Author's response to reviews

Title: Efficacy of different strategies to treat anemia in children: A randomized clinical trial.

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Author's response to reviews: see over
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Editorial Office
Nutrition Journal

Attached please find the revised version of the manuscript entitled “Effectiveness of different strategies to treat anemia in children: A randomized clinical trial” as a potential original contribution for publication in the Nutrition Journal. The study evaluates the efficacy of several worldwide recognized strategies to treat anemia, and also evaluates child’s acceptance to the different treatments.

Regarding the possible conflict of interest mentioned by the editor, Dr. Odio was working at P&G Co. at the time of the study; however, he declared that there is no financial or commercial interest of this company in the present study. Also Dr Odio was only involved in design of the study but he did not participate in data analysis or interpretation of the study.

Below you will find the responses to each reviewer comments and suggestions. We believe that with the recommended changes the paper has improved considerably for publication in the Nutrition Journal.

Sincerely yours,

Jorge L Rosado, PhD.
Reviewer 1

Major Compulsory Revisions

1. Major grammar revisions are required throughout the abstract and manuscript to improve understanding and readability. As written, the writing is not acceptable or suitable for a scientific peer reviewed journal. Information presented is difficult to interpret due to poor and excessive wording. I have specific suggested editing comments and will be glad to forward to the authors if requested; however, I will not list all of them in detail within this review. Author needs to avoid writing in the first person throughout.

The paper has been revised and edited according with reviewer’s suggestions.

Abstract:

2. Within results section, include the major significance values rather than providing only generalities.

Abstract was modified and now includes specific values and their significance.

3. The objective stated throughout the abstract and manuscript should be consistent throughout the paper, ie abstract pg 2 line 5 and background pg4 line 12.

Objectives in both sections are consistent; they are presented here for clarification as follows:

   Abstract: To evaluate the efficacy and child acceptance of several recognized strategies to treat anemia.

   Background: In this study we evaluated the efficacy and the child acceptance of several strategies that have recently been recommended to treat anemia.
Methods:

4. this section needs considerable help in organization to ensure that the correct methodology is all contained within the appropriate subheading sections. Currently there is significant overlap and the methods are mixed among sections. This section was reviewed as suggested by reviewer and arrangements were made in organization and content to improve readiness.

5. pg 5 consistently use for Hb g/dl (units need to be consistent and correct throughout). Talk about cut off levels of Hb in section where you are talking about blood collection. In subject selection section just refer to those that were anemic vs not anemic. Explain what you mean by “10 blocks” in line 21. Hb is now consistent along the manuscript. All information about methods and cutoff values are now in the biochemical measurements section. The randomization process was rewritten in the manuscript to make it clear.

6. Clarify on pg 5 line 23 that siblings with anemia were treated the same unless you actually treated all children in the household even if they were not anemic. Also need to clarify if only one or multiple children from within a household were included in the study. If more than one child was included from a single household, need to adjust for this in data analysis. All siblings from an anemic child within the same age range of subjects in the study were treated regardless if they were anemic or not; this is now explained in the text. Siblings with the same treatment were included in the analyses if they were anemic. We did adjust for siblings in previous analyses, obtaining similar results in a GEE model that accounts for the
siblings correlation. However, we decided to present results from the univariate ANOVA that accounts for the community random effect that explains more variability than the siblings variable.

7. Pg 7 line 4&5. Move the discussion about iron and ferritin to biochemical measurements section.

As suggested by the reviewer, the discussion about iron and ferritin was moved to biochemical section.

8. Pg 7 line 5&6. Move the height weight statement down to the evaluation of weight and ht section. Line 8 is a repeat of line 5&6.

The sentence was removed from the text since it was repeated in the next paragraph.

9. Line 13: specify age cut offs for use of supine length (WHO anthro standards use age 2 as the cut off). The way it currently reads implies that only children that could not stand were measured lying down. If this is so and it was not based on age then data interpretation based on WHO anthro would not be correct.

The WHO calculations in the SPSS syntax, also allow for choosing length or height, and so it was specified in the manuscript. We did not have an age criteria, whenever the child was able to walk we measured height and if the child was not able to walk we measured length.

10. Pg 7 line 21: suggestion- blood samples to determine iron and ferritin were collected....

Text was inserted as suggested.
11. Pg 8 line one should be moved to the end of the paragraph since all info on iron should be kept together and all info on Hb kept together. Methods for iron, ferritin and Hb should all be described separately and not mixed throughout methods section

The text was moved as suggested and this section is now reorganized by type of analysis.

12. Pg 8 line 8 C-reative protein cut-off values used need to be included.

The C Reactive protein method cited in the reference refers to a qualitative technique, and there are no cut-off values. This was clarified in the text.

13. Pg 9 line 6-8 should be moved to biochemical analysis section within methods

The text was moved as suggested.

14. Pg 9 line 14 verify how treatment rejection was computed. “Percentage of weeks” is not clear and is not a typical way to compute percentage.

The questionnaire that refers to treatment rejection was administered once a week; each response of rejection corresponded to the children average behavior of the previous 7 days. This part of the methodology was more extensively explained.

15. Pg 9 line 17 is where the analysis section should actually begin. Previous information is appropriate for the methods
As mentioned, the methods section was reorganized according with the suggestions by the reviewer.

Results:
16. Need to add statement as to whether the groups were the same or significantly different at baseline for the main variables. If that is what was being verbalized in pg 10 line 13, it is not worded correctly since that is stating that treatments were not significantly different.
   **The sentence to indicate that there were not significant differences between groups at baseline was re-written to make it clear.**

17. Pg 10 line 17 states that data is not shown. The data should be included since within the methodology pg 10 line 8&9 it states that the children were stratified by age (cut-off of 24 points) and by gender. Line 17 states that the data is remarkable- define remarkable based on whether it is statistically significant or not.

   **Sub-group analyses was not a main objective of our study, however we carried out those analysis because in randomized clinical trials it is recommended to carry out sub-group analysis, and we did not find significant differences. The word remarkable was eliminated from the text to avoid confusion.**

18. Pg 11 line 1. Remove the statement (data not shown).
   **Statement was removed.**

19. Pg 11 line 10: for viral diseases do you mean viral infections? Also is this
sentence fact based on actual medical diagnosis or was it information reported by parents or your inference? - this sentence needs to be clarified.

The diagnosis was determined by a physician with the morbidity questionnaire that included questions about several symptoms and their duration. The sentence was clarified.

Discussion:
20. Pg 12 line 7. “Even at the same dose of iron”. This is not correct since you previously explained that the various treatments tested did not have the same amount of iron.

The text “Even at the same dose of iron” was removed from the paragraph to avoid confusion.

21. Pg 13 line 17 need to add reference number for Sachdev et al at the end of the sentence.

The reference was moved to the end of the sentence.

22. Pg 14 line 1. “due to the…” I would suggest rewording since you did not actually evaluate the why to be able to state definitively that it was a result of the high concentrations of minerals.

Text was reworded to indicate that the high amount of minerals in the supplements could be a possible contribution to the final taste of the solutions.

Conclusion:
23. pg 14 line 20 #3 needs rewording to improve clarity. Within the conclusion
need a sentence to state what this research has contributed to science. Add a suggestion for the next step. Include a statement on how to best apply this knowledge.

**The conclusion now includes a sentence of the potential applications of our findings.**

List of abbreviations:
24. add IDA, WHO and UNICEF

**IDA, WHO and UNICEF were added to the list of abbreviations**

Tables and Figures
25. Table 2. need to add Hb <11.7 and elevated C-reactive protein based on cut off used. Also point out any statistically significant differences between groups. Again utilize consistent units ie Hb g/dl.

We believe the reviewer refers to table 3, not table 2. Units for Hb were changed. The reviewer suggests to “add Hb<11.7 and elevated C Reactive Protein”. Hb<11.7 would refer to the percent of children that remained anemic after treatment, this information is given in figure 2; C-reactive-protein was not one of the outcomes investigated, it was used to avoid false positive results in the iron and ferritin analysis, so we do not think it should be included in this table.

26. Figure 2. spell out percentage along left hand of table vs using % sign. Need to reword title- it is not clear. Are end of treatment data presented? Use of a&b is not clear.
Titles and footnotes of Figure 2 were changed according with reviewer´s suggestions

Minor Essential Revisions
1. Abstract: Suggest continuing with acronyms within the abstract conclusion for consistency and clarity.
   Use of acronyms in abstract are now consistent.

2. Background: Line 13. Requires reference following the word population.
   Reference was added.

3. Also ensure capitalization is used correctly throughout. Ie. Anemia in line 16.
   Capitalization was revised and corrected where necessary.

4. UNICEF needs to be spelled out the first time used.
   UNICEF was spelled the first time used.

5. Do not begin sentences with acronyms.
   Sentence that began with acronyms were corrected.

6. If you have previously spelled out an acronym then it should be used consistently rather than reverting back and forth (except spell out at the beginning of a sentence). Ie hemoglobin vs Hb pg 11 line 22
   The use of acronyms was modified accordingly to be consistent in the document.
Discretionary Revisions:

1. Figure 2. Since the prevalence of anemia is of interest, I suggest that the anemia prevalence be at the bottom of the column along with the Percentage value listed rather than having the not anemic prevalence listed.

Figure 2. The figure was changed as suggested by reviewer.
Reviewer 2

Major compulsory revisions/issues to address:

1) In the title this paper is referred to as an "effectiveness" trial, while in the body of the paper the "efficacy" of the intervention is referred to. Clarification is required as to how the authors perceive their own study.

   The study compares treatments that already have been approved for their effectiveness, thus the study evaluates their relative efficacy among them. Effectiveness in title was changed to efficacy.

2) There is too much emphasis on morbidity and anthropometry throughout the paper (methods, results, discussion) when the study is not powered to see differences in these outcomes.

   Sample size was calculated to detect a difference in hemoglobin change, which was the main outcome variable. Morbidity and anthropometry were included in the objectives as part of the efficacy evaluation along with the hemoglobin status. We are cautious to indicate that the evaluation of growth and morbidity corresponds to the period of observation.

3) Additionally, there is too little information in the paper on how compliance/rejection was assessed considering this was part of the rationale for the study.

   Explanation on how compliance and rejection to treatments were assessed was expanded in the method's section.

4) Given that all the treatment regimens provided different amounts of iron, the
authors should examine iron intake (compliance rate x supplement Fe content) in one of their models to help describe the impact of each supplement on change in Hb. Moreover, the authors should provide a rationale for what they think is the best possible intervention strategy given their findings. (Is the MMS to be recommended if compliance is somewhat affected? Are the more palatable interventions effective enough?)

Iron intake was calculated as suggested by the reviewer and included in the model as an additional control variable for measuring the effects of treatment on hemoglobin and anemia. Text was modified accordingly to describe the additional control variable. Recommendations on best possible interventions, given our results, were included.

Methods section:

5) Was the sample size chosen before the study or calculated post hoc to justify the number of participants, as the wording seems to imply?

Sample size was calculated before the study. Text was changed to describe how sample size was calculated.

6) Were IS, IFS, and MMS delivered to homes in liquid form? How were mothers advised to administer? For which of the interventions were the treatments "counted"? (page 6, line 25) To what precision were they weighed, using what, where (eg. in the home)?

The way supplements were administered and delivered at the home of the children and how mothers were instructed to prepared them was explained more extensively to make it clear.
7) More detail is required on the methods for collecting adherence/rejection information. What were the questions that were asked? The paper reports that "treatment rejection was computed as the percent of weeks that the mother reported any difficulty when giving the treatment to the child...", which does not seem like a very discriminatory variable--was this more likely to happen when children were sick, tired, full, etc? It seems that if the treatment regimen was not well defined (eg. always give at the beginning of a meal) there are many factors other than the supplement itself that could lead to rejection.

**Since the questionnaire was administered once a week, data is presented as the number of times that the mother reported any difficulty when administering the treatment to the child. In addition, another question (child dislike of the treatment) that we did not include before because seemed redundant is now included to make clear that the rejection was due to palatable characteristics. We now provide more information about the specific questions asked regarding evaluation of treatment adherence and rejection.**

8) Page 9, line 1 onward--The authors should clarify whether children who did not complete the treatment were included in the analysis as long as a follow-up blood sample was available. It seems this would be the appropriate "intent-to-treat" analysis. Figure 1 implies that children were lost to follow up because they EITHER did not follow the treatment regimen (the most extreme form of treatment "rejection"--and thus ideally kept in the analysis if a blood sample is available) OR because they did not give a blood sample. Is this the correct interpretation? Or were children who discontinued the treatment never asked for a follow-up blood sample?
Children that did not finish the study did not accept to provide a blood sample, which was one of the reasons why we had some lost-to-follow-up cases. In addition, children who discontinued the treatment were not asked for a blood sample. Thus, the intent to treat analysis was not possible in this study. We analyze the percent of children that did finish the study per treatment (table 5) and we did not find any statistical difference, meaning that the lost-to-follow-up cases were not related to any treatment.

9) The description of the data analysis using compliance data is not clear.
Compliance is now better explained for clarity in the section describing evaluation of adherence, compliance and rejection to the treatment.

Minor essential revisions
10) Units for Hb are wrong throughout--should be g/dL
Units of Hb were corrected.

11) Page 7, line 4-5: "Also, height or length..." does not fit here since it is addressed in the next paragraph
The sentence was removed since it was repeated.

12) Page 8, line 15: The questionnaire is introduced on page 6, line 25, which is more appropriate placement since it is a high priority objective of the study to evaluate compliance. It is repetitive to reintroduce it here without providing substantially more information on how it is used.
Text was reorganized to avoid repeating the methodology and more information about the questionnaire and its administration is given in the appropriate section.

13) Page 9, line 8: morbidity data expressed per what unit time?
The unit was frequency of disease experienced within the trial period; the text was modified to clarify.

14) Results, page 11, line 1: "When comparing adherence rates...", there weren't significant differences in what? The sentence is too vague.
The sentence was reworded.

Discretionary revisions
15) Results, page 10, line 17: why not show the results for the effect modification with age?
We believed it was not necessary. Subgroup analysis was not a major focus of the study, however we carried out sub-group analyses as it is recommended for randomized clinical trials. The differences in this sub-groups were not statistically significant, thus we decided not to show those results to avoid confusion.
Reviewer 3
Answer to general comment of reviewer.
The reviewer’s concern is not having a specified reference group; this is because we did not intend to measure the effect of treatments versus a standard of reference. In fact there is not such a “standard of care” to treat anemia. The objective of this study was to compare several worldwide recognized strategies to treat anemia among themselves. Besides, a placebo group was not possible due to ethical reasons, since all children were anemic and needed treatment.

Regarding the reviewer’s comment about the literature not mentioned in the paper, a literature revision was made to include relevant studies in the introduction section. It was not the purpose of the paper to review all literature on iron supplementation.

Background
1. P3, Lines 9-10 incorrectly states that there have been few studies of how to treat micronutrient deficiencies in children. There is actually a large body of work on this topic. For a review of iron deficiency, see Iannotti et al (2006). Iron supplementation in early childhood: health benefits and risks. AJCN; 84(6):1261-76.

The sentence was re-written to make clear the kind of studies we believe are needed to contribute to a better definition of strategies to treat anemia.

2. P4, Lines 1-4: Please describe the results of the previous supplementation trials in Mexico as they directly relate to this study population.
These lines refer to a national supplementation program with a micronutrient fortified porridge. Unfortunately the effectiveness of this supplement to reduce anemia has not been evaluated; only few studies have evaluated the effect of the whole program on hemoglobin concentration.

3. P4, Lines 19-20: How far in advance of the trial was the census conducted? Please provide dates of the trial and the census.

**Dates are now included; the census was conducted 2 months before the study started. The purpose of the census was to plan the logistic of the study, and all inclusion criteria were verified at baseline.**

4. Why were breastfeeding children excluded? What is the justification for the exclusion? This would then indicate that these findings are only generalizeable to non-breastfed children.

**Breastfeeding children were excluded since anemia is higher after breastfeeding and supplementation is recommended after breastfeeding. Moreover, we did not want the supplementation to interfere with breastfeeding.**

5. P5, Lines 10-15: Please use consistent units throughout—g/dL are reported in some instances and mg/dL in others.

**Units of Hb are now consistent.**

6. P6, Line 22: What was the frequency of dosing (daily or weekly)?

**Treatments were administered daily; this is now clear in the text.**
7. P9, Lines 17-23: You stated earlier that siblings were included in the study. How many siblings and how was clustering addressed in the analysis?

We did carry out analyses adjusted for siblings, obtaining similar results in a GEE model that accounts for the siblings correlation. However, since GEE model does not include random factors, we decided to present results from the univariate ANOVA that accounts for the community random effect that explains more variability than the siblings variable.

Results
1. Figure 1 is difficult to follow because both a flow chart and a table have been used. Can the table below be converted into text boxes to complete the flow chart?

The flowchart was completed and modified to make it easier to follow.

2. Table 2: There appear to be some large differences in baseline characteristics between groups. In particular, the prevalence of low serum ferritin and low serum iron appears to be quite large between groups. What test of significance was used to compare groups? This needs to be stated in the footnote of Table 2 and in the methods.

We used ANOVA to compare means and Chi square to compare prevalence and found no differences among groups at baseline. As suggested, this is now stated in the table’s footnote and methods section.

3. P10, Lines 16-17: If the interaction is described is significant, the data should be presented. Otherwise, this statement should be omitted. What about the interaction with gender? This was described in the methods but not presented in
the results. If the data is not to be presented, then the mention of it should be dropped.

Possible interactions were tested and resulted non-significant then there are no interactions including interactions with gender. We clarify this in the data analysis section to avoid confusion.

4. P10, Line 19: Here, the authors have stated that they used a chi-squared test, but this was not described in the methods. What method was actually used to test between-group differences?

   The chi square analysis is now mentioned in the data analysis section.

5. P10, Line 25 – P11, Line 1: The growth and morbidity data is not shown in any way in the paper. Was the study powered to look at morbidity rates as an outcome? Also, the short intervention period would be unlikely to have much of an effect on change in growth. These two sentences should thus be dropped.

   Sample size was calculated to detect differences in Hb, which was the main outcome variable. Although the study was not designed to test an effect of treatments on growth we believe that it is important to mention that in the period of observation there was not any difference in growth between groups. On the other hand, morbidity data did not show significant differences because none of the treatments have an important effect on morbidity. We included a new table with the anthropometry data and the morbidity results are now in the text of results section.

6. Figure 2: It would be more informative to have the prevalence of anemia on the bottom and exclude the sections of the bars representing the non-anemic
subjects. This is implied since the definition of anemia is dichotomous. Why do the numbers in the text not match the numbers in the figure?

The figure was changed as suggested. Numbers in the text were corrected..

7. Table 3: Some of the numbers in this table appear to be unusual. For example, the change in serum ferritin in the IFS group was reported to go from 70.69 at baseline to 50.31 ug/dL at follow-up, which is a change of -20.38 ug/dL. The reported adjusted difference is only -1.88 ug/dL, however. Similarly, in the FCF group, the change in ferritin goes from 57.95 to 60.45 ug/dl from baseline to follow-up—a difference of only 2.5 ug/dl. Yet, the reported difference is 20.24 ug/dL. Can the authors explain?

This table now includes the unadjusted change and adjusted changes. It is true that these two values differ. The reason why both values look different in some treatments is because the initial ferritin concentrations differ between treatment groups. In fact, the ANOVA significance is 0.07, and initial mean values go from 22mcg/dL to 72mcg/dL. When including the initial ferritin concentration as covariable, mean values are modified due to the strong correlation between the initial ferritin concentration and ferritin change. The adjusted model shows the mean values independently from the initial ferritin concentration.

8. Table 4: in the text, the compliance rates at 80 or 90% are reported to be not significantly different. In the table the compliance rate of 100% is reported. It would be clearer if the authors would report consistent numbers between the text and the table.

The text was modified to agree with the table.
9. Table 4: The methods of analysis reported below the table report that the difference in means are compared, yet the table contains only proportions. Can the authors clarify what methods were used?

*Individual proportions can also be analyzed in the same way as numbers, obtaining a mean of proportions. However, to avoid confusion, the subject values are now shown as number of times that the mother reported treatment rejection or dislike.*

Discussion

1. P12, Line 7: This sentence isn’t clear because the study has tested a variety of different dosages of iron. Which one is being referred to as having the “same dose of iron” as the food developed for the Oportunidades Program?

*Sentence was removed to avoid confusion.*

2. P12, Lines 16-18: Most of the references for this sentence are for adolescent or adult women, yet this study is about young children. It would be more useful to cite the papers referring to micronutrient sprinkles for children. See, for example: Menon et al (2007). J Nutr; 137(4):1023-30 or Christofides et al (2006). Matern Child Nutr; 2(3):169-80. There are others as well that would be more relevant here.

*References were update in this part of the text as well as in other parts of the document as suggested by the reviewer.*

3. P12, Lines 25- P13, Line 20: The morbidity and growth data is not presented in this paper. Therefore, this whole section should be dropped.
As mentioned before, one of the objectives was to evaluate the effect of the treatments on morbidity and growth, we included the growth results in table 4 and the morbidity results in the text of results section.

4. P14, Lines 15-23: Because there was no placebo control group, it is impossible to tell if the change in Hb seen in this study was due to the supplements or due to secular changes in the intervention communities. Therefore, the first conclusion statement is too strong and should be dropped. The treatment groups can only be compared to each other or, ideally, to whichever one is considered the reference group.

**Conclusion 1 was changed to state that all treatment groups decreased Hb concentration which is one of our findings. Conclusion 2 and 3 refer to comparison among treatments. We already addressed the issue of not having a placebo group due to ethical reasons. This is also addressed in the text of the paper for clarity.**