INTRODUCTION

The food fish production continues to rise worldwide, and the share of production is increasingly based on farming of fed food fish (FAO, 2018). In order to improve future sustainability and food security, new ingredients from marine and terrestrial origins are constantly searched for to satisfy the growing demand of fish feed industry (Shepherd et al., 2017; Tocher, 2015). Camelina seed (Camelina sativa) and its fractions have been studied in the diets of Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss) both as a source of oil and protein due to its robustness in farming and high lipid and n-3 fatty acid content (Anderson et al., 2018; Bullerwell et al., 2016; Collins et al., 2018; Hixson, Parrish, & Anderson, 2014a, 2014b; Hixson et al., 2016; Fraser et al., 2017; Ye et al., 2016). Camelina oil lacks long-chain n-3 fatty acids such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are important in maintaining health in humans and animals. Therefore, camelina may slightly impair the absorption of vitamin D3 (p = 0.055). Camelina diets decreased (p < 0.001) the proportion of monounsaturated fatty acids and increased (p < 0.001) polyunsaturated fatty acids and n-3 fatty acids, particularly α-linolenic acid, in fish muscle. Camelina seed proved to be a potential plant-based ingredient for rainbow trout feed.
(DHA, 22:6n-3), but distinct from other commonly used terrestrial plant oils it contains exceptionally high proportion of α-linolenic acid (18:3n-3) (Turchini et al., 2011). Rainbow trout has the ability to de-saturate and elongate 18:3n-3 to longer fatty acids of the n-3 series (Buzzini et al., 1996; Hixon et al., 2014a; Owen et al., 1975), but the ability is limited and tissue levels of long-chain n-3 fatty acids generally decrease when fish oil, rich in long-chain n-3 fatty acids, is replaced with vegetable oils (Hixon et al., 2014a; Pettersson et al., 2009; Thanuthong et al., 2011).

Inclusion of camelina in rainbow trout feed may be limited by antinutritional factors, such as glucosinolates (GSL), sinapine, phytic acid and condensed tannins, which are found in camelina seeds (Matthäus & Zubr, 2000; Russo & Reggiani, 2012; Schuster et al., 1998). Antinutrients are concentrated when seeds are processed further to products with higher protein content. Even though rainbow trout has been shown to be less sensitive for antinutritive effects of GSL than Atlantic salmon, it can still suffer from compromised growth when intake of GSL increases (Burel et al., 2000). However, due to the high crude lipid content, beneficial fatty acid composition and moderately high contents of protein, whole camelina seeds could fit the diets better than camelina meals and cakes after oil removal. The lack of various processing steps needed when using whole seed makes it cost effective raw material for feed.

Important fish quality factors for humans are nutritionally beneficial fatty acids and vitamin D. Many of the health benefits of eating fish have been linked to n-3 fatty acids, especially to 20:5n-3 and 22:6n-3. In clinical trials, fish oils have been shown to prevent arteriosclerosis, type 2 diabetes and memory disorders in the elderly (Fard et al., 2018). The fat content and the fatty acid composition of fish depend on the species, age, nutrition, body part and maturity of the fish as well as on season and living environment in general (Välimaa et al., 2019).

Fish and fish products are regarded as the most important dietary source of vitamin D for humans. Vitamin D is a hormone-like vitamin, and its deficiency in humans is common globally. The best-known function of vitamin D is its antirachitic property, but it has also numerous noncalcemic functions in the body (Autler et al., 2014). The predominant vitamin D compound in fish is cholecalciferol (vitamin D₃), which occurs in highly variable concentrations in different species. No correlation between fat or vitamin D₃ content of feed and vitamin D₃ content of the rainbow trout muscle has been found (Mattila et al., 1997, 1999). However, the transfer efficiency of vitamin D₃ from feed to rainbow trout muscle may be hampered due to the general composition of the feed (Ferreira et al., 2020).

The aim of this study was to examine the usability of camelina seeds as a component of rainbow trout feed and its effects on fish performance and nutritional composition of fish muscle including fatty acid composition and vitamin D₃ contents. To maintain the raw composition of the diet constant upon increasing camelina seed level, a mixture containing faba bean (Vicia faba), wheat (Triticum aestivum) gluten meal and rapeseed (Brassica rapa subsp. Oleifera) oil was replaced by camelina seed accordingly.

### Table 1 Nutritional composition of camelina seed, dehulled faba bean and wheat gluten meal

| Nutrient (% of air-dry weight) | Camelina seed | Faba bean | Wheat gluten meal |
|-------------------------------|--------------|----------|------------------|
| Moisture                      | 7.0          | 5.6      | 4.8              |
| Crude protein                 | 27.3         | 30.9     | 82.0             |
| Crude fat                     | 39.5         | 2.3      | 4.9              |
| Crude fibre                   | 25.9         | 8.6      | 0.6              |
| Ash                           | 4.4          | 2.3      | 1.0              |
| Glucosinolates (mmol kg⁻¹ of air-dry weight) | 35.6 | na | na |
| Total                         |              |          |                  |
| 9-methylsulfinylnononylglucosinolate | 7.6 | na | na |
| 10-methylsulfinyldecylglucosinolate | 22.6 | na | na |
| 11-methylsulfinylundecylglucosinolate | 5.4 | na | na |
| Phytic acid (% of air-dry weight) | 2.1 | na | na |

Abbreviations: na, not analysed.

### Materials and Methods

#### 2.1 Ethical statement

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. The use of animals in scientific experimentation was in line with Directive 2010/63/EU, and the study followed the protocols approved by the Regional State Administrative Agency, Helsinki, Finland.

#### 2.2 Experimental diets

A mixture of three plant ingredients, that is faba bean, wheat gluten meal and rapeseed oil, was replaced by increasing concentrations of camelina seeds (variety Calena, cultivated in Finland, Table 1). The mixture had a similar crude protein and crude fat content than camelina seed.

Three isonitrogenous and isolipidous experimental diets were formulated to meet the nutrient requirements of rainbow trout by using ingredients typically used in commercial rainbow trout feed (National Research Council (NRC), 2011; Tables 2 and 3). In all diets, 80% of the added oil was from a plant source (rapeseed oil or camelina seed oil) and 20% from fish oil. In the test diets, the mixture of plant ingredients was gradually replaced by camelina seeds (camelina seed in diet; CS0 = 0%, CS10 = 10% and CS20 = 20%).

When rapeseed oil, faba bean and wheat gluten meal were partially replaced with camelina seed, the amino acid composition of the diets showed only minor variation (Table 3). Adequate and standardized
levels of methionine, lysine and threonine were achieved by their supplementation. The proportion of oleic acid (18:1n-9) in the diets decreased while the proportion of 20:1n-9 and 18:3n-3 increased (Table 4). All diets contained similar proportions of fish meal and fish oil resulting in similar proportions of long-chain polyunsaturated fatty acids (PUFA), such as 20:5n-3, docosapentaenoic acid (DPA, 22:5n-3) and 22:6n-3. However, there was a small increase in 20:3n-3 when camelina seed was added to the diets. Changes in the proportions of other individual fatty acids were minor. The analysed vitamin D₃ contents of the feeds were higher than expected (Table 2) after vitamin premix addition (2550 IU kg⁻¹, i.e. 64 µg kg⁻¹ added). The total GSL concentration of camelina seed was 35.6 mmol kg⁻¹ (Table 1). Phytic acid concentration in the camelina seed was 20.5 g kg⁻¹. The diet CS0 did not contain GSL, and in CS10 and CS20, the total GSL content was 3.28 mmol kg⁻¹ and 5.03 mmol kg⁻¹ respectively (Table 2).

Dry feed ingredients were weighed, mixed, ground using a hammer mill (M82A, MP Pehrsson, Lapinjärvi, Finland) and conditioned 15 minute at 40–45°C using a dough kneader (Karhu, Metos) with 100°C water to get a 25% moisture content in the feed mash. Diets were extruded with a twin-screw extruder (BC-45, Creusot Loire) at 120°C barrel temperature to 5 mm pellets and dried for 12 h at 35–40°C. The pellets were top-dressed with fish and rapeseed oil mixture under a vacuum in a vacuum coater (Pegasus vacuum lab mixer, Dinnissen). Diets were stored in a cold room (+4°C).

2.3 | Fish and experimental conditions

The study was carried out between November 2018 and March 2019 in a recirculation aquaculture system (RAS) at the Parainen research unit of the Natural Resources Institute Finland (Luke). The

| TABLE 2 Ingredients and analysed nutrient composition of the experimental diets |
|---------------------------------------------|-----------------|-----------------|-----------------|
| Composition                                | Dietᵃ            | CS0             | CS10            | CS20            |
| Ingredients (% as fed)                      |                 |                 |                 |
| Camelina seed                              | 0.0             | 10.0            | 20.0            |
| Faba bean (dehulled)                        | 11.8            | 7.3             | 2.9             |
| Wheat gluten                                | 12.5            | 11.0            | 9.4             |
| Rapeseed oil                                | 19.8            | 15.9            | 12.1            |
| Soya protein meal                           | 20.0            | 20.0            | 20.0            |
| Wheat (seed)                                | 9.5             | 9.5             | 9.5             |
| Fishmeal                                    | 12.0            | 12.0            | 12.0            |
| Fish oil                                    | 5.2             | 5.2             | 5.2             |
| Blood meal                                  | 4.0             | 4.0             | 4.0             |
| Vitamin and micronutrient premix            | 1.7             | 1.7             | 1.7             |
| L-lysine sulphate                           | 1.1             | 1.1             | 1.1             |
| Monocalcium phosphateᵇ                      | 0.96            | 0.81            | 0.66            |
| Astaxanthin premix                          | 0.55            | 0.55            | 0.55            |
| L-threonine                                 | 0.44            | 0.50            | 0.56            |
| DL-methionine 100%                          | 0.26            | 0.27            | 0.27            |
| Organic selenium premixᶜ                    | 0.15            | 0.11            | 0.08            |

| Composition (% as fed unless otherwise stated) |                 |                 |                 |
| Moisture                                    | 3.3             | 3.4             | 3.3             |
| Crude protein                               | 43.3            | 43.9            | 43.8            |
| Crude fat                                   | 26.8            | 25.6            | 25.3            |
| Ash                                         | 4.87            | 4.96            | 5.08            |
| Vitamin D₃ (µg kg⁻¹ as fed)                  | 104             | 107             | 97              |

| Glucosinolates (mmol kg⁻¹ as fed)            |                 |                 |                 |
| Total                                        | nd              | 3.28            | 5.03            |
| 9-methylsulfinylnonyl-gluconosinate           | nd              | 0.73            | 1.06            |
| 10-methylsulfinyldecyl-gluconosinate          | nd              | 2.04            | 3.18            |
| 11-methylsulfinylundecyl-gluconosinate        | nd              | 0.52            | 0.80            |
| Phytic acid (% as fed)                       | 0.9             | 1.0             | 1.2             |

Abbreviations: nd, not detected (<0.05 mmol kg⁻¹ of air-dry weight).
ᵃCamelina seed in the diet: 0%, 10% or 20%.
ᵇTarget for total phosphorus content was set to 0.8% for all diets. Monocalcium phosphate was added accordingly.
ᶜSelenium content of the diets was balanced by reducing the amount of added selenium premix with the increasing inclusion level of camelina seed.

| TABLE 3 Analysed amino acid composition of the experimental diets (% as fed basis) |
|---------------------------------------------|-----------------|-----------------|-----------------|
| Essential amino acids                       | Dietᵃ            | CS0             | CS10            | CS20            |
| Arginine                                    | 2.45            | 2.52            | 2.68            |
| Histidine                                   | 1.08            | 1.09            | 1.15            |
| Isoleucine                                  | 1.65            | 1.70            | 1.77            |
| Leucine                                     | 3.17            | 3.22            | 3.33            |
| Lysine                                      | 2.94            | 3.00            | 3.16            |
| Methionine                                  | 0.94            | 0.99            | 0.93            |
| Phenylalanine                               | 2.00            | 2.04            | 2.13            |
| Threonine                                   | 2.09            | 2.20            | 2.27            |
| Tryptophan                                  | 0.53            | 0.53            | 0.55            |
| Valine                                      | 1.92            | 2.01            | 2.14            |

| Non-essential amino acids                    |                 |                 |                 |
| Alanine                                      | 1.88            | 1.97            | 2.07            |
| Aspartic acid                                | 3.37            | 3.53            | 3.87            |
| Cysteine                                     | 0.57            | 0.60            | 0.70            |
| Glutamic acid                                | 8.87            | 8.83            | 8.86            |
| Glycine                                      | 1.79            | 1.87            | 1.96            |
| Proline                                      | 2.80            | 2.69            | 2.74            |
| Serine                                       | 2.01            | 2.00            | 2.12            |
| Tyrosine                                     | 1.33            | 1.34            | 1.39            |

ᵃCamelina seed in the diet: 0%, 10% or 20%.
fish used were 0+ hatchery-reared all female rainbow trout (initial weight 155.1–156.5 g), originating from a private company (Hanka-Taimen). Acclimation to the experimental conditions commenced 4 weeks before the experiment started and during that time the fish were kept in four tanks and fed a commercial trout diet (Hercules 5.0 mm, Raisioaqua). Then, the fish were randomly divided into 12 round, 500 L fibreglass tanks, and 50 fish were placed in each tank to enable three treatments with four replicate tanks each.

The RAS consisted of 12 similar bottom-drained rearing tanks with one common water treatment system: a drum filter (mesh size of 60 µm) for solids removal, two moving bed and one fixed bed bioreactors, cascade aeration column to remove dissolved carbon dioxide, a forced-ventilated cascade to add pure oxygen and UV-disinfection unit. The brackish water (salinity 6–7 ppm) from coastal area of Baltic Sea was used as the clean replacement water at the relative water renewal rate of about 4 000–5000 L kg\(^{-1}\) feed fed.

During the experiment, the water temperature (15.3°C ± 0.15, mean ± SD) and oxygen content (9.9 mg L\(^{-1}\) ± 0.58) from one tank were automatically recorded continuously by Oxycard pond master (Arvo-Tec).

During acclimatization and experiment, fish were fed twice a day (50% and 50% of the daily dose at 05.00–05.15 and 11.00–11.15) by computer-controlled feeding system (Arvo-Tec) using published restricted autumn-feeding table of Raisioaqua. The feeding system calculates feeding and biomass growth based on tank biomass, feeding level (% of biomass) and feed conversion ratio (FCR). The feeding activity of fish was observed daily during the second feeding session and if the feeding activity began to decline, the feeding level was restricted to ensure that all the feed offered was eaten. The fish were exposed to continuous illumination provided by artificial overhead lighting. The use of animals in scientific experimentation was in line with Directive 2010/63/EU.

### 2.4 Sampling and chemical analyses

Fish were fasted for two days prior to each sampling. Biomasses were recorded at the beginning, three times during the experiment and at the end of the experiment. After each intermediate weighing, the amount of feed fed was recorded and FCR was calculated and adjusted in the feeding system accordingly if needed. A random sample of 10 fish and five fish per tank was taken at the beginning and at the end of the experiment, respectively, for chemical analysis of fish body and muscle. A skinless sample of muscle (approximately 20 g per fish) was taken between the pelvic fin and the lateral line. The remaining fish body was used for whole-body composition analysis. The individual samples were pooled to provide one common initial body sample, and 12 final samples (one per each tank) of body and muscle. The whole-body samples were minced twice using meat mincers (Tre spade and Hallde), and subsamples (approximately 200 g) were taken after that. The whole body and muscle samples were freeze-dried (Heto drywinner DW3) to a constant weight and ground to a fine powder (Grindomix GM 100, Retsch). All samples were stored in freezer at −20°C.

Samples of camelina seed, faba bean, wheat gluten meal and experimental diets were freeze-dried, ground to a fine powder and

### Table 4 Analysed fatty acid composition of the experimental diets

| Fatty acids (g/100 g fatty acids) | Diet\(^a\) | CS0 | CS10 | CS20 |
|---------------------------------|----------|-----|------|------|
| 14:0                            |          | 1.07| 1.10 | 1.15 |
| 15:0                            |          | 0.13| 0.14 | 0.14 |
| 16:0                            |          | 9.05| 9.38 | 9.78 |
| 18:0                            |          | 1.77| 1.90 | 2.06 |
| 20:0                            |          | 0.44| 0.60 | 0.77 |
| 22:0                            |          | 0.25| 0.26 | 0.27 |
| 24:0                            |          | 0.13| 0.11 | 0.14 |
| 16:1n-10 + 16:1n-9              |          | 0.10| 0.11 | 0.11 |
| 16:1n-7                         |          | 1.31| 1.32 | 1.35 |
| 16:1n-5                         |          | 0.09| 0.09 | 0.08 |
| 18:1n-9                         |          | 47.74| 40.60| 33.09|
| 18:1n-7                         |          | 3.23| 2.84 | 2.43 |
| 20:1n-9                         |          | 1.36| 3.50 | 6.10 |
| 20:1n-7                         |          | 0.10| 0.22 | 0.35 |
| 22:1n-9                         |          | 0.17| 0.68 | 1.24 |
| 24:1n-9                         |          | 0.40| 0.50 | 0.57 |
| 18:2n-6                         |          | 18.14| 17.78| 17.08|
| 18:3n-6                         |          | 0.01| 0.02 | 0.02 |
| 20:2n-6                         |          | 0.20| 0.50 | 0.83 |
| 20:3n-6                         |          | 0.02| 0.02 | 0.03 |
| 20:4n-6                         |          | 0.13| 0.13 | 0.14 |
| 22:4n-6                         |          | 0.01| 0.01 | 0.01 |
| 22:5n-6                         |          | 0.06| 0.06 | 0.06 |
| 18:3n-3                         |          | 7.85| 11.56| 15.09|
| 18:4n-3                         |          | 0.53| 0.52 | 0.54 |
| 20:3n-3                         |          | 0.06| 0.32 | 0.58 |
| 20:4n-3                         |          | 0.10| 0.10 | 0.10 |
| 20:5n-3                         |          | 1.79| 1.84 | 1.90 |
| 22:5n-3                         |          | 0.15| 0.15 | 0.16 |
| 22:6n-3                         |          | 2.96| 3.00 | 3.12 |
| 24:5n-3                         |          | 0.01| 0.01 | 0.01 |
| 24:6n-3                         |          | 0.01| 0.01 | 0.01 |
| Σ Saturated fatty acids         |          | 12.9| 13.6 | 14.4 |
| Σ Monounsaturated fatty acids   |          | 55.0| 50.4 | 45.9 |
| Σ Polyunsaturated fatty acids   |          | 32.1| 36.0 | 39.7 |
| Σ n-3 fatty acids               |          | 13.5| 17.5 | 21.6 |
| Σ n-6 fatty acids               |          | 18.6| 18.5 | 18.2 |
| n-6/n-3 ratio                   |          | 1.38| 1.06 | 0.84 |

\(^a\)Camelina seed in the diet: 0, 10 or 20%.
stored in freezer until analysed. All other analyses except fatty acid composition and contents of vitamin D₃, phytic acid and GSL were analysed in Eurofins laboratories.

Fatty acid methyl esters (FAME) of lipid in freeze-dried experimental diet samples (200 mg) were prepared in a one-step extraction-transesterification procedure using chloroform and 2% (v/v) sulfuric acid in methanol (Shingfield et al., 2003). The lipids in freeze-dried trout samples (80 mg) were extracted with methanol, MQ-water and methyl tert-butyl ether and transesterified to FAME using acetylchloride in methanol (1:9) (Ostermann et al., 2014). Fatty acid content was determined using tritridecanoin (T-135; Nu-Chek-Prep) as an internal standard and tripalmitin (T-5888; Sigma-Aldrich) as an external standard. The FAME were quantified using a gas chromatograph (model 6890 N; Agilent Technologies) fitted with a CP-Sil 88 column.

The fatty acid composition as weight percentages of 2.1 mL min⁻¹ of 206.8 kPa and nominal initial flow rate of 2.1 mL min⁻¹. The fatty acid composition was calculated using theoretical response factors (Wolff et al., 1995).

An internal standard method previously described by Mattila et al. (1992) and Mattila et al. (1999) with some modifications was used to analyse contents of cholecalciferol (vitamin D₃) in the experimental diet and fish samples. This method consists of saponification, extraction, normal-phase high-performance liquid chromatography (HPLC) purification and reversed-phase HPLC quantification. With an accuracy of 0.01 g, ca. 1 g of freeze-dried fish or ca. 2 g of feed sample was weight into a 50 ml narrow flask. Then, 0.478 µg of ergocalciferol (vitamin D₃, 99.8%, 47768, Supelco) in 1 ml of ethanol was added as an internal standard along with 0.1 g of ascorbic acid (1018, J.T. Baker) in 2 ml of distilled water, 5 ml of 50% KOH solution (pa, 1.05028, Merck) and 10 ml of ethanol (Aas, Altia). The flask was sealed and hydrolysation conducted overnight at room temperature while stirring with magnetic stirrer. Next, 15 ml of n-hexane (HPLV, 24575.320, VWR Chemicals) was added to the flask, and the flask was stirred 15 min with magnetic stirrer. The phases were left to separate, and hexane layer was transferred to the 50 ml centrifuge tube. The extraction was repeated with 15 ml of n-hexane. The combined hexane phases were washed with 20 ml of 5% NaCl (27810.262, VWR Chemicals) in water and centrifuged (Hermle Z513, 2500 rpm, 10 min, HERMLE Labortechnik GmbH), and the hexane layer was transferred to the 100 ml evaporation flask and evaporated to dryness. The residue was transferred with n-hexane to a 10 ml test tube, evaporated to dryness under nitrogen and dissolved in 1 ml of n-hexane. The extract was then purified using a semipreparative HPLC (Shimadzu LC-8a, communications bos module CBM-20A, DAD SPD-M20A, autosampler SIL-10AP, Shimadzu Europa GmbH). The mobile phase consisted of 1.2% iso-propanol (8067 J.T. Baker) in n-hexane, and the injection volume was 600 µL. After collection and evaporation, the sample was diluted to 150 µl of methanol (83638.320 VWR Chemicals). The HPLC quantification was performed using Agilent 1100 Series (US device). The injection volume was 100 µl. Otherwise, the conditions were the same as described by Mattila et al. (1992).

The recovery of spiked cholecalciferol was 110% as calculated using internal standard method. The coefficient of variation of triplicated performed analyses varied between 3.3% and 10.1%.

Glucosinolates were analysed as their desulphonated forms as described by Mattila et al. (2018). The three main GSLs in came-lina, namely 9-methylsulfinyl-nonyl-glucosinolate, 10-methysulfinyldecyl-glucosinolate and 11-methylsulfinylundecyl-glucosinolate, were quantitated by sinigrin standard with response factor 1.00. Determination of phytic acid content consisted of ferric precipitation, release of the phytate by addition sodium hydroxide and determination of the phosphorous content by the ICP-OES (Mattila et al., 2018; Plaumi & Kumpulainen, 1991).

2.5 Calculations and statistical analysis

Average initial and final weight of fish (wet weight, WW), feed intake (FI %), specific growth rate (SGR %), thermal growth coefficient (TGC × 1000), FCR and protein efficiency ratio (PER) were calculated as follows.

\[
\text{WW}_{\text{initial or final}} = \text{weight of tank biomass} \times \text{fish number in tank}^{-1}
\]

\[
\text{FI} \% = 100 \times \left( \frac{\text{Cumulative feed intake of tank biomass}}{\text{average tank biomass}^{\frac{1}{2}} \times t^{-1}} \right),
\]

where \( t \) is the number of feeding days.

\[
\text{SGR} \% = 100 \times \left( \ln (\text{final WW}) - \ln (\text{initial WW}) \right) \times t^{-1}
\]

\[
\text{TGC} \times 1000 = 1000 \times \left( \frac{\text{final WW}^{\frac{1}{2}} - \text{initial WW}^{\frac{1}{2}}}{t \times \text{average water temperature}} \right)^{-1}
\]

\[
\text{FCR} = \frac{\text{Cumulative feed intake of tank biomass}}{\text{weight gain of tank biomass}}
\]

\[
\text{PER} = \frac{\text{Weight gain of tank biomass}}{(\text{cumulative feed intake of tank biomass} \times \text{crude protein \% of feed})^{-1}}
\]

Protein retention efficiency (PRE %) describes the gain of whole-fish protein as a percentage of the protein intake from diets. It was calculated as follows: \( \text{PRE protein \%} = 100 \times \left( \frac{\text{final tank biomass} \times \text{final crude protein \% of fish}}{\text{initial tank biomass} \times \text{initial crude protein \% of fish}} - 1 \right) \times \left( \text{total feed intake of tank biomass} \times \text{crude protein \% of feed} \right)^{-1} \). In calculation of lipid retention efficiency (LRE %), the crude protein values of carcass and diets were substituted for corresponding values of crude fat (%).

Statistical analyses were performed using SYSTAT statistical software (SYSTAT, 2009). Differences among treatments were tested using an ANOVA model, and Tukey’s test was used to make
post hoc comparisons between sample means. The $p < 0.05$ was taken as the level of significance. The percentage values were arcsine transformed prior to statistical analysis. To analyse linear trends of dietary camelina seed on growth performances, a linear regression analysis was performed. The model was as follows: $A \times CS + B$, where $A$ and $B$ are constant and $CS$ is the amount (%) of dietary camelina seed.

3 | RESULTS

3.1 | Fish performance

Fish in all treatment groups had similar initial body wet weight (155.1–156.5 g), and one fish died during the experiment (group CS20). By the end of the trial, fish in all dietary groups more than tripled their initial wet weights (504.9–517.5 g). At the end of the experiment, the dietary treatments did not have statistically significant effects on the feed intake and growth performance (Table 5). However, there was a weak numerical trend that SGR %, TGC, PER and PRE % decreased whereas FCR and LRE % increased with increasing addition of camelina seeds. The PER, PRE (%) and LRE (%) were the most sensitive parameters and, as estimated by linear regression models, 10 percentage unit changes in these values would be reached with 42%, 36% and 30% addition of camelina seed respectively.

The initial moisture, crude protein and crude fat content of fish were 72.4%, 16.6% and 9.2% respectively. The final moisture content was statistically significantly lower ($p = 0.021$) in CS20 group (62.8%) than in other dietary groups (63.8%–64.5%). There were no differences in crude protein (17.1%–17.3%) or crude fat (16.7%–16.9%) content of fish body or total fatty acid content (3.5%–4.3%) of fish muscle between dietary groups (Table 6).

3.2 | Effect of camelina seeds on the vitamin D$_3$ content and fatty acid composition in rainbow trout muscle

Feeding with CS0, CS10 and CS20 resulted in 7.7, 6.1 and 6.0 µg kg$^{-1}$ vitamin D$_3$ in rainbow trout muscle samples respectively. There was a tendency that camelina enriched diets led to lower vitamin D$_3$ concentrations in the muscles ($p = 0.055$; Table 7).

Feeding CS20 resulted in the highest ($p < 0.001$) proportion of saturated fatty acids (SFA) in the muscle of rainbow trout compared with CS0 and CS10 (Table 7). The increased proportion of SFA was a result of an increase in mainly 16:0 and to a lesser extent in 18:0 and 20:0 ($p = 0.003$, $p = 0.005$ and $p < 0.001$ respectively) when camelina seed was fed in comparison with CS0. Camelina seed diets decreased ($p < 0.001$) the proportion of monounsaturated fatty acids (MUFA) compared with CS0, the values being the lowest with CS20. This decrease was attributed to the lowered ($p < 0.001$) proportions of 16:1n-7 + 16:1n-9, 18:1n-9 and 18:1n-7 in rainbow trout fed increasing amounts of camelina seed. On the contrary, 20:1n-9 ($p < 0.001$), 20:1n-7 ($p < 0.001$) and 22:1n-9 ($p < 0.001$) increased with the increased amount of camelina seed in the diet. In addition, 16:1n-7 increased ($p = 0.027$) with CS20 compared with CS0 but was not different between camelina diets or between CS0 and CS10, whereas camelina seed diets increased 24:1n-9 ($p < 0.001$) compared with CS0.

Contrary to MUFA, the proportion of PUFA increased ($p < 0.001$) in muscle from rainbow trout fed camelina diets compared with CS0, being the highest with CS20. Compared with CS0, rainbow trout fed camelina seed contained higher proportions of total n-3 fatty acids ($p < 0.001$) and the highest values were observed with CS20. Regarding the individual n-3 series fatty acids, the proportions of 18:3n-3 ($p < 0.001$), 18:4n-3 ($p < 0.001$), 20:3n-3 ($p < 0.001$), 22:5n-3 ($p < 0.001$) and 24:5n-3 ($p < 0.001$) increased with increasing amounts

### Table 5: Performance of the rainbow trouts fed the experimental diets (mean ± standard deviation, n = 4)

|                | CS0     | CS10    | CS20    | ANOVA p-value | Linear regression $R^2$ p-value |
|----------------|---------|---------|---------|---------------|---------------------------------|
| Survival (%)   | 100.0   | 100.0   | 99.5    | 0.405         |                                 |
| Initial weight (g) | 155.1 ± 2.83 | 152.9 ± 3.83 | 156.5 ± 1.03 | 0.323         |                                 |
| Final weight (g) | 517.5 ± 21.1 | 504.9 ± 25.6 | 509.6 ± 24.6 | 0.810         | -0.395 0.017 0.683             |
| Cumulative feed intake (%) | 1.73 ± 0.02 | 1.73 ± 0.03 | 1.74 ± 0.02 | 0.711         | 0.001 1.723 0.063 0.430        |
| Specific growth rate (%) | 1.45 ± 0.05 | 1.44 ± 0.04 | 1.42 ± 0.06 | 0.778         | -0.001 1.452 0.054 0.469      |
| Thermal growth coefficient × 1000 | 2.1 ± 0.09 | 2.1 ± 0.08 | 2.0 ± 0.10 | 0.822         | -0.002 2.086 0.041 0.531      |
| Feed conversion ratio | 1.18 ± 0.06 | 1.19 ± 0.05 | 1.22 ± 0.07 | 0.613         | 0.002 1.174 0.091 0.341      |
| Protein efficiency ratio | 1.96 ± 0.09 | 1.92 ± 0.08 | 1.87 ± 0.10 | 0.493         | -0.005 1.958 0.134 0.242      |
| Protein retention efficiency (%) | 34.7 ± 1.4 | 33.2 ± 1.2 | 32.7 ± 1.5 | 0.253         | -0.095 34.48 0.241 0.105      |
| Lipid retention efficiency (%) | 64.5 ± 3.3 | 65.7 ± 4.8 | 68.8 ± 3.0 | 0.390         | 0.215 64.20 0.178 0.172      |

*Camelina seed in the diet: 0, 10 or 20%.

$^b$Linear regression model, $A \times CS% + B$, where CS% is the dietary amount of camelina seed.
of camelina seed in the diet. Fatty acid 20:4n-3 was higher \( (p = 0.002) \) with CS20 compared with CS10 and CS0, whereas 20:5n-3 increased \( (p = 0.01) \) in camelina fed rainbow trout compared with CS0. However, the proportions of 22:6n-3 and 24:6n-3 in the muscle of rainbow trout were not different between diets. The proportion of total n-6 fatty acids lowered when camelina seed was fed \( (p < 0.001) \), being the lowest with CS20. This resulted in lower \( (p < 0.001) \) n-6/n-3 ratio in rainbow trout fed camelina seed compared with CS0. Regarding the proportions of individual n-6 fatty acids, 18:2n-6 decreased \( (p = 0.001) \) with increasing amounts of camelina seed in the diet, while 20:2n-6 increased \( (p < 0.001) \). Fatty acid 18:3n-6 was lower \( (p = 0.005) \) with camelina seed diets compared with CS0, but not different between CS10 and CS20. Fatty acid 20:3n-6 was lower with CS20 than with CS0 \( (p = 0.004) \), whereas 20:4n-6 was lower \( (p = 0.004) \) with CS20 compared with CS0 and CS10. No differences were observed between diets in the proportion of 22:5n-6.

4 | DISCUSSION

4.1 | Fish performance and antinutrient content of feeds

Camelina seed tested proved to be safe and potential feed ingredient. The effect of using camelina seed as a component of rainbow trout feed on the large (150–500 g) fish seems to be rather neutral, not detrimental nor beneficial. Inclusion of camelina seed did not result in statistically significant decrease in fish performance measures even though the total GSL content of diets (CS10 3.28 mmol kg\(^{-1}\) and CS20 5.03 mmol kg\(^{-1}\)) exceeded the safe upper limit of GSL intake for rainbow trout of 1.4 mmol kg\(^{-1}\) (Burel et al., 2000; NRC, 2011).

The main GSL in camelina seed is 10-methylsulfinylecyl-glucosinolate (50%–60% of the total GSLs), while the amounts of 9-methylsulfinylmonyl-glucosinolate and 11-methylsulfinylundecyl-glucosinolate are smaller. The total GSL content of camelina seed was 35.6 mmol kg\(^{-1}\) (i.e. 58.8 mmol kg\(^{-1}\) in oil-free material), which is higher than reported by Russo and Reggiani (2012) (15.2–24.6 mmol kg\(^{-1}\)) or by Russo and Reggiani (2017) (19.6–40.3 mmol kg\(^{-1}\) in oil-free material). However, Schuster et al. (1998) have reported similarly high GSL values in some camelina samples (13.2–36.2 mmol kg\(^{-1}\)).

The GSL themselves are not considered biologically active molecules but their degradation products are active and known for their various harmful biological effects. The seeds contain myrosinase enzyme, which can be released when the seeds are mechanically damaged, and the released enzyme can hydrolyse GSL into different harmful degradation products. On the other hand, heat treatment, such as extrusion, can inactivate myrosinase enzyme (Fenwick et al., 1986; Tripathi & Mishra, 2007). The GSL contents in the CS10 and CS20 diet were 15% and 35% lower, respectively, than the theoretically calculated amounts. This indicates that the GSLs in camelina seeds were not efficiently decomposed by intrinsic myrosinase during the preparation and processing of the feed. The period of time between grounding of diet mixture including camelina seeds and extruding it to pellets was 45–60 min, which probably resulted in partial decomposition of the GSL and minor effects of dietary GSL on the growth performances of fish.

Phytic acid concentration in the camelina seed was 20.5 g kg\(^{-1}\), which was slightly less than reported by Russo & Reggiani, 2012 (25.4–32.3 g kg\(^{-1}\)). The processing used in this study did not reduce the phytic acid content. The phytic acid contents of all experimental diets were rather similar (9.0–12.0 g kg\(^{-1}\)), so the possible effects of phytic acid to the results cannot be perceived. However, based on the current data, higher than 10% inclusion rate of camelina should be thoroughly considered as there appears to be a numerical tendency for decreasing growth indicators with increasing inclusion percentage. The results are in accordance with earlier reports of Bullerwell et al. (2016) and Anderson et al. (2018). They reported that 10%–15% dietary addition of ground camelina seed had only a small effect on the performances of fingerling (3–110 g) rainbow trout. At the commercial production scale and production cycles, these small differences might turn economically significant in practise.

4.2 | Fatty acid composition of rainbow trout muscle

As expected, the dietary fatty acid composition was reflected in the rainbow trout muscle. This has been shown in many earlier reports (Caballero et al., 2002; Hixon et al., 2014a; Pettersson et al., 2009; Thanuthong et al., 2011). However, due to the metabolism of fatty acids, such as chain elongation, desaturation, \( \beta \)-oxidation and de novo synthesis occurring in fish tissues, the fatty acid composition of feed is not directly proportional to the fatty acid composition of fish tissues (Tocher, 2003). In the present study, the proportion of 18:1n-9 was much higher in CS0 diet (47.7%) compared to fish muscle on diet CS0 (41.9%). This was
probably a result of the relatively rapid oxidation of 18:1n-9 for energy in rainbow trout muscle (Kiessling & Kiessling, 1993) when fish were fed CS0 rich in 18:1n-9. Thus, the partial replacement of rapeseed oil, faba bean and wheat gluten meal with camelina seed reduced the proportion of 18:1n-9 by 15 and 31% in CS10 and CS20 diets, respectively, whereas the decrease in the proportion of 18:1n-9 in rainbow trout muscle was 12 and 23% for CS10 and CS20 respectively.

| Fatty acids (g/100 g fatty acids) | Diet\(\dagger\) | CS0 | CS10 | CS20 | ANOVA | p-value |
|-----------------------------------|-----------------|-----|------|------|--------|---------|
| Vitamin D\(_3\) (µg kg\(^{-1}\) fresh muscle) | 7.7 ± 0.9 | 6.1 ± 1.0 | 6.0 ± 0.6 | 0.055 |
| 14:0 | 1.2 ± 0.0\(\text{ab}\) | 1.1 ± 0.0\(\text{b}\) | 1.2 ± 0.0\(\text{a}\) | 0.039 |
| 16:0 | 12.1 ± 0.3\(\text{b}\) | 12.7 ± 0.4\(\text{a}\) | 13.4 ± 0.3\(\text{a}\) | 0.003 |
| 18:0 | 2.5 ± 0.0\(\text{b}\) | 2.7 ± 0.1\(\text{a}\) | 2.9 ± 0.1\(\text{b}\) | 0.005 |
| 20:0 | 0.2 ± 0.0\(\text{c}\) | 0.3 ± 0.0\(\text{b}\) | 0.4 ± 0.0\(\text{a}\) | <0.001 |
| 22:0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.346 |
| 16:1n-10 + 16:1n-9 | 0.4 ± 0.0\(\text{a}\) | 0.4 ± 0.0\(\text{b}\) | 0.3 ± 0.0\(\text{c}\) | 0.002 |
| 16:1n-7 | 1.7 ± 0.1\(\text{b}\) | 1.7 ± 0.1\(\text{a}\) | 2.0 ± 0.1\(\text{a}\) | 0.027 |
| 16:1n-5 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.841 |
| 18:1n-9 | 41.9 ± 0.4\(\text{a}\) | 36.9 ± 0.8\(\text{b}\) | 32.1 ± 0.9\(\text{c}\) | <0.001 |
| 18:1n-7 | 3.1 ± 0.0\(\text{a}\) | 2.8 ± 0.0\(\text{b}\) | 2.5 ± 0.1\(\text{c}\) | 0.001 |
| 18:1n-9 | 2.3 ± 0.0\(\text{c}\) | 3.0 ± 0.1\(\text{b}\) | 4.3 ± 0.1\(\text{a}\) | <0.001 |
| 20:1n-9 | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | <0.001 |
| 22:1n-9 | 0.2 ± 0.0 | 0.4 ± 0.0 | 0.7 ± 0.0 | <0.001 |
| 24:1n-9 | 0.4 ± 0.0\(\text{b}\) | 0.5 ± 0.0\(\text{a}\) | 0.5 ± 0.0 | <0.001 |
| 18:2n-6 | 14.4 ± 0.1\(\text{a}\) | 13.8 ± 0.3\(\text{b}\) | 13.2 ± 0.3\(\text{c}\) | 0.001 |
| 18:3n-6 | 0.23 ± 0.0\(\text{a}\) | 0.21 ± 0.0\(\text{b}\) | 0.18 ± 0.0\(\text{b}\) | 0.005 |
| 20:2n-6 | 1.1 ± 0.0\(\text{c}\) | 1.2 ± 0.1\(\text{b}\) | 1.4 ± 0.0\(\text{a}\) | <0.001 |
| 20:3n-6 | 0.5 ± 0.0\(\text{b}\) | 0.5 ± 0.0\(\text{a}\) | 0.4 ± 0.0\(\text{b}\) | 0.004 |
| 20:4n-6 | 0.4 ± 0.0\(\text{a}\) | 0.4 ± 0.0\(\text{a}\) | 0.3 ± 0.1\(\text{b}\) | 0.004 |
| 22:5n-6 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.310 |
| 18:3n-3 | 4.8 ± 0.1\(\text{a}\) | 6.8 ± 0.1\(\text{b}\) | 9.2 ± 0.2\(\text{a}\) | <0.001 |
| 18:4n-3 | 0.7 ± 0.0\(\text{c}\) | 0.9 ± 0.0\(\text{b}\) | 1.0 ± 0.1\(\text{a}\) | <0.001 |
| 20:3n-3 | 0.4 ± 0.0\(\text{c}\) | 0.6 ± 0.0\(\text{b}\) | 1.0 ± 0.1\(\text{a}\) | <0.001 |
| 20:4n-3 | 0.4 ± 0.0\(\text{b}\) | 0.5 ± 0.0 | 0.7 ± 0.1\(\text{a}\) | 0.002 |
| 20:5n-3 | 1.4 ± 0.0\(\text{b}\) | 1.7 ± 0.1\(\text{a}\) | 1.7 ± 0.2\(\text{a}\) | 0.010 |
| 22:5n-3 | 0.4 ± 0.0\(\text{c}\) | 0.5 ± 0.0\(\text{b}\) | 0.5 ± 0.0\(\text{a}\) | <0.001 |
| 22:6n-3 | 7.5 ± 0.2 | 8.6 ± 0.7 | 8.3 ± 1.0 | 0.180 |
| 24:5n-3 | 0.10 ± 0.0\(\text{c}\) | 0.11 ± 0.0\(\text{b}\) | 0.13 ± 0.0\(\text{a}\) | 0.001 |
| 24:6n-3 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.3 ± 0.0 | 0.084 |
| Σ Saturated fatty acids | 16.5 ± 0.3\(\text{b}\) | 17.3 ± 0.5\(\text{b}\) | 18.3 ± 0.4\(\text{a}\) | 0.001 |
| Σ Monounsaturated fatty acids | 50.7 ± 0.4\(\text{a}\) | 46.5 ± 0.9\(\text{b}\) | 43.1 ± 1.2\(\text{c}\) | <0.001 |
| Σ Polyunsaturated fatty acids | 32.8 ± 0.2\(\text{c}\) | 36.2 ± 0.6\(\text{b}\) | 38.6 ± 0.8\(\text{a}\) | <0.001 |
| Σ n-3 fatty acids | 15.9 ± 0.3\(\text{a}\) | 19.9 ± 0.7\(\text{b}\) | 22.9 ± 1.0\(\text{a}\) | <0.001 |
| Σ n-6 fatty acids | 16.9 ± 0.1\(\text{a}\) | 16.3 ± 0.2\(\text{b}\) | 15.6 ± 0.2\(\text{c}\) | <0.001 |
| n-6/n-3 ratio | 1.1 ± 0.0\(\text{a}\) | 0.8 ± 0.1\(\text{b}\) | 0.7 ± 0.1\(\text{c}\) | <0.001 |

Note: \(a,b,c\)Values within rows that are denoted by different superscripts are significantly different from each other, \(p < 0.05\).

†Camelina seed in the diet: 0%, 10% or 20%.
However, 1.4- and 1.9-fold higher proportions of 18:3n-3 in muscle tissue were observed with CS10 and CS20, respectively, compared with CS0, which is consistent with the 1.5- and 1.9-fold increases in 18:3n-3 in CS10 and CS20 diets respectively. Salmonids have complete pathways to produce 20:5n-3 and 22:6n-3 from 18:3n-3, and the increased intake of 18:3n-3 and, to a lesser extent, 20:3n-3 increased the availability of these n-3 substrates for desaturase and elongase enzymes converting them to 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3 (Tocher, 2015). The proportions of 20:5n-3 and 22:5n-3 in the camelina seed diets were similar to CS0 as the content of fish meal and fish oil was similar in all diets, but in the muscle of rainbow trout, the increases were 1.2-fold for 20:5n-3 with both camelina seed diets and 1.2- and 1.3-fold for 22:5n-3 with CS10 and CS20 respectively. In addition, the proportions of 18:4n-3 and 20:4n-3 in the diets were similar. However, 1.3- to 1.5-fold increase in 18:4n-3 was observed in muscle with CS10 and CS20, respectively, compared with CS0, and a 1.5-fold increase in 20:4n-3 was observed with CS20 compared with CS0. This further demonstrates the bioconversion of 18:3n-3 to longer-chain PUFA. The proportion of 22:6n-3 in the fish muscle was not different between diets indicating that the conversion of 18:3n-3 to 20:5n-3 and 22:5n-3 was slightly more efficient than conversion to 22:6n-3. 22:6n-3 was only numerically but not statistically significantly increased in camelina diets compared with CS0, which implies that in this study rainbow trout was not able to convert 18:3n-3 and 20:3n-3 efficiently to 22:6n-3 after a certain threshold level of 18:3n-3 and 20:3n-3 intake. Nevertheless, the proportion of 24:5n-3, but not 24:6n-3, was increased with increases in camelina seed in the diet. Both 24:5n-3 and 24:6n-3 are intermediate products in the 22:6n-3 synthesis pathway (Buzzi et al., 1996).

The previous studies suggest that the bioconversion of 18:3n-3 to 22:6n-3 is active in rainbow trout and that the concentrations of n-3 long-chain PUFA increase with the increase in dietary supply of 18:3n-3 even though the bioconversion to 22:6n-3 is insufficient to compensate the reduced intake of 22:6n-3 (Thanuthong et al., 2011; Turchini and Francis, 2009), which is consistent with the present results. It has also been shown that the Δ6 desaturase has higher affinity towards 18:3n-3 than 18:2n-6 and Δ5 desaturase has greater affinity towards 20:4n-3 over 20:3n-6 in rainbow trout (Thanuthong et al., 2011). In addition, in Atlantic salmon the expression of Δ5 and Δ6 desaturases increased when vegetable oils replaced fish oil in the diet (Jordal et al., 2005; Zheng et al., 2005) and Δ6 desaturase had a preference for 18:3n-3 over 18:2n-6 (Zheng et al., 2005). In the present study, the proportions of 18:2n-6 and total n-6 fatty acids were slightly decreased in the diets with increasing amount of camelina seed, suggesting that the competition between n-6 and n-3 substrates in rainbow trout tissues was even lower in CS10 and CS20 compared with CS0. This could result in increasing the efficiency of chain elongation and desaturation of n-3 fatty acids in camelina seed diets.

The decrease in the proportion of total n-6 fatty acids, especially 18:2n-6, and the increase in total n-3 fatty acids in rainbow trout muscle when rapeseed oil, faba bean and wheat gluten meal were replaced with camelina seed resulted in lower n-6/n-3 ratio. In addition, although the total SFA increased and MUFA decreased in camelina fed rainbow trout, the total PUFA increased together with n-3, which is beneficial to human health. Fish are the primary source of the highly unsaturated n-3 PUFA for humans, and regarding the eating quality of cultured rainbow trout and cultured fish in general, the quantity of long-chain n-3 fatty acids in the fish muscle is an important attribute. The long-chain n-3 PUFA in seafood reduce the risk for congestive heart failure, coronary heart disease, ischaemic stroke and sudden cardiac death (Rimm et al., 2018). Despite being subtle, the increases in long-chain n-3 PUFA in rainbow trout fed camelina seed in the present study indicate increased nutritional quality inducing positive implications on human health. The results also show that by feeding camelina seeds, rainbow trout can be used to produce health promoting very long-chain n-3 PUFA to consumers. However, vegetable oils rich in 18:3n-3 cannot fully replace fish oil in the feed of cultured rainbow trout as the pathway of long-chain n-3 PUFA biosynthesis is not efficient enough to maintain the same level of 20:5n-3 and 22:6n-3 in vegetable oil-fed fish as in fish oil-fed fish (Tocher, 2015).

4.3 | Vitamin D

The vitamin D$_3$ levels in rainbow trout muscle samples (Table 7) were very low (6.0–7.7 µg kg$^{-1}$) compared with earlier published values. According to Finnish National Food Composition Table of The Finnish Institute for Health and Welfare (THL) (2020), Stancheva et al. (2013) and Jakobsen and Smith (2017) the mean contents of vitamin D$_3$ in commercial rainbow trout fillets were 51, 114 and 50 µg kg$^{-1}$ respectively. Recently, Ribeiro et al. (2017) and Ferreira et al. (2020) reported concentrations of 60 and 123 µg kg$^{-1}$ vitamin D$_3$ in fillet, respectively, in rainbow trout fed commercial diets. In our earlier study (Mattila et al., 1999), the contents of vitamin D$_3$ in individual rainbow trouts varied from 57 to 156 µg kg$^{-1}$ fillet. The low levels observed in the present study may be due to the differences in the composition of the experimental diets and the fish farming systems. For example, in Mattila et al. (1999), the feeds contained mostly ingredients of fish origin and the experiment was conducted outdoors in earthen ponds during summer. In the present study, most of the feed ingredients were of vegetable origin and the experiment was performed indoors in round, 500 L fibreglass tanks using RAS.

According to our previous studies, the origin of vitamin D$_3$ in fish is not easily explained. We have shown that vitamin D$_3$ contents vary greatly between different fish species and within the same species caught from different places (Mattila et al., 1995, 1997). Further, individual variation between fish of the same species may be great, even if they are caught from the same place. This individual variation was not explained by the season, fat content, weight, sex or age of the fish (Mattila et al., 1997, 1999). The study of Mattila et al. (1997) gave indication that the diet would be a likely factor which causes high variation. However, later we showed that the content of vitamin
D₃ in rainbow trout diet did not correlate with the vitamin D₃ content of the fillet (Mattila et al., 1999). Accordingly, in the present study, the experimental diets contained over target levels of vitamin D₃ (Table 2) and still the vitamin contents in fish muscle were low. However, one important factor may be the general composition of fish feeds. Rainbow trout feeds have changed significantly during the last 20 years. Fish-derived ingredients have been replaced with plant-derived ones (faba bean, rapeseed oil, etc.). These plant ingredients contain antinutrients which may hamper the transfer efficiency of vitamin D₃ from feed to muscle. There was indication in the present study that replacing part of faba bean, wheat gluten and rapeseed oil in the fish diet with camelina seeds may impair the transfer efficiency of vitamin D₃ to the fish muscle even more (p = 0.055).

Another factor affecting vitamin D₃ content in rainbow trout may be illumination. Quite recently Pierens and Fraser (2015) showed that rainbow trout can produce vitamin D₃ when exposed either to full spectrum simulated solar light (290–1200 nm) or to visible blue light in the wavelength range of 380–480 nm. Sunlight simulation produced more vitamin D₃ than blue light. According to this study, instead of obtaining vitamin D via diet, some fish, such as rainbow trout, may need visible light from the sun to induce vitamin D₃ production in skin to meet their vitamin D requirements. In the present study, the rainbow trout was kept indoors and was exposed to normal artificial industry lighting. In addition to feed composition, this can cause the generally low vitamin D₃ levels in the fish muscle samples.

Vitamin D deficiency is a global public health problem, especially in northern countries and amongst older people and ethnic minority groups. Generally, the daily recommendations vary between 5 and 20 µg in Europe depending on country, age and physiological condition (Mendes et al., 2020; Spiro & Buttriss, 2014). Only few foods contain naturally vitamin D, and fish is one of the most important (Spiro & Buttriss, 2014). It seems that the conditions used in this study led to very low vitamin D₃ content in rainbow trout muscle which is undesirable in terms of vitamin D intake of consumers. Currently, RAS is not widely used for rainbow trout farming but there is a tendency to increase its use due to environmental concerns as it causes less eutrophication compared to conventional fish farming practices.

5 | CONCLUSIONS

Camelina seed proved to be a potential plant-based ingredient for rainbow trout feed. No negative effects on productivity indicators were observed upon intermediate to relatively high inclusion rates. The present study confirms the earlier findings that plant oils rich in 18:3n-3 increase slightly the proportion of long-chain n-3 fatty acids in rainbow trout, which increases the quality of the fish in terms of human nutrition. This can play an important role in the future when marine resources are needed more, and they need to be utilized more sparingly. The vitamin D₃ contents in rainbow trout muscle samples were very low. The reason for low levels may be poor transfer efficiency of vitamin D₃ from feed to muscle due to great concentration of antinutrient containing plant components in feeds, non-optimal illumination conditions during fish growth or both. This requires further research not only in RAS but also in conventional cultivation system to ensure vitamin D-rich rainbow trout for consumers. Camelina production is competitive as it is a robust plant, and when it is used as seed, no elaborate processing is required. Currently high demand of camelina oil, however, might limit the availability of whole camelina seeds for fish feeding.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION

The experiment was planned by Juha Koskela, Heidi Leskinen, Pirjo Mattila, Susanna Airaksinen, Marketta Rinne and Anne Pihlanto. Heidi Leskinen, Juha-Matti Pihlava and Pirjo Mattila planned and conducted the sample preparations, method development, and the chemical analyses. The manuscript was drafted by Juha Koskela and Heidi Leskinen. Juha Koskela, Heidi Leskinen, Pirjo Mattila, Susanna Airaksinen, Marketta Rinne, Juha-Matti Pihlava and Anne Pihlanto critically examined and revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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