1 ^c	1 ± 0.1 ^b	12.1 ± 0.5 ^a	0.13 ± 0.007 ^a
V30	17.2 ± 0.3	3.1 ± 0.2	2 ± 0.1	0.3 ± 0.01	3.7 ± 0.04	0.8 ± 0.03	26.5 ± 9.8	2.4 ± 0.3 ^{cd}	0.9 ± 0.1 ^b	13.1 ± 0.1 ^{ab}	0.13 ± 0.004 ^a
V60	17.6 ± 1.0	3.1 ± 0.1	2.2 ± 0.3	0.3 ± 0.02	3.6 ± 0.06	0.8 ± 0.02	26 ± 5.5	2.5 ± 0.2 ^{cd}	1 ± 0.2 ^b	13.8 ± 0.2 ^b	0.14 ± 0.005 ^b
V100	17.2 ± 1.0	3.1 ± 0.2	2.2 ± 0.4	0.3 ± 0.07	3.5 ± 0.07	0.8 ± 0.03	21.4 ± 2.5	2.8 ± 0.2 ^d	0.9 ± 0.1 ^b	13.8 ± 0.6 ^b	0.16 ± 0.002 ^c
V150	17.4 ± 1.0	3.2 ± 0.1	2.2 ± 0.3	0.3 ± 0.06	3.6 ± 0.06	0.9 ± 0.02	18.5 ± 2.4	4 ± 0.4 ^e	0.9 ± 0.1 ^b	16.4 ± 0.5 ^{cd}	0.18 ± 0.006 ^d
M0	17.6 ± 0.7	3.3 ± 0.16	2.4 ± 0.28	0.3 ± 0.01	3.7 ± 0.04	0.9 ± 0.02	14.4 ± 0.23	0.9 ± 0.12 ^a	0.7 ± 0.04 ^a	15.4 ± 0.51 ^c	0.3 ± 0.01 ^e
M100	18.4 ± 0.8	3.4 ± 0.14	2.6 ± 0.18	0.3 ± 0.01	3.7 ± 0.1	0.9 ± 0.04	22.5 ± 5.58	1.4 ± 0.35 ^b	1.2 ± 0.09 ^c	17.3 ± 0.4 ^d	0.3 ± 0.01 ^e
<i>p</i> -value	.1	.09	.24	.17	.03	.2	.67	.00	.01	.01	.01

V0, Basal plant ingredient-based diet; M0, Basal fishmeal-based diet; V30, V60, V100 and V150, plant ingredient-based diets with 3, 6, 10 and 15 g/kg inclusion of micromineral premix; M100, Fishmeal-based diet with 10 g/kg inclusion of micromineral premix. Data are expressed as mean ± SD. *p*-value indicates statistical significance as obtained through ANOVA. Values within the same column with different superscripts are statistically significant.

Cu levels (Ogino & Yang, 1980). Reports on Cu requirement for several other fish species range from 3.1 to 12 mg/kg DM (Cao et al., 2014; Gatlin & Wilson, 1986; Lin, Shie, & Shiao, 2008; Lorentzen, Maage, & Julshamn, 1998; Shao et al., 2010, 2012; Shiao & Ning, 2003; Tan, Luo, Liu, & Xie, 2011). In our study, the minimal dietary Cu concentration of 8.7 mg/kg DM (3.2 mg/kg DM on available basis) was sufficient to maintain normal body Cu concentration. The present estimate on available basis (3.2 mg/kg DM) was higher by 7% compared to the estimate of Ogino and Yang (1980) in rainbow trout, but actually this difference could be larger as the latter estimate of 3 mg/kg DM is on total basis. Although the diet used in the study of Ogino and Yang (1980) was based on purified ingredients, 100% availability may not be realistic, as assumed by NRC. Certain microminerals, especially Cu, are very often reported to accumulate in the body when exposed to high dietary or aqueous supply (Kamunde, Grosell, Higgs, & Wood, 2002). Even in the present study, it was observed that accumulation rate of Cu in the body increased steeply when dietary Cu was in excess of 13.2 mg/kg DM (>4 mg/kg DM on available basis). This increase in whole body Cu was concurrent with the significant reduction in AAC of Cu in V fed fish possibly indicating a regulatory mechanism in response to the dietary Cu overload, as observed in Atlantic salmon (Berntssen, Lundebye, & Maage, 1999). Moreover, despite the level of Cu in the M and V diets being comparable, the V diet fed fish had almost twice the level of whole body Cu compared to M fed fish, at both 0 and 100% premix inclusion levels. Our previous findings also show that fish fed V diets tend to accumulate more Cu in body (Antony Jesu Prabhu et al., 2015, 2016). In view of this, an optimal range of 8.7–13.2 mg/kg DM total dietary Cu (3.2–4 mg/kg DM on available basis) could be considered for rainbow trout fed the V diets to avoid excess accumulation of Cu in the body.

The Mn requirement of rainbow trout ranges from 12 to 13 mg/kg DM in purified diets on total basis, with growth and tissue Mn levels as response criteria (Ogino & Yang, 1980). Similar to Cu, this estimate was obtained from testing only two dietary Mn levels. In the present study, fish fed the basal V0 diet with a total Mn content of 89 mg/kg DM (6.5 mg/kg DM on available basis) were able to maintain normal whole body Mn level, presumably due to the contribution from lupin meal. Lupin meal is reported to contain very high levels of Mn, up to 1390 mg/kg, as fed basis (NRC, 2011). Nevertheless, the estimate of 6.5 mg/kg on available basis, which was sufficient to maintain body Mn level in the present study, was lower by 46% than the estimate (12 mg/kg DM) recommended by NRC (2011) based on data from Ogino and Yang (1980). This could possibly be due to (i) over estimation of Mn requirement by Ogino and Yang (1980), as only two dietary levels were tested; (ii) present estimate is on available basis, while the earlier was on total basis as explained for Cu; (iii) possibility of an underestimated Mn availability in the present study due to high total dietary Mn concentration in the basal V0 diet and biliary excretion of absorbed Mn making a significant contribution to faecal Mn load (Sugiura et al., 1998). However, vertebral Mn concentration would have provided a more robust indicator to evaluate whether the Mn supplied by diet V0 was sufficient to rainbow trout, as shown by Satoh, Takeuchi, and Watanabe (1991); in view of this, supplementation of 6–10 mg/kg inorganic Mn to the V diet may be beneficial.

Zinc and selenium concentrations are naturally low in plant-derived ingredients as compared with fishmeal (Lall, 2002). Moreover, the availability of both Zn and Se can be reduced by phytic acid (Cheng & Hardy, 2002) and inclusion of inorganic P or Ca salts (Antony Jesu Prabhu et al., 2014; Kousoulaki, Fjellidal, Aksnes, & Albrektsen, 2010). Zinc is by far the most studied among the microminerals essential to fish, and rainbow trout in particular. NRC (2011) recommends the Zn requirement of rainbow trout as 15 mg/kg DM; while NRC (1993) recommended 30 mg/kg DM, both based on the range of 15–30 mg/kg DM reported for maximal growth and body Zn levels when fed purified diets (Ogino & Yang, 1978). In the present study, the whole body levels of Zn in both M and V diet fed fish were lower compared to the initial levels. Tri-calcium phosphate (TCP) at 7% (equivalent to levels present in fishmeal) resulted in complete unavailability of Zn to rainbow trout even with inclusion of 20 mg/kg ZnSO₄ (Satoh, Tabata, Izume, Takeuchi, & Watanabe, 1987). Thereby, supplementation of 40 mg/kg Zn (80 mg/kg total dietary Zn) was required to maintain whole body or vertebral Zn content when fed white fishmeal-based practical diets (Satoh, Takeuchi, & Watanabe, 1987; Satoh, Tabata, et al., 1987). In diets containing 4% TCP, dietary available Zn level of 15.7 mg/kg DM satisfied the growth and body Zn requirements of rainbow trout (Satoh, Tabata, et al., 1987). Thereby, Zn supplementation higher than NRC (2011) recommendation is required in rainbow trout feeds to maintain normal Zn balance in the body, as suggested from the results of present study and Read et al. (2014).

As described earlier, the Se levels in plant ingredients are low compared to fishmeal. The basal V0 diet had a total Se concentration of 0.24 mg/kg DM which is about threefold to fourfold lower than observed with a fishmeal-based diet. NRC (2011) recommends a Se requirement of 0.15 mg/kg DM based on weight gain as response criterion; while NRC (1993) had recommended 0.35 mg/kg DM which was based on maximal plasma GPx activity in rainbow trout reported by Hilton, Hodson, and Slinger (1980). Maximal weight gain at 0.2 mg/kg DM and Se accumulation in tissues beyond this level was reported in hybrid striped bass (Cotter, Craig, & McLean, 2008). With increasing dietary Se levels, while a fixed proportion was defecated, excess Se was excreted by the kidneys (Hilton, Hodson, & Slinger, 1982). Data on AAC and Se balance in the present study are in line with aforementioned study (Hilton et al., 1982) with a constant proportion (18%–20%) of ingested Se being eliminated through faeces and the non-faecal loss increasing from 30% in V0 fed fish to 50% in fish fed diet V150. Regarding the optimal inclusion level of dietary Se to fish, some of the recent reports advocate high levels of dietary Se (in mg/kg DM): 0.9–1.2 for hybrid striped bass (Jaramillo, Peng, & Gatlin, 2009); 0.8 for cobia (Liu, Wang, Ai, Mai, & Zhang, 2010); 1.18 for gibel carp (Han et al., 2011); 1.6 for largemouth bass (Zhu et al., 2012); 5.56 for yellowtail kingfish (Le & Fotedar, 2013); and 1.6 for grouper (Lin, 2014) under normal health conditions. The findings of the present study suggested that dietary supply of 0.54 mg/kg DM (0.4 mg/kg DM on available basis) was not sufficient to maintain body Se balance. Fontagné-Dicharry et al. (2015) also reported similar results in whole body Se content of rainbow trout fry fed similar V-based diets and also on GPx activity. Therefore, higher inclusion of Se in plant ingredient-based diets is recommended to maintain normal Se balance and GPx levels in the body. Supranutritional

levels of Se fortification in the form of Se-met or Se-yeast are reported to be beneficial in improving resistance to abiotic stress (Ribeiro et al., 2012; Rider et al., 2009; Saleh et al., 2014) and pathogen challenge (Le & Fotedar, 2013, 2014), particularly in early life stages.

To conclude, the mineral levels in the V0 (basal) diet covered 98.1%, 92.9%, 53.9%, 115% and 130.2% of the minimal dietary inclusion levels of Fe, Cu, Mn, Zn and Se to rainbow trout, respectively, with reference to NRC (2011) recommendations. However, data on whole body mineral contents showed that normal levels were maintained only for Cu and Mn through supply from basal V0 diet. For Zn and Se, available supply even from the highest supplemented diet (V150) was not sufficient to maintain normal body mineral levels of rainbow trout in the present study. On the whole, optimal dietary inclusion levels of microminerals are altered while using fishmeal-free diets for rainbow trout.

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