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ORIGINAL RESEARCH

Effects of diets containing synbiotics on the gut microbiota of critically ill septic patients: a pilot randomized controlled trial

Kontee Wongseree[1], Kamonnut Singkhamanan[2], Supattra Uppanisakorn[3], Veerapong Vattanavanit[4,*]

[1]Division of Internal Medicine, Faculty of Medicine, Prince of Songkla University, 90110 Hat Yai, Songkhla, Thailand
[2]Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, 90110 Hat Yai, Songkhla, Thailand
[3]Clinical Research Center, Faculty of Medicine, Prince of Songkla University, 90110 Hat Yai, Songkhla, Thailand
[4]Critical Care Medicine Unit, Division of Internal Medicine, Faculty of Medicine, Prince of Songkla University, 90110 Hat Yai, Songkhla, Thailand

***Correspondence**
vveerapong@gmail.com
(Veerapong Vattanavanit)

Abstract

The effects of synbiotics on gut microbiota have not been thoroughly clarified in critically ill patients with sepsis. In this present study, we aimed to evaluate the effects of synbiotics in a commercial diet on the gut microbiota of mechanically ventilated septic patients. This double-blind, randomized controlled clinical trial was conducted on septic patients under mechanical ventilation in a university-affiliated hospital in southern Thailand from February 2019 to March 2021. The patients were randomly divided into 2 groups stratified by sepsis stages and given commercial enteral feeding with synbiotics or standard commercial feeding for 7 days. The primary outcome was fecal microbial diversity measured as alpha and beta diversity. The secondary outcomes included ventilator-associated pneumonia, nosocomial diarrhea, ventilator days, length of hospital stay, and mortality. Twenty-four patients, 12 on a synbiotic diet and 12 on a non-synbiotic diet, completed this study. On day 3 of feeding, no significant difference was observed in their alpha fecal microbial diversity. However, significantly greater beta diversity was observed in the non-synbiotics group compared with the synbiotic group (Bray Curtis distance, $p = 0.001$; Jaccard's distance, $p = 0.001$; unweighted UniFrac, $p = 0.001$; weighted UniFrac, $p = 0.029$). The secondary outcomes were not significantly different between the two groups. In critically ill septic patients, feeding with a commercial diet containing synbiotics did not significantly improve fecal microbial diversity. Due to the small sample size, further study is required.

Keywords

Gut microbiota; Microbial diversity; Sepsis; Synbiotics; Intensive care unit

1. Introduction

Sepsis, defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection, is a concerning public health problem [1]. It is also one of the most common causes of mortality in critically ill patients [2]. Some studies have reported that critically ill patients have a depleted gut microbiota in terms of gut microbiota diversity, called dysbiosis [3, 4]. It was shown that critically ill patients had decreased alpha and beta gut microbiota diversity, characterized by the quantity, concentration and differences between two samples of microbes [4, 5]. The use of broad-spectrum antibiotics, antacids and stress can alter the gut microbial balance and bacterial translocation [6]. Dysbiosis increases susceptibility to nosocomial infections such as ventilator-associated pneumonia (VAP) and poorer outcomes [7]. The loss of gut microbiota diversity was associated with an increased risk of mortality in critically ill patients [8, 9]. Thus, therapeutic interventions that modulate gut microbiota diversity could improve the outcomes of critically ill septic patients.

Probiotics are live microbial food supplements, while prebiotics are non-digestible food ingredients that beneficially affect the growth of the gut microbiota. Synbiotics, such as a combination of probiotics and prebiotics, have been reported to promote immunity against infection. In recent years, the importance of the composition of the gut microbiota in physiological and pathophysiological processes has become more evident, with an increasing number of clinical trials dealing with the benefit of gut microbiota being published. The enteral administration of probiotics and synbiotics can benefit the recipient in terms of reducing the incidence of VAP [10, 11] and nosocomial diarrhea [12]. Synbiotics may help maintain the gut microbiota following systemic inflammatory response syndrome [13]. However, there is limited research on synbiotics addressing the effects of diet on bacterial diversity in septic patients.

Based on the current understanding on synbiotics, we hypothesized that they could improve the gut microbial diversity of critically ill septic patients. Therefore, we designed this trial to investigate the effects of a commercial diet containing syn-

biotics on the gut microbiota of critically ill septic patients and other clinically important outcomes, including the incidence of ventilator-associated pneumonia (VAP), nosocomial diarrhea, ventilator days, hospital length of stay and mortality.

2. Materials and methods

2.1 Study design

We conducted a pilot randomized controlled trial between February 2019 and March 2021 at the intensive care unit (ICU), cardiac care unit (CCU), respiratory care unit (RCU) and other general medical wards of the Songklanagarind Hospital (Hat Yai, Thailand), an 800-bed hospital and the largest university-based tertiary care center in southern Thailand.

2.2 Participants

The study inclusion criteria were: (1) aged 18 years or older, (2) received invasive mechanical ventilation, (3) were fed via a nasogastric tube, (4) diagnosed with sepsis, severe sepsis or septic shock, as defined by the sepsis diagnostic criteria from the American College of Chest Physicians/Society of Critical Care Medicine consensus conference 1991 [14], and (5) after admission, they received antibiotics less than 48 hours prior to inclusion, and provided informed consent. Those in end-stage or palliative care; had lower gastrointestinal bleeding or diarrhea; were pregnant or lactating; had a history of bowel surgery, medical diet allergy, synbiotic or probiotic use in the last month, and; were immunocompromised or had undergone prior steroid, chemotherapy, targeted therapy or immunotherapy within one month were excluded.

2.3 Randomization and blinding

The investigators evaluated patients for eligibility, obtained informed consent, and enrolled the participants. After inclusion, the patients were randomly assigned without restriction in a block of 4 at a 1:1 ratio stratified by sepsis, severe sepsis or septic shock according to a computer-generated randomization table derived from www.randomization.com, by a research nurse assistant who had no role in patient management. The research nurse assistants not otherwise involved in the study administered both study diets. The attending physicians, nursing care teams, research investigators, participants, and their family members were blinded to treatment allocation. The diets were prepared by a nutritionist who had no other role in the trial. The diets were packaged in identical bottles labeled with sequential numbers that contained a specific diet volume depending on the attending physician's orders. The patients who received the symbiotic diet were assigned to the synbiotics group, and those who received the non-synbiotic diet were assigned to the non-synbiotics group.

2.4 Study procedure

The synbiotics group received the commercial polymeric formula Boost optimum® (Nestle Japan Co. Ltd., Tokyo, Japan; 1 kcal/mL; 49:16:35 ratio of carbohydrate, protein, and fat; 310 mOsm/L), which contained probiotics, such as 1 billion colony forming units (CFUs) of *Lactobacillus paracasei* NCC 2461 (ST11) per 500 kcal and 12.3 gm/L of prebiotics consisting of 70% fructooligosaccharide (FOS) and 30% inulin [15]. The non-synbiotics group received the commercial polymeric formula Ensure® (Abbott Japan Co. Ltd., Tokyo, Japan; 1 kcal/mL; 55:16:29 ratio of carbohydrate, protein, and fat; 433 mOsm/L). Enteral feeding was initiated as soon as possible through a nasogastric tube, which did not determine the volume or rate of feeding depending on the attending physician's decision. The feeding protocol strictly adhered to the Songklanagarind Hospital feeding protocol (**Supplementary Fig. 1**). Enteral feeding with the study diet was continued for 7 days or until the patient asked for another diet, such as soft food or a regular diet.

Fecal samples were acquired from the subjects before or within 1 day after starting the study enteral feeding and before day 3. The samples were collected using a specific stool collection tube with a defined quantity of feces, stored in a 4 ℃ sample collection box, preserved in a DNA/RNA Shield (209501, Zymo Research, Irvine, CA, USA) and transferred to a −80 ℃ refrigerator within 1 hour of collection. Bacterial DNA was extracted using the ZymoBIOMICS^{TM} DNA Miniprep kit (211447, Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. The DNA samples were sequenced using the Illumina® MiSeq™ platform at V3–V4 of 16s rRNA with the ZymoBIOMICS® Targeted Sequencing Service for Microbiome Analysis (Zymo Research, Irvine, CA, USA), with international quality control. After sequencing, all sequence reads were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (KnightLab, La Jolla, CA, USA) to generate data on alpha diversity, beta diversity and bacterial composition.

2.5 Outcomes

The primary endpoint of the study was fecal microbial diversity, defined by the alpha (within-sample) and beta (between-sample) diversity.

The alpha diversity was represented by the number of different taxa (richness) and their distribution (evenness) in fecal samples. Here is an illustration of alpha diversity using two samples (groups 1 and 2). The samples from groups 1 and 2 contained four distinct taxa each, suggesting that the two samples' richness levels were equivalent. The distribution of taxa in the sample of group 2 was 94% for taxa one, 1% for taxa two, 2% for taxa three, and 3% for taxa four, compared to 25% for each of the four taxa in the sample of group 1. Thus, compared with group 2, group 1 exhibited greater diversity and was regarded as having higher alpha diversity. Richness and evenness within fecal samples were assessed using Shannon's index. Faith's phylogenetic diversity was used to qualitatively measure richness using phylogenetic relationships within the fecal samples. Pielou's evenness measured the evenness of taxa distribution [16].

Beta diversity was used to measure the differences in the community composition between the samples. It was calculated and analyzed using principle coordinated analyses, with the derivation of Bray-Curtis distance, Jaccard's distance, unweighted UniFrac distance matrices and weighted UniFrac distance matrices. Bray-Curtis distance measured the dis-

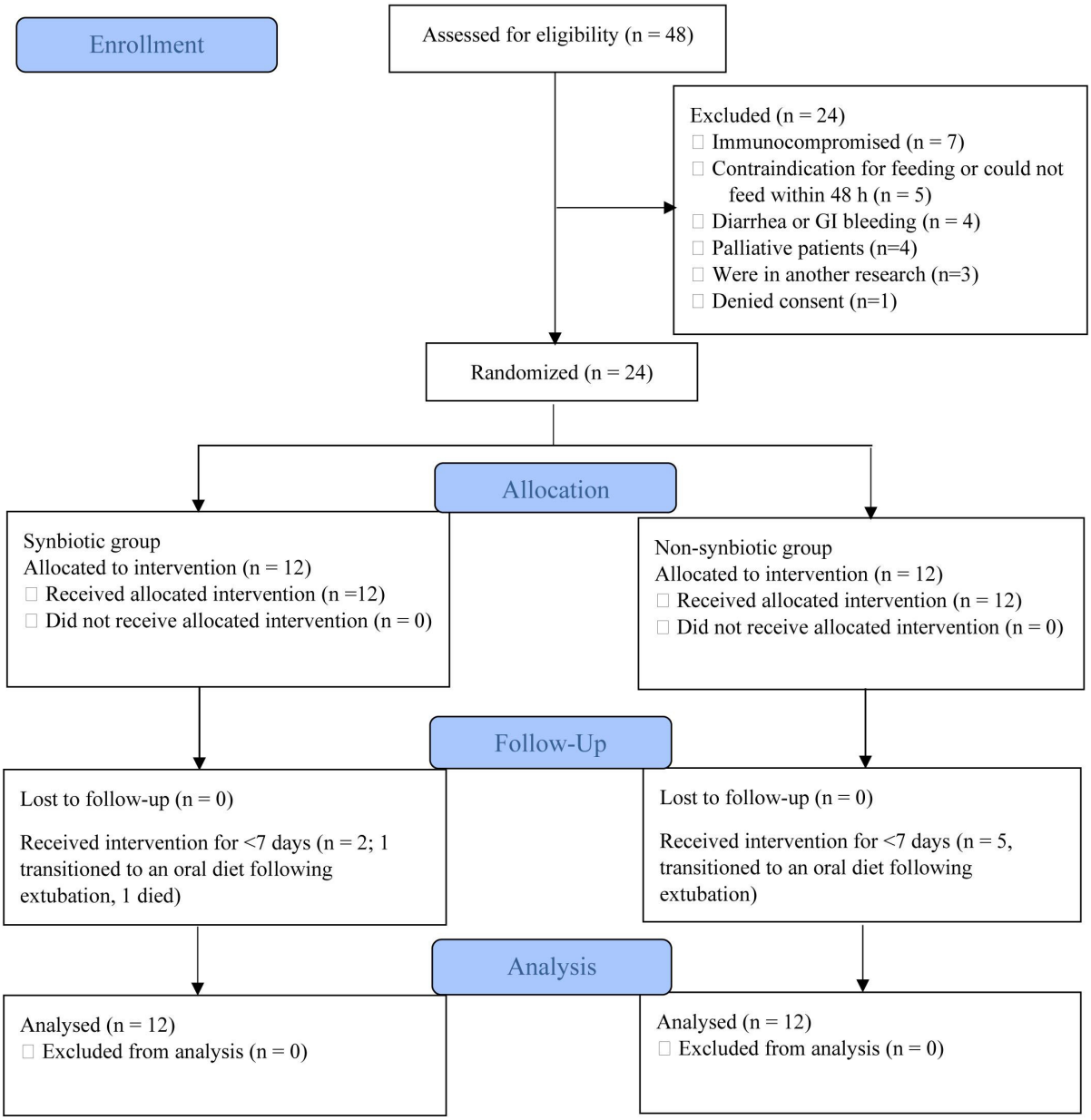


FIGURE 1. Flow diagram describing the screening, recruitment, and randomization of this study. Abbreviation: GI, gastrointestinal.

similarity in microbial composition among the samples by taking abundance into account. Jaccard's distance measured the number of members in a community that only contained the shared genera number, unrelated to their abundance. Unweighted UniFrac was used to detect the presence or absence of lineages and was calculated based on the relative abundances of lineages within communities. The beta diversity values varied from zero to one, of which a higher value denoted lesser similarity between two groups, while a lower value denoted greater similarity [16]. Based on these calculations, we could determine the association of the given diet with a change in gut microbiota diversity by comparing the alpha and beta diversity indices in two groups of fecal specimens.

The secondary endpoints included VAP, nosocomial diarrhea, ventilator days, length of hospital stay, and mortality. VAP was defined as pneumonia occurring 48 hours after intubation according to the 2005 American Thoracic Society guidelines, which included new infiltration based on the chest film and clinical worsening of secretions, respiration, or fever [17]. Nosocomial diarrhea was defined as acute diarrhea 3 days after admission [18].

Baseline data, including age, gender, comorbidities, the sequential organ failure assessment (SOFA) score, lactate level, sepsis characteristics, including the source of infection and organism, and the use of antibiotics, were collected. For adverse events related to feeding, we monitored feeding intolerance and other feeding issues, including bowel ischemia and gut obstruction. Feeding intolerance was defined as either gastric residual volume (GRV) $\geq$250 mL or feeding symptoms, including vomiting, ileus, abdominal pain and abdominal distension resulting in feeding interruption.

All patients were followed up until discharge or death, whichever occurred first.

2.6 Statistical analyses

No previous studies have investigated the effects of a commercial diet containing synbiotics on gut microbial diversity. For this pilot study, the minimum sample size of the patients required to show the optimal effect size was determined to be 24 [19]. No planned interim analysis was conducted in this trial.

The study was conducted on an intention-to-treat basis. No imputation was performed. The Shapiro-Wilk test was used to assess the normal distribution of the continuous variables. Continuous data are described as mean and SD or median and interquartile range (IQR), depending on the data distribution. Numbers and percentages are used to describe the categorical variables. The differences in patient characteristics and outcomes between the two groups were compared using the Wilcoxon-rank sum test, Fisher exact test, and χ^2 test, as appropriate.

For the statistical analysis of alpha diversity, the Kruskal-Wallis test was used to calculate statistically significant differences using the QIIME2 plugin. For beta diversity, the permutational multivariate analysis of variance (PERMANOVA) test was used to determine significant differences using the QIIME2 plugin based on 999 permutations.

All statistical analyses were performed with Stata version 16 (StataCorp, College Station, TX, USA), and a p-value < 0.05 was used to indicate statistical significance for all comparisons.

3. Results

From February 2019 to March 2021, 48 patients who had been intubated and had sepsis were assessed for eligibility (Fig. 1), and after excluding 24 patients because they did not match the eligibility criteria, 24 patients were randomized into the synbiotics group and non-synbiotics group in a 1:1 ratio. They were treated and analyzed using a modified intention-to-treat basis.

The baseline characteristics of the patients are shown in Table 1. Their average age was 77 years. Of them, 58.3% were men and 41.7% were women. Patients from the two groups were fed at a median volume of 860 mL/day or 19.1 kcal/kg/day, and no significant differences were observed between them. Of the investigated cohort, 11 patients (45.8%) were diagnosed with sepsis, three (12.5%) with severe sepsis and 10 (41.7%) with septic shock. The median SOFA score was 6 (3–9). The mean serum lactate level was 2.3 (1.4–3.1). The most common source of infection was respiratory tract infection (75%).

3.1 Primary outcome

We found no significant difference in alpha diversity between the synbiotics and non-synbiotics groups after feeding for three days, as measured by Shannon's index, Faith's phylogenetic diversity and Pielou's evenness. However, a significant difference was observed in beta diversity between the two groups, whereby the non-synbiotics group was found to have a greater beta diversity than the synbiotics groups (Bray Curtis distance, $p = 0.001$; Jaccard's distance, $p = 0.001$; unweighted UniFrac, $p = 0.001$; weighted UniFrac, $p = 0.029$) (Table 2, Figs. 2,3, **Supplementary Fig. 2**).

The fecal microbial diversity compared between day 0 (before starting feeding) and day 3 (after feeding) in both groups showed decreased alpha and beta diversity at day 3. Alpha diversity was significantly affected in terms of richness based on Faith's physiologic diversity. In addition, beta diversity was significantly affected, as indicated by the Bray Curtis distance and Jaccard's distance (Table 3 and Figs. 4,5).

3.2 Secondary outcomes

The comparison of secondary outcomes between the two groups is shown in Table 4. Although the synbiotics group showed a numerically lower incidence in VAP and ventilator days than the non-synbiotics group, the difference was not statistically significant. Further, no significant differences were observed between the two groups in terms of the incidence of nosocomial diarrhea and other outcomes, including ICU length of stay, hospital stay, and hospital mortality.

3.3 Adverse events

There were no reports of major adverse events related to the administration of synbiotics (Table 5). Only three patients in the synbiotics group had feeding intolerance during the study compared to two in the non-synbiotics group, but the difference was not statistically significant. No incidence of ischemic bowel of bowel obstruction was observed in this study.

4. Discussion

In this pilot randomized controlled trial, feeding with a synbiotic diet did not remarkably improve fecal microbial diversity in critically ill septic patients. In addition, although the synbiotics data tended to show a reduced incidence in VAP and number of ventilator days, the difference between the symbiotic and non-synbiotic groups was not statistically significant. There was no difference in the incidence of nosocomial diarrhea or other outcomes.

In previous studies, significant benefits were reported for probiotics and synbiotics in terms of improving gut dysbiosis and clinical outcomes [20–22]. We believed that the differences in these results could be related to the type and dose of probiotics used. For instance, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* were commonly used in a meta-analysis performed on critically ill patients [7]. In a previous study, synbiotics containing *Lactobacillus paracasei* NCC 2461, which were used in critically ill pediatric patients, were associated with an increased fecal bacterial composition [23]. Each species of probiotic organism showed a beneficial effect on different conditions or diseases. Multi-strain probiotics (VSL#3) significantly decreased proinflammatory cytokines in children with sepsis [24]. Most of these studies used probiotic doses ranging from 4×10^9 to 10^{11} CFU, whereas only about 10^9 CFU were used per day in our present study. The higher dose of probiotics might have stronger effects on gut microbiome alterations. Another explanation might be due to the inadequate number of calories received. Although the median feeding duration was 7 days, the total calories

TABLE 1. Baseline characteristics of the critically ill septic patients.

Variables	Total (n = 24)	Synbiotics (n = 12)	Non-synbiotics (n = 12)	*p*-value
Demographic data				
Sex (male)	14 (58.3)	8 (66.7)	6 (50.0)	0.408
Age (years)	77 (64–86)	73 (64–85)	77 (67–88)	0.452
BMI (kg/m^2)	21.6 (17.6–24.4)	22.5 (18.8–24.1)	19.3 (17.0–24.7)	0.419
Underlying diseases				
Hypertension	11 (45.8)	4 (33.3)	7 (58.3)	0.219
Dyslipidemia	6 (25.0)	2 (16.7)	4 (33.3)	0.640
DM	3 (12.5)	0	3 (25.0)	0.217
Stroke	3 (12.5)	1 (8.3)	2 (16.7)	1.000
Lung diseases	8 (33.3)	3 (25.0)	5 (41.7)	0.667
CKD	5 (20.8)	2 (16.7)	3 (25.0)	1.000
Solid malignancy	4 (16.7)	2 (16.7)	2 (16.7)	1.000
Feeding data				
Amount (mL/day)	860 (656–1116)	1007 (589–1268)	851 (676–1033)	0.453
Calories (kcal/day)	899 (656–1293)	1106 (627–1469)	860 (676–1033)	0.386
Calories (kcal/kg/day)	19.1 (14.7–26.0)	18.9 (12.2–21.5)	19.6 (15.6–26.0)	0.922
Feeding duration (days)	7 (6–7)	7 (7–7)	7 (5–7)	0.331
Severity				
Sepsis	11 (45.8)	5 (41.7)	6 (50.0)	0.682
Severe sepsis	3 (12.5)	2 (16.7)	1 (8.3)	0.537
Septic shock	10 (41.7)	5 (41.7)	5 (41.7)	1.000
SOFA	6 (3–9)	5 (3–9)	7 (3–10)	0.235
Lactate level (mmol/L)	2.3 (1.4–3.1)	2.3 (1.6–3.1)	2.3 (1.2–4.4)	0.829
Organ-specific source				
Respiratory tract	18 (75.0)	9 (75.0)	9 (75.0)	1.000
Urinary tract	3 (12.5)	1 (8.3)	2 (16.7)	1.000
Bloodstream	3 (12.5)	1 (8.3)	2 (16.7)	1.000
Intraabdominal source	1 (4.2)	0	1 (8.3)	1.000
Skin and soft tissue	1 (4.2)	1 (8.3)	0	1.000
CNS	1 (4.2)	0	1 (8.3)	1.000
Sample positive				
Sputum	10 (41.7)	6 (50.0)	4 (33.3)	0.408
Blood	8 (33.3)	3 (25.0)	5 (41.7)	0.667
Urine	2 (8.3)	1 (8.3)	1 (8.3)	1.000
Wound	1 (4.2)	0	1 (8.3)	1.000
Body fluid	1 (4.2)	0	1 (8.3)	1.000
Organism				
S. aureus	1 (4.2)	1 (8.3)	0	1.000
S. pneumoniae	3 (12.5)	1 (8.3)	2 (16.7)	1.000
Enterococcus spp.	2 (8.3)	1 (8.3)	1 (8.3)	1.000

TABLE 1. Continued.

Variables	Total (n = 24)	Synbiotics (n = 12)	Non-synbiotics (n = 12)	*p*-value
E. coli	3 (12.5)	0	3 (25.0)	0.217
K. pneumoniae	5 (20.8)	1 (8.3)	4 (33.3)	0.317
Other Gram-negative	6 (25.0)	3 (25.0)	3 (25.0)	1.000
Fungus	1 (4.2)	1 (8.3)	0	1.000
Antibiotics				
Cephalosporins	14 (58.3)	7 (58.3)	7 (58.3)	1.000
Piperacillin/tazobactam	5 (20.8)	2 (16.7)	3 (25.0)	1.000
Carbapenem	7 (29.2)	4 (33.3)	3 (25.0)	1.000
Fluoroquinolones	8 (33.3)	3 (25.0)	5 (41.7)	0.667
Other antibiotics	6 (25.0)	3 (25.0)	3 (25.0)	1.000

Notes: Data are presented as median (interquartile range) or n (%).
Abbreviations: BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; CNS, central nervous system; DM, diabetes mellitus; SOFA, sequential organ failure assessment.

TABLE 2. Fecal microbial diversity (alpha diversity) in the synbiotics and non-synbiotics groups on day 3 after feeding.

Fecal microbial diversity	Synbiotics (n = 12)	Non-synbiotics (n = 12)	*p*-value
Shannon's index	5.65 (5.55–5.78)	6.10 (5.62–6.32)	0.263
Faith's phylogenetic diversity	6.63 (4.97–9.39)	7.04 (5.92–8.55)	0.690
Pielou's evenness	0.89 (0.88–0.90)	0.89 (0.86–0.91)	0.720

Notes: Data are presented as median (interquartile range).

TABLE 3. Alpha diversity on day 0 (pre-treatment) and day 3 (post-treatment) of feeding.

Fecal microbial diversity	Day 0 (n = 24)	Day 3 (n = 24)	*p*-value
Shannon's index	5.78 (5.43–6.06)	5.53 (5.32–5.92)	0.216
Faith's phylogenetic diversity	8.51 (6.89–9.39)	6.43 (5.51–8.30)	0.013
Pielou's evenness	0.91 (0.89–0.92)	0.91 (0.89–0.92)	0.560

Notes: Data are presented as median (interquartile range).

TABLE 4. Secondary outcomes of this study.

Secondary outcomes	Total (n = 24)	Synbiotics (n = 12)	Non-synbiotics (n = 12)	*p*-value
VAP	4 (16.7)	1 (8.3)	3 (25.0)	0.273
Nosocomial diarrhea	3 (12.5)	1 (8.3)	2 (16.7)	0.537
Ventilator days (days)	12 (4–18)	7 (3–17)	15 (8–22)	0.164
ICU length of stay (days)	5 (0–10)	3 (0–16)	5 (1–9)	0.744
Hospital stays (days)	22 (14–28)	17 (11–37)	23 (16–28)	0.563
In-hospital mortality	5 (20.8)	3 (25.0)	2 (16.7)	1.000

Notes: Data are presented as median (interquartile range) or n (%).
Abbreviations: VAP, ventilator-associated pneumonia; ICU, intensive care unit.

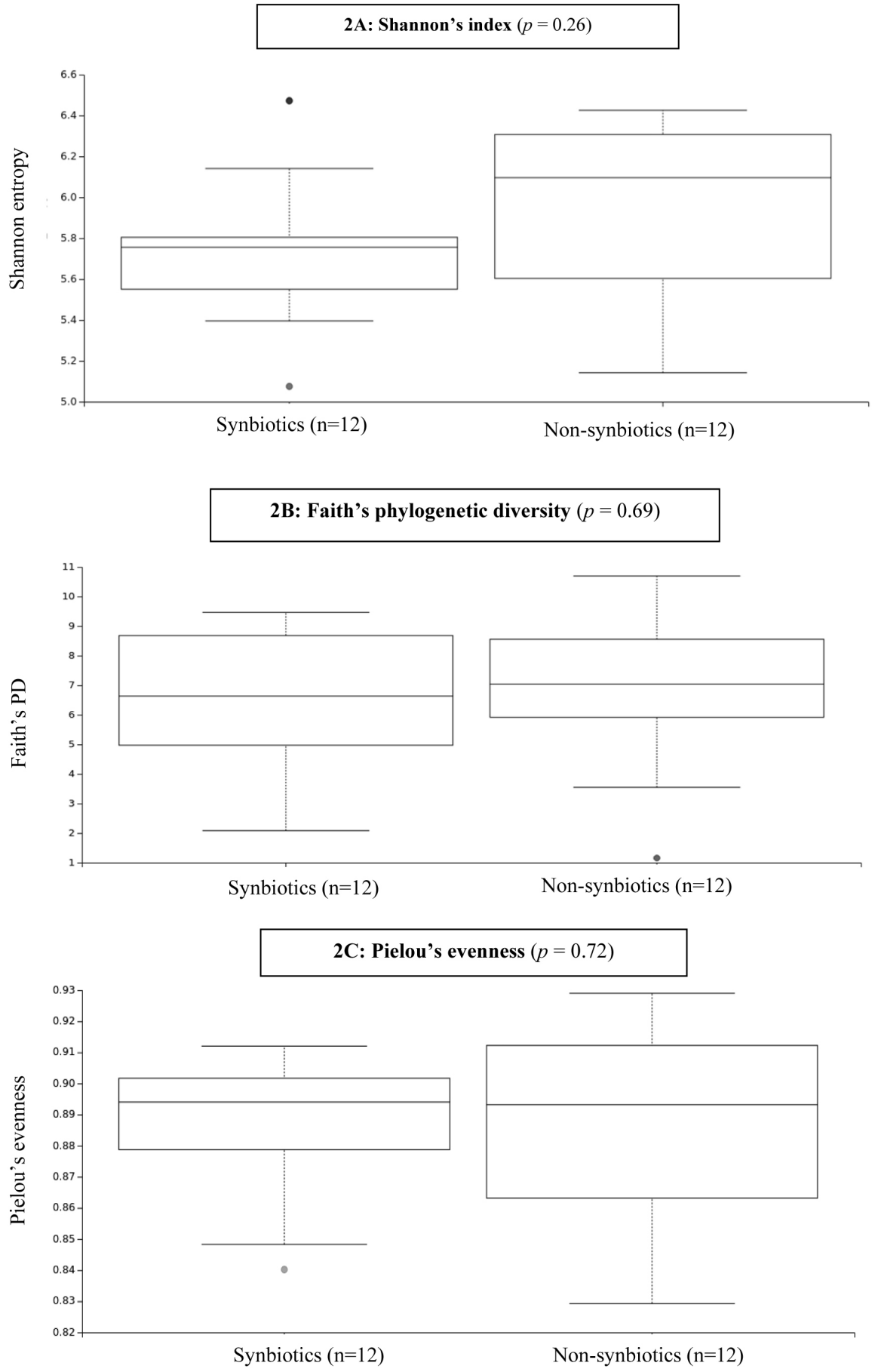


FIGURE 2. Alpha diversity in the synbiotics and non-synbiotics groups on day 3 of feeding. 2A: Shannon's index. 2B: Faith's phylogenetic diversity. 2C: Pielou's evenness. Abbreviation: PD, phylogenetic diversity.

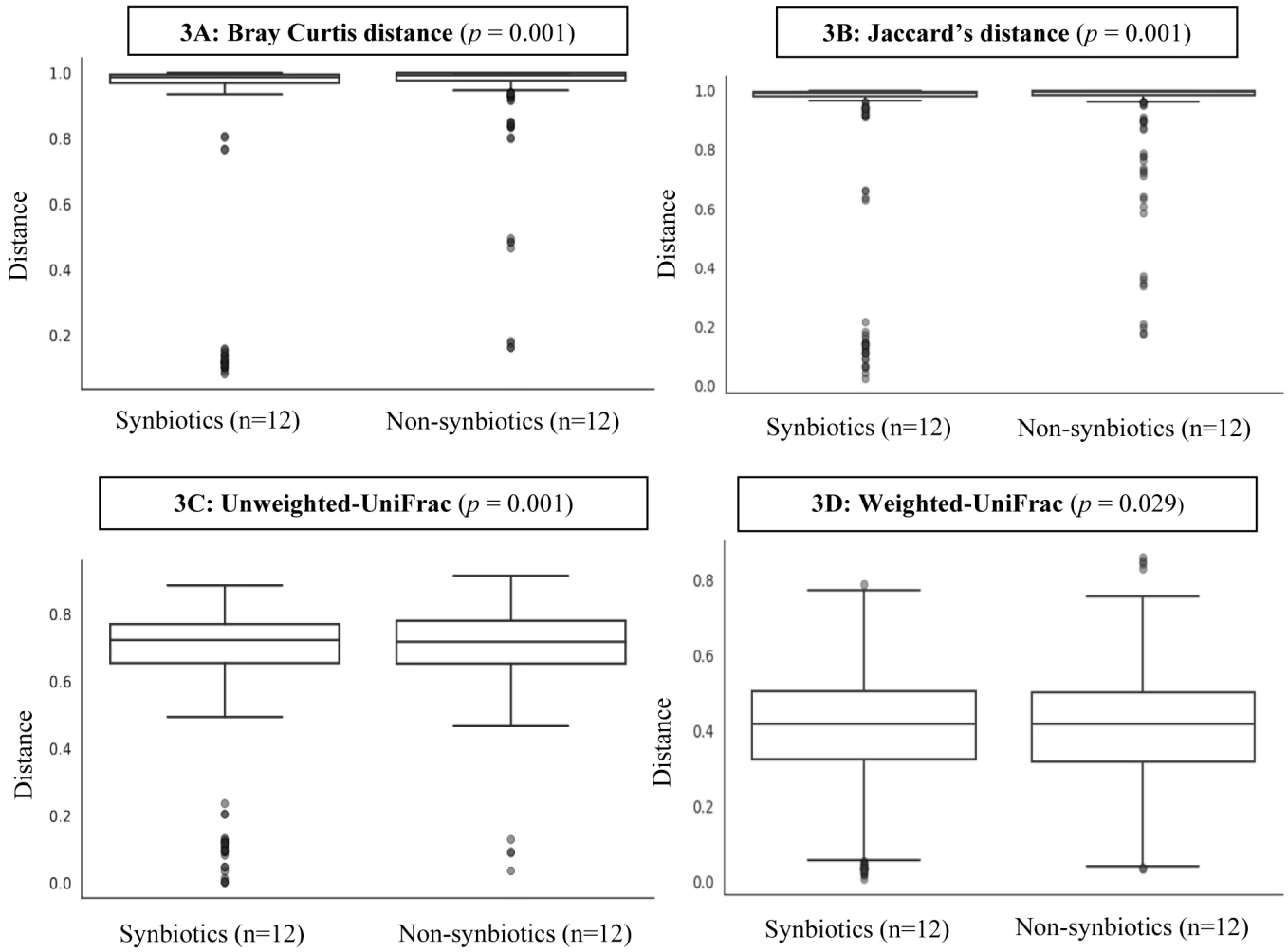


FIGURE 3. Beta diversity in the synbiotics and non-synbiotics groups on day 3 of feeding. 3A: Bray Curtis distance. 3B: Jaccard's distance. 3C: Unweighted-UniFrac. 3D: Weighted-UniFrac.

TABLE 5. Adverse outcomes.

Outcomes	Synbiotics (n = 12)	Non-synbiotics (n = 12)	*p*-value
Feeding intolerance	3 (25.0)	2 (16.7)	0.615
Bowel ischemia	0	0	NA
Gut obstruction	0	0	NA

Notes: Data are presented as n (%).
Feeding intolerance was defined as either GRV ≥250 mL, vomiting and/or ileus, or abdominal distension due to feeding interruption.
Abbreviations: GRV, gastric residual volume; NA, nonapplicable.

per day did not achieve the standard daily requirements in critically ill patients [25], which might also explain the non-significant difference in microbiome between the two groups on intervention day 3. In addition, inadequate caloric intake might have also caused gut microbiome alterations [26].

Sepsis is a complex condition that might result from altered gut microbiota and antibiotics used. The most common pathogens in our study were Gram-negative bacteria and the most common antibiotics were cephalosporins and fluoroquinolones. One study revealed that Proteobacteria was reduced by 10-fold following fluoroquinolone administration [27].

Other findings from our study confirmed the significant gut microbial diversity depletion on day 3 after admission due to sepsis or septic shock according to alpha and beta diversity. Dysbiosis can occur in critically ill patients over time [3, 5]. Moreover, not only the gut but also the skin, trachea and urine were reported to show decreased bacterial diversity [5].

Our study had several strengths. First, this was a randomized control trial with blinded investigators, research nurse assistants, attending physicians and patients. The methodology was well-designed, with high protocol adherence and no

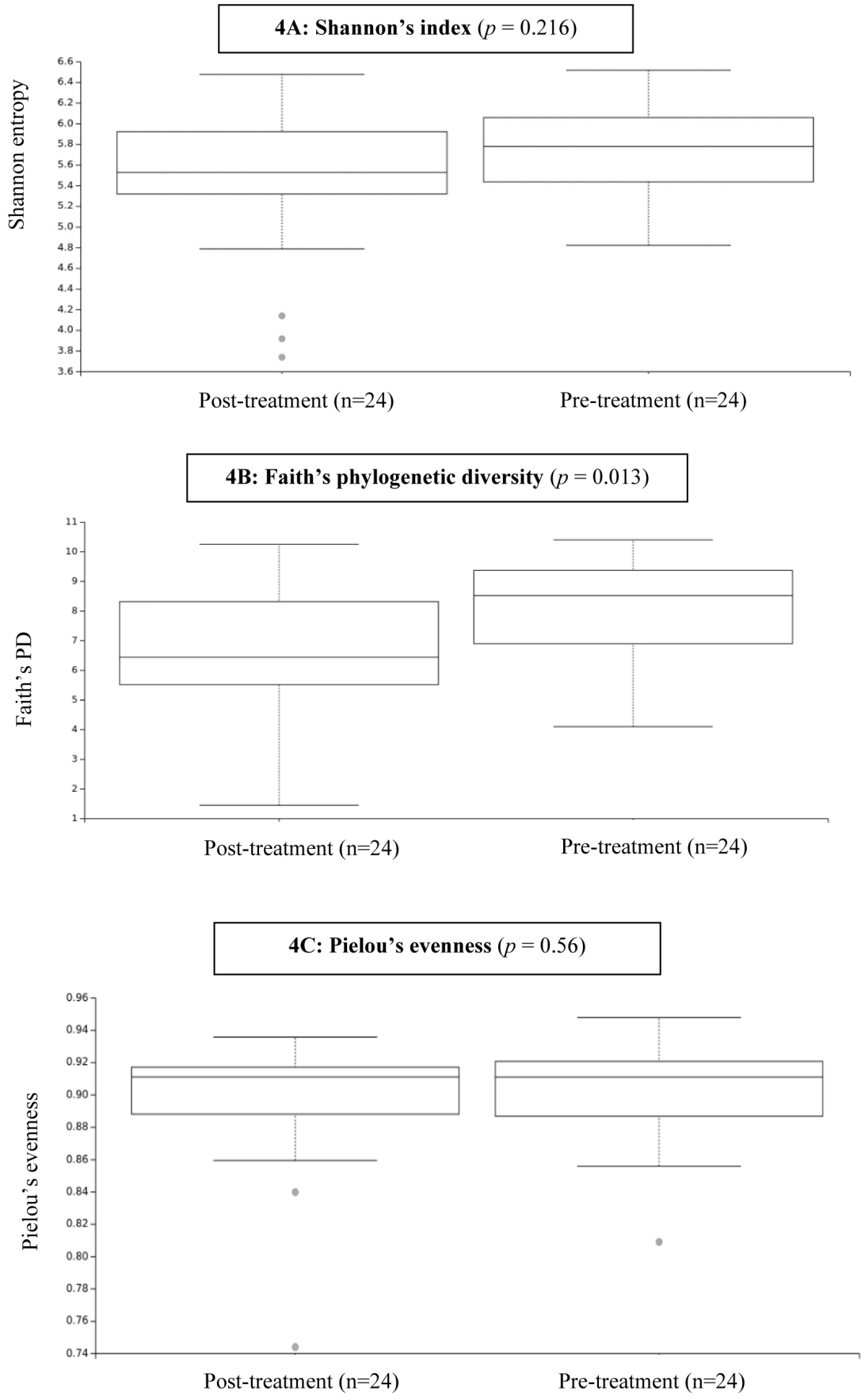
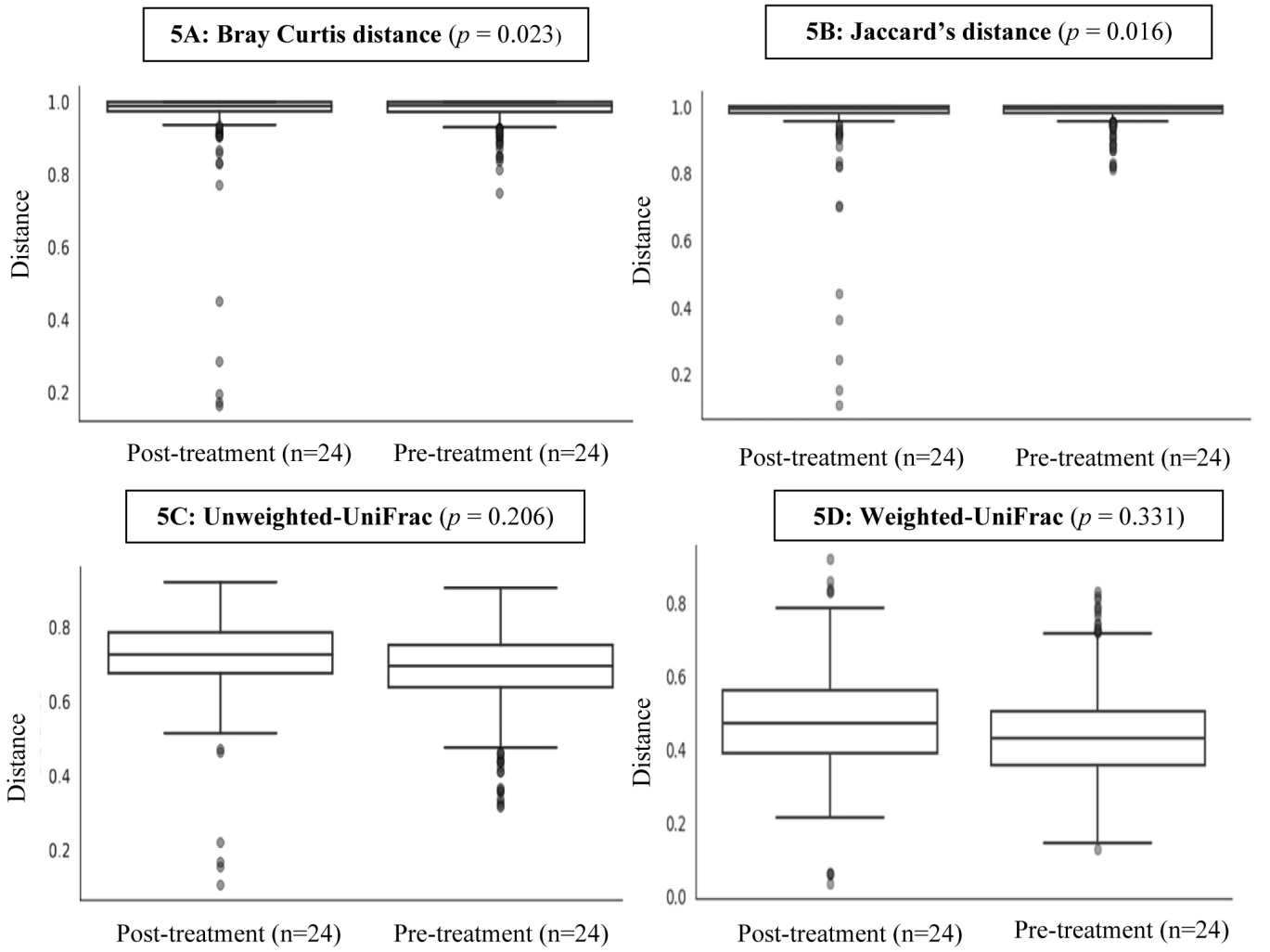


FIGURE 4. Alpha diversity on day 0 (pre-treatment) and day 3 (post-treatment) of feeding. 4A: Shannon's index. 4B: Faith's phylogenetic diversity. 4C: Pielou's evenness. Abbreviation: PD, phylogenetic diversity.



F I G U R E 5. Beta diversity between day 0 (pre-treatment) and day 3 (post-treatment) of feeding. 5A: Bray Curtis distance. 5B: Jaccard's distance. 5C: unweighted-UniFrac. 5D: weighted-UniFrac.

loss to follow-up or treatment crossover. Second, the dietary sources in both groups were commercial enteral diets with proven long-term safety and extensive use. Third, our study assessed the microbiome using next-generation sequencing, which can identify the 16s rRNA sequences of both culturable and nonculturable bacteria and correlate the findings with the ZymoBIOMICS® targeted sequencing service for microbiome analysis, which has an international license.

However, there were also some limitations that should be acknowledged. First, the explanation for the differences between the results of our trial and previous studies might also be due to the study sample size. Our trial was a pilot study performed in a single center and included only 24 patients. Thus, the non-significant difference observed in this trial might be possibly related to the small sample size, which might have also resulted in a lack of power to show statistical significance and generalizability, suggesting that these results should be cautiously interpreted. Second, some samples were obtained from rectal enemas (**Supplementary Table 1**). Although there were limited data to confirm whether samples obtained from a rectal enema affected the microbiota, one study revealed that intestinal lavage fluid contained more abundant pathogenic microbiota than feces [28]. When these samples were extracted, DNA sequencing was performed using the 16S metagenomics method, so the fecal microbiota obtained might not reflect the total microbiota of that sample. Third, the patients in this study were critically ill patients with sepsis; thus, the study results might not apply to surgical or post-operative patients. Fourth, our source of synbiotics was Boost optimum® (Nestle Japan Co. Ltd., Tokyo, Japan), which cannot illustrate other diets containing synbiotics. Therefore, the interpretation and applications of our pilot study should be carefully considered, and further larger clinical studies are required.

5. Conclusions

In critically ill septic patients, a commercial diet containing synbiotics might not significantly improve fecal microbial diversity during hospitalization, although we observed a trend toward reduced ventilator-associated pneumonia and ventilator days in those given synbiotics for 7 days. Additional studies with a larger sample size are needed to clarify our findings and confirm the clinical efficacy of diets containing synbiotics on the gut microbiota of critically ill septic patients.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

KW, SU, KS and VV—Conceptualization. KW, SU, KS and VV—Investigation. KW, SU, KS and VV—Data curation. KW, KS and VV—Formal analysis. KW and VV—Funding acquisition. KW, SU, KS and VV—Methodology. KW, KS and VV—Project administration. KS and VV—Supervision. KW, SU, KS and VV—Writing-original draft. KW, SU, KS and VV—Writing-review & editing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University (protocol code 62-050-14-1, 28 June 2019). Informed consent to participate was obtained from participants who met the eligibility criteria before study initiation or from their proxies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.signavitae.com/mre-signavitae/article/1694257979159658496/attachment/Supplementary%20material.docx.

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