Author's response to reviews

Title: Influence of the Calcium Concentration in the Presence of Organic Phosphorus on the Physicochemical Compatibility and Stability of All-In-One Admixtures for Neonatal Use

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Author's response to reviews: see over

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Rio de Janeiro, July 18, 2009.

FROM: Dr[a] Valéria Pereira de Sousa
TO: Nutrition Journal Editorial Team

Subject: MS. ref. No.: MS: 7471245592728961

We appreciate the detailed review and the suggestions. We have accepted all the suggestions and would like to resubmit our revised paper. Below is a point-by-point explanation of the changes included in the paper.

Reviewer 1:

The sentences were corrected as suggested (l. 346 and 348).

Reviewer 2:

All the suggestions were accepted and were changed in the text as marked.

1-2- The sentence was rewritten (lines 66-67 and 68-72).

3 and 5- The references were included (l. 85 and 97).

4 - In USA is not permitted the use of organic phosphate in TPN admixtures. The sentence was rewritten (l. 91-92)

6- These lines were deleted (l. 102-107).

7- The multilayered bags is a 3-layered bag composed by polyester, polypropylene and polyethylene. All the nutrients are mixed in the same bag and the order of addition is now presented in table 1 (l. 110-115 and 224-230).

8- The lots are prepared in separately bags. All the experiments were carried out with at least 2 different lots and in most cases 3 or 4 to verify the reproducibility of the data (l. 118-121).

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9- The crystalline precipitation was evaluated visually and microscopically. The sentence was rewritten (l. 150-151 and 254-257).

10 e 11 - The figures and table were moved to the results section (l. 224-231, 252 and 257).

12- The sentence was rewritten (l. 211-213).

13- Many authors have showing that FOX method is appropriated for the measurement non-specifically of lipid hydroperoxide and hydrogen peroxide in TPN admixtures. The linearity of this method was also extensively showed (jiang et al., 1992, 1994; Wolff et al., 1994; Colon 2002; Silvers et al., 2001). Calibration with standard peroxides, such as hydrogen peroxide is utilized (jiang et al., 1992; Nourooz-Zadeh et al, 1995; Colon et al.,2002; Silvers et al., 2001), as also linoleic hydroperoxide, *t*-butyl hydroperoxide, and cumene hydroperoxide, and present apparent extinction coefficient similar (Jiang et al., 1992; Nourooz-Zadeh et al., 1995; Gay et al., 1999; Yin et al., 2003)

In our experiments we prepared each day a standard curves in the range 1 - 100 μM. But, the calculations were made using the average of 3 standard curves prepared using a lower range (0-7 μm), next to the values found.
We recalculated the peroxide values measured using the standard curve obtained at the same day of the experiment (l. 214-216). This procedure is more accurate.

14 and 16- The values showed no significant differences, so the correct is P> 0.05, as remarked by the reviewer (l. 247 and 252).

15- The sentence was deleted.

17- The sentence was rewritten without a safe limit for peroxide level as many articles has been showing deleterious effects with low concentrations as remarked by the reviewer (l. 377-382).

18- In our experiments the bags were not exposed directly to natural light. The laboratory has artificial illumination and is protected to avoid sunlight. When the bags were stored at 4 and 37 ºC the formulation was completed photoprotected, kept in dark room (l. 387-420). Our peroxide values are similar with Neuzil et al. (1995) and Silvers et al. (2001), when the TPN was kept totally photo protected.

19- The discussion was reformulated and focused more on the interpretation of the findings (l. 410-416; 330-334; 295-299; 300-310).

- Despite Silvers et al. (2001) has observed a protective effect of vitamin in the formation of peroxides, many works have been showing that addition of vitamins cause more peroxidation. However, the lipids in the TPN protect MVI degradation in

the light exposure, maybe due the shielding of light by the opaque lipid emulsion (Silvers et al., 2001; Lavoie et al., 2005). In our case we photo protected the bags to study only the influence of temperature and excipients on the peroxide formation.

- We described the problems associated to lipid emulsion and TPN with increased fat globules at line 330-334. The benefits vs. problems associated with TPN are explained at lines 306-310.

- The selection of the calcium/phosphorus ratio is discussed at lines 288-310.

- Steger et al. (2000) demonstrated by in vitro studies the increase in peroxidation in TPN admixtures associated to TE, but these results may be due to the long period of analysis (30 days). Research shows that by assessing the presence of peroxides in the urine in preterm infants, the use of TE did not affect peroxide production (Bassiouny 2009). In vitro studies also corroborate this data (Huston et al., 1982 and 1987, Lavoie et al., 1997).

20- The sentence was deleted.

21- The sentence was corrected (l. 301-303).

22 and 23- The sentence was deleted.

24- The sentence was corrected (l. 338).

25- The filter usually recommended has 1.2 µm of pore diameter (Driscoll, 1996) (l. 340-342).

26, 27 and 28- The sentences were corrected as suggested (l. 344, 346 and 355).

29- This segment was deleted.

30- We attributed the low amount of peroxide measured to several factors. First, we use total protection from light for admixtures stored at 4 °C and 37 °C or partial exposure to artificial light (25 °C). It was shown that formulations kept covered with aluminum foil presented very low peroxide values (Neuzil et al., 1995; Silvers et al., 2001). Furthermore, the type of bag used has low permeability to oxygen and keeps little air inside. It is also possible that the peroxides generated were quenched by oxidation of the amino acids and vitamins present in the admixtures (Bathia et al., 1980 and 1983, Lavoie et al., 1997) (l. 394-422).

However, any work demonstrates peroxide values using the same conditions (photoprotection, multilayered bag) and formulation (calcium/phosphorus ratio, organic source, lipids, amino acids, trace elements and vitamins together) used in this work.

31- The volume is now indicated on the first line of the Table 1 and the sequence follows the order of addition in the bag. All the vitamins are now provided in the same unit.

32- The table 4 was corrected. The correct value is 8.7.

33- The letters A, B and C were replaced by the temperatures in the panels.

Yours sincerely,

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