Dear Dr. Gobet,

We thank you and the reviewers for your effort and time on the detailed revision and suggestions for the improvement of our manuscript. We provide below a point-by-point response (in red letters) to all these comments. We have addressed all the comments, and we believe that the manuscript has been improved.

On behalf of all the authors,
K. Kormas & S. Stefanos Katsoulis-Dimitriou

Dear Mr Katsoulis-Dimitriou and co-authors,

Thank you for submitting your manuscript for a recommendation from PCI microbiology. I apologize for the time it took to hand in the recommendation. Please see below some comments and suggestions from Dr Liu, an anonymous reviewer, and I. Please address them as much as you can and I look forward to receiving a revised version of the manuscript.

General comments
Overall, this is an interesting study with a good potential to better understand the effect of algal-based diets on the gut microbiota of the sea bream. However, I feel that the manuscript needs some work and rewriting before publication.

In the introduction, some rearrangements may be needed but the information is there. In the **materials and methods**, more details are needed to understand better what has been done in the study and for it to be reproducible. More details on **the interpretation of the results** are also needed, please spend **more time on describing** the figures. Also, for each paragraph in the results, one sentence to introduce the content would help the reading and the description of the figures and tables must be **much more detailed by giving values**. In the discussion, the authors may also consider that the diet likely comes along with its own microbiota, for instance see some literature on the phycosphere.
---We have followed your advice accordingly. Regarding the suggestion on the phycosphere we have added the following sentence in the DISCUSSION "As it has been shown that the phycoshpere of cultivated microalgae used as feed might affect the fish gut microbiota in early developmental stages (e.g. Nikouli et al. 2019), in the future the impact of the bacteria contained in the microalgae to be incorporated in the aquafeed should be investigated as well." [L. 324-327 of the revised manuscript]

Throughout the manuscript, please verify that all taxonomic names are in italic. Also, the manuscript may be carefully re-read by the authors to rewrite several sentences to ease the reading.
---Checked and corrected. We have changed, mostly shortened, some sentences as well.

Specific comments
Abstract
To introduce the aim of the study, I would add one or two sentences of context.
---It now reads "It is well known that the gut microbiome and its interaction with the host influence several important factors for fish health such as nutrition and metabolism. Diet is one of the main factors influencing the composition of the gut microbiome in reared fish.

Microalgae, due to their high fatty acid content, appear to be a promising alternative for replacing fish oil in aquafeed. Thus, the aim of this work was to evaluate the effects of dietary microalgae blends as fish oil replacers on the midgut bacterial microbiota of gilthead sea bream (*Sparus aurata*).” [L. 18-24 of the revised manuscript].

L23: There is a typing mistake: “Mic**rochloropsis”.
---Corrected.

L25-27: As a teaser to the audience, the authors may add some details on the differences between FO and the other diets, maybe the differences in taxonomic composition?
---It now reads “The midgut bacterial community composition of the experimental diets was altered compared to the control diet. There were 11 operational taxonomic units (OTUs) which were highly abundant in FO compared to the three experimental diets (FO,MI,SP) and two OTUs that were found in high abundance in both FO and the experimental diets in all comparisons (FO-MI, FO-PI, FO-SP). Most of the highly abundant OTUs in the experimental diets were unique to each experimental diet, with two OTUs being found in common between FO-MI and FO-PI.” [L. 28-34 of the revised manuscript].

L28: There is a typing mistake: “suggest*ed*”, description of results should be in the past tense.
---Corrected.

L30: Please be more specific on the importance of fucose. Is fucose a storage carbohydrate? Part of the cell wall? Part of a specific metabolism?
---It now reads “The overexpressed pathway was related to the metabolism of fucose, a major cell wall exopolysaccharide of several microalgal species.” [L. 36-38 of the revised manuscript].

L34-35: Give some names of the genera potentially beneficial?
---It now reads “The MI feed seems to promote several beneficial bacteria with potential probiotic abilities in the fish gut, belonging to the *Pseudoalteromonas, Pseudomonas, Bacillus* and *Rhodopseudomonas* genera.” [L. 42-45 of the revised manuscript].

Introduction
In the introduction, the authors may consider putting the 2nd paragraph first, and the first as second which would more smoothly introduce the third paragraph.
---Thank you for this suggestion, we see now that it fits better.

L47: Rewriting suggestion: “…fish stocks and thus reducing their sustainability.”.
---Corrected as sugested.

L47-48: Please replace “For this” by “As a result”.
---Corrected.

L50-51: Please rephrase: “Among these alternatives, microalgae are suitable and sustainable…”. Also add some details/examples about the promising results and do not forget about oomycetes.
---It now reads “Among these alternatives, microalgae and oomycetes are suitable and sustainable feed ingredients. Relevant studies showed that microalgae/oomycetes containing feeds have high digestibility, positive effect on the growth rate of aquatic species, high

quantities of important pigments like carotenoids and phycobiliproteins, polysaccharides with potential antiviral and antibacterial properties, potential immunostimulant and probiotic effects and low carbon footprint for their production (Shah et al. 2017, Ahmad et al. 2022, Ma & Hu 2024)." [L. 71-77 of the revised manuscript].

L52: Please rephrase: "In *reared* fish,…".
---Corrected.

L56: I would remove "in".
---Corrected.

L60: I believe you can talk about dysbiosis here. There are several articles/reviews on the topic: https://onlinelibrary.wiley.com/doi/full/10.1111/raq.12862
https://www.frontiersin.org/journals/immunology/articles/10.3389/fimmu.2020.00114/full
---Added "Gut microbiota dysbiosis is common in aquaculture species and is associated with changes in microbial structure, an increase in pathogenic microorganisms and/or decreases in the abundance of beneficial taxa (Xavier et al., 2023)." [L 59-63 of the revised manuscript].

L63-66: These sentences may be kept at the end of the introduction before presenting the objectives of the study.
---Corrected as sugested and we have adapted the last paragraph accordingly [L. 78-87 of the revised manuscript]

L91: Please replace "However" by "To our knowledge".
---Corrected.

Materials and methods
L102-105: The authors may add more background on the way the fishes were reared in the feeding trial and also for instance, their age, sex, the feeding diet during the trial, their health state. This would help understand how homogeneous the chosen specimens were before the current study.
L105-106: Following the previous comment, details on the composition/homogeneity of each group may be informative here.
---As previously mentioned, detailed information about fish rearing is provided in another manuscript that has been submitted for publication. In response to the reviewer's comment, we have added in the "Sampling" section of the "Materials and methods" a more descriptive text about fish rearing. Please let us inform you that gilthead seabream is a protandric hermaphrodite and it is meaningful to state its sex in the text [L. 116-139 of the revised manuscript].

L115-116: ten individuals were randomly collected from each dietary group but at the beginning of the paragraph it is said that 40 specimens were put into 4 groups. So, if I understand this well, all fishes were collected for each diet, right?
---Yes.

L116: The concentration of the anesthetic may be added here.
---Corrected to "…of the anesthetic 2-phenoxyethanol (450 mg l^{-1}, 15 min) and placed…" [L. 140-141 of the revised manuscript].

L118: There is a typing mistake, please replace “its” by “each”.
--- Corrected.

L124: There is a typing mistake, please add “gene” in “16S rRNA gene amplicon sequencing”. Please check the article for this mistake throughout the article.
---Corrected and checked throughout the manuscript.

L126: If the sequencing platform gave the information, maybe add at least the Tm of the PCR cycle.
---Added [L. 151 of the revised manuscript]

L129-130: A couple of lines indicating what the Mothur MiSeq SOP is doing may help the reader.
---Added “The 16S rRNA gene sequencing raw data were processed using the MOTHUR MiSeq standard of protocol procedure (Schloss et al. 2013); MOTHUR is a stand-alone bioinformatics platform covering the entire procedure from raw sequencing data to bacterial taxa 16S rRNA gene abundances.” [L. 155-158 of the revised manuscript]

L131: There is a typing mistake, please replace “() with PRJNA1068122 BioProject accession number” by “under the BioProject accession number PRJNA1068122”.
---Corrected. We also addd the webpage of SRA [L.168 of the revised manuscript]

L133: Were sequence identified as non-bacterial groups removed? (e.g. chloroplasts, mitochondria, eukaryotes). Please specify.
---We added the sentence “Sequences assigned as mitochondria or chloroplasts were removed from subsequent analysis.” [L. 164 of the revised manuscript]

L133-135: Here, add some details on the parameters chosen to use blastn and also the accession numbers of the closest relatives should be written somewhere (as supplementary information for instance?).
---Details added “The search was conducted with the following parameters: Standard databases, highly similar sequences (Megablast) and closest relatives were considered with percentage identity higher than 90%.”. [L. 170-172 of the revised manuscriot]. Accession numbers added on Table S4.

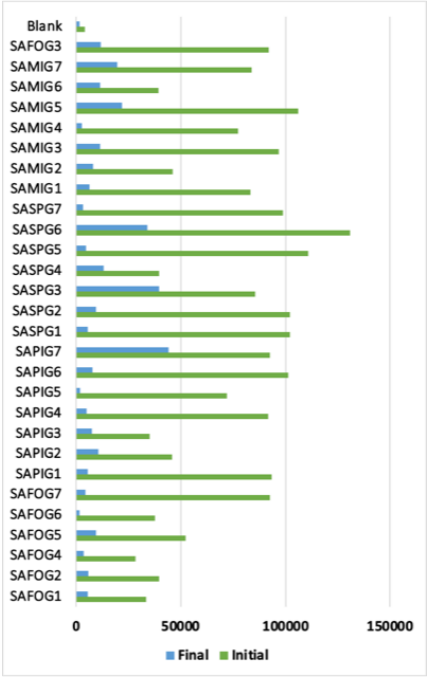
L136: Add the version of PAST. Also add details on the table used for further calculations, as well as on the alpha diversity and multivariate analyses done.
---It now reads “The statistical analysis and graphic illustrations were performed using PAleontological STudies (PAST) software v.4.16 (Hammer **et al.** 2001). **T**he input matrix for all statistical analyses was the OTUs table of each sequenced sample.” [L. 173-175 of the revised manuscript].

L137-138: Add the version of PICRUSt2.
---Added [L. 176 of the revised manuscript]

L143: There is a typing mistake, please replace “have” by “had”.
---Corrected.

Results
L149-151: a table with the number of sequences in each sample for each step of the Mothur

procedure would help checking for the quality of the sequencing and it would illustrate the results in the sentence.
---We think that such detail is not necessary in the manuscript, so we provide here the number of reads from the initial to the final steps of our MOTHUR analysis.



L151-153: Details are needed on the way the dataset was normalized, on the reason of the choice of 2859 reads per sample, and why two samples were removed.
---It now reads "The 'sub.sample' command was used and the data were normalized to a depth of 2859 reads per sample maintaining, thus, a sufficient number of reads per sample but at the sample time excluding only two samples with ≤2000 reads" [L. 165-167 of the revised manuscript].

L154-155: Do you mean taxonomically assigned?
---It now reads "The total number of OTUs to which the reads were taxonomically assigned was 519." [L. 193-194 of the revised manuscript].

L156: Please check the literature on Taxa_S, Shannon_H, Simpson_1-D and Chao-1 indices as they are not all indices. Taxa_S is probably OTU richness and Chao 1 is an estimator of the diversity (it calculates the potentially missed diversity using the presence of singletons and doubletons). Please rephrase the text accordingly.
---It now reads "The Shannon_H and Simpson_1-D indices were calculated to assess the alpha diversity of the gut microbiota of gilthead sea bream in the four groups (Table 1). Also, Taxa_S was calculated for the estimation of the OTUs richness and Chao-1 to estimate the diversity by calculating the potentially missed diversity using the presence of singletons and doubletons (Table 1)." [L. 195-199 of the revised manuscript].

L161-162: More information is needed here or in the methods part to explain why the Bray-Curtis index and PERMANOVA were used here.
---The Bray-Curtis PERMANOVA is among the most widely used tests for checking statistically significant differences in animal microbiota, most likely due to its better performance on data sets with varying dispersions within groups (Anderson MJ, Walsh DCI (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous

dispersions: What null hypothesis are you testing? Ecological Monographs 83:557-574) such as animal gut microbiota datasets. We have added the number of permutations used (9999).

L164-165: Please give details on the statistical test used + the P value for each comparison.
---Added in L. 206 of the revised manuscript.

L168: There is a typing mistake, please replace “is” by “was”.
---Corrected.

L174-182: In this paragraph, the authors may add the correspondence of shared/unique OTUs in read numbers. It usually gives interesting information on the number of reads actually shared between conditions.
This is a proposition but, to help compare the microbiota composition between the 4 diets, the authors could add the taxonomic composition of the shared/unique OTUs to the figure. This could be complementary to the paragraph “Most important OTUs” L205-221.
---At this part we only wish to show the degree of overlap in terms of OTUs numbers. Commenting on the taxonomic composition of these large numbers of OTUs might make the text more difficult to follow.

L175: Please do not start a sentence by a number
---It now reads “In total, 11.2% of the OTUs were found in all groups (FO, PI, SP, MI).” [L. 217-218 of the revised manuscript]

L183-194: Please verify that all taxonomic names are in italic.
---Checked and corrected. We use italics for the genera and species taxa.

L183-184: Please rephrase, it seems that there is a word missing.
---It now reads “Proteobacteria and Actinobacteriota were within the three most abundant phyla in all dietary groups (Figure S2).” [L. 225-226 of the revised manuscript]

L188: Please explain what means this ratio.
---It now reads “the ratios of the phyla abundances were quite close (Figure S2).” [L. 230 of the revised manuscript].

L195-L204: Please give values to the description of the results.
---Values added in this section.

L210: Please replace: “was among the most dominant* OTUs of* FO…”.
---Corrected.

L218-220: Please rephrase.
---It now reads “Furthermore, among the highly abundant OTUs, two of them (OTU001 and OTU003) were observed in all treatments.” [L. 263-264 of the revised manuscript]

L221: Please rewrite: “being common *to* FO-MI and FO-PI.”.
---Corrected.

L222-230: Please give values to describe the figures and tables cited in the paragraph.
---Added.

Discussion

L241: Please correct: “the Shannon index ** decreased in fish fed *with* the Schizochytrium…”.

---Corrected

L242: Please correct: “but ** slightly increased in fish fed *with* the…”.

---Corrected.

L243-249: Please rewrite this sentence in shorter sentences.

---It now reads “Jorge et al. (2019) using *M. gaditana* for fishmeal replacement reported a higher, but insignificant to the control, intestinal richness and diversity of the microbiota. Using a microalgae blend of *Tisochrysis lutea* (member of the *Isochrysis* spp. group), *M. gaditana* and *Scenedesmus almeriensis* at 5%, 15% and 25% dietary inclusion levels, Garcia-Marquez et al. (2023) found that the microalgae blend induced an increase in bacterial species diversity and a distinct shift in microbiota fingerprinting as inclusion levels increased. In our study, compared to the control (FO), the diet containing *M. gaditana* (MI) showed lower alpha diversity indices, but this was not statistically significant. Similar lack of statistically significance has been reported in the *Sparus aurata* gut microbiota after the inclusion of 5% hydrolysed *M. gaditana* (Cerezo-Ortega et al. 2021).” [L. 292-302 of the revised manuscript].

L249: “In our results*,* the”.

---Corrected.

L249-251: Proposition to rephrase the sentence: “Compared to the control (FO), the diet containing M. gaditana (MI) showed lower alpha diversity indices, but this was not statistically significant.”.

---Corrected.

L255: Please correct: “also showed vari*ying*”.

---Corrected.

L257: I would be careful comparing Chao indices as the calculation depends on the way the original dataset was treated, i.e. whether rare OTUs such as singletons and doubletons were removed or not.

---We removed the Chao comparisons.

L270-276: The authors may also consider that the diet likely comes along with its own microbiota (metabarcoding the diet would have given extra information on this, maybe to consider for a next experiment).

---It now reads “Overall, the effect on gut microbiota diversity is primarily determined by the different species of microalgae, the fish as the host and the levels of inclusion of each microalga in the diet. The literature as discussed above in combination with the results of our study shows the various changes that occur as the gut microbiome adapts to the different components added by microalgae to the diet. However, differences in diversity alone cannot show the overall effects and investigation of changes at the level of bacterial phyla, families, and specific OTUs that modulate the diversity is required. As it has been shown that the phycoshpere of cultivated microalgae used as feed might affect the fish gut microbiota in early developmental stages (e.g. Nikouli et al. 2019), in the future the impact of the bacteria contained in the microalgae to be incorporated in the aquafeed should be investigated as well.” [L. 318-327 of the revised manuscript].

L296: Please correct: “p*h*ylogenetically”.
---Corrected.

L324-325: Please correct and maybe rewrite this way to be more careful : “search of the KEGG database confirm*ed* that most of the important bacterial genera found in *are affiliated to genomes with* five enzymes to carry out the L-fucose degradation.”.
---Corrected.

L342-343: If the data are available, why not showing them?
---We have added Table S7 where we depict the unique OTUs in the overexpressed OTUs is the SP treatment and we have added this in the DISCUSSION “Finally, SP was the only treatment with unique overexpressed pathways along with the highest number of unique OTUs compared to FO which, however, only one of the treatment’s unique OTUs are included in these overexpressed pathways (Table S7).” [L. 395-397 of the revised manuscript].

L343-344: Please give some possible perspective, do the authors have specific experiments in mind to complete the study?
---It now reads “This microalgal feed inclusion requires further investigation with in vitro experiments using specific isolates and growth media to test the functionality of these presumptive metabolic pathways.” [L. 397-399 of the revised manuscript]. However, the possible specific experimental approaches remain quite numerous.

L349: Please correct: “inclusion *were*s inferred”.
---Corrected.

L333-344: To conclude on this paragraph, the authors may add that the putative underexpression of peptidoglycan synthesis with the 3 diets compared to FO is likely representative of the higher presence of gram negative strains.
---It now reads “The only pathway that was considerably under-expressed in the three experimental feeds compared to FO is peptidoglycan biosynthesis. The cell wall of some microalgae species contains peptidoglycan (Agboola et al. 2019, Machado et al. 2022), but the species used in our research do not seem to contain it (Domozych et al. 2012, Le Costaouëc et al. 2017, Nadzir et al. 2023). Peptidoglycan forms around 90% of the dry weight of Gram-positive bacteria but only 10% of Gram-negative strains (Malanovic & Lohner 2016). The relative abundance of Gram-negative phyla and families like Vibrionaceae in PI and MI and Bacteroidota in SP is higher than FO. Consequently, the putative under-expression of peptidoglycan synthesis with the three diets compared to FO is likely representative of the higher presence of Gram- taxa.” [L. 385-394 of the revised manuscript]

References
Please read references carefully, especially taxonomic names that are not written conventionally (upper/lower cases, italic).
---Checked and corrected.

Figures and tables
Table 1: Replace “OTUs” by “number of OTUs”. Please check the literature on Taxa_S, Shannon_H, Simpson_1-D and Chao-1 indices as they are not all indices and then please rephrase the text legend accordingly.

---The legend now reads “”**Table 1.** Alpha diversity metrics (Shannon H and Simpson 1-D), OTUs richness and Chao-1 values for each treatment. OTUs: operational taxonomic units.”.

Figure 1: Please rephrase « The MI samples have the largest range, PI does not coincide with FO and SP.”. Give details of the diet acronyms in the legend. Here, it seems that the 10 samples per diet (as written in the methods part) were not considered, were some samples missed (in addition to the two lower than 2859 reads)?
---See revised paragraph “Beta diversity”.
---For the DNA extraction and sequencing we used 7 gut samples of each feeding group. It is written in the methods part in the paragraph “DNA extraction and sequencing”.

Figure 2: Please rephrase “Relative abundances were calculated adding the relative abundance (calculated based on the average samples reads) of all OTUs belonging to each family.”. Give details of the diet acronyms in the legend. Please add the corresponding abundance table as supplementary data, it will help reading the relative abundances.
---The legend now reads “**Figure 2.** Taxonomic composition of the bacterial families in each dietary group. Relative abundances were calculated adding the relative abundance (calculated based on the average samples reads) of all OTUs belonging to each family. Only the families with relative abundance > 1% were considered for the diagram. Families with relative abundance < 4% are depicted in grey. FO: Fish Oil, MI: *Microchloropsis* + *Isochrysis*, SP: *Schizochytrium* + *Phaeodactylum*, PI: *Phaeodactylum* + *Isochrysis*.”.

Figure 3: Give details of the diet acronyms in the legend.
---The legend now reads “**Figure 3.** Most dominant OTUs in each dietary group (cumulative relative dominance > 80% and closest relative (Nucleotide BLAST). Relative abundances are % of reads. FO: Fish Oil, MI: *Microchloropsis* + *Isochrysis*, SP: *Schizochytrium* + *Phaeodactylum*, PI: *Phaeodactylum* + *Isochrysis*.”.

Figure 4: Give details of the diet acronyms in the legend. Please describe the dark blue plots.
---The legend now reads “**Figure 4.** Relative abundance of the FO reads versus their respective values of the PI, MI and SP. OTUs in green dots are in ≥1% relative abundance in PI or MI or SP but not in FO, OTUs in red dots are in ≥1% relative abundance in FO and PI, MI and SP concomitantly, OTUs in pale blue dots are in ≥1% relative abundance in FO but not in PI, MI and SP and OTUs in dark blue dots are in ≤1% relative abundance in any treatment. The limit set (orange point) is the log of 1% of the total reads. FO: Fish Oil, MI: *Microchloropsis* + *Isochrysis*, SP: *Schizochytrium* + *Phaeodactylum*, PI: *Phaeodactylum* + *Isochrysis*.”.

Figure 5: Give details of the diet acronyms in the legend.
---The legend now reads “**Figure 5**. Scatter plots with every pathway in x axis and the log of the ratio PI/FO, MI/FO and SP/FO of the Taxon function abundance in y axis. Pathways > 1.5 are considered overexpressed in PI, MI and SP respectively compared to FO and pathways < -1.5 are considered underexpressed. FO: Fish Oil, MI: *Microchloropsis* + *Isochrysis*, SP: *Schizochytrium* + *Phaeodactylum*, PI: *Phaeodactylum* + *Isochrysis*.”.

Supplementary tables and figures
Please give details of the diet acronyms in the legends.
---Added.

Table S3: Please explain the column “No. of dominant OTUs”.
---Added.

Table S4: Please give the accession numbers of the closest relatives.
---Added.

Table S6: Please explain what means “x” and “-“.
---We have replaced “x” with “+” and explained its meaning.

Figure S1: The “a” and “b” are missing on the figure. Do the percentages indicate the proportion in number of OTU or in number of reads?
---“a” and “b” added. Number of OTUs.

Figure S2: The title may be removed as the description is already in the text legend. % may be removed in the axis title “Relative abundance”. Please explain in the methods part in the main text how the ratio has been calculated, I do not understand. If the representation in the figure comes from the calculation of a ratio I wonder if the obtained results should still be in %? Please add the corresponding table as supplementary data, it will help reading the data.
---Figure corrected and values added in the corresponding table.

Review by Yaqiu Liu, 03 Mar 2024 14:45
This study investigated the effects of dietary microalgae blends as fish oil replacers on the midgut bacterial microbiota of gilthead sea bream, which provide valuable information for the development of a new type of feed for gilthead sea bream. The topic analyzed is very interest. Experiment design is good. In terms of manuscript appear sufficient to satisfy the journal parameters. I think there are some issues I may remind the author to improve the study before publication.
These are my comments
1. Abstract section is well prepared. I only recommend the authors refine the content and highlight the theme make this section more attractive to the reader, and extract the objective of this study.
---We added some sentences to highlight the theme better before the aim of the study, as per other suggestions.

2. The MS fails to explain the major patterns leading to hypothesis in the Introduction part, which is helpful for readers understanding the research objectives easily. Moreover, I recommend the authors supply more information of microalgae blends and gilthead sea bream to enrich research background.
3. In the Materials and methods part, the nutrient content of the experimental diets (e.g. microalgae blends) should be added.
---A relevant table with the dietary formulation (g/Kg) and proximate composition (%) of the diets has been used in another manuscript that has been submitted for publication and deals with the dietary effects on fish growth and physiology. Therefore, such a Table cannot be used in the present manuscript. In response to the reviewer’s comment, we have added this information in the “sampling” section of Materials and Methods.

4. I believe method of ‘The 16S rRNA sequencing raw data analysis’ is too simple, I suggest author adding more details in the MS, which is good for readers understanding. Line 131, missing information should be added in ‘()’.

---We have included more details in this part.

5. I suggest that author can provide gut bacterial community assembly process of gilthead sea bream in the different groups. Meanwhile, author can also do some analysis (e.g. gut bacterial community stability, which evaluated by average variation degree (AVD)), which is calculated using the deviation degree from the mean of the normally distributed OTU relative abundance among different the groups.
---We partially agree with the suggested analysis, but we believe that these would make much appropriate if we had higher numbers of replicates.

9. I recommend that author can estimate mean abundances of key putative enzymes related to the use of L-Fucose degradation in the gut bacterial community and do ANOVA test for finding significant difference in the mean proportion of genes coding for putative L-Fucose degradation in the different test groups.
---We do not think that the PICRUST data should undergo extensive and rigorous quantitative tests as they are based on taxa with known genomes and might not represent the whole communities metabolic potential.

8. Discussion. The content of discussion is well prepared. But there are still several parts that are not well exposed with confusing links or elements. Relevance between references and your work is not unclear, especially in this part references should be well used to discuss your result.
---We have rephrased several parts in our manuscript, and we believe that the text runs more smoothly now. If the reviewer wished to depict specific parts we can correct them.

Review by anonymous reviewer 1, 21 Mar 2024 13:53
The Manuscript contains important evidence about dietary effects on microbiota. The aim of this work was to evaluate the effects of dietary microalgae blends as fish oil replacers on the midgut bacterial microbiota of gilthead sea bream (Sparus aurata). The midgut bacterial community composition and the dominant OTUs indicated that the sea bream midgut bacterial communities were altered compared to the control diet. Additional evidence from the presumptive bacterial functional pathways suggests that the microalgae-based diets resulted in one overexpressed and one underexpressed pathway. The overexpressed pathway was related to the metabolism of fucose, a major carbohydrate of these microalgae species. This suggests that a new gut microbiota profile was selected due to the microalgae inclusion in the provided diet.
Title and abstract
The introduction would be enhanced if it explicitly included the rationale behind the specific combinations of algae used in the study. This would provide a clear understanding of the strategic choices made regarding algae selection and their intended synergistic effects or benefits.e clearly reflect the content of the article? Yes.
Does the abstract present the main findings of the study? Yes
--We have rewritten and rearranged the abstract and the introduction accordingly to all the suggestions.

Introduction
Are the research questions/hypotheses/predictions clearly presented?
Yes. However, I suggest that 1st paragraph mention the value of microalgae in terms of source of lipids, to focus the attention in lipid replacement not protein.

The introduction would be enhanced if it explicitly included the rationale behind the specific combinations of microalgae used in the study. This would provide a clear understanding of the strategic choices made regarding microalgae selection and their intended synergistic effects or benefits.
---We think that this is now clear in the revised introduction.

Materials and methods
Are the methods and analyses sufficiently detailed to allow replication by other researchers?
The Materials and Methods section needs a comprehensive description of the diets, including a detailed composition table. This should particularly emphasize the contribution of algae in terms of EPA/DHA content, as well as the amount of carbohydrates, to ascertain whether the diets are isoenergetic.
---Please see the revised text in the "Sampling" section of the Materials and Methods.

Are the methods and statistical analyses appropriate and well described? Yes.
My major concern is there is not replicate in the dietary treatment, each experimental diet was applied on a single tank during 80 days.
---The number of replicated tanks and the tanks which we could sample were dictated by the scope of the whole experiment (which was not focused on microbiome analysis), available resources and the number of fish we can sample for microbiota analysis, i.e. we sampled the maximum possible number of specimens so it did not disrupt the rest of the required analyses.

No data about effect in fish growth is reported.
---We added the following sentence in the "Sampling" section of the revised manuscript: "The body mass growth parameter L/W^3 of the sampled fish based on weight (W) and length (L) ranged between 0.013±0.001 (FO) and 0.012±0.002 (MI) (Karapanagiotidis & Kormas unpubl. data)." [L. 141-143 of the revised manuscript].

Results
Are the results described and interpreted correctly?
Yes. I suggest clarifying whether the relative abundance value is an average percentage in Fig.3.
---Added.

Fig.3 : Relative abundance considering Genus level ?
---Yes, added in the legend.

Fig.5. The manuscript would greatly benefit from the inclusion of an additional table that explicitly reveals the codes of the pathways studied. This table should provide a comprehensive and accessible reference for readers, detailing each pathway's specific code.
---Each pathway can be easily retrieved by their code name at the MetaCyc database. However, we provide here the requested table

pathway	description
PWY-3781	aerobic respiration I (cytochrome c)
PWY-7111	pyruvate fermentation to isobutanol (engineered)
PWY-5101	L-isoleucine biosynthesis II
ILEUSYN-PWY	L-isoleucine biosynthesis I (from threonine)
VALSYN-PWY	L-valine biosynthesis
PWY-7013	L-1,2-propanediol degradation

BRANCHED-CHAIN-AA-SYN-PWY	superpathway of branched amino acid biosynthesis
PWY-7663	gondoate biosynthesis (anaerobic)
PWY-5667	CDP-diacylglycerol biosynthesis I
PWY0-1319	CDP-diacylglycerol biosynthesis II
NONOXIPENT-PWY	pentose phosphate pathway (non-oxidative branch)
TCA	TCA cycle I (prokaryotic)
FASYN-ELONG-PWY	fatty acid elongation -- saturated
PWY-5973	cis-vaccenate biosynthesis
PWY-2942	L-lysine biosynthesis III
PWY-7094	fatty acid salvage
PWY-5103	L-isoleucine biosynthesis III
PHOSLIPSYN-PWY	superpathway of phospholipid biosynthesis I (bacteria)
PWY-6969	TCA cycle V (2-oxoglutarate:ferredoxin oxidoreductase)
SER-GLYSYN-PWY	superpathway of L-serine and glycine biosynthesis I
PWY-7229	superpathway of adenosine nucleotides de novo biosynthesis I
PWY-5097	L-lysine biosynthesis VI
PWY-7208	superpathway of pyrimidine nucleobases salvage
PWY-7219	adenosine ribonucleotides de novo biosynthesis
PWY4FS-7	phosphatidylglycerol biosynthesis I (plastidic)
PWY4FS-8	phosphatidylglycerol biosynthesis II (non-plastidic)
PWY-5695	urate biosynthesis/inosine 5'-phosphate degradation
PWY-7220	adenosine deoxyribonucleotides de novo biosynthesis II
PWY-7222	guanosine deoxyribonucleotides de novo biosynthesis II
FAO-PWY	fatty acid β-oxidation I
PWY-6126	superpathway of adenosine nucleotides de novo biosynthesis II
PWY-7228	superpathway of guanosine nucleotides de novo biosynthesis I
CALVIN-PWY	Calvin-Benson-Bassham cycle
PWY-3001	superpathway of L-isoleucine biosynthesis I
PWY-6121	5-aminoimidazole ribonucleotide biosynthesis I
PWY-6125	superpathway of guanosine nucleotides de novo biosynthesis II
PENTOSE-P-PWY	pentose phosphate pathway
PWY-6122	5-aminoimidazole ribonucleotide biosynthesis II
PWY-6277	superpathway of 5-aminoimidazole ribonucleotide biosynthesis
PWY-841	superpathway of purine nucleotides de novo biosynthesis I
PWY-7221	guanosine ribonucleotides de novo biosynthesis
GLUCONEO-PWY	gluconeogenesis I
PWY0-162	superpathway of pyrimidine ribonucleotides de novo biosynthesis
PWY-5686	UMP biosynthesis
ANAGLYCOLYSIS-PWY	glycolysis III (from glucose)

PWY-6163	chorismate biosynthesis from 3-dehydroquinate
PWY-5104	L-isoleucine biosynthesis IV
RIBOSYN2-PWY	flavin biosynthesis I (bacteria and plants)
P105-PWY	TCA cycle IV (2-oxoglutarate decarboxylase)
COMPLETE-ARO-PWY	superpathway of aromatic amino acid biosynthesis
HEMESYN2-PWY	heme biosynthesis II (anaerobic)
THRESYN-PWY	superpathway of L-threonine biosynthesis
PWY-7539	6-hydroxymethyl-dihydropterin diphosphate biosynthesis III (Chlamydia)
PWY-5659	GDP-mannose biosynthesis
PWY-5189	tetrapyrrole biosynthesis II (from glycine)
PWY-5188	tetrapyrrole biosynthesis I (from glutamate)
GLYCOLYSIS	glycolysis I (from glucose 6-phosphate)
ARO-PWY	chorismate biosynthesis I
PEPTIDOGLYCANSYN-PWY	peptidoglycan biosynthesis I (meso-diaminopimelate containing)
TRPSYN-PWY	L-tryptophan biosynthesis
PWY-6387	UDP-N-acetylmuramoyl-pentapeptide biosynthesis I (meso-diaminopimelate containing)
DAPLYSINESYN-PWY	L-lysine biosynthesis I
DTDPRHAMSYN-PWY	dTDP-L-rhamnose biosynthesis I
PWY-6385	peptidoglycan biosynthesis III (mycobacteria)
PWY-6386	UDP-N-acetylmuramoyl-pentapeptide biosynthesis II (lysine-containing)
REDCITCYC	TCA cycle VIII (helicobacter)
PWY-7197	pyrimidine deoxyribonucleotide phosphorylation
FOLSYN-PWY	superpathway of tetrahydrofolate biosynthesis and salvage
GLUTORN-PWY	L-ornithine biosynthesis
SULFATE-CYS-PWY	superpathway of sulfate assimilation and cysteine biosynthesis
PWY-5121	superpathway of geranylgeranyl diphosphate biosynthesis II (via MEP)
SO4ASSIM-PWY	sulfate reduction I (assimilatory)
PWY-6147	6-hydroxymethyl-dihydropterin diphosphate biosynthesis I
PWY-5484	glycolysis II (from fructose 6-phosphate)
COA-PWY	coenzyme A biosynthesis I
1CMET2-PWY	N10-formyl-tetrahydrofolate biosynthesis
NONMEVIPP-PWY	methylerythritol phosphate pathway I
PWY-7560	methylerythritol phosphate pathway II
P221-PWY	octane oxidation
PWY-6123	inosine-5'-phosphate biosynthesis I
PWY-5913	TCA cycle VI (obligate autotrophs)
PWY-6612	superpathway of tetrahydrofolate biosynthesis
PANTO-PWY	phosphopantothenate biosynthesis I
HISTSYN-PWY	L-histidine biosynthesis

PWY-7211	superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis
TCA-GLYOX-BYPASS	superpathway of glyoxylate bypass and TCA
PANTOSYN-PWY	pantothenate and coenzyme A biosynthesis I
POLYISOPRENSYN-PWY	polyisoprenoid biosynthesis (E. coli)
GLYCOLYSIS-E-D	superpathway of glycolysis and Entner-Doudoroff
ARGSYNBSUB-PWY	L-arginine biosynthesis II (acetyl cycle)
SALVADEHYPOX-PWY	adenosine nucleotides degradation II
PWY-7184	pyrimidine deoxyribonucleotides de novo biosynthesis I
PWYG-321	mycolate biosynthesis
GLYCOLYSIS-TCA-GLYOX-BYPASS	superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass
PWY-5345	superpathway of L-methionine biosynthesis (by sulfhydrylation)
PROTOCATECHUATE-ORTHO-CLEAVAGE-PWY	protocatechuate degradation II (ortho-cleavage pathway)
TRNA-CHARGING-PWY	tRNA charging
PWY-6609	adenine and adenosine salvage III
UDPNAGSYN-PWY	UDP-N-acetyl-D-glucosamine biosynthesis I
ANAEROFRUCAT-PWY	homolactic fermentation
PWY-7664	oleate biosynthesis IV (anaerobic)
HSERMETANA-PWY	L-methionine biosynthesis III
PWY0-166	superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (E. coli)
GLYOXYLATE-BYPASS	glyoxylate cycle
PWY-7237	myo-, chiro- and scillo-inositol degradation
P42-PWY	incomplete reductive TCA cycle
PWY-6608	guanosine nucleotides degradation III
GLYCOCAT-PWY	glycogen degradation I (bacterial)
PWY-6737	starch degradation V
PWY-5989	stearate biosynthesis II (bacteria and plants)
ARGSYN-PWY	L-arginine biosynthesis I (via L-ornithine)
PWY-7400	L-arginine biosynthesis IV (archaebacteria)
PWY-5154	L-arginine biosynthesis III (via N-acetyl-L-citrulline)
PWY-6282	palmitoleate biosynthesis I (from (5Z)-dodec-5-enoate)
OANTIGEN-PWY	O-antigen building blocks biosynthesis (E. coli)
PWY-7234	inosine-5'-phosphate biosynthesis III
GLYCOGENSYNTH-PWY	glycogen biosynthesis I (from ADP-D-Glucose)
P161-PWY	acetylene degradation
PYRIDNUCSYN-PWY	NAD biosynthesis I (from aspartate)
PWY-6353	purine nucleotides degradation II (aerobic)
PWY0-862	(5Z)-dodec-5-enoate biosynthesis
P4-PWY	superpathway of L-lysine, L-threonine and L-methionine biosynthesis I
PWY-5918	superpathay of heme biosynthesis from glutamate
PWY-6467	Kdo transfer to lipid IVA III (Chlamydia)
PWY-1861	formaldehyde assimilation II (RuMP Cycle)
PWY0-1061	superpathway of L-alanine biosynthesis

PWY0-1586	peptidoglycan maturation (meso-diaminopimelate containing)
PYRIDNUCSAL-PWY	NAD salvage pathway I
P108-PWY	pyruvate fermentation to propanoate I
PWY-6897	thiamin salvage II
MET-SAM-PWY	superpathway of S-adenosyl-L-methionine biosynthesis
PWY0-781	aspartate superpathway
HEME-BIOSYNTHESIS-II	heme biosynthesis I (aerobic)
PWY-5347	superpathway of L-methionine biosynthesis (transsulfuration)
FASYN-INITIAL-PWY	superpathway of fatty acid biosynthesis initiation (E. coli)
PWY-6700	queuosine biosynthesis
NAGLIPASYN-PWY	lipid IVA biosynthesis
PWY-6545	pyrimidine deoxyribonucleotides de novo biosynthesis III
PWY-7254	TCA cycle VII (acetate-producers)
PWY-1269	CMP-3-deoxy-D-manno-octulosonate biosynthesis I
PWY-7323	superpathway of GDP-mannose-derived O-antigen building blocks biosynthesis
PWY-7199	pyrimidine deoxyribonucleosides salvage
PWY-6628	superpathway of L-phenylalanine biosynthesis
PWY-7200	superpathway of pyrimidine deoxyribonucleoside salvage
HOMOSER-METSYN-PWY	L-methionine biosynthesis I
PWY-5022	4-aminobutanoate degradation V
PWY-6630	superpathway of L-tyrosine biosynthesis
COBALSYN-PWY	adenosylcobalamin salvage from cobinamide I
PWY0-1261	anhydromuropeptides recycling
PWY0-1297	superpathway of purine deoxyribonucleosides degradation
PWY-6317	galactose degradation I (Leloir pathway)
PWY-7196	superpathway of pyrimidine ribonucleosides salvage
PWY-7392	taxadiene biosynthesis (engineered)
PWY-5920	superpathway of heme biosynthesis from glycine
RUMP-PWY	formaldehyde oxidation I
PWY-6151	S-adenosyl-L-methionine cycle I
DENOVOPURINE2-PWY	superpathway of purine nucleotides de novo biosynthesis II
PRPP-PWY	superpathway of histidine, purine, and pyrimidine biosynthesis
HISDEG-PWY	L-histidine degradation I
P124-PWY	Bifidobacterium shunt
LEU-DEG2-PWY	L-leucine degradation I
PWY-5855	ubiquinol-7 biosynthesis (prokaryotic)
PWY-5856	ubiquinol-9 biosynthesis (prokaryotic)
PWY-5857	ubiquinol-10 biosynthesis (prokaryotic)
PWY-6708	ubiquinol-8 biosynthesis (prokaryotic)

COLANSYN-PWY	colanic acid building blocks biosynthesis
PWY-6703	preQ0 biosynthesis
FERMENTATION-PWY	mixed acid fermentation
PWY0-1415	superpathway of heme biosynthesis from uroporphyrinogen-III
ASPASN-PWY	superpathway of L-aspartate and L-asparagine biosynthesis
PWY-6519	8-amino-7-oxononanoate biosynthesis I
PPGPPMET-PWY	ppGpp biosynthesis
BIOTIN-BIOSYNTHESIS-PWY	biotin biosynthesis I
P562-PWY	myo-inositol degradation I
PWY0-1296	purine ribonucleosides degradation
UBISYN-PWY	superpathway of ubiquinol-8 biosynthesis (prokaryotic)
PWY-7187	pyrimidine deoxyribonucleotides de novo biosynthesis II
THISYN-PWY	superpathway of thiamin diphosphate biosynthesis I
PWY-7328	superpathway of UDP-glucose-derived O-antigen building blocks biosynthesis
PWY-6404	superpathway of mycolyl-arabinogalactan-peptidoglycan complex biosynthesis
PWY-5505	L-glutamate and L-glutamine biosynthesis
PWY-7431	aromatic biogenic amine degradation (bacteria)
PWY-4984	urea cycle
PWY-5100	pyruvate fermentation to acetate and lactate II
PWY0-1533	methylphosphonate degradation I
PWY-5838	superpathway of menaquinol-8 biosynthesis I
P23-PWY	reductive TCA cycle I
PWY-7007	methyl ketone biosynthesis
PWY-5897	superpathway of menaquinol-11 biosynthesis
PWY-5898	superpathway of menaquinol-12 biosynthesis
PWY-5899	superpathway of menaquinol-13 biosynthesis
PWY-5180	toluene degradation I (aerobic) (via o-cresol)
PWY-5182	toluene degradation II (aerobic) (via 4-methylcatechol)
PWY0-1298	superpathway of pyrimidine deoxyribonucleosides degradation
PWY-5840	superpathway of menaquinol-7 biosynthesis
PWY0-1277	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation
PWY0-1479	tRNA processing
PWY-6383	mono-trans, poly-cis decaprenyl phosphate biosynthesis
PWY-5971	palmitate biosynthesis II (bacteria and plants)
PWY-6901	superpathway of glucose and xylose degradation
PYRIDOXSYN-PWY	pyridoxal 5'-phosphate biosynthesis I
PWY-181	photorespiration
PWY-6948	sitosterol degradation to androstenedione
PWY-3661	glycine betaine degradation I
PWY-6396	superpathway of 2,3-butanediol biosynthesis

PWY-5384	sucrose degradation IV (sucrose phosphorylase)
PWY-5861	superpathway of demethylmenaquinol-8 biosynthesis
TYRFUMCAT-PWY	L-tyrosine degradation I
PWY-621	sucrose degradation III (sucrose invertase)
HCAMHPDEG-PWY	3-phenylpropanoate and 3-(3-hydroxyphenyl)propanoate degradation to 2-oxopent-4-enoate
PWY-6690	cinnamate and 3-hydroxycinnamate degradation to 2-oxopent-4-enoate
P125-PWY	superpathway of (R,R)-butanediol biosynthesis
ARGORNPROST-PWY	arginine, ornithine and proline interconversion
PWY-6895	superpathway of thiamin diphosphate biosynthesis II
PWY-5509	adenosylcobalamin biosynthesis from cobyrinate a,c-diamide I
PWY-7376	cob(II)yrinate a,c-diamide biosynthesis II (late cobalt incorporation)
PWY-5863	superpathway of phylloquinol biosynthesis
ARG+POLYAMINE-SYN	superpathway of arginine and polyamine biosynthesis
PWY-7255	ergothioneine biosynthesis I (bacteria)
PWY-7242	D-fructuronate degradation
PWY-5837	1,4-dihydroxy-2-naphthoate biosynthesis I
PWY-5845	superpathway of menaquinol-9 biosynthesis
PWY-5850	superpathway of menaquinol-6 biosynthesis I
PWY-5896	superpathway of menaquinol-10 biosynthesis
PWY1G-0	mycothiol biosynthesis
GLUCOSE1PMETAB-PWY	glucose and glucose-1-phosphate degradation
PWY-6892	thiazole biosynthesis I (E. coli)
PWY-6269	adenosylcobalamin salvage from cobinamide II
PWY-6397	mycolyl-arabinogalactan-peptidoglycan complex biosynthesis
PWY0-1241	ADP-L-glycero-β-D-manno-heptose biosynthesis
PWY-5747	2-methylcitrate cycle II
PWY0-845	superpathway of pyridoxal 5'-phosphate biosynthesis and salvage
PWY-5028	L-histidine degradation II
P122-PWY	heterolactic fermentation
PWY0-42	2-methylcitrate cycle I
PWY-5860	superpathway of demethylmenaquinol-6 biosynthesis I
PWY-5862	superpathway of demethylmenaquinol-9 biosynthesis
GALACTUROCAT-PWY	D-galacturonate degradation I
PWY-5415	catechol degradation I (meta-cleavage pathway)
CRNFORCAT-PWY	creatinine degradation I
PWY-5676	acetyl-CoA fermentation to butanoate II
PWY-5741	ethylmalonyl-CoA pathway
PWY-5420	catechol degradation II (meta-cleavage pathway)
POLYAMSYN-PWY	superpathway of polyamine biosynthesis I
P101-PWY	ectoine biosynthesis
KDO-NAGLIPASYN-PWY	superpathway of (Kdo)2-lipid A biosynthesis

GLUCUROCAT-PWY	superpathway of β-D-glucuronide and D-glucuronate degradation
PWY-5531	chlorophyllide a biosynthesis II (anaerobic)
PWY-7159	chlorophyllide a biosynthesis III (aerobic, light independent)
P441-PWY	superpathway of N-acetylneuraminate degradation
PWY-5419	catechol degradation to 2-oxopent-4-enoate II
PWY-7332	superpathway of UDP-N-acetylglucosamine-derived O-antigen building blocks biosynthesis
PWY-6891	thiazole biosynthesis II (Bacillus)
PWY-6507	4-deoxy-L-threo-hex-4-enopyranuronate degradation
PWY-5705	allantoin degradation to glyoxylate III
PWY-1541	superpathway of taurine degradation
P164-PWY	purine nucleobases degradation I (anaerobic)
PWY-622	starch biosynthesis
SUCSYN-PWY	sucrose biosynthesis I (from photosynthesis)
RHAMCAT-PWY	L-rhamnose degradation I
CATECHOL-ORTHO-CLEAVAGE-PWY	catechol degradation to β-ketoadipate
PWY-6562	norspermidine biosynthesis
POLYAMINSYN3-PWY	superpathway of polyamine biosynthesis II
GLCMANNANAUT-PWY	superpathway of N-acetylglucosamine, N-acetylmannosamine and N-acetylneuraminate degradation
PWY-5529	superpathway of bacteriochlorophyll a biosynthesis
PWY-6641	superpathway of sulfolactate degradation
PWY-6565	superpathway of polyamine biosynthesis III
NAD-BIOSYNTHESIS-II	NAD salvage pathway II
PWY-6906	chitin derivatives degradation
NADSYN-PWY	NAD biosynthesis II (from tryptophan)
PWY-2941	L-lysine biosynthesis II
PWY-1622	formaldehyde assimilation I (serine pathway)
PWY0-321	phenylacetate degradation I (aerobic)
GALACT-GLUCUROCAT-PWY	superpathway of hexuronide and hexuronate degradation
AST-PWY	L-arginine degradation II (AST pathway)
PWY-6581	spirilloxanthin and 2,2'-diketo-spirilloxanthin biosynthesis
PWY-5417	catechol degradation III (ortho-cleavage pathway)
PWY-5431	aromatic compounds degradation via β-ketoadipate
PWY-5266	p-cymene degradation
PWY-5273	p-cumate degradation
ALL-CHORISMATE-PWY	superpathway of chorismate metabolism
PWY-5910	superpathway of geranylgeranyldiphosphate biosynthesis I (via mevalonate)
PWY-6182	superpathway of salicylate degradation
CHLOROPHYLL-SYN	chlorophyllide a biosynthesis I (aerobic, light-dependent)

PWY-6588	pyruvate fermentation to acetone
P461-PWY	hexitol fermentation to lactate, formate, ethanol and acetate
PWY-7347	sucrose biosynthesis III
PWY-6471	peptidoglycan biosynthesis IV (Enterococcus faecium)
PWY-5651	L-tryptophan degradation to 2-amino-3-carboxymuconate semialdehyde
PWY-6263	superpathway of menaquinol-8 biosynthesis II
PWY-922	mevalonate pathway I
PWY-5654	2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate
HEXITOLDEGSUPER-PWY	superpathway of hexitol degradation (bacteria)
PWY-6185	4-methylcatechol degradation (ortho cleavage)
PWY-6107	chlorosalicylate degradation
PWY-5655	L-tryptophan degradation IX
ENTBACSYN-PWY	enterobactin biosynthesis
DENITRIFICATION-PWY	nitrate reduction I (denitrification)
PWY-6944	androstenedione degradation
PWY-6505	L-tryptophan degradation XII (Geobacillus)
P281-PWY	3-phenylpropanoate degradation
PWY-5181	toluene degradation III (aerobic) (via p-cresol)
PWY490-3	nitrate reduction VI (assimilatory)
PWY-5647	2-nitrobenzoate degradation I
PWY-6071	superpathway of phenylethylamine degradation
PWY-5178	toluene degradation IV (aerobic) (via catechol)
PWY-7616	methanol oxidation to carbon dioxide
PWY-6590	superpathway of Clostridium acetobutylicum acidogenic fermentation
ORNDEG-PWY	superpathway of ornithine degradation
PWY-5304	superpathway of sulfur oxidation (Acidianus ambivalens)
PWY-5430	meta cleavage pathway of aromatic compounds
METHYLGALLATE-DEGRADATION-PWY	methylgallate degradation
3-HYDROXYPHENYLACETATE-DEGRADATION-PWY	4-hydroxyphenylacetate degradation
PWY-7391	isoprene biosynthesis II (engineered)
CENTFERM-PWY	pyruvate fermentation to butanoate
VALDEG-PWY	L-valine degradation I
PWY-6210	2-aminophenol degradation
GALLATE-DEGRADATION-I-PWY	gallate degradation II
PWY-6876	isopropanol biosynthesis
PWY-7371	1,4-dihydroxy-6-naphthoate biosynthesis II
GALLATE-DEGRADATION-II-PWY	gallate degradation I
PWY-5941	glycogen degradation II (eukaryotic)

PWY-6478	GDP-D-glycero-α-D-manno-heptose biosynthesis
PWY0-1338	polymyxin resistance
LACTOSECAT-PWY	lactose and galactose degradation I
PWY-7295	L-arabinose degradation IV
FUCCAT-PWY	fucose degradation
P184-PWY	protocatechuate degradation I (meta-cleavage pathway)
PWY-7377	cob(II)yrinate a,c-diamide biosynthesis I (early cobalt insertion)
PWY-6992	1,5-anhydrofructose degradation
PWY-5392	reductive TCA cycle II
PWY-7374	1,4-dihydroxy-6-naphthoate biosynthesis I
PWY-6338	superpathway of vanillin and vanillate degradation
PWY-7097	vanillin and vanillate degradation I
AEROBACTINSYN-PWY	aerobactin biosynthesis
PWY-7003	glycerol degradation to butanol
PWY-5005	biotin biosynthesis II
KETOGLUCONMET-PWY	ketogluconate metabolism
PWY-7446	sulfoglycolysis
PWY-7090	UDP-2,3-diacetamido-2,3-dideoxy-α-D-mannuronate biosynthesis
GOLPDLCAT-PWY	superpathway of glycerol degradation to 1,3-propanediol
PWY-6728	methylaspartate cycle
P261-PWY	coenzyme M biosynthesis I
PWY-6470	peptidoglycan biosynthesis V (β-lactam resistance)
PWY-7098	vanillin and vanillate degradation II
PWY-5183	superpathway of aerobic toluene degradation
TEICHOICACID-PWY	teichoic acid (poly-glycerol) biosynthesis
PWY-6339	syringate degradation
PWY-7456	mannan degradation
P163-PWY	L-lysine fermentation to acetate and butanoate
CODH-PWY	reductive acetyl coenzyme A pathway
GLUCARDEG-PWY	D-glucarate degradation I
GALACTARDEG-PWY	D-galactarate degradation I
GLUCARGALACTSUPER-PWY	superpathway of D-glucarate and D-galactarate degradation
P162-PWY	L-glutamate degradation V (via hydroxyglutarate)
LIPASYN-PWY	phospholipases
P381-PWY	adenosylcobalamin biosynthesis II (late cobalt incorporation)
PWY-5677	succinate fermentation to butanoate
PWY-7527	L-methionine salvage cycle III
PWY-5177	glutaryl-CoA degradation
P621-PWY	nylon-6 oligomer degradation
METHGLYUT-PWY	superpathway of methylglyoxal degradation
PWY-1361	benzoyl-CoA degradation I (aerobic)
PWY-7315	dTDP-N-acetylthomosamine biosynthesis
PWY-722	nicotinate degradation I

PWY-4361	S-methyl-5-thio-α-D-ribose 1-phosphate degradation
ARGDEG-PWY	superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation
ORNARGDEG-PWY	superpathway of L-arginine and L-ornithine degradation
PWY-6957	mandelate degradation to acetyl-CoA
PWY-6749	CMP-legionaminate biosynthesis I
PWY-6174	mevalonate pathway II (archaea)
PWY-1501	mandelate degradation I
PWY-7024	superpathway of the 3-hydroxypropanoate cycle
PWY-7373	superpathway of demethylmenaquinol-6 biosynthesis II
PWY-3941	β-alanine biosynthesis II
PWY-5507	adenosylcobalamin biosynthesis I (early cobalt insertion)
PWY-5265	peptidoglycan biosynthesis II (staphylococci)
P241-PWY	coenzyme B biosynthesis
PWY-5198	factor 420 biosynthesis
PWY-5744	glyoxylate assimilation
PWY-2221	Entner-Doudoroff pathway III (semi-phosphorylative)
PWY-5743	3-hydroxypropanoate cycle
PWY-7046	4-coumarate degradation (anaerobic)
PWY-1882	superpathway of C1 compounds oxidation to CO2
PWY-7210	pyrimidine deoxyribonucleotides biosynthesis from CTP
PWY-7031	protein N-glycosylation (bacterial)
PWY-6143	CMP-pseudaminate biosynthesis
PWY-1422	vitamin E biosynthesis (tocopherols)
PWY-7198	pyrimidine deoxyribonucleotides de novo biosynthesis IV
FUC-RHAMCAT-PWY	superpathway of fucose and rhamnose degradation
PWY-6572	chondroitin sulfate degradation I (bacterial)
PWY-5088	L-glutamate degradation VIII (to propanoate)
GLYCOL-GLYOXDEG-PWY	superpathway of glycol metabolism and degradation
METH-ACETATE-PWY	methanogenesis from acetate
THREOCAT-PWY	superpathway of L-threonine metabolism
ECASYN-PWY	enterobacterial common antigen biosynthesis
P341-PWY	glycolysis V (Pyrococcus)
DHGLUCONATE-PYR-CAT-PWY	glucose degradation (oxidative)
PWY-7002	4-hydroxyacetophenone degradation
PWY-7398	coumarins biosynthesis (engineered)
PWY-6713	L-rhamnose degradation II

Discussion

The discussion section would be enhanced by delving deeper into the potential taxa that have the capability to degrade the sugars present in the microalgae used in the diet.

Additionally, the discussion would improve by providing data on the sugar composition of the different microalgae used, and exploring what occurs with their various combinations.

---In this paper the presumptive bacterial pathways indicated that fucose was degraded by these bacteria. Even if we added information on other sugars of all the used algae, this would remain with no/little relevance to our findings related to the major metabolic pathways. For this, we prefer to comment only on fucose.