Cover letter for revision of the manuscript

September 5, 2020

Dear Editor,

Thank you for giving me the opportunity to revise and resubmit the manuscript number 56700 entitled "Optimal Hang Time of Enteral Formula at Standard Room Temperature and High Temperature" I appreciate the careful review and constructive suggestions provided by reviewer and editor. The manuscript has certainly benefited from these insightful revision suggestions. I look forward to hearing from you regarding our submission and to respond to any further questions and comments you may have.

Following this letter are the reviewer's comments and the editor's comments with our specifically responses to each suggestion in italics, including how and where the text was modified. Changes made in the manuscript are marked using yellow highlighted text.

Please address all correspondence concerning this manuscript to me at Narisorn.L@chula.ac.th

Sincerely,

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Reviewer: 1

Comments to the Author

I read with interest the article by Lakananurak and colleagues who did the study to investigate optimal hang time of two types of formula at standard room temperature and high temperature. This is a very interesting study, with a very current topic. No previous study has investigated hang time between standard room temperature and high temperature in enteral formulas, which is crucial for food safety of EN administration. This will potentially be of interest to a wide number of clinicians. However I also think the study has major shortage and needs some major changes that may help improving the manuscript:

(1) "Materials and Methods" section, the details of the study method was poor, please report the methods in detail.

Answer: *The reviewer's comment is noted. We have edited and described the study method in details, including enteral formula preparation, administration, and sample collection and culture. This was described on page 5-6 as follow:*

*"**Materials and Methods***

Enteral formulas

Blenderized and reconstituted powdered formulas were prepared by a trained dietitian using aseptic techniques. The blenderized diet was made from cooked ingredients (rice, chicken, pumpkin, eggs, and vegetable oil). The dietitian prepared, mixed, and blended all ingredients in sterile containers. Sterile water was used to dilute the formula to achieve caloric density of 1 calorie per 1 mL. The caloric ratio for carbohydrate:protein:fat for the standard blenderized diet at the King Chulalongkorn Memorial Hospital was 55%: 15%: 30%.

A polymeric formula (Neomune®) and sterile water were used to make the reconstituted powdered formula. The same dietitian prepared and mixed the reconstituted powdered formula in a sterile container. Caloric density of the formula was also 1 calorie per 1 mL, and the caloric distribution for carbohydrate:protein:fat of the reconstituted powdered formula was 50%: 25%: 25%.

Both formulas were put into a 500 mL sterile feeding bag (Nutri-Bag®) and were immediately transferred and used after preparation.

Enteral nutrition administration

In order to precisely control the temperature, simulated administration was done in an incubator. The feeding bag was connected to a feeding tube and an infusion pump with aseptic

techniques to mimic EN administration in patients. Standard enteral feeding pump and infusion tubing (Kangaroo®) were used to deliver both formulas. The pump does not have heat preservation function which might have an effect on the temperature control. Standard room temperature was set at 25 degrees Celsius, and high temperature was set at 32 degrees Celsius, based on the average temperature in Thailand. Both formulas were delivered at the rate of 80 mL/hr to mimic standard continuous feeding rate in most patients. A sterile container was used to receive enteral formulas, and there was no contact between the feeding tube and the container.

Sample collection and culture

Five milliliters of formula were collected from the tip of the feeding tube at 0, 2, 4, and 6 hours. All samples were sent for aerobic culture using blood agar and MacConkey agar. A colony count and bacterial identification were done by a microbiologist, who was blinded from the experiment.

FDA criteria were used to determine unacceptable levels of contamination. Given that no previous study has investigated this issue before and at least 3 specimens were needed according to the FDA criteria, we decided to conduct a pilot study by evaluating 5 specimens of each formula at 25 degrees Celsius and 32 degrees Celsius, resulting in a total of 20 specimens (Table 1). The protocol for this study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB number 645/59)."

(2) What type of infusion pump was used in this study? As some infusion pumps have heat preservation function which might influence results of the study.

Answer: *The standard enteral infusion pump (Kangaroo®) was used in the current study. This pump does not have heat preservation function, which might have an effect on temperature control. This was described on page 6 under "Enteral Nutrition Administration" as follow:*

"Standard enteral feeding pump and infusion tubing (Kangaroo®) were used to deliver both formulas. The pump does not have heat preservation function which might have an effect on the temperature control."

(3) "Statistical analysis" section was poor. Please report it in detail.

Answer: *We have revised "Statistical analysis" section and described it in detail on page 7 as follow:*

*"**Statistical analysis***

Descriptive statistics were used to describe the optimal hang time, type and quantity of bacterial growth in each culture positive specimen. Optimal hang time was described as number of hours without unacceptable bacterial contamination of each formula at both temperatures as per the FDA criteria. Bacterial growth was described as colony-forming unit (CFU) and specific types of bacteria. The number and percentage of specimens with unacceptable levels of bacterial contamination for each enteral formula at standard room temperature and high temperature were also described."

(4) The study sample size was small, and only 20 species in total. In my opinion, the sample size needs to increase. As small sample size might lead to big bias.

Answer: *Thank you for your valuable comment. Given that no previous studies have investigated the optimal hang time of blenderized and reconstituted powdered formulas at standard room temperature and high temperature, a preliminary study to evaluate this crucial issue is necessary. By using the standard and acceptable criteria (the FDA criteria), at least 3 specimens of each formula were needed. As a result, we decided to conduct a small-scale study by evaluating 5 specimens of each formula at 25 degrees Celsius and 32 degrees Celsius, resulting in a total of 20 specimens.*

However, we agree with your suggestion, and therefore the small sample size was mentioned as one of the limitations of this study on page 9 as follow:

"Finally, even though the sample size is adequate to evaluate the unacceptable bacterial contamination in enteral formula according to the FDA recommendation, this study has a small sample size that can result in the bias results."

This is the first study to directly evaluate optimal hang time at both room and high temperatures. Hence, we believe that the findings from this study will help establish and provide the groundwork for large-scale studies in the future.

Company Editor-in-Chief

Comments to the Author

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Clinical Cases, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. However, the quality of the English language of the manuscript does not meet the requirements of the journal. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend: https://www.wjgnet.com/bpg/gerinfo/240.

Answer: *Thank you for your valuable comment. We have revised the quality of English language in the manuscript by using a professional English language editing company in which professional medical writer was used. We also provided a new English editing certificate with the manuscript.*