Reviewer's report

Title: Gene expression profiling of flaxseed in mouse lung tissues-modulation of toxicologically relevant genes

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Reviewer: Matthew Philip Greig Barnett

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Generally this is an interesting study, with some potentially important data, and it is of relevance to the target journal. However, there are two major, and several, minor points which I believe need to be addressed before it is suitable for publication.

Major Compulsory Revisions

1. The discussion and conclusion (at almost 2,500 words) is too long. While the study is interesting, and I appreciate the difficulty of dealing with large numbers of differentially expressed genes, the authors need to focus this section more (for example, on inflammation, protective enzymes such as phase I and II, and perhaps one or two other points). At the moment, there is too much detail discussing individual genes.

2. There does not appear to be any reference to the array data having been submitted to a public repository (for example, Gene Expression Omnibus). This must be completed prior to acceptance of the paper for publication.

Minor Essential Revisions

1. The term flaxseed, when first used within the background, should be in full followed by the abbreviation (i.e., Flaxseed (FS), a nutritional supplement……)

2. Are lignans really closely related to lignin? And if so, is this particularly relevant? I would also suggest a little more information than: “Lignans are widely occurring plant compounds..”. For example, that they are phytoestrogens could be of interest.

3. In the background, it is stated: “In this first ever reported study of genomic profiling of flaxseed in lung tissues”. This implies that flaxseed was profiled in lung tissues, which I don’t believe was the case. I would suggest this sentence is changed, for example: “In this first reported study of genomic profiling of lung tissues in response to dietary flaxseed”.

4. Methods: Diets and dietary treatments: Do the authors have any evidence that 4degC is sufficiently cold to prevent any potential degradation of the diet, in particular the lipid component? In similar studies, -80degC (or at least -20) appears to be used to prevent such degradation.

5. Methods: Why are the methods for arrays described before the methods for tissue sample collection and RNA extraction? It would seem more sensible to
describe the collection and isolation of RNA before describing the arrays on which the RNA was used.

6. Methods: Quantitative RT-PCR: Validation of Selected Genes: What was the rationale for the selection of the six genes for validation? Also, in the third line: “differences of both unregulated and down regulated genes” – do the authors mean “up-regulated and down-regulated” here?

7. Within the results, changes in protein expression are referred to in the context of microarray data. Changes in mRNA expression are not necessarily reflected by changes in the encoded protein, so the authors should make it clear that the data they refer to is based on mRNA expression in these instances.

8. Throughout the manuscript there are a number of minor typographical and grammatical errors. I suggest that the authors thoroughly proof-read the article and correct these errors.

9. Discussion: “we observe for the first time all significant biological impacts attributed to FS.” This is clearly not correct, as there are many biological impacts (epigenetic effects, metabolites, phenotypic outcomes) which the authors have not measured. This statement should be moderated.

10. Discussion: “modulated the gene expression profile of several proteins implicated in…..”. I would suggest altering this statement (and others like it, see the earlier point about protein expression inferred from mRNA levels) as follows: “..modulated the expression profile of several genes which encode proteins implicated in…..”.

11. Discussion: “The gene E2F3 was substantially up regulated in the FS diet treatment by 3.9-fold.” Having given the actual fold-change, it is not necessary to refer to this value as “substantially”.

12. Conclusion: “In conclusion, microarray study of a FS-diet has provided unique insights..” The microarray study was not of a FS-diet, it was of mouse lung tissue in response to a FS-diet. There is quite a big difference between the two.

Discretionary Revisions

1. In the background, the authors assert that FS oil contains “…but only 16% linoleic acid (LA)”. The use of “but only” implies there is some importance associated with this statement, but this is not clear from the subsequent text. It seems that the presence of 52% ALA is more relevant than having “only” 16% LA. Only in comparison with what?

2. Methods: I think control is a more suitable term than placebo for the standard AIN-93G diet. A placebo can be defined as “prescribed or given to reinforce a patient’s expectation”, thus its use in the context of a mouse trial is not accurate, as I would doubt a mouse has any expectation of a treatment effect.

3. Abstract and results: “3,713 genes (12.8%) were significantly (p<0.05) differentially expressed, of those 2,088 had a >1.5-fold change.” This should read “of which 2,088 had a >1.5-fold change.”

4. Discussion: That authors state: “It is therefore important to determine the
molecular mechanisms by which dietary flaxseed exerts its therapeutic action”, following a sentence describing the various beneficial effects that FS has been shown to have. However, it is not made clear why determining the mechanisms is important.

5. Discussion: “One of the most exciting observations…”. I would suggest that the authors leave it to the reader of the article to decide whether this observation is exciting or not, or else use another word, e.g., important, relevant, informative.

6. Discussion: “One of the surprising effects of FS treatment is its ability to regulate the expression of a number…”. Why is this surprising? It is relatively well known that compounds such as omega-3 fatty acids can influence, for example, gene expression via interaction with specific transcription factors.

7. Discussion: “Interestingly, our gene array data suggest that T cells…”. If, as is stated in the next sentence, this hypothesis is novel, then why is it particularly interesting?

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests