White Button Mushroom, *Agaricus bisporus* (Agaricomycetes), and a Probiotics Mixture Supplementation Correct Dyslipidemia without Influencing the Colon Microbiome Profile in Hypercholesterolemic Rats

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ABSTRACT: Consumption of foods rich in dietary fiber has attracted considerable attention for lowering blood cholesterol and triglycerides through attenuation of gut microbiome. Diets rich in fiber may provide substrates for microbes to digest and proliferate. In response, products of microbial digestion enter systemic circulation and support host energy homeostasis. In the present study, rats with hypercholesterolemia (HC) were supplemented with probiotics (PB) and *Agaricus bisporus* mushroom to examine the antidyslipidemia effects. Forty adult rats were divided into five treatment groups. The rats in the control group were fed only a chow maintenance diet (CON; n = 8), whereas an atherogenic diet (chow diet supplemented with 1.5% cholesterol and 0.5% cholic acid) was offered to the remaining rats to induce hypercholesterolemia (HC group; n = 32). Rats developed HC following a 24-day continuous supplementation with the atherogenic diet. From day 25 onward, the HC group was further divided into HC-CON, HC-PB (supplemented with PB at 1 mg/rat/day), HC-AB (supplemented with *A. bisporus* at 5% of diet), and HC-AB.PB (supplemented with both *A. bisporus* and PB). After 6 weeks of supplementation, rats were killed to collect blood to determine serum lipid profile, oxidative stress, and for metagenomics analysis of colon contents. Results showed that all supplementations corrected HC-induced oxidative stress. Furthermore, *A. bisporus* supplementation corrected HC-induced dyslipidemia ($P \leq .05$). *Blautia* and *Bifidobacterium* were the most dominant bacterial genera in HC-AB and HC-PB groups, respectively. Phylum Firmicutes and class Clostridia predominantly occupied the gut microbiome in all groups. However, no significant differences were observed in microbiome diversity and clustering patterns among study groups. In conclusion, supplementation of *A. bisporus* mushroom and probiotics can lower oxidative stress and dyslipidemia with partial effects on the phylogenetic makeup in the gut microbiome.

KEY WORDS: 16S sequencing, *Agaricus bisporus*, dyslipidemia, medicinal mushrooms, microbiome, oxidative stress, probiotics

ABBREVIATIONS: AB, *Agaricus bisporus*; **ANOSIM**, analysis of similarities; **ABCG5/G8**, ATP-binding cassette subfamily G-transporters; **CMD**, chow maintenance diet; **CON**, control group; **CYP7A1**, cholesterol 7-α-hydroxylase 1; **GIT**, gastrointestinal tract; **HC**, hypercholesterolemia group; **HD-cholesterol**, high-density cholesterol; **LD-cholesterol**, low density cholesterol; **LDA**, linear discriminant analysis; **LDL**, low-density lipoprotein diet; **PB**, probiotics; **OTU**, operational taxonomic unit

I. INTRODUCTION

The collective cell number or the genetic content in the gastrointestinal microbiome outnumbers the human body cell count and their gene content. A large fraction of this microbial community, approximately 100 trillion, is made up of bacteria.[1] This microbial community constantly interacts and communicates with host cells and modulates host metabolism and immunity. The gut microbiome interacts with host

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genetics and encodes several essential proteins required for host homeostasis.[2] Any transient change in the microbiome can disrupt the physiological homeostasis of the host and may result in a metabolic disorder.[3] Diet is one of the many factors that alters taxonomy and functional contents of the microbiome. Western style, energy-rich foods lower the microbial diversity and trigger autoimmunity and inflammation that may lead to metabolic diseases.[4] On the other hand, foods rich in fiber improve bacterial diversity and functional contents that help in the management of glucose and lipid homeostasis.[5] Continuous consumption of a particular type of food for a few days can alter the phylogenetic structure and functional capacity of the gut microbiome.[6] Particularly, consumption of plant fibers, which are fermented by microbes for production of short chain fatty acids (SCFAs), has shown to improve gut wall integrity, immunity, and metabolic homeostasis.[7]

White button mushroom, *Agaricus bisporus* (J. Lange) Imbach (Agaricaceae, Agaricomycetes), is a culinary-medicinal mushroom that is commonly cultivated. This mushroom contains a high level of protein, vitamins, minerals, and dietary fiber. These compounds have antioxidant, antiinflammatory, hypoglycemic, and hypocholesterolemic effects.[8] Earlier studies have shown that *A. bisporus* supplementation reduced serum glucose, cholesterol, and triglyceride concentrations as well as the activities of liver enzymes (alanine aminotransferase and aspartate aminotransferase).[9,10] Furthermore, Fukushima et al.[9] reported that the mushroom supplementation improved colon fermentation profiles as represented by higher levels of SCFAs production. These findings suggest that the health-promoting activities of *A. bisporus* are partially linked with the gastrointestinal microbiome and metabolic functions.[11]

A review of the literature reveals that *A. bisporus* supplementation in poultry improves cercal *Lactobacillus* spp. and *Bifidobacterium* spp. loads.[12] However, no studies were found wherein the gut microbial ecology of rodents supplemented with *A. bisporus* has been examined. The present study is, therefore, designed to explore the gut microbiome profile of hypercholesterolemic rats supplemented with *A. bisporus* and probiotics mixtures.

II. MATERIALS AND METHODS

A. Animals and Research Design

Forty mixed sex Wistar rats, weighing approximately 200 g, were acquired from the Laboratory Animal Research Center, Department of Physiology, Government College University, Faisalabad, Pakistan. The rats were kept in individual cages, maintained at 24 ± 2°C, under a 12-h light/dark schedule, and offered *ad libitum* food and water. After a 1-week acclimation period, rats were weight-matched and allocated to dietary groups. The rats in the control group were fed a basic chow maintenance diet (CMD; CON group; n = 8). The other rats (hypercholesterolemia [HC] group; n = 32) were fed an atherogenic diet (containing cholesterol [1.5% w/w] and cholic acid [0.5% w/w]) for 24 days to induce dyslipidemia. On day 25, dyslipidemia was confirmed by measuring cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) concentrations using commercially available kits (Cholesterol liquicolor, REF# 10017, 10094, 10084; HUMAN, Gesellschaft für Biochemica und Diagnostica mbH, Max-Planck, Wiesbaden, Germany). In HC rats, a significant rise in the concentrations of cholesterol and LDL cholesterol, compared with the control, was considered as dyslipidemia.[13] Thereafter, dyslipidemic rats were divided into four groups. The HC-CON group was fed only the hypercholesterolemic diet for the entire experimental period. The supplemented groups were fed the HC diet and were further supplemented with either *A. bisporus* mushroom (5% w/w; HC-*A. bisporus* group), a probiotics mixture (1.8×10^5 CFU/rat; HC-PB group; see information below), or a combination of both AB and the probiotics mixture (HC-AB.PB). These dietary supplementations were continued from day 25 to day 66. Food intake and body weight were recorded weekly. At the termination of this study, the rats were killed by cervical dislocation, and trunk blood and colon digesta were

collected. Due permission was sought, and rats were monitored for signs of injury or disease in accordance with the guidelines of the Animal Care and Research Ethics Committee of Government College University, Faisalabad, Pakistan (Reference no. GCUF/ERC/132).

B. Diet Composition and Supplements

The basal diet was composed of the following ingredients (g/100 g): starch 76, soybean oil 10, casein 10, and vitamin-mineral premix 4. Supplements and atherogenic compounds were added to replace starch in the supplemental groups. A commercially available probiotics mixture (Protexin; Probiotics International Ltd., Somerset, UK), composed of 14 live bacterial strains at a minimum of 6×10^7 CFU per gram, was added to the ration pellets. *A. bisporus* was procured from a local market. The fruiting bodies of the mushroom were washed, stems were removed, and caps were sliced into equally sized pieces. Sliced mushrooms were air-dried and then ground with a mortar and pestle to make a powder.

C. Serum Biochemistry

At the end of the trial, blood serum was separated by centrifuging blood at $1500 \times g$ for 15 min. Serum triglycerides, total cholesterol, HDL, LDL, and markers of oxidative stress (total oxidant status [TOS] and total antioxidant capacity [TAC]) were determined using enzymatic colorimetric methods as described previously.[14–16] In brief, commercially available kits (HUMAN, Gesellschaft für Biochemica und Diagnostica mbH, Max-Planck, Wiesbaden, Germany) were used to perform enzymatic reactions, and color changes were observed using a semiautomatic bioanalyzer (Biosystem BTS-330, Costa Brava, Barcelona, Spain). The TOS was determined in serum samples as equivalent to H_2O_2. A standard curve was made with 0.39, 0.78, 1.56, 3.12, and 6.25 μmol/L of H_2O_2. The minimum detectable range of the test was 0.13 to 200 μmol H_2O_2 equivalent/L, with < 3% precision and an intraassay coefficient of variance (CV) below 10%.[16]

Similarly, vitamin C was used for constructing a standard curve for TAC at concentrations of 0.3, 0.6, 0.9, 1.2, and 1.5 mmol/L. The minimum detectable range of this assay was 0.18 to 6 mmol of vitamin C equivalent/L, with 3% precision.[16]

D. Microbiome Analysis

Digesta collected from the colon was subjected to DNA extraction using QIAmp DNA Mini Kit (QIAgen Corporation, Valencia, CA, USA). Double-stranded DNA concentrations and purity (A260/280 and A260/230, respectively) were measured using a NanoDrop spectrophotometer (ND 1000; Wilmington, DE, USA). For 16S rRNA gene sequencing, amplicon libraries for the V4 hypervariable region were prepared using the 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) primer pair. The libraries were washed using Agencourt AMPure XP beads (A63881; Beckman Coulter, Indianapolis, IN, USA) and indexed in another PCR run using the Nextera XT Index Kit (FC-131-1001; Illumina, San Diego, CA, USA). All DNA libraries were pooled together and denatured before running them on MiSeq. Sequencing was performed by MR DNA (Shallowater, TX, USA) using an Illumina MiSeq instrument.

Raw sequence data were obtained through BaseSpace as paired R1 and R2 reads. QIIME 1.9 was used for data alignment and statistical analysis. Raw data obtained were denoised, trimmed, and chimera depleted for improving Phred quality score of the data (≥ 30). All sequences were demultiplexed into their respective samples and clustered as operational taxonomic units (OTUs). Clustering was performed at 97% similarity using default settings.

E. Statistical Analysis

Mean values and standard deviations were calculated for all serum biochemistry readings. Statistical differences among mean values were calculated using analysis of variance and Tukey's multiple-comparison test. Default QIIME scripts were used for alpha and beta diversity analysis of metagenomics data. An even sampling depth of 19,793 sequences per sample was used for these analyses. Statistical significance among groups was analyzed using the analysis of similarity (ANOSIM) at $P \leq .05$. Kolmogorov–Smirnov test was used to assess normal distribution of the data. Nonparametric ANOVA (Kruskal–Wallis H test) was applied on the metagenomics data and significance was observed at $P \leq .05$.

III. RESULTS

A. Serum Biochemistry

The results of the serum biochemistry analysis are presented in the Table 1 as mean ± standard error (SE). The total cholesterol decreased significantly ($P \leq .05$) in the HC-AB group as compared to the HC-CON group only; the rest of the groups were not found to be significantly different from each other. Similar results were observed for HDL and LDL cholesterol, in which HDL increased significantly and LDL decreased significantly ($P \leq .05$) in the HC-AB group in comparison to the HC-CON group only. However, a significant increase ($P \leq .05$) in triglycerides was observed in the HC-CON group compared to all other groups. These results signify that the lipid profile was influenced by various treatments compared to the untreated hypercholesterolemic group. The TOS was significantly decreased ($P \leq .05$) in all treated groups as compared to the HC-CON group. Conversely, TAC showed an increase ($P \leq .05$) in the treated groups compared to the HC-CON group, and conclusively demonstrated the oxidative stress-lowering impact of the various treatments with the mushroom and the probiotics mixture.

B. Stool Microbiome

High throughput amplicon sequencing yielded 1,767,765 good-quality reads corresponding to 48,721 OTUs. These OTUs belonged to 21 phyla, 41 classes, 74 orders, 141 families, and 258 genera. Phylum Firmicutes constituted more than 60% of the sequences in all treatment groups. The Kruskal–Wallis

TABLE 1: Serum Biochemistry of Rats Supplemented with *Agaricus bisporus* and a Commercial Probiotics Mixture

Parameters	CON	HC-CON	HC-AB	HC-PB	HC-AB.PB	*P* value
Cholesterol (mg/dL)	$228^{ab} \pm 12.6$	$257^{a} \pm 1.7$	$197.9^{b} \pm 12.1$	$207.1^{ab} \pm 13.9$	$228.1^{ab} \pm 14.6$	0.04
HDL (mg/dL)	$67.9^{ab} \pm 5.5$	$65.6^{b} \pm 5.3$	$83.8^{a} \pm 1.8$	$66.9^{ab} \pm 3.5$	$70.6^{ab} \pm 4.6$	0.02
LDL (mg/dL)	$179.2^{ab} \pm 13.2$	$206.1^{a} \pm 3.5$	$148.5^{b} \pm 17.3$	$193.8^{ab} \pm 11.2$	$177.4^{ab} \pm 6.8$	0.03
TG (mg/dL)	$176.6^{b} \pm 8.3$	$254.8^{a} \pm 21.9$	$162.5^{b} \pm 19.3$	$167.9^{b} \pm 9.1$	$177.7^{b} \pm 21.2$	0.01
TOS (μMol/L)	$19.4^{ab} \pm 0.8$	$21.8^{a} \pm 0.8$	$15.7^{b} \pm 1.2$	$17.2^{b} \pm 0.7$	$15.6^{b} \pm 0.8$	0.00
TAC (mMol/L)	$2.7^{ab} \pm 0.3$	$1.9^{b} \pm 0.7$	$2.9^{a} \pm 0.6$	$2.6^{ab} \pm 0.2$	$2.74^{a} \pm 0.1$	0.04

Mean values within a row lacking a common superscript are significantly different ($P \leq .05$).
CON, control group; HC-CON, hypercholesterolemic control group; HC-AB, hypercholesterolemic group supplemented with *A. bisporus*; HC-PB, hypercholesterolemic group supplemented with the probiotics mixtutre; HC-AB.PB, hypercholesterolemic group supplemented with *A. bisporus* and the probiotics mixture; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; TOS, total oxidant status; TAC, total antioxidant status.

H test revealed significant differences ($P \leq .05$) among Actinobacteria, Euryarchaeota, and Proteobacteria sequence counts in different treatment groups (Table 2). Similarly, Gloeobacteria, Methanobacteria, and class Actinobacteria and their respective lineages significantly differed ($P \leq .05$) among different groups. Rarefaction curves drawn on an even sampling depth of 19,793 revealed no differences ($P > .05$) in microbiome diversity or species abundance across the five study groups. All statistical parameters used for alpha diversity presented P values > 0.05 (Fig. 1). Similarly, microbiota community composition, compared as the total/shared OTUs among the groups by ANOSIM and weighted-UniFrac, illustrated no clear clustering pattern. Principal coordinate analysis (PCoA) revealed significant overlapping of the bacterial clades with no observed unique clustering of any group (Fig. 2). Most of the microbial communities overlap each other and reflect a noncomparable clustering pattern (ANOSIM $P > .05$).

TABLE 2: Percentage* Abundance of Predominant Bacterial Taxa Identified in Rats Supplemented with *Agaricus bisporus* and a Commercial Probiotics Mixture

Phyla	CON	HC-CON	HC-AB	HC-PB	HC-AB. PB	SEM	*P* value
Bacteroidetes	1.4	1.9	1.3	0.9	1.0	0.35	0.74
Actinobacteria	11.1[b]	25.0[a]	13.1[b]	19.3[a]	4.3[c]	3.05	0.00
Firmicutes	76.5	61.5	76.8	73.4	73.0	5.59	0.25
Euryarchaeota	4.2[b]	4.8[b]	6.1[b]	4.7[b]	11.1[a]	2.54	0.03
Proteobacteria	2.2[a]	0.4[c]	0.3[c]	1.3[b]	1.1[b]	0.69	0.03
Class							
Erysipelotrichia	4.6	16.3	7.1	5.0	7.8	2.02	0.08
Bacteroidia	1.4	1.9	1.3	0.9	1.0	0.35	0.90
Clostridia	63.4	33.8	62.6	50.1	55.1	5.78	0.09
Deltaproteobacteria	1.9	0.2	0.2	1.0	0.9	0.62	0.09
Methanobacteria	4.1[b]	4.8[b]	6.1[b]	4.6[b]	11.1[a]	2.56	0.03
Actinobacteria	11.1[b]	25.0[a]	13.1[b]	19.3[a]	4.3[c]	3.08	0.02
Bacilli	1.6	0.5	2.6	5.0	1.7	1.51	0.11
Order							
Clostridiales	63.4	34.0	63.0	50.2	55.2	5.78	0.13
Bacteroidales	1.4	2.0	1.3	1.0	1.0	0.36	1.00
Desulfovibrionales	2.0	0.2	0.2	1.0	1.0	0.66	0.14
Bifidobacteriales	8.3	16.0	10.0	17.4	2.8	5.31	0.24
Lactobacillales	1.4	0.4	2.6	4.8	1.7	1.48	0.13
Erysipelotrichales	4.6	16.3	7.0	5.0	7.8	4.25	0.11
Methanobacteriales	4.2[b]	4.8[b]	6.1[b]	4.7[b]	11.0[a]	1.5	0.03
Coriobacteriales	1.6[b]	6.7[a]	2.7[b]	0.9[b]	1.65[b]	1.07	0.01
Family							
Eubacteriaceae	7.6[b]	1.9[c]	2.8[c]	9.8[b]	16.4[a]	2.24	0.00
Lachnospiraceae	41.6	27.6	49.3	35.5	35.8	3.20	0.08
Desulfovibrionaceae	1.9	0.2	0.2	0.9	0.9	0.62	0.09

TABLE 2: (*continued*)

Phyla	CON	HC-CON	HC-AB	HC-PB	HC-AB.PB	SEM	*P* value
Family							
Ruminococcaceae	3.4	3.5	5.8	2.9	4.2	1.00	0.64
Erysipelotrichaceae	4.6	16.3	7.1	5.0	7.8	4.24	0.08
Porphyromonadaceae	0.9	0.8	1.1	0.4	0.8	0.22	0.45
Lactobacillaceae	1.1	0.2	0.8	2.6	0.8	0.80	0.04
Bifidobacteriaceae	8.3	15.9	9.6	17.5	2.8	2.30	0.18
Coriobacteriaceae	1.6^{bc}	6.7^{a}	2.7^{b}	0.9^{c}	1.7^{bc}	1.07	0.01
Clostridiaceae	4.9	3.2	3.5	4.2	4.9	0.70	0.75
Methanobacteriaceae	4.1^{b}	4.8^{b}	6.1^{b}	4.7^{b}	11.0^{a}	2.51	0.03
Genus							
Bifidobacterium	8.3	15.9	9.6	17.4	2.8	2.31	0.18
Collinsella	1.3^{c}	6.0^{a}	2.5^{b}	0.7^{d}	1.3^{c}	1.92	0.00
Ruminococcus	2.7	2.9	5.2	2.0	3.2	1.07	0.52
Blautia	26.0^{b}	22.5^{b}	43.8^{a}	23.6^{b}	19.8^{b}	4.56	0.04
Desulfovibrio	1.9	0.2	0.1	0.9	0.9	0.64	0.08
Methanobrevibacter	4.2^{b}	4.8^{b}	6.1^{b}	4.7^{b}	11.0^{a}	2.50	0.03
Allobaculum	34.0	12.3	6.2	4.6	7.2	5.87	0.37
Clostridium	4.8	3.0	3.4	4.0	4.4	0.65	0.77
Lactobacillus	0.8	0.2	0.4	2.3	0.4	0.76	0.10
Dorea	4.1^{b}	0.7^{c}	0.7^{c}	8.4^{a}	3.1^{b}	1.83	0.02
Eubacterium	7.5^{b}	1.8^{c}	2.8^{c}	9.7^{b}	16.2^{a}	1.59	0.00
Lachnoclostridium	7.0^{a}	2.4^{b}	2.7^{b}	2.7^{b}	5.2^{b}	0.80	0.32

Mean values within a row lacking a common superscript are significantly different ($P \leq 0.05$).

*Groups presented had more than one percent sequences abundance in at least one of the treatment groups.

CON, control group; HC-CON, hypercholesterolemic control group; HC-AB, hypercholesterolemic group supplemented with *A. bisporus*; HC-PB, hypercholesterolemic group supplemented with the probiotics mixture; HC-AB.PB, hypercholesterolemic group supplemented with *A. bisporus* and the probiotics mixture.

IV. DISCUSSION

The present study was conducted to elucidate potential health benefits of the medicinal mushroom *A. bisporus* and a commercial probiotics mixture in atherogenic rats. The mushroom and live bacterial strains, when supplemented in low doses, were reported to influence lipid metabolism.[14,17] A review of the literature revealed that these supplements may be beneficial in lowering oxidative stress and may act as inexpensive natural health remedies for many metabolic and idiopathic diseases.[13,14] The elevated serum triglyceride and LDL cholesterol concentrations are well-known risk factors for cardiovascular diseases.[18] Associated oxidative stress and inflammatory processes further add to the complications.[19]

In the current study, dyslipidemia was induced in rats by feeding them an atherogenic diet. After confirmation of hypercholesterolemia, the rats were supplemented with low doses of the mushroom and the probiotics mixture. These supplementations, partially or completely, lowered total cholesterol, LDL cholesterol, and triglycerides concentrations. Particularly, the mushroom supplementation decreased total cholesterol,

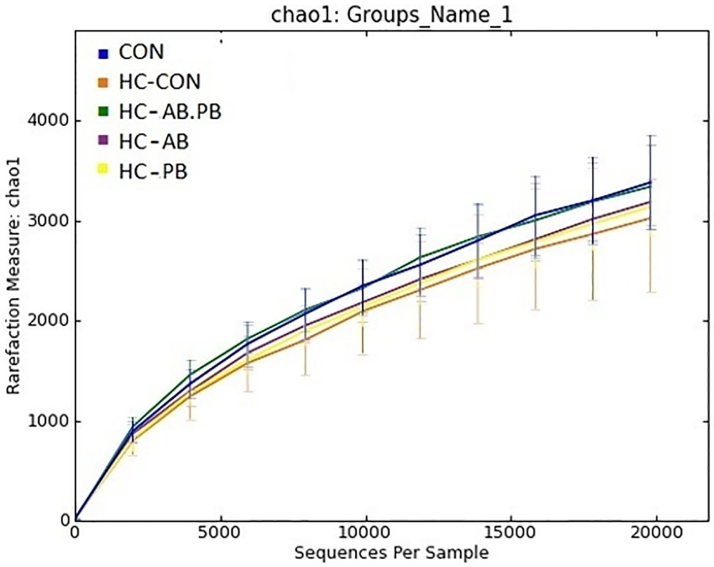

FIG. 1: Chao1 alpha diversity measured at 19,793 sequences per sample obtained from colon microbiome samples of rats supplemented with *Agaricus bisporus* and a commercial probiotics mixture. Rare faction curves indicate changes in diversity as the sequencing depths increases. The standard error bars at each sampling depth indicate sampling distribution. CON, control group; HC-CON, hypercholesterolemic control group; HC-AB, hypercholesterolemic group supplemented with *A. bisporus*; HC-PB, hypercholesterolemic group supplemented with the probiotics mixture; HC-*A. bisporus*. PB, hypercholesterolemic group supplemented with *A. bisporus* and the probiotics mixture.

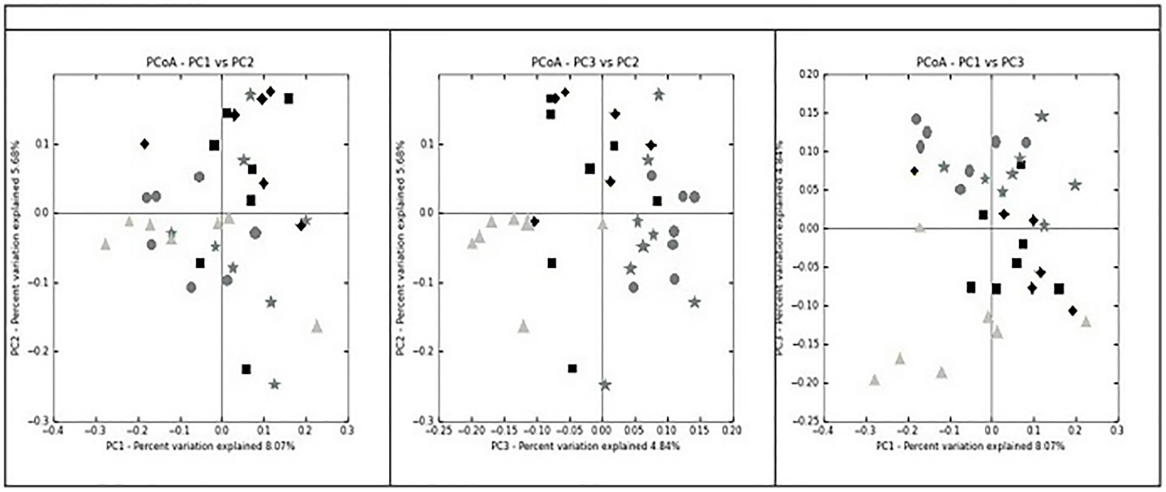

FIG. 2: Principal coordinate analysis plots of the weighted Unifrac distance matrix of microbiome profiles of rats supplemented with *A. bisporus* and a commercial probiotics mixture. Pairwise anosim analysis indicates no difference ($P \leq .05$) between treatment groups. Pairwise anosim analysis indicates no difference ($P \leq 0.05$) between treatment groups. Diamond, CON; star mark, HC-CON, hypercholesterolemic control group; rectangle, HC-PB, hypercholesterolemic group supplemented with the probiotics mixture; turquoise circle, HC-AB, hypercholesterolemic group supplemented with *A. bisporus*; triangle, HC-AB.PB, supplemented with *A. bisporus* and the probiotics mixture group.

LDL cholesterol, and triglycerides concentrations. The biological active ingredients of the mushroom are potent antiinflammatory and antioxidant agents that decrease blood cholesterol levels and improve cardiovascular health.[20] Caz et al.[21] demonstrated that dietary fibers obtained from edible mushrooms could lower expression levels of Dgat1, a key metabolic enzyme that is associated with obesity and cholesterol metabolism. Similarly, de Miranda et al.[22] observed an increase in the expression of CYP7A1, ATP-binding cassette subfamily G-transporters (ABCG5/G8), and LDL cholesterol in mushroom-supplemented rats. In the present study, lipid profiles in groups given the mushroom alone and the mushroom in combination with the probiotics mixture were improved compared to the no-treatment control group, but these changes were not statistically significant ($P > .05$). A review of the literature showed that outcomes concerning the effect of probiotics supplements on lipid profile or oxidative stress are highly diverse.[18,23,24] Ostadrahimi et al.[23] reported that cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides concentrations did not improve following probiotics supplementation in diabetic subjects. A more pronounced improvement in the lipid profile and oxidative stress parameters in the mushroom-supplemented group may be due to the presence of several biologically active ingredients (vitamins, minerals, poly unsaturated fatty acids) in the mushroom.[25]

It was also proposed that dietary fibers or live bacterial hydrolase deconjugation of bile salts in the intestine prevent reabsorption of bile into systemic circulation.[26] No significant changes were seen in the current study in microbiome diversity between different treatment groups; therefore, observations from this study do not support microbiome-associated changes in the lipid profile. The only differences observed in microbiome diversity were seen in less representative bacterial clades, while the major bacterial groups, such as Phylum Firmicutes and Class Clostridia, which constitute more than 80% of the bacterial phylogeny, remained constant among the groups. These observations reflected that the core microbiome among all groups stayed the same, irrespective of the supplementation regimen. Findings regarding the lack of any association between the probiotics mixture- or mushroom-supplemented changes in microbiome diversity may be supported by results from a few randomized controlled clinical trials in human.[27] Furthermore, it can be argued that the dyslipidemia-alleviating mechanisms of supplemented mushrooms and probiotics may not be associated with their gut microbiota modulating functions, but rather related to the genes expression phenomena previously discussed. Efficacy of these supplements for improving systemic metabolism and gut microbiota remained controversial[28]; however, findings from the current study clearly define mechanisms supporting the hypocholesterolemic effect of these supplements, which are consistent with published clinical trials and nonclinical data.[29–31] The lack of microbiome changes and associated cholesterol-lowering effects seen in the current study, and in other studies as well, may be attributed to subjective parameters such as chemical or microbial composition of the supplements, mode and volume of supplements, sample size, or host metabolic state.

V. CONCLUSIONS

A. bisporus supplementation significantly improved lipid profiles and elicited an oxidative stress-lowering response. Supplementation with *A. bisporus* and the probiotics mixture completely or partially corrected dyslipidemia; however, the study lacks power to detect associations between dyslipidemia and the microbiome. Future studies supplementing better-defined dose rates and larger sample sizes in dyslipidemia models are indicated.

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