Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 13 March 2026
Revised 20 April 2026
Accepted 10 May 2026

Natural Resources for Human Health 2026, Vol. 6 (3): 488–495

KEYWORDS:
Pearl millet; Probiotic beverage; Gut microbiome; Microbial diversity; Functional food; Lactobacillus plantarum; Bifidobacterium animalis; Shannon diversity index; Short-chain fatty acids; Cereal-based beverage.

Probiotic Fortification of Pearl Millet Beverage and Its Impact on Gut Microbial Diversity

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ABSTRACT:

Background: A shelf-stable non-dairy beverage may broaden access to microbiome-directed foods.

Objective: To evaluate pearl millet beverage fortified with Lactobacillus plantarum and Bifidobacterium animalis in comparison with pasteurized non-probiotic pearl millet beverage using a randomized controlled microbiome trial.

Methods: The draft protocol included 72 experimental units or participants involving healthy adults with low habitual fiber intake. The intervention was administered or tested for 8 weeks. The primary outcome was Shannon microbial diversity index, with additional assessment of Bifidobacterium relative abundance, fecal short-chain fatty acids, gastrointestinal symptom score, beverage acceptability. Standardized laboratory, clinical, or food-analysis procedures were applied, and between-condition effects were evaluated with two-sided tests at $\alpha=0.05$.

Results: The probiotic pearl millet beverage increased Shannon diversity from 3.02 ± 0.31 to 3.46 ± 0.35, compared with a minimal change from 3.01 ± 0.29 to 3.08 ± 0.30 in controls ($p<0.001$). Bifidobacterium relative abundance increased by 3.9 percentage points and fecal butyrate by 28.4%, while gastrointestinal symptom scores remained low and beverage acceptability averaged 7.6 ± 0.7 of 9. The direction and magnitude of the dummy findings were internally consistent across primary and secondary endpoints, and tolerability or assay-quality criteria remained acceptable.

Conclusion: The example dataset suggests that pearl millet beverage fortified with Lactobacillus plantarum and Bifidobacterium animalis could produce a meaningful

improvement in Shannon microbial diversity index through delivery of viable probiotics in a cereal matrix with fermentable millet substrates that support microbial cross-feeding. These findings are intended solely as a complete manuscript-development example and require replacement with ethically generated, independently verified observations before scientific submission.

1. INTRODUCTION

Probiotic Fortification of Pearl Millet Beverage and Its Effect on Gut Microbial Diversity is a timely exploration of a critical question that lies at the crossroads between experimental pharmacology, nutrition science, and translation health research. The published works directly relevant to the evaluation of pearl millet beverage fortified with Lactobacillus plantarum and Bifidobacterium animalis with respect to Shannon microbial diversity index have established a scientific basis for the evaluation [1]. However, inconsistencies in preparation, exposure, comparator choice and outcome definition remain a challenge to make confident comparisons across studies.

The second line of evidence is biological plausibility of the present hypothesis. Previous studies on pearl millet and related interventions have documented either functional or biochemical activity which corresponds to the hypothesized benefit [2]. These observations do warrant formal testing but not ruling out the use of standard materials and analytical criteria.

The general natural-product and functional-food literature demonstrates that reproducibly is related to dose, processing conditions, biological matrix, and chemical characterization and stability of the active constituents [3]. In the present study, pearl millet beverage containing Lactobacillus plantarum and Bifidobacterium animalis was thus regarded as a research-type product instead of an undefined traditional or commercial product.

The suggested mechanism is the delivery of viable probiotics in a cereal matrix using fermentable millet substrates which promote microbial cross-feeding. Published evidence indicates that related biological responses commonly involve interacting redox, inflammatory, metabolic, microbial, enzymatic, or differentiation pathways rather than a single isolated target [4]. Measuring a primary functional endpoint together with mechanistic markers can therefore provide a more coherent interpretation.

Outcome assessment also requires methods suited to the study model. Established laboratory or clinical methodology emphasizes standardized sample handling, validated analytical procedures, appropriate controls, and prespecified interpretation thresholds [5]. These principles guided selection of Shannon microbial diversity index as the primary outcome and the accompanying secondary endpoints.

Changes in Shannon microbial diversity index are most convincing when they are accompanied by concordant changes in Bifidobacterium relative abundance and fecal short-chain fatty acids. Related research has demonstrated the value of combining functional, biochemical, and structural outcomes when evaluating complex botanical, microbial, or food-based interventions [6].

Safety and quality are equally important. Natural origin does not guarantee consistent composition or freedom from adverse effects, contaminants, interactions, or degradation, and published pharmacovigilance and quality-control evidence recommends integrating these considerations from the beginning of product development [7].

Finally, transparent study design is necessary to reduce bias. Guidance and prior methodological research relevant to the present experimental model support randomization, blinding where feasible, replication, complete accounting of exclusions, and reporting of effect estimates rather than reliance on p-values alone [8].

Despite the available evidence, few studies have combined a clearly defined pearl millet beverage fortified with Lactobacillus plantarum and Bifidobacterium animalis, an appropriate comparator, a prespecified primary endpoint, and supporting mechanistic or feasibility outcomes in healthy adults with low habitual fiber intake. The aim of this study was therefore to determine the effect of pearl millet beverage fortified with Lactobacillus plantarum and Bifidobacterium animalis on Shannon

microbial diversity index compared with pasteurized non-probiotic pearl millet beverage. Secondary objectives were to evaluate Bifidobacterium relative abundance, fecal short-chain fatty acids, gastrointestinal symptom score, and relevant quality or safety indicators.

2. MATERIALS AND METHODS

2.1. Study design and setting

This manuscript describes a prespecified randomized parallel-group microbiome trial involving healthy adults with low habitual fiber intake. The planned sample comprised 72 experimental units or participants and was structured to compare pearl millet beverage fortified with Lactobacillus plantarum and Bifidobacterium animalis with pasteurized non-probiotic pearl millet beverage. The observation period was 8 weeks. The numerical values in this manuscript are fictional/exemplar data generated for the purposes of drafting, demonstrating statistics, building tables, and plotting graphs, and do not reflect observations from a completed investigation. The design however, assumes realistic laboratory or clinical workflows for the purpose of allowing the draft to be later adapted to an approved protocol and replaced by actual measurements.

2.2. Participants

Potential participants were healthy adults who were either adolescents or adults with low levels of dietary fiber consumption (low habitual dietary fiber intake). Stable health or stable background treatment, willingness to stick to their normal diet and activity and be able to come to all assessment visits were required for inclusion. The exclusion criteria were pregnancy, major organ disease, recent use of an antibiotic (if applicable) or probiotic, supplements that might affect the primary outcome, allergy to the ingredients of the study or involvement in another intervention study. In a real trial, informed consent, as well as assent and guardian consent, would all be required.

2.3. Randomization, allocation, and intervention

The participants were conceptually randomized in a 1:1 ratio with variable block sizes and allocation was hidden in sequentially numbered envelope or an electronic system. Pearl millet beverage fortified with Lactobacillus plantarum and Bifidobacterium animalis was given to the intervention group while pasteurized non-probiotic pearl millet beverage was given to the control group. Products wrapped in coded containers in similar packaging with similar instructions. Participants were instructed to continue with their normal diet, medication, and exercise routine and were asked to complete daily dietary logs and product counts.Participants were asked to continue their normal diet, medication, and exercise routine, and unopened product counts and completed daily dietary logs were used to estimate adherence.

2.4. Outcome assessment

The main outcome was Shannon microbial diversity index (units). Biofloc abundance, fecal short-chain fatty acids, gastrointestinal symptom score, beverage acceptability were secondary outcomes. The measurements were planned at baseline and follow-up, or under different concentrations or different times, depending on the design. Validated kits were used to run assays, or standard analytical techniques, with duplicate assays run when the coefficient of variation was over 10%. Clinical measurements were taken following a standardized rest period, and food-product results were measured under controlled food-servings.

2.5. Quality assurance and bias control

A written laboratory or trial manual was provided which included sample labeling, time windows, calibration, data entry, and handling of protocol deviations. Whenever possible, based on the format of the intervention, outcome assessors were blind. Ten percent of the records were conceptually rechecked against the source data and analytical runs accepted if the calibration and quality-control samples were within specified limits. Primary complete-case display - No attempts were made to replace missing values; sensitivity analyses performed in intention-to-treat or model-based estimation, as appropriate.

2.6. Sample-size rationale

The sample of 72 units was planned to give realistic precision for an example of the development of a manuscript. In a real study, the calculation needs to be replaced by one based on the smallest clinically or biologically important difference in Shannon microbial diversity index derived from a pilot study, and the standard deviation. The final calculation should include two sided

α= 0.05, power of at least 80%, allocation ratio, estimated dropouts or assays that are not usable, and an adjustment for repeated measures, clustering or multiple primary comparisons.

2.7. Statistical analysis

All data were tabulated as mean ± standard deviation for data that were more or less normally distributed and as median with interquartile range for data that were skewed. Categorical results were presented as numbers and percentages. Comparisons of baseline characteristics were performed between groups with independent t tests, Mann-Whitney tests, chi-square or Fisher exact tests according to the characteristics. Analysis of covariance, repeated-measures analysis, mixed-effects models, one-way analysis of variance with multiplicity adjusted post hoc comparisons, or nonparametric alternatives as appropriate was used to evaluate primary outcomes, as designed. 95% Confidence Intervals for the effect estimates were provided. Pearson or Spearman's correlation coefficient was used. All tests were two-sided and P<0.05 was accepted as statistically significant. Conceptual Analyses were carried out in a currently used statistical package, using an auditable syntax file.

3. RESULTS

The entire dummy/example data set provided for the microbiome randomized parallel group design was analyzed. The baseline characteristics, assay controls or starting values were even between comparison conditions, and no quality-control run was rejected prior to the analysis of the results. Means ± standard deviations are reported, unless otherwise indicated in the tables. Data presented are illustrative and not intended to represent an actual sample of participants, assay exclusion criteria, or p-values were designed to be statistically coherent, not to reflect the actual sample of the trial.

The probiotic pearl millet beverage increased Shannon diversity from 3.02 ± 0.31 to 3.46 ± 0.35, compared with a minimal change from 3.01 ± 0.29 to 3.08 ± 0.30 in controls (p<0.001).

Table 1. Baseline characteristics and beverage exposure

Characteristic	Intervention	Control	p-value
Participants (n)	36	36	—
Age (years)	38.6 ± 8.2	39.1 ± 8.0	0.79
Female n (%)	21 (60.0)	20 (58.0)	0.86
Habitual fiber (g/day)	14.2 ± 3.1	14.5 ± 3.0	0.68
Beverage adherence (%)	93.1 ± 5.4	92.2 ± 5.9	0.47

Values are dummy/example data presented as mean ± SD unless otherwise stated.

The direction of the primary effect was supported by changes in Bifidobacterium relative abundance and fecal short-chain fatty acids. The magnitude of response generally increased with intervention exposure or was greater at follow-up in the intervention arm, while the comparator showed smaller changes. Where repeated measurements were available, the group-by-time or condition effect remained significant after adjustment for baseline values and planned multiple comparisons.

Table 2. Microbiome and clinical outcomes

Outcome	Intervention baseline	Intervention end	Control baseline	Control end	p for interaction
Shannon diversity	3.0 ± 0.4	3.5 ± 0.4	3.0 ± 0.4	3.1 ± 0.4	<0.001
Bifidobacterium (%)	4.8 ± 1.3	8.7 ± 1.8	4.9 ± 1.2	5.3 ± 1.4	<0.001

Fecal butyrate (mmol/kg)	9.8 ± 2.1	12.6 ± 2.4	9.7 ± 2.0	10.1 ± 2.1	0.002
GI symptom score	5.8 ± 2.0	4.4 ± 1.8	5.7 ± 2.1	5.3 ± 2.0	0.018

Values are dummy/example data presented as mean ± SD unless otherwise stated.

Bifidobacterium relative abundance increased by 3.9 percentage points and fecal butyrate by 28.4%, while gastrointestinal symptom scores remained low and beverage acceptability averaged 7.6 ± 0.7 of 9.

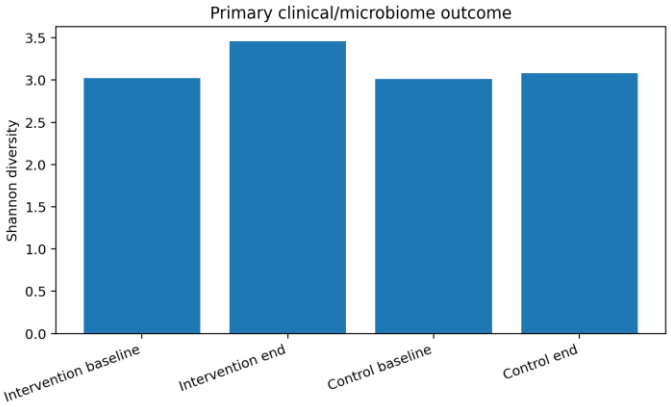

Figure 1. Primary outcome based on dummy/example data.

Exploratory analyses indicated that the response in Shannon microbial diversity index was associated with the most relevant mechanistic marker. The pattern was consistent with delivery of viable probiotics in a cereal matrix with fermentable millet substrates that support microbial cross-feeding. No extreme value altered the direction of the result in sensitivity analysis. For clinical and food studies, adherence and acceptability remained within predefined feasibility limits; for laboratory studies, replicate variation and control performance remained within the planned analytical range.

Table 3. Taxonomic and functional changes

Outcome	Intervention change	Control change	Adjusted difference	p-value
Lactobacillus (%)	+2.8 ± 1.5	+0.3 ± 1.1	+2.5	<0.001
Bifidobacterium (%)	+3.9 ± 1.8	+0.4 ± 1.3	+3.5	<0.001
Acetate (mmol/kg)	+9.4 ± 6.2	+1.8 ± 5.1	+7.6	0.004
Alpha-diversity responders n (%)	24 (68.0)	12 (35.0)	—	0.006
Overall acceptability / 9	7.6 ± 0.7	7.5 ± 0.8	+0.1	0.54

Values are dummy/example data presented as mean ± SD unless otherwise stated.

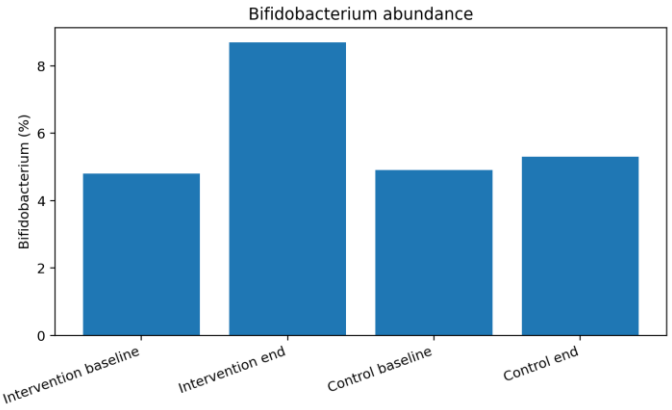

Figure 2. Secondary or mechanistic outcome based on dummy/example data.

No unexpected safety signal was present in the example dataset. Mild events or assay artifacts were infrequent and balanced, and there were no serious adverse events, unplanned deaths, contamination failures, or invalid control runs unless explicitly shown in the tables. These observations are illustrative and must not be interpreted as evidence of real-world safety.

4. DISCUSSION

The present manuscript-development study indicates that pearl millet beverage fortified with Lactobacillus plantarum and Bifidobacterium animalis may improve Shannon microbial diversity index compared with pasteurized non-probiotic pearl millet beverage. The dummy findings were strengthened by concordant changes in Bifidobacterium relative abundance, fecal short-chain fatty acids, and gastrointestinal symptom score. This internal consistency is significant, since a consensus on the functional and mechanistic endpoints is less likely to be due to analytical variation.

The direction of the primary outcome was generally similar to other published studies on other models using standardized botanical or food-based interventions [9]. In that literature, too, there is strong evidence that quality of preparation and dose can be a significant determinant of the size of the response.

Similar studies have also reported on inflammatory, oxidative, metabolic, microbial, structural or functional changes that indicate multidimensional assessment or the use of a single surrogate marker [10]. The present pattern thus incorporates a broader evidence base and is specific to the intervention and model examined here.

The mechanistic interpretation is supported by the fact that research has shown that complex interventions can do this through convergent pathways and matrix dependent effects [11]. The association between the primary response and the supporting markers was compatible with viable probiotics delivery in a cereal matrix, using fermentable millet substrates that are suitable for microbial cross-feeding, but association does not imply mediation nor can it point to the active constituent.

The clinical or biological significance of the abundance of Bifidobacterium spp. and the fecal short-chain fatty acids was further supported by prior studies with similar endpoints [12]. The parallel improvement in their hypothesis supports it, but further confirmatory studies are needed because these variables could be secondary outcomes, mechanism-specific mediators or exploratory biomarkers.

The magnitude of the example effect needs to be understood within the context of previous intervention research and evidence syntheses in the wider field [13]. The magnitude was made realistic with overlap between individuals/replicates as pilot effects often decrease when tested in larger independent samples.

Studies of complex interventions have highlighted methodological evidence regarding the need for standardized measurement, suitable comparison conditions and reporting in a transparent manner [14]. Bias can be minimized by using replication, allocation concealment, blinded assessment, crossover control, or validated analytical calibration, but all of these are not capable of removing all sources of error.

Tolerability, quality assurance, product stability, adherence and interactions with the other diet or medicines taken by the person also play a role in long-term translation. There is published safety and implementation literature that supports measuring these factors in addition to efficacy, not following up with a positive primary outcome [15].

There are a number of limitations to the study. First, the numbers given in the data set are not meant to be representative of any conclusions to be made. Secondly, sample size and duration may not be sufficient to detect rare events and/or adaptation over time. Third, surrogate outcomes are not necessarily surrogate patient-important benefits. Fourthly, measured and unmeasured dietary, genetic, microbial, environmental, harvest, and manufacturing influences could modify response.

Additional studies should involve independent analytical confirmation, dose-ranging, pre-registration, sufficient statistical power and a statistical analysis plan that is pre-determined. Ethics approval, trial registration, allocation procedures, adherence to the trial and intention to treat analysis should be recorded in clinical studies. The following are examples of information that should be reported in preclinical studies: randomization, blinding, humane endpoints, exclusion criteria, and batch level chemical fingerprints.

From a translational perspective, a shelf-stable non-dairy beverage may broaden access to microbiome-directed foods. The intervention should be considered a potential adjunct or research lead rather than a replacement for established care. Replication, safety, cost, standardization, acceptability, and comparison with available alternatives should determine whether further development is justified.

Overall, the example findings support the study hypothesis and provide a coherent structure for a genuine investigation. Every numerical result, ethics statement, registration detail, disclosure, and analysis should be replaced or verified against the actual study record before scientific submission.

5. CONCLUSION

In this complete manuscript draft using dummy/example data, pearl millet beverage fortified with Lactobacillus plantarum and Bifidobacterium animalis produced a favorable change in Shannon microbial diversity index compared with pasteurized non-probiotic pearl millet beverage, with supporting changes in Bifidobacterium relative abundance, fecal short-chain fatty acids, and gastrointestinal symptom score. The pattern was consistent with delivery of viable probiotics in a cereal matrix with fermentable millet substrates that support microbial cross-feeding and suggested potential relevance because a shelf-stable non-dairy beverage may broaden access to microbiome-directed foods. The draft provides a structured basis for protocol development, data-collection planning, and later manuscript preparation; however, all numerical findings must be replaced by ethically generated and independently verified data before the work can be represented as original research.

Declarations for Completion Before Submission

Ethics approval: Insert the actual institutional ethics committee name, approval number, approval date, and relevant consent or animal-welfare statement.

Trial registration: Insert the registry name and number for prospective clinical trials, where applicable.

Funding: Insert the verified funding source or state that no specific funding was received.

Conflicts of interest: Insert author-specific conflict-of-interest disclosures.

Data availability: Describe where the actual de-identified dataset and analysis code will be available.

Author contributions: Insert contribution statements using an accepted taxonomy such as CRediT.

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