**Editorial comments:**  
  
1. Please revise step 1.3 to avoid the use of the word "kill." Please use a less graphic term such as "euthanize."

**This has been revised as suggested (see step 1.3).**  
  
2. Please describe how to remove the liver in step 1.4. If these details have been reported elsewhere, please provide the appropriate citation.

**This has been described as suggested (see step 1.4).**  
  
3. Please indicate how long one can store the samples in all steps where samples are stored (e.g., steps 2.5, 2.7, etc.)

**Storage time recommendations have now been included as suggested (see step 2.5, 2.7, 3.6, 3.9, 3.11, 3.13, 3.19, 3.21, 4.9, 5.9, 6.2)**  
  
4. Please re-write step 7.5 in imperative tense.

**This has been revised as suggested (see step 7.5)**  
  
5. You have only highlighted ~ 0.75 pages of your protocol to be filmed. Please note that highlighting is used by our script writers to translate your protocol into a script and plan the video portion of your article. Any information that is not highlighted will therefore not be included in your video. Your video should contain enough detail such that an entire, coherent story is presented to the viewer. Additionally, the highlighting should include complete statements and complete actions and not portions of sentences or actions. For example, steps 2.5 and 2.7 are highlighted while step 2.6 is not highlighted. In order for the protocol to make sense in the video, please also highlight step 2.6.   
  
Please re-evaluate your protocol considering the highlighting guidelines above and adjust your highlighting as you see fit to tell a complete, coherent story to be included in your video. You may highlight as much as 2.75 pages of text to be filmed.

**The protocol has been re-evaluated, and further sections highlighted as suggested for a more coherent story.**  
  
**Reviewer #2:**   
*Minor Concerns:*  
I have just few comments (listed below), which I would appreciate if they will be addressed by the authors:  
- p. 5 line 220: it is step 3.19, not 2.19.

**This has now been corrected (see step 4.1)**

- p. 7 line 273: step 6.2 is ambiguous. What do you mean by repeating step 5.1 and then combine the two hexane fractions? Maybe you mean repeat all the step 5 (from 5.1 to 5.9) or maybe repeat just step 6.1? Please, shine more light on this particular step of the protocol.

**This has now been revised to make the instructions more clear (see step 6.2).**

- p. 7 line 280: the temperature method of the GC instrument starts at 15 °C, I think it is 150 °C.

**This has now been amended to 115 oC (see step 7.1).**

- p. 8 line 345: the authors affirm that 16:0 and 18:2n6 are the most identifiable peaks within the samples expressed in Figure 2. If I am correct, I see other two peaks that present a higher intensity at ~ 9 and 12 minutes, which fatty acid methyl esters are they?

**We can confirm that the two main peaks are 16:0 and 18:2n-6, and the ‘box’ on the figure has been adjusted to fall into the correct position. We have also included peak identities for the other major fatty acids within this sample (see Figure 2).**

- p. 9 line 364: 20:4n6 content (close to 8% of the total) is not present in figure 5 so I would add in the caption also this fatty acid. The five (not four) major fatty acids within CE that are not shown.

**Figure 5’s title has been corrected to state that the five major fatty acids within CE are not shown.**

- By examination of the GC run, I have noticed that retention time in Figure 1 is shortened with respect to the others present in Figure 2-4. It is matter of fact that RT changes its value run by run, for this reason I suggest the authors to label with the fatty acid name at least the six main peaks in each figure in order to help the reader to analyze the chromatograms.-

**All figures have now been revised to ensure that the main peaks on each chromatogram are identified (see figure 2, 3 and 4).**

Moreover, the peak intensity in the chromatograms is different in all the figures, suggesting a different fatty acid composition of the analyzed sample. Maybe it could be of reader's interest adding to each caption which kind of sample it is shown (FAME of CE coming from virgin or pregnant rat? type of diet?).

**Details of the sample used to generate each chromatogram are now included in the title of figures 1, 2 and 4 as suggested.**   
  
*Additional Comments to Authors:*  
The present paper is focused on n6-n3 PUFA content and these values are reported in Table 1. As said by the authors, there are other fatty acids that are the major ones, which give also the higher contribution to the total fatty acid composition (methyl palmitate, stearate and oleate). Which is their contribution in terms of relative percentage? Perhaps it might be interesting to add their value to Table 1.

**Table 1 now also includes data for saturated and monounsaturated fatty acids as suggested.**  
  
**Reviewer #3:**   
1. The authors should include more details of the GC instrument conditions all of which can markedly impact separation of peaks. These include: the type of gas used (nitrogen, hydrogen or helium) and the volume of gas flow through the column; the injector and detector temperatures; the makeup gas and flow rate.

**Further details of GC instrument conditions have now been incorporated into step 7.1 as suggested.**

2. It would be helpful to the reader to provide a representative trace of either a standard mixture of fatty acids or an actual sample, in which all relevant peaks are identified. This could well replace Figure 2.

**Figure 2 has now been amended to include peak identities for all relevant peaks, and includes both chromatograms from a standard mixture of fatty acids, and an actual sample.**