Author's response to reviews

Title: Effect of daily versus weekly home fortification with multiple micronutrient powder on haemoglobin concentration of young children in a rural area, Lao People's Democratic Republic: a Randomized Trial

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Author's response to reviews: see over
RESPONSE TO COMMENTS FROM Reviewers

Manuscript Number: 1919577385523630

Manuscript Title: Effect of multiple micronutrient supplements for improving anemia of young children in a rural Lao People's Democratic Republic: a randomized trial

Submitted to Nutrition Journal

Dear Dr. Donnen and Dr. Arlappa,

Thank you very much for your valuable time for revising our manuscript and give very good comments. It is very much appreciated. We did going through your comments then revised accordingly on the manuscript. Below are point by point responses to the comments.

Response to Reviewer: Philippe Dr Donnen
Version: 1 Date: 28 April 2011
Reviewer's report:

Major compulsory revisions
- The text should be reviewed by an English native speaker;
  - I do apologize for my Laos English, I did ask a native English speaker to go through the text before re-submission
- There are quite a lot of methodological weaknesses (see below); and Discussion is not enough developed;
  - Thank you very much for this good comments, we tried to extensively edit in the line of your comments.
- Title
  The title is confusing as all children included are not anemic;
  - You are right. The introduction of multiple vitamins and minerals powder in Laos is to correct micronutrient deficiency in particular anemia which is a severe public health problem in Lao PDR, why we used a term anemia in the title.
  - We could change as “Effect of daily versus weekly home fortification with multiple micronutrient powder on haemoglobin concentration of young children in a rural area, Lao People’s Democratic Republic: a randomized trial”
Abstract
P2
L6: Stop the sentence after “recommended” and start a new one with “However”.
- Yes we revised accordingly

P3
L1-2: The improvement of Z-score WFA is very small and not SS!
- Thank you very much, it is true

Background
P3
-L1: The authors should first present the problem at the world’s level and in a second time for Laos.
You are right, we deleted the first part of introduction and we added the sentence to present the problem at the world’s level and in a second time for Laos. As well as additional information about the problem

“According to UNICEF/WHO/WFP, micronutrient or vitamin and mineral deficiencies affect approximately 2 billion people worldwide [1]. The adverse effects of micronutrient deficiencies during childhood are substantial. Micronutrient deficiencies have negative effects on growth and development, and cause reduced psychomotor performance, and increased morbidity and mortality [2-8].
The main cause of the multiple deficiencies is a poor quality diet, often as the result of an inadequate intake of animal sources of foods.”

-L1-4: it is contradictory! 10.8% from 40.9% is about a quarter, it seems not to be the major cause;
- Not to confuse, we deleted this information.

-L4-5: a major risk factor is also infection and especially parasitosis;
We added the risk factors accordingly

“ Additionally, non-nutritional factors such as parasitic infections, genetic hemoglobinopathies, malaria, and infectious diseases also impair nutritional status and health as well as alter the metabolism of multiple micronutrients[9].

Infant and young children are particularly at risk of VMDs. From the ages of 6 months, the nutrient needs of infants need to be met by breast milk and complementary foods. Even though possible modifications of the recipes were considered such as adding extra animal source foods (ASFs) and reductions absorption-inhibiting components such as phytate, it would still be unlikely to meet requirements for certain micronutrients such as iron, calcium, and zinc. Additionally ASF alone may not provide enough vitamins and minerals for young children [10-11]. Therefore, micronutrients are very likely to be limited in the diets of young children between ages 6 and 23 months old in developing countries. This fact applies also in Lao PDR where the quality of complementary food that is predominantly cereal-based is very poor[12]. Thus, for children aged 6-23 months, extra vitamin and minerals need to be added to their diets to improve development and growth and reduce morbidity and mortality[9].
Anemia, which is mainly due to iron deficiency, is one of the major micronutrient deficiencies in developing countries. In Laos PDR, the prevalence of anemia among children 6-59 months of age in Lao PDR based on Multiple Indicators Clusters Survey & National Nutrition Survey in 2006 was 41%. The prevalence was even much higher among children aged 6-23 months at 63.5%\textsuperscript{[13]}. The prevalence over 40% indicates a very severe public health problem according to the WHO\textsuperscript{[14]}.

-L7: “decreased interaction with the environment”: what does this means exactly?
We deleted the sentence

P4
-L5: “micronutrient supplementation”: the article is about home fortification not supplementation;
We edited this term

-L7: individual status of what?
We deleted this sentence

-L12: 2 sachets per week: it is not clear if both sachets should be taken the same day or one sachet at two different days;

Our primary aim was to compare the effects of 2 sachets that will be given at two separate days during the week with a daily sachet of multivitamins and minerals powder (MMP) on haemoglobin concentration, the prevalence of anaemia, and the growth of infants and young children. We also assessed MMP compliance for the two regimens and the acceptability of the product among mothers or care takers of the study subjects.

-L13: MMP should be written entirely the first time it appears;
-The authors should explain why they gave multiple micronutrient home fortification and not only iron;

We added the sentence on the four paragraph of the introduction part to explain why we gave multiple micronutrient home fortification and not only iron

“in addition with multiple deficiencies coexist, home-fortification with multivitamins and minerals in powdered form or known as sprinkles has been widely promoted as a way to address IDA and micronutrient deficiencies. [17-25].”

-Nothing is said about the epidemiology and control of malaria in this region though it has been recently demonstrated to be an important factor to take into account when dealing with iron supplementation;

Thank you for this very good comment. In fact, the control of malaria in the study areas was successfully announced ten years before the study commenced.
We added the following sentence to the fourth paragraph on the study area part under Material and Methods

“A malaria control program was successfully executed in all the villages 10 years ago. Materials and methods”

P5
-L2: why is the production of other foodstuffs not sufficient? no data on consumption of food potentially rich in iron?
All of villagers are farmers. The main economic income is from the production of rice, cotton, and watermelon. At the same time textile is the second source of income. We added the new sentence to the second paragraph on the study area part under Material and Methods

“The Lahanam area has relatively high production of rice, cotton and watermelon and most villagers have access to adequate amounts of rice. However, other foodstuff is seasonally available from nearby small patchy forests and market town (with 10 kilometers from Lahanam area). Therefore, the intake levels of fat, calcium, iron, and retinal in the area were very low compared with the recommended dietary allowance of Thailand and the WHO/FAO[29].”

-L3: food supplement is not a right term; complementary food is better;
- Thank you, yes you are right We changed the term

-L10-13: it is necessary to increase the number to compensate the lost to follow up; what is the evaluation of lost to follow up?
- Yes, when we calculated the sample size, we did plus 10% for lost of follow up, but on the first draft we did not put this information. We added to the first paragraph under Study design and randomization

“The study was a randomized trial. A statistical power test (80% power and significance of p = 0.05) showed that to reduce anaemia by 20% (from 41% to 21%), to improve haemoglobin concentration by 0.56 g/L, and plus 10% to compensate the lost to follow up, a sample size of 110 children is needed in each group”.

P6
-L1: how was children’s age established?
Data collectors did check the family book of each household that recorded date of birth (DOB) of children (Administratively, all new born children are registered 1) to the village head; 2) to district authority that can put a new child’s name on the family book, 3) in case that above 1) & 2) did not performed, the village head will inform to the vaccination outreach team when they come routine to the village every 2 mo, they will record on the yellow card for vaccination about child’s name, DOB, weight, height, and vaccination received). Then we can check child’s age through family book and yellow card (vaccination card).
We recruited children from 6 mo as they already received complementary food that can fortified and up to 53 mo as we have to follow up 6 mo then children 53 mo will reach 59 when we completed the study.

-L2-3: exclusion criteria: what if the children had received iron or other micronutrient supplementation before the study? What if baseline Hb levels were very low?

In fact we did have 2 more criteria but we did not add at the first draft as the lowest Hb level of our subjects at baseline is 72 (only one case) and no one received any supplementation.

We revised to the third paragraph under Study design and randomization

“367 eligible pre-school age children were identified from the HDSS database of the National Institute of Public Health (NIOPH). Inclusion criteria of subjects were, (i) age 6 to 53 months at the time of recruitment, (ii) willingness to participate, (iii) receiving complementary food in addition to breast milk, and (iv) apparently healthy. Exclusion criteria of subjects were having fever or any illnesses during the day of enrolment, baseline level of haemoglobin is less than 70 g/L, and children currently receive iron supplementation. Out of original 367 children who met the criteria, 17 were absent at the time of enrolment, and 14 were excluded for having infections with fever at the day of enrolment. Therefore, a total of 336 children were enrolled in the study.”

-L9-11: This is an unusual way of doing randomization; could the authors provide a reference?

We did revised this paragraph for more understandable to the fourth paragraph under Study design and randomization.

“In advance of recruitment, we used an simple computer program (a random number generator) for the randomization process, which was done by household. At baseline, we then enrolled 336 eligible children and randomly allocated them to 3 groups as follows: control (n=111), twice weekly multiple micronutrients supplementation (n=115), and daily multiple micronutrients supplementation (n=111). One family had two children and more who fulfilled the requirement to participate in the study; therefore all children were included and treated as separate cases, but they were allocated to the same group control or intervention. There were 215 one child families; 49 families with 2 children, and 3 families with 4 children.”

-L16: it should be immediately precised that the sachets will be given at two separate days during the week;

Yes, we did add this information on the introduction part and clarify more in the method section.

P7

-How were the doses of micronutrients calculated? The age of the children varies considerably and for examples, iron needs are very different for children between 6 and 12 months compared with children older than 12 months. The authors should justify their choice.
Thank you for this comment. We added the information to the second paragraph under Micronutrient supplements:

“The doses of micronutrients used in this study, were calculated based on the World Health Organization recommendation on the dosage schedules for iron supplementation to prevent iron deficiency anaemia[14]. Indication for supplementation is where the diet does not included foods fortified with iron or where an anaemia prevalence is above 40%, children from 6 to 23 months of age should receive 2 mg of iron per kg body weight per day with duration from 6 mo up to 23 mo of age and children from 24 to 59 mo of age should receive the same dose calculation up to 30 mg per day for 3 months. The dose of iron in the multivitamins and minerals powder used in this study is 10 mg and this is in the range of daily recommended dosage of iron for young children according to the America’s Food and Drug Administration. A total number of 168 sachets were provided for daily supplementation group and 48 sachets for twice weekly supplementation group.

- Why did the authors not used a placebo controlled group? It is not difficult to manufacture sachets of powder without micronutrients? Why was the study not double blind?

The sachets of sprinkles used in this study were provided by UNICEF that imported from Switzerland. In Lao PDR, it is not easy to manufacture any of the products, in particular concerning packing, and so on. We still have many difficulties and constraints both technically and financially. Therefore, MOH allowed us to conduct the study as we need evidence based information for implementing the pilot distribution of the product to correct anemia and micronutrients deficiencies in the affected areas of flood in 2008 as well as Typhoon Ketsana in 2009, as we mentioned on the last paragraph of the introduction part that “However, the effect of providing sprinkles to home-prepared foods may depend on local food culture and acceptance by local people. Therefore, multivitamins and minerals powder need to be tested locally to clarify the optimal starting point, the duration, and acceptability [27].”

P8
-L8: NIOPH should be written entirely the first time it appears;
Thank you, yes, we did correction

P10
-The way the supplements were given to the mothers and children should be explained before;

Thank you, yes, we did correction

- How exactly was the compliance measured? Following what mothers said? Counting the empty sachets? Other?
Under the Monitoring forms (Compliance and acceptability), we added this information to the last sentence of the paragraph “the total number of empty sachets was used to measure compliance.”

**Results**
P11
-L3: having diarrhoea is a reason to drop out?
I know that this is not a main reason to stop, but according to consent form for each participants that they could stop at any time if they wish and are not sure that they want to continue. Those who refused to continue the study thought that because of sprinkles, their children got diarrhea, even our team tried to convince them with many explanation, but they still wanted to discontinue.
-L4: is this reference 28 really essential?
- It might be not necessary, we deleted this reference

P12
-L11: what does this means?
We deleted the sentence

L12-14: why not indicating the SD for baseline measures?
We added the SD to all baseline measures

P13
-L5: The means and changes of anthropometric Z-scores between baseline and week 24 are shown in table 3;
Thank you, we did correction

-L6-7: Weight for height Z-scores decreased in all groups!
Thank you, we did correction

**Discussion**
P14
-L5-7: in the result section, the authors present evolution of anaemia and haemoglobin for the groups as a whole but without statistical tests. It is thus not possible to conclude that the interventions were effective;

In fact, we did the statistical test for anemia we used McNemar test that showed the following Group 123
1 vs 2 ns 0.043 0.039
1 vs 3 ns <0.001 <0.001
2 vs 3 0.012 <0.001 <0.001 so the control group only shows a significant change between 12 and 24 weeks whereas the TWS and DS show significant changes on all three occasions
For Hb concentration we used the repeated measures with all 3 groups and just with the DS & TWS
  - All together no difference between the 2 lines
  - For anaemic only borderline significance p=0.054

-L8-9: this affirmation corresponds to which results?
It is a mistake of writing, we did revise the phrase

-L10-12: the authors do not comment the fact that for severely to moderately anaemic children haemoglobin changes were similar for TWS and control groups;

We did revise the paragraph

P15
-L1-2: was the change statistically significant?
-L2-5: what does this means?
We did revise the sentence

-L9-12: this sentence is unclear; if we talk about compliance, it was much higher in the TWS group; if we talk about efficacy in reducing anaemia, DS might be better; but the authors should not give program implications as their work is not enough strong in its methodology to give valid results;
Thank you for this good comments, we did revise the paragraph

-L13-16: these subgroups analysis may give unvalid results because of confusion and the sample size of the subgroups may be too small;
Yes, you are right, we deleted this sentence

The authors don’t at all discuss the eventual benefits of multiple micronutrients compared with iron alone (interactions between micronutrients absorption and utilization). They also don’t discuss about the doses of micronutrients given.
Thank you, we added more information to discuss this issue

Figure 1
-Why did the randomization led to unequal groups in terms of number of children? (110/111 and 115)
As in some selected household had more than 2 children and all children were included in the same group but treated separately, I think this is a reason why number of subject in each group is differed

Table 1
-The n for the variables in the 3 groups should be removed; they can be calculated from the total n in the subtitle. Ex: Sex, male in the control group: 34.5% of 110 make 38;
Thank you, we removed n for the variables
- “mothers working outside” was not defined in the methodology section; 
  We added the information in the methodology section

- High SES2 the footnote 2 does not refer to SES; 
  We added the information in the methodology section

- Breastfeeding status: what represent the % ? The breast fed infants?, exclusive?  
  This showed % of breastfed infants, we added the information

- What is the interest of presenting weight and height as WHZ, HAZ and WAZ are presented?  
  To see the full range of measurement

Table 2
- Title is incomplete; 
  We edited the title

- What is the significance of AB joined? 
  Mistake of typing

- The problem with these subgroups analysis is that they may be confusion factors so as 
  nutritional status, age, etc. Interpretation of these results should therefore be prudent; 
  Thank you, you might be right

Table 4 - It is table 3; Yes 
- The line with the n should be placed at the right position; Thank you 
- b: which tests have been performed? When Anova is SS, comparison 2 by 2 can be done but 
  using correcting factors (ex Bonferroni). Was this done?

**Level of interest:** An article of limited interest
**Quality of written English:** Not suitable for publication unless extensively edited

This study is very important for the MOH of Lao PDR, we are still young in doing research and 
publication, and we have very few publications at international level by our own researchers as 
first author, we do hope that after this extensive editing, you will consider our paper for 
publishation. We are very happy to revise if there will have any weaknesses.

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.
2. Reviewer's report

Title: Effect of Multiple Micronutrient Supplements for Improving Anaemia of Young Children in a Rural Area, Lao People's Democratic Republic: a Randomized Trial

Version: 1  Date: 27 April 2011

Reviewer: N Arlappa

Reviewer's report:
Major Compulsory Revisions

1. The children are randomly allocated to three different groups. Thus, it is clear that children of control group and other two intervention groups are residing in the same village. In addition, the authors mentioned that the mothers and field workers are not masked. Then, how the authors rule out contamination between children of three different groups that influence the feeding pattern of others groups. Similarly, how can they rule out the sharing of MMP sachets, especially as control group not received any supplementation?

Thank you very much for this very valuabe comments, it is not easy to answer, but we tried to revised the method section to clarify the way of randomization, intervention, monitoring and follow up

2. As the authors mentioned that though the increment in haemoglobin concentration by week 24 was relatively higher in DS group, the difference between the groups was not statistically significant in moderate to severe anaemia group.

The analysis showed that in anemic only subjects, the average increases in Hb in 3 groups was SS. We added the following sentence to the discussion part.

“The reduction of anaemia was also observed in the control group, but relatively smaller than DS & TWS group. The average increases in haemoglobin concentrations in the DS & TWS group was 18.6 & 15.6 g/L, respectively, higher than the control 9.3 g/L (P<0.001).”

Could we attribute it poor compliance DS group as only 44% children in DS group consumed all sachets and 27% of children consumed # 4 sachets per week? Then how the authors authentically interpret daily supplementation of MMP is effective as the compliance is poor compared to twice-weekly supplementation.

I would like to explain in the following sentence, is it make sense or not? We need further comments if this is an answer to your comment

“The daily multiple micronutrients supplementation group received 70 mg Fe when calculated on a weekly basis, whereas the TWS group received 20 mg Fe. Over 24 weeks of supplementation, 43.6% of the daily group received 1680 mg Fe (168 sachets×10 mg), and only 27.3% of the daily group received 960 mg Fe (96 sachets×10mg). This amount was sufficient to produce a haemoglobin response. The recommended amount by the Global sprinkles initiative for preventing anaemia among children under five is 600 mg.
The daily and the weekly multiple micronutrient supplements showed dose-response, if a daily dose were used with a full compliance, it should be possible to eliminate anaemia among study subjects during the 6 mo of supplementation”.

3. Authors need to mention the reasons for low compliance in DS group.

We added the reason for low compliance in the daily group.

“The most common reason for not taking powder in the DS group was illness, such as diarrhea (n=20), cough (n=10) and forgetting to take supplements (n=32).”

4. Authors need to mention the proportion children experienced side effects in two experimental groups.

We added the side effect information under Compliance and acceptability section

“According to the monitoring report, mothers in both intervention groups reported that their children had constipation or dark stool. The control, DS and TWS groups did not differ significantly in reported illness (diarrhoea or cough) (32.7%, 39.1%, and 34.2%, respectively; P=0.587). In the intervention group, 42.1% (93/221) of mothers reported that sprinkles changed the colour of their children’s food and 43.9% (97/221) of mothers reported that they had an unpleasant smell or taste. Some mothers mixed the sprinkles in liquids such as juice or milk. Mothers felt MMP increased their child’s appetite (31.7%) and playfulness (48.4%).”

5. Provide distribution of compliance of consumption of MMP by age group in DS group as only 44% children in DS group consumed all sachets.

The below table showed the distribution of children’s age based on the compliance.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of subjects</th>
<th>4 sachets</th>
<th>5 Sachets</th>
<th>6 sachets</th>
<th>7 sachets</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-18 mo</td>
<td>6</td>
<td>3 (50.0)</td>
<td>0</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>19-31 mo</td>
<td>29</td>
<td>5 (17.2)</td>
<td>2 (6.9)</td>
<td>5 (17.2)</td>
<td>17 (58.6)</td>
</tr>
<tr>
<td>32-44 mo</td>
<td>29</td>
<td>9 (31.0)</td>
<td>6 (20.7)</td>
<td>4 (13.8)</td>
<td>10 (34.5)</td>
</tr>
<tr>
<td>45-53 mo</td>
<td>46</td>
<td>13 (28.3)</td>
<td>9 (19.6)</td>
<td>4 (8.7)</td>
<td>20 (43.5)</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>30 (27.3)</td>
<td>17 (15.5)</td>
<td>15 (13.6)</td>
<td>48 (43.6)</td>
</tr>
</tbody>
</table>

6. The increase in height for age Z score and the decrease in weight for height Z score were significantly greater in the control and DS groups than in the TWS groups. The authors need to
explain how it is possible that there is increase in height, since increment in height needs longer duration. Similarly, authors need to explain why the weight of the children decreased over time.

Thank you for this good comments, we did revised the sentence and add more discussion on the issue

**Minor Essential Revisions**

7. As the authors mentioned (page-13), the Table 3 is not appeared in the text. They might have wrongly mentioned Table 3 as Table 4.
   Yes, we did correction

8. Did the authors collect the information on morbidity pattern during the study period?
   Yes, we did collect the information. We added to the result section

**Level of interest:** An article whose findings are important to those with closely related research interests
   Thank you very much for your positive comment

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:** 'I declare that I have no competing interests’

We hope that with these changes our manuscript can be accepted for publication in the Nutrition Journal.

We look forward to your feedback.

With best wishes

Yours sincerely

Sengchanh Kounnavong