DOI: 10.1111/are.13678

ORIGINAL ARTICLE

WILEY Aquaculture Research

Partial replacement of soybean meal with *Methylobacterium extorquens* single-cell protein in feeds for rainbow trout (*Oncorhynchus mykiss* Walbaum)

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Funding information
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Abstract

A feeding trial was conducted with juvenile rainbow trout (15–16 g initial weight) to assess the effects of including single-cell protein (SCP) produced from *Methylobacterium extorquens* in trout feeds. Three isonitrogenous and isoenergetic diets were produced: a control diet and two experimental diets containing 5% or 10% bacterial protein meal replacing soybean meal. Triplicate tanks, each containing 35 fish, were fed each diet to apparent satiation in a constant-temperature (15°C), flow-through tank system for 12 weeks. No statistically significant differences in final fish weight or other fish growth parameters were observed. Similarly, feed efficiency parameters showed no significant differences among groups. Nutrient retention indices (protein, fat, energy) were relatively high and similar among fish in each dietary treatment group, as were whole body proximate compositions. Fish survival was high, with a small but statistically significant increase for the 10% SCP diet. Overall, results demonstrate that SCP from *M. extorquens* is a safe and effective alternative protein for rainbow trout diets at the low inclusion levels tested. Slightly lower weight gain in fish fed the 10% SCP diet was largely due to lower feed intake, suggesting that adding palatability-enhancing ingredients to feeds may allow higher levels of *M. extorquens* SCP to be used without compromising fish growth.

KEYWORDS
alternative proteins, rainbow trout, single-cell protein, sustainable feeds

1 | INTRODUCTION

For many years, fishmeal was the primary source of protein in formulated feeds for salmonids due to its competitive price relative to other protein ingredients as well as its contribution of a range of nutrients to feeds. However, a major disruption in global supply associated with an El Niño event in Peru and Chile in 1972–73 caused fishmeal prices to triple (Kolhonen, 1974). This disruption resulted in higher fish feed prices, stimulating research to identify and evaluate alternative protein ingredients for their suitability as fishmeal replacements. Among the alternate proteins evaluated at the time were SCPs from yeast and bacteria (Beck, Gropp, Koops, & Tiews, 1979; Kaushik & Luquet, 1980; Matty & Smith, 1978; Nose, 1974; Spinelli, Mahnken, & Steinberg, 1979). Collectively, studies showed that SCPs were suitable ingredients to supply a portion of dietary protein in trout feeds, although appropriate inclusion levels in feeds depended on the source of the SCP (yeast or bacteria), the substrate upon which it was grown, which fish species and life history stage was tested, and how the experimental feeds were formulated. SCP use in livestock and poultry feeds was limited by the nucleic acid content of SCPs, but trout have high levels of uricase that allows them to

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metabolize nucleic acids (Rumsey, Hughes, Smith, Kinsella, & Shetty, 1991).

Bacterial SCP produced using methane as a feedstock was first evaluated in rainbow trout feeds by Kaushik and Luquet (1980), who reported that up to 80% of fishmeal could be replaced without compromising fish performance. Skrede et al. (1998) and Storebakken et al. (1998) examined digestibility of meal produced from methanotrophic bacteria and reported values slightly lower than those for high-quality fishmeal. Perera, Carter, and Houlihan (1995) found that bacterial SCP could be included in rainbow trout diets at 17.4%, replacing 25% of the fishmeal, without adverse effects on feed consumption (based on radiography), feed efficiency or growth rate. However, these authors reported that 17.4% dietary bacterial SCP reduced protein efficiency ratio and increased nitrogen excretion, and lowered protein digestibility (85% vs. 79.9%). These effects were attributed, in part, to the nucleic acid content of bacterial SCP. Nucleic acids contribute to dietary nitrogen content but are non-protein nitrogen compounds. It is difficult to draw generic conclusions from these studies, however, as different animals have distinct nutritional and immune responses, and the distinct formulations utilized represent the varied goals of these studies. Furthermore, there can be substantial differences in the composition of a bacterial SCP depending upon the species being utilized. These differences arise in the gross macromolecular composition (e.g., the percentage of protein, lipids and nucleic acids), intracellular storage compounds (e.g., glycogen vs. poly-β-hydroxybutyrate), surface molecules that may interact with the immune system (e.g., extracellular polysaccharides and lipopolysaccharides), and many other attributes.

Methylobacterium extorquens is a non-pathogenic, plant epiphyte notable for the ability to consume methanol as well as a variety of multi-carbon substrates such as ethanol, glycerol or many organic acids (Ochsner, Sonntag, Buchhaupt, Schrader, & Vorholt, 2015). Methanol is a particularly attractive feedstock due to its abundant availability from either geological or biological sources of natural gas. Because of this, methanol-based biotechnology has seen considerable development in the past decade (for review Schrader et al., 2009) and the vast majority of this has occurred in *M. extorquens* (Bélanger et al., 2004; Tlusty et al., 2017) because of its genetic tractability (Marx, 2008; Marx & Lidstrom, 2001; Schada von Borzyskowski, Remus-Emsermann, Weishaupt, Vorholt, & Erb, 2014) and proven track record for high-density fermentation (Bélanger et al., 2001; Bourque, Pomerleau, & Groleau, 1995; Tlusty et al., 2017). As a model system, the nutritional value of new SCP products can be manipulated to enhance levels of targeted essential amino acids, alter levels of major biomass constituents or to produce other high value molecules.

KnipBio is a US Corporation that has recently demonstrated that *M. extorquens* (KnipBio meal, or KBM) can serve as a feed ingredient in diets for a variety of aquatic organisms (Tlusty et al., 2017). Initial studies with KBM in fish and shrimp feeds reported positive growth results with Pacific white shrimp (*Litopenaeus vannamei*) and smallmouth grunts (*Haemulon chrysargyreum*). Feeds with KBM had no effect on sensory attributes of Pacific white shrimp. Interestingly, addition of KBM to feeds did not lead to significant changes in the shrimp gut microbiome, as is sometimes the case with alternative feeds (Zhou, Ringø, Olsen, & Song, 2017). KBM was also reported to be highly digestible to Atlantic salmon (*Salmo salar*) (Tlusty et al., 2017) and to rainbow trout (https://www.ars.usda.gov/pacific-west-area/aberdeen-id/small-grains-and-potato-germplasm-research/docs/fish-ingredient-database/).

The objective of the current study was to evaluate KBM as a feed component for rainbow trout (*Oncorhynchus mykiss*) in a laboratory-scale feeding trial, focusing on fish growth and survival. The length of the trial was of sufficient duration to assess the apparent nutritional quality of feeds in which soybean meal (SBM) was partially replaced with KBM while other protein source levels were kept constant to avoid confounding effects associated with feed formulation differences.

2 | MATERIALS AND METHODS

2.1 | Experimental feeds

KBM supplied by Knipbio was analysed for proximate composition as described below prior to feed formulation (Table 1). *M. extorquens* (strain KB203) was grown by aerobic fermentation at 30°C with standard procedures for assuring purity of the seed cultures, as described previously (Bélanger et al., 2004; Tlusty et al., 2017). The defined CHOI4 medium and trace metals stock solution were used throughout the process, ultimately culminating in growth in a 1,500 L fermenter as before (Bélanger et al., 2004; Tlusty et al., 2017). The collected biomass was spray-dried (Tlusty et al., 2017) to generate a dry powder or meal (KBM) that was used in the feeds.

Three diets, a control diet plus two experimental diets, were formulated using software (WinFeed 2.8, Cambridge, UK) to contain 45% crude protein, 18% lipid, 3.1% lysine and 1% methionine (as-is basis, Table 2). The diets were as follows: Diet 1: Control – standard level of fishmeal in commercial trout feeds;

Diet 2: 5% KBM replaced SBM on a crude protein basis; and Diet 3: 10% KBM replaced SBM on a crude protein basis.

All diets met or exceeded the minimum nutrient requirements of rainbow trout (NRC, 2011). Experimental feeds were produced by extrusion pelleting at the Bozeman Fish Technology Center, Bozeman, MT. All ingredients were ground to a particle size of <200 μm using an air-swept pulverizer (Model 18H, Jacobsen, Minneapolis, MN, USA) and processed into pellets using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with a ~25 s exposure to 127°C in the extruder barrel (average across 5 sections). Pellets were dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland) for 20 min at 102°C with a 10 min cooling period, resulting in final moisture levels of less than 10%. Added oil was top-coated using a vacuum-coater (AJ Mixing, Ontario, CA, USA). Diets were placed in plastic-lined paper bags, shipped to the University of Idaho's Hagerman Fish Culture Experiment Station and stored at room temperature until fed.

TABLE 1 Proximate and amino acid composition of bacterial protein meal (% as-is basis unless mentioned otherwise)

Nutrient	Percentage
Dry matter	96.33
Crude protein (N × 6.25)	50.88
Crude fat	0.84
Ash	4.12
Organic matter	92.21
Gross energy (MJ/kg)	21.2
Essential and semi-essential amino acids	
Arginine	3.28
Histidine	1.13
Isoleucine	1.72
Leucine	3.32
Lysine	2.5
Methionine	0.88
Cysteine	0.34
Phenylalanine	2.03
Tyrosine	1.44
Threonine	1.97
Valine	2.75
Non-essential amino acids	
Alanine	3.75
Aspartic acid	4.39
Glutamic acid	6.32
Glycine	2.52
Hydroxyproline	0.02
Proline	1.87
Serine	1.88

2.1.1 | Fish and feeding

The feeding trial was conducted at the Hagerman Fish Culture Experiment Station. Rainbow trout fingerlings, hatched from eggs purchased from a commercial source (TroutLodge, Sumner, WA, USA) were used in the study. Thirty-five fish (initial average weight: 15.6 g) were stocked into each of nine 145-L tanks. Each tank received 10–12 L/min of constant temperature (15°C) spring water supplied by gravity. Using a completely randomized design, three triplicate tanks were assigned to each of the three experimental diets. Each diet was fed by hand by trained staff three times per day to apparent satiation, 6 days per week, for 12 weeks. Photoperiod was held constant at 14 hr light: 10 hr dark with fluorescent lights on electric timers. Tanks were cleaned daily and any mortality was removed and recorded when first noticed. Fish were bulk-weighed and counted every 3 weeks for the duration of the study. Twenty fish from the initial population used in the study were sacrificed and frozen at −20°C for later whole-body proximate analysis. At the end of 12 weeks, five fish per tank were removed and euthanized with MS-222 (250 mg/L). Fish were pooled by tank and frozen at −20°C for proximate analysis. Experimental protocols used in the growth trial were approved in advance by the University of Idaho's Institutional Animal Care and Use Committee.

TABLE 2 Ingredient and proximate composition of experimental diets with graded levels of bacterial protein meal fed to juvenile rainbow in the growth trial (g/kg, as-fed basis unless mentioned otherwise)

Ingredients	Diet 1 Control	Diet 2 5% KBM	Diet 3 10% KBM
Fishmeal, sardine	300.00	300.00	300.0
Poultry by-product meal, feed grade	120.5	120.5	120.5
Soybean meal, dehulled & solvent-extracted	160.0	108.0	56.0
Bacterial protein meal	0.0	50.0	100.0
Soy protein concentrate, Profine VF	90.0	90.0	90.0
Wheat gluten meal	20.0	20.0	20.0
Wheat flour	161.1	160.7	160.7
Dicalcium phosphate	4.2	6.5	8.4
Trace mineral mix, Trouw Nutrition[a]	1.0	1.0	1.0
Vitamin premix, ARS 702[b]	10.0	10.0	10.0
Choline chloride (60%)	6.0	6.0	6.0
Vitamin C (Stay-C, 35%)	2.0	2.0	2.0
Fish oil	125.2	125.3	125.4
Proximate composition (analysed)			
Moisture	25.3	24.9	20.7
Crude protein (N × 6.25)	486.3	486.2	485.8
Crude fat	148.0	153.6	156.2
Ash	101.9	103.5	101.2
Total phosphorus (calculated)	14	14	14
Gross energy (MJ/kg)	21.5	21.6	21.9

[a]Trace mineral premix supply the following to the diet (mg/kg diet): Zn (as $ZnSO_4$ $7H_2O$), 50; Mn (as $MnSO_4$), 7.5; Cu (as $CuSO_4$ $5H_2O$), 2.5; I (as KIO_3), 1; selenium, 0.05.

[b]Vitamin premix supply the following to the diet (mg/kg diet): D calcium pantothenate, 46.47; pyridoxine (pyridoxine HCl), 13.68; riboflavin, 9.58; niacinamide, 21.78; folic acid, 2.49; thiamine (thiamine mononitrate), 9.1; inositol, 599; biotin, 0.33; vitamin B_{12}, 0.03; menadione sodium bisulphite complex, 1.1; vitamin E (DL α-tocopherol acetate), 131.9 IU; vitamin D_3 (stabilized), 6594 IU; vitamin A (vitamin A palmitate, stabilized), 9641 IU; ethoxyquin, 198.

2.1.2 | Chemical analysis

Proximate composition of bacterial meal, experimental feeds and whole-body fish samples were determined using AOAC (2002) procedures. Fish samples pooled by tank were pureed using an industrial food processer. Briefly, samples were dried in a convection oven at 105°C for 12 hr to determine per cent moisture. Dried samples were finely ground by mortar and pestle and analysed for crude protein (total nitrogen × 6.25) using the combustion method with a nitrogen determinator (TruSpec N, LECO Corporation, St. Joseph,

TABLE 3 Growth performance and feed utilization of juvenile rainbow trout fed diets with graded levels of bacterial protein meal for 12 weeks[1,2]

	Diet 1 Control	Diet 2 5% KBM	Diet 3 10% KBM
Initial weight (g/fish)	15.6 ± 0.2	15.8 ± 0.1	15.5 ± 0.1
Final weight (g/fish)	236 ± 1	233 ± 7	220 ± 5
Weight gain (g/fish)	220 ± 1	218 ± 7	204 ± 5
Mean weight gain (%)	1417 ± 11	1381 ± 35	1318 ± 21
Specific growth rate (SGR, %/d)	3.24 ± 0.01	3.21 ± 0.03	3.16 ± 0.02
Feed consumed (g/fish)	187 ± 2	180 ± 5	172 ± 4
Feed conversion ratio	0.85 ± 0.01	0.82 ± 0.01	0.84 ± 0.00
Protein efficiency ratio (PER)	2.42 ± 0.04	2.49 ± 0.02	2.44 ± 0.01
Survival (%)[3]	96.2 ± 0.9[b]	98.9 ± 0.9[ab]	100[a]

[1]Mean ± *SE* (n = 3) in the same row that share the same superscript or do not have superscripts are not statistically different ($p > .05$; Completely Randomized Design, One-factor ANOVA; Tukey's HSD Test).
[2]All calculations were performed on an average fish weight basis.
[3]Each treatment group consisted of 105 fish (three tanks, 35 fish per tank).

MI, USA). Crude fat in the extruded feeds was measured after acid hydrolysis with an ANKOM HCL hydrolysis system (ANKOM Technology, Macedon, NY, USA) by extraction with petroleum ether using an ANKOM XT15 extractor. For other samples, crude fat was analysed without the acid hydrolysis step. Ash was analysed by incineration at 550°C in a muffle furnace for 5 hr. Energy contents of samples were determined using an isoperibol bomb calorimeter (Parr 6300, Parr Instrument Company Inc., Moline, IL, USA). All analyses were conducted in duplicate.

2.1.3 | Calculations

Using the live-weight and feed consumption data, the following indices were calculated as per Hardy and Barrows (2002):

$$\text{Weight gain (g/fish)} = (\text{g mean final weight} - \text{g mean initial weight});$$

$$\text{Mean weight gain (\%)} = [(\text{g mean final weight} - \text{g mean initial weight})/\textit{g mean initial weight}] \times 100;$$

$$\text{Specific growth rate (SGR, \%/d)} = [(\ln \text{mean final weight} - \ln \text{mean initial weight})/\text{number of days}] \times 100;$$

$$\text{Feed consumed (g/fish)} = \text{g total feed consumed/number of surviving fish};$$

$$\text{Feed conversion ratio (FCR)} = \text{g feed consumed per fish/g wet } \textit{weight gain perfish};$$

$$\text{Survival(\%)} = (\text{number of fish at the end of the trial/number } \textit{of fish at the beginning}) \times 100;$$

$$\text{Protein efficiency ratio (PER)} = \text{g weight gain/g protein consumed};$$

$$\text{Nutrient retention(\%)} = (\text{g nutrient gain/g nutrient consumed}) \times 100; \text{and}$$

$$\text{Energy retention (ER, \%)} = (\text{MJ energy gain/MJ energy consumed}) \times 100$$

2.1.4 | Statistical analysis of data

Tank mean values were used as units of observation for statistical analysis. Fish growth performance, feed utilization, body composition and nutrient retention data were tested for normality and homogeneity of variance prior to one-way Analysis of Variance (ANOVA). When significant differences were found, data were subjected to Tukey's HSD test to separate the means at a significance level of $p < .05$. All statistical tests were performed with SAS 9.4 software for personal computers (SAS Institute Inc. Cary, NC, USA).

3 | RESULTS

The crude protein content of KBM was 51%; fat and ash levels were low in the product (Table 1). The gross energy was 21.2 MJ/kg. The sum of amino acids in the product indicated that approximately 9% of the product was non-protein nitrogen. The proximate composition and energy content of diets used in the growth trial are presented in Table 2. Moisture was low in the diets (2.07%–2.53%). Crude protein content was similar across the diets (~48.6%) and higher than expected. Measured crude fat levels ranged from 14.8% (Diet 1, Control) to 15.62% (Diet 3, 10KBM). Dietary ash levels were similar (10.12%–10.35%), as were gross energy levels, 21.5 MJ/kg in the control diet to 21.9 MJ/kg in diet 3.

After 12 weeks of feeding, mean final weights of fish were similar (220–236 g) with no statistically significant differences among treatment groups (Table 3). Mean feed intake (g/fish) decreased with increasing percentages of KBM in the diet. Feed conversion ratios were low and similar (0.82–0.85) among the dietary treatments. Protein efficiency ratio varied from 2.42 (control diet) to 2.49 (diet 2). There were no significant differences among the dietary groups with respect to the feed utilization indices. Whole-body proximate composition and energy content of fish did not differ significantly among the dietary treatments (Table 4). There were no significant differences among the dietary groups in nutrient retention values (Table 4).

Survival was high across the treatments (96.2%–100%). Fish fed the 10KBM diet had a small, but statistically significant increase in survival compared to those fed the control diet. The fish fed the 5KBM diet had intermediate survival not statistically separable from either of the other two treatments.

4 | DISCUSSION

The results of this study demonstrate that KBM produced from *M. extorquens* is a safe and effective dietary ingredient in trout feeds

TABLE 4 Whole-body proximate composition and nutrient retention of juvenile rainbow trout (average initial weight, 15.6 g) fed experimental diets containing graded levels of bacterial protein meal for 12 weeks (%, wet basis)[a,b]

	Initial fish[c]	Diet 1 Control	Diet 2 5% KBM	Diet 3 10% KBM
Proximate composition				
Dry matter	23.5	31.0 ± 0.4	31.0 ± 0.3	31.2 ± 0.4
Crude protein	13.8	16.6 ± 0.1	17.0 ± 0.1	16.7 ± 0.1
Crude fat	7.07	11.9 ± 0.5	11.6 ± 0.3	12.2 ± 0.5
Ash	2.13	2.00 ± 0.01	1.91 ± 0.07	2.00 ± 0.11
Gross energy (MJ/kg)	6.03	8.61 ± 0.16	8.56 ± 0.14	8.72 ± 0.19
Nutrient retention				
Fat		97.3 ± 3.4	94.3 ± 2.0	95.3 ± 4.3
Protein		40.7 ± 0.8	43.0 ± 0.7	41.3 ± 0.4
Energy		48.2 ± 0.6	49.0 ± 0.6	48.4 ± 1.1

[a]Mean ± *SE* (n = 3) in the same row that share the same superscript or do not have superscript are not statistically different ($p > .05$; Completely Randomized Design, One-factor ANOVA).
[b]Five fish from each tank were pooled for analysis.
[c]Initial fish composition values were not included for statistical analysis.

at relatively low levels of inclusion. Although growth indices numerically decreased with increased inclusion of KBM, the absolute decrease (g) was nearly equivalent to a decrease in feed intake. Given equivalent feed conversion ratios among dietary treatment groups, differences in feed intake explain the weight gain trend with increasing KBM dietary percentage. Kaushik and Luquet (1980) also recorded a decrease in feed intake in trout fed diets with increasing levels of meal produced from methanotrophic bacteria. Similarly, Kiessling and Askbrandt (1993) noted a decrease in feed intake and trout weight gain when fish were fed a diet containing bacterial protein meal produced from *Corynebacterium glutamicum* at only 4% of the feed, but not when fish were fed a diet with up to 16% bacterial protein meal produced from *Brevibacterium lactofermentum*. In contrast, other studies with trout or Atlantic salmon have not reported a reduction in feed intake associated with dietary level of meal produced from methanotrophic bacteria (Aas, Grisdale-Helland, Terjesen, & Helland, 2006; Aas et al., 2006). While the source of bacterial protein meal and the process by which it was dried likely affect feed intake, the primary factor accounting for these differences are the feed formulations used in various trials. For example, Aas et al. (2006) did not observe reductions in weight gain of trout fed diets containing up to 27% methanotrophic bacteria meal over the course of a 76-day feeding trial. However, fishmeal levels in feeds were high, 63.5% in the control diet and 40.7% in the diet containing the highest level of bacterial meal increased. This contrasts with the study of Kaushik and Luquet (1980) in which the fishmeal level was 35% in the control diet and other protein sources were used in experimental feeds. The conclusion by Kaushik and Luquet that 80% of fishmeal could be replaced with methanotrophic bacteria meal must be tempered by the fact that the highest level of bacterial meal in their study was 35%, and that feed intake and fish performance were reduced at levels above 21%. In a study in which feeding rate was held constant, three levels of bacterial protein were used to replace fishmeal in diets for rainbow trout (Perera et al., 1995). At each level of bacterial protein in diets (17.4%, 43.5% and 69.5%), fish weight gain was significantly reduced. In this context, it is notable that increasing levels of KBM did not alter either the feed conversion ratio or the protein efficiency ratio. These results confirm that the nutritional quality of the diets was not affected by KBM inclusion.

In the present study, nitrogen retention values were similar to those found in Atlantic salmon (Aas et al., 2006) and rainbow trout (Aas et al., 2006). Inclusion of KBM did not affect nitrogen retention in rainbow trout. Research with methanotrophic bacterial SCP in feed for Atlantic salmon produced results similar to those found with rainbow trout. Aas et al. (2006) fed diets containing 0%, 4.5%, 9%, 18% and 36% methanotrophic bacteria SCP that replaced fishmeal in diets for salmon. The highest and lowest levels of fishmeal in diets were 65% and 35.4%. Over the course of the 52 day study, fish approximately doubled their initial weight (170 g). In contrast with the results of Storebakken, Baeverfjord, Skrede, Olli, and Berge (2004), who had found decreased growth for bacterial SCP above 20% inclusion, fish weight gain increased with increasing levels of methanotrophic bacteria meal in the diet. Nitrogen retention also increased with increasing bacterial SCP in the diet, despite the fact that digestibility values for nitrogen, sum of amino acids, lipid and energy decreased as the bacterial SCP level in the diet increased. Increased retention suggests that diets containing higher levels of bacterial SCP resulted in more efficient metabolism of these nutrients rather than more efficient digestion, resulting in lower catabolic losses. A challenge for interpretation of these studies, however, is the very high and variable percentages of fishmeal used. Importantly, fishmeal levels in the current study were held constant at 30% across the three experimental diets, with KBM replacing soybean meal. This formulation thus provides a more realistic assessment of KBM as an alternative feed ingredient for salmonids than many previous studies that used high and impractical fishmeal levels higher than commonly used in commercial trout feeds.

Although cumulative mortality was low throughout the feeding trial, 10% KBM inclusion led to a small but statistically significant increase in survival compared to the control diet (with the 5% inclusion treatment group being intermediate). Additional work will be required to confirm this effect, but it should be noted that there is precedent for SCP sources to increase survival and to reduce gastroenteritis (Banerjee, Azad, Vikineswary, Selvaraj, & Mukherjee, 2000; Dabrowski, 1984; Laranja et al., 2014; Romarheim, Øverland, Mydland, Skrede, & Landsverk, 2011) and thus this may represent a significant value added even at a modest inclusion rate.

The gross energy of KBM measured in this study was 21.1 MJ/kg, of which 11.045 MJ/kg is accounted for by protein and lipid in the KBM. Other constituents in KBM, primarily poly-β-hydroxybutyrate and non-protein nitrogen, contributed the remaining amount of gross energy, approximately 10.15 MJ/kg. The apparent digestibility coefficient values of energy and protein in KBM meal are reported to be 58% and 73% respectively ((https://www.ars.usda.gov/pacific-west-area/aberdeen-id/small-gra

ins-and-potato-germplasm-research/docs/fish-ingredient-database/). This implies that the poly-β-hydroxybutyrate and non-protein nitrogen fractions of KBM meal contribute digestible energy to rainbow trout.

These preliminary findings that KBM is a safe and suitable protein ingredients for trout open the door to a variety of future innovations. As it has been found that bacterial protein products' quality and nutrient profile vary widely due to substrate and conditions of fermentation, type of bacteria and processing after fermentation (Øverland, Tauson, Shearer, & Skrede, 2010), there exists a wide space of design choices that can be exploited to produce KBM that might further enhance the growth or survival traits conferred. Future developments may include process conditions or gene deletions that affect macromolecular composition, such as per cent of protein or poly-β-hydroxybutyrate, but can also include the inclusion of novel genes to produce high-value molecules that can be supplied directly in the feed ingredient.

5 | CONCLUSION

The present study evaluated KBM as an alternative protein source in feeds for juvenile rainbow trout during a 12-week growth trial. Results showed that KBM can be included up to 10% replacing soybean meal in a rainbow trout diet without significantly affecting growth performance, feed utilization, nutrient retention or fish health. A small but statistically significant increase in survival under benign rearing conditions warrants further investigation to assess if KBM increases fish survival under production conditions.

ACKNOWLEDGMENTS

KnipBio Inc. provided the single-cell protein meal used in the study and funding to conduct the study at the University of Idaho. KnipBio was involved in designing the study and in manuscript preparation but had no role in conducting the study or in data collection, analyses or interpretation. Catherine Pujol-Baxley, Christopher J. Marx and Lawrence Feinberg are employees of KnipBio Inc., Lowell, MA 01451, USA.

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How to cite this article: Hardy RW, Patro B, Pujol-Baxley C, Marx CJ, Feinberg L. Partial replacement of soybean meal with *Methylobacterium extorquens* single-cell protein in feeds for rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquac Res*. 2018;00:1–7. https://doi.org/10.1111/are.13678