Response to Review Comments  
(Manuscript ID nutrients-552980)

Reviewers' comments:
Reviewer #2: This paper nicely discusses the quantification of nervonic acid in human milk over the first month of lactation and compare with the level of different artificial milk. The manuscript covers the aim of the journal and the subject investigated is of worldwide interest. However, some points need to be addressed and the manuscript needs re-editing of some fragments.  
The authors highly appreciate the reviewer’s comments, which the results and value of this work have been precisely summarized.

Overall  
As stated by Authors, “eight healthy Chinese mothers were included to the study”, however, there are a lot of scientific reports which highlight the interindividual variation of different factors among lactating mothers, also by the authors themselves, so in my opinion, the obtained results, due to the low number of lactating mothers, should be reported as preliminary and this should be reflected in the title of the manuscript.  
More information has been added into the title of the manuscript.

Was the total fat concentration determined in the analyzed milk? For example, by using Miris Milk Analyser. The results should be correlated with the total fat content. This would benefit and refine the obtained results. Moreover, I have doubts whether the calculation of statistical differences among individual mothers (at the subsequent days of lactation) is justified (see Table 1 – last column, p value). Under the Table, the details concerning the statistical test used are missing. Moreover, the detailed data in Table 1 should be presented as supplementary file.  
The human milk fat was extracted using classic Röse-Gottlieb method [1] and the fat contents were analyzed as our previous studies [2-6]. Follow the reviewer’s comments, we have added the fat content of eight samples during lactation day as one table in Supplementary file (Table S2). Table 2 has been moved to the supplementary file, as suggested (Table S3).

Line 46-47  
“NA concentrations in colostrum have been found to range from 2- to 6-fold higher than those in mature milk [13,14].”  
It benefits the reader if the Authors will added information “how mature milk”, namely weeks or months.  
The information of the lactation day has been added as suggested. NA concentrations in colostrum (≤1 week postpartum) have been found to range from 2- to 6-fold higher than present in mature milk (>2 weeks and ≤16 weeks).

Line 70-79  
2.2. Human milk samples  
Some very important details concerning collections of milk are missing (i.e. using an electric breast pump, sampling of a single breast, etc.).
The detailed description is needed since there are a lot of data which point out the significant differences in human milk lipid fraction during single feeding (fat levels changing foremilk to hindmilk) as well as over the day.

The authors agree with the reviewer’s comments. The detailed information about the sample collection has been added (line 87-89).

Line 111
2.5. Quantitation of nervonic acid
“The NA concentration in human milk fat...” – the method used by Authors is semi-quantitative, so it would be more appropriate to use the word “level”.

We thank the reviewer’s comments. We have provided two formats of NA in human milk. One is the relative content which expressed as a percentage by weight of total fatty acids [7], which is easy to compare with other studies and provide more information of NA in infant formula production. Another is the absolute NA concentration. Here we used ‘concentration’ for a better understanding and distinguishing from the relative content.

Results
Some subsections contain the sentences which should be transferred to the Discussion. I suggest that the authors should modified these fragments:

Line 138-144
“NA occurs naturally in human milk; however, it has not been routinely reported in the global FA profile of human milk [24]. The main reason...”

Line 152-155
“The limits of detection and quantitation were lower than previous studies by Barros et al. [28] and Arcari et al. [8]. In general, ...”

Line 164-166
“The results of the NA concentration in human milk during the three stages, that is, colostrum, transitional milk, and mature milk, are similar to those found by Xiang [30].”

Line 186-189
“The OA content measured in this study is in accordance with Qi’s results (30.66-32.71%) [20], but inconsistent with Weiss’s study (43.96-48.21%) [31]. OA content was stable in this study, which is contrary to the study by Lopez-Lopez et al., who claimed that OA content decreased as lactation progressed [32].”

Line 195-197
“Additionally, the percentage of EiA, EA, and NA decreased with the progression of lactation, which agrees with the results obtained by Nyuar [11].”

Line 207-210
“It has been demonstrated that the heart may be damaged by EA, and EA is undesirable for human
consumption [33]. So, it is recommended that EA content in infant formulae be below 1% of the total fat content [8].”

The authors appreciate the reviewer’s comments. These fragments have been moved into the discussion where related.

Line 157
The title of Figure 1 “Gas chromatograms obtained from human milk” should be more informative. Modified to as suggested.

Line 158-159
“The NA concentrations in human milk according to GC analysis are presented in Table 1. The NA concentration in all human milk samples decreased significantly (p < 0.05) during the first month of lactation.”
The value of the corelation coefficient (r=) should be given.
The authors thank review’s comments. The correlation coefficient (r=-0.822, p < 0.001) has been added into the manuscript (line 176).

Line 160
The Authors reported the results as mg/g or mg/g fat?
“The average NA concentration on day 3 of lactation was about 5 times higher than that 160 on day 30 (p < 0.001), with values of 1.00 ± 0.24 mg/g and 0.18 ± 0.03 mg/g fat, respectively”
Was the total fat concentration determined in the analyzed milk? The results should be correlated with the total fat content. This would benefit and refine the obtained results (Table 1).
The fatty acid in this study is expressed as mg/g milk fat. However, the reason we use mg/g milk fat instead of mg/mL is that we think this report format is more accurate. Although we have followed a widely used lipid extraction method and have repeated several times, the fat content analysis is not accurate, which is largely influenced by the experimental operation. Besides, fat content is the most variable constituent in human milk [9,10]. Therefore, we think the NA concentration presented as mg/g milk fat is better. As the response above, we have provided the fat content of all samples during lactation. Therefore, we can calculate the fatty acid concentrations using these data.

Discussion
Line 232
“The difference could be due to analytical methods, as well as differences between individuals from different locations.”
It is worth adding that also from which day of lactation the samples originated since there is a difference between the 3rd and the 6th day of lactation - both colostrum.
Added as suggested (line 251).

Line 235 - Table 3
It benefits the reader if the Authors will added additional citation to the Table.
It is very nice work. The reference has been cited as suggested (reference 39).

Line 264 The “top” of the Figure 4 – Omega-9” is misleading, please correct.
We deleted the “top” in this Figure 4.

Minor
Line 39 - Is “fatty acid (FAs)” – should be “fatty acids (FAs)”
Corrected as suggested.

References