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Dear Editors and Reviewers:

Thank you very much for giving us the opportunity to revise our manuscript entitled "Effects of early enteral nutrition on Th17/Treg and IL-23/IL-17 in septic patients (**Manuscript NO: 47395, Name of journal: World Journal of Gastroenterology**)". We appreciate our editor very much for your valuable and constructive comments on our manuscript! We are also pleased to know that our study is of general interest for the readers of **World Journal of Gastroenterology**. We have revised our manuscript in accordance with the reviewers' comments, made point-by-point responses, and detailed the changes. All changes were highlighted with **red color** in the text so that they may be easily identified. Hope these will make it more acceptable for publication.

Thank you very much for your attention and consideration.

Sincerely yours,

Xiang Wang

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Responses to Reviewers' comments

Name of journal: World Journal of Gastroenterology

Manuscript NO: 47395

Title: Effects of early enteral nutrition on Th17/Treg and IL-23/IL-17 in septic patients

Reviewer's code:03475636

SPECIFIC COMMENTS TO AUTHORS

1. Please describe more details on Randomization; block randomization? Mixed block randomization?

Responses: Thanks very much for the reviewer's suggestion. We used the simple randomization or complete randomization: the randomization was based on the remainder grouping method of random numbers. After generating random numbers with a computer, the remainder grouping method (divided by 2) was performed to decide which group (EEN or DEN) the patients would be assigned into. We modified and added the description into "**Study design**" section of "**Materials and Methods**".

2. It will be crucial that treatment allocation is concealed in order for you to publish this study and it should be clear how this was ensured.

Responses: Thanks very much for the reviewer's comments. The treatment allocation was concealed before patient was included in this study, and patients would not be removed from the study after treatment allocation become known. The main intervention difference of the two groups was the initial time of enteral feeding, therefore, it was not possible to blind treating physicians to the treatment allocation. The inclusive patients were blind to the treatment allocations. Therefore, this is a single-blind clinical trial. We added these descriptions into "**Study design**" section of "**Materials and Methods**".

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3. All clinical trials should be registered in clinicaltrials.gov

Responses: Thanks much for the reviewer's suggestion. This study was registered at Clinical Trials.gov, the ID was NCT03385850, we had described that in "**Study design**" section of "**Materials and Methods**".

4. Methods and laboratory to measure Th17/Treg, IL-23, and IL-17 should all be describe in details.

Responses: Thanks much for the reviewer's suggestions. Th17 and Treg lymphocytes were measured by flow cytometry. After human peripheral blood mononuclear cells were isolated, the proliferation analysis of Th17 cell subpopulations was performed by using a BD PharmingenTM Human Th17 Phenotyping Kit (BD Biosciences, USA), and the Treg cell subpopulation was detected by using CD4 (antigen presenting cells), CD25 (PE), and Foxp3 (Fluorescein isothiocyanate)-labeled antibodies (e-Bioscience, USA). Serum IL-17, IL-23, IL-6, and IL-10 cytokines were detected by commercial available Human Quantikine enzyme-linked immunosorbent assays (ELISA) kits (R&D Systems, Bio-Techne Corporation, USA) according to the manufacturer's instructions. The sensitivity of ELISA kits was 15 pg/mL for IL-17, 16.3 pg/mL for IL-23, 0.7 pg/mL for IL-6, and 3.9 pg/mL for IL-10. The assay range of ELISA kits was 31.2 - 2000 pg/mL for IL-17, 39.0 - 2500 pg/mL for IL-23, 3.1 - 300 pg/mL for IL-6, and 7.8 - 500 pg/mL for IL-10. We modified and added these descriptions into "**Data collection**" section of "**Materials and Methods**".

5. "clinical randomized trial" should be "randomized clinical trial"

Responses: Thanks much for the reviewer's suggestion. We have replaced all of "clinical randomized trial" with "**randomized clinical trial**" in our manuscript.

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Reviewer's code:01370434

SPECIFIC COMMENTS TO AUTHORS

The author reported the beneficial effect of early enteral nutrition on the Th17/Treg ratios and IL-23/IL-17 in septic patients. This study is interesting, but there are some questions to be clarified.

Major comment:

1. The authors used two groups EEN and DEN. Why did you choice 4 days delayed enteral nutrition as DEN groups?

Responses: Thanks very much for the reviewer's comment. According to recent guidelines[1-3], early enteral nutrition (EEN) should be initiated within the first 24-48h after intensive care unit admission (ICU) in critically ill patients. However, the initial time of delayed enteral nutrition (DEN) was still be discussing. Chourdakis M et al[4] reported that DEN was performed during 2-5 days after ICU admission, whereas our previous studies[5-6] reported that DEN was performed from the 7th day after ICU admission in critically ill patients. Moreover, our recent study[7] reported that DEN was initiated on the 4th day or later after ICU admission in septic patients. Therefore, we choice 4 days delayed enteral nutrition as DEN groups.

2. EEN can decrease APACHII and SOFA score on 7th day after admission. Which factors was decrease mainly by EEN?

Responses: Thanks very much for the reviewer's comment. The APACHII and SOFA scoring items include infectious parameters, oxygenation conditions, hemodynamic parameters, and organ function markers. We investigated the differences on WBC, Hb, TBil and albumin levels between the two groups, and we found that EEN had a

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tendency of decreasing WBC counts (9.57±3.12 V.S. 12.03±5.53, *P* =0.051) and Tbil (14.04±11.06 V.S. 20.14±18.21, *P* =0.146) on the 7th day after admission. Moreover, EEN also had a tendency of increasing albumin levels (33.51±3.75 V.S. 31.47±3.82, *P* =0.055) on the 7th day after admission. Moreover, our results also found that the duration (days) of MV and ICU stay of the EEN group were shorter than those of the DEN group ($P < 0.05$). In addition, our previous study[5-7] revealed that EEN could increase antioxidant activity and modulate the inflammatory and sepsis response, and reduce the incidence of systemic inflammatory response syndrome and subsequent MODS. These may be part of the reasons why EEN can decrease APACH II and SOFA score on 7th day after admission.

3. The EEN can decrease T17/Treg, IL-17 and IL-23 on 7th day after admission, However, no effect on the 28-day mortality was observed, Why did this discrepancy occur? The author should be more clarified this point.

Responses: Thanks very much for the reviewer's comments. The EEN can decrease T17/Treg, IL-17 and IL-23 levels, as well as APACHII and SOFA score on 7th day after admission. These results indicated that EEN can suppress the immune overactivation and improve the clinical disease severity during early stage of sepsis. However, the main intervention difference of the two groups was the initial time of enteral feeding in this study, thus patients would received same treatments (especilly enteral nutrtion) for sepsis after the acute stage. Therefore, no difference about the mortality was observed on 28th day after admission. Moreover, this discrepancy occured probably because this small sample trail was not powered to detect a difference in mortality, and we also look forward to work in collaboration with dear reviewers in future. We had modified and added these descriptions into **"Discussion".**

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4. Are there any beneficial effect of EEN on other parameter such as WBC, Hb, TP and albumin, etc. ?

Responses: Thanks much for the reviewer's suggestions. The effects of EEN on WBC, Hb, TBil and albumin were investigated, we found that EEN had a tendency of decreasing WBC counts (9.57±3.12 V.S. 12.03±5.53, *P* =0.051) and Tbil (14.04±11.06 V.S. 20.14±18.21, *P* =0.146) on the 7th day after admission. Moreover, EEN also had a tendency of increasing albumin levels (33.51±3.75 V.S. 31.47±3.82, *P* =0.055) on the 7th day after admission. No similar tendency on Hb (106.73±16.53 V.S. 105.56±23.60, *P* =0.835) was found during the 7 days after admission between the two groups. It may be part of the reasons why EEN can decrease APACH II and SOFA score on 7th day after admission. We modified and added these descriptions into **"Data collection", "Results", and "Discussion".**

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Thank you very much for your suggestive comments again!