CHAPTER 5

RESULTS

Clinical findings link changes in the gut microbial environment to a variety of psychiatric disorders. Most of the hype around the human microbiome's ability to influence mental health derives from a long-held hypothesis that gut bacteria communicate with the central nervous system (CNS) by affecting brain chemistry involved in stress response, anxiety, and memory.

There is compelling evidence that manipulating the gut microbiota improves the host's health. Prebiotics, probiotics, antibiotics, and biogenic metabolites produced by milk fermentation open up new avenues for psychological therapies by altering the intestinal microbiota axis.

Therefore, present research focuses on determining biogenic metabolites in prebiotic enriched cereal buttermilk based fermented beverage and its impact on curbing mild to moderate depression. Supplementation role of prebiotic (FOS) and buttermilk in building favourable gut microbiome and ultimately improving the state of depression was also investigated. This chapter discusses the findings, statistical analysis, interpretation, and discussion of data obtained during the research process. The current study's findings are divided into eight phases based on the objectives.

PHASE -I: Quantification of Biogenic metabolites (B Casomorphin and Casoxin c) in ambil using High Performance Liquid Chromatography.

PHASE II: Snap -Shoting the presence of mild to moderate depression among the subjects in the age group of 19-30 in the Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda, Vadodara.

PHASE -III: Impact evaluation of intervention trials with ambil (prebiotic enriched fermented beverage) on the fecal microbial counts (Lactobacillus, Bifidobacteria and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.

PHASE -IV: Impact evaluation of intervention trials with fructooligosaccharide on the fecal microbial counts (Lactobacillus, Bifidobacteria and Enteric pathogens),

depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.

PHASE -V: Impact evaluation of intervention trials with fresh buttermilk on the fecal microbial counts (Lactobacillus, Bifidobacteria and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.

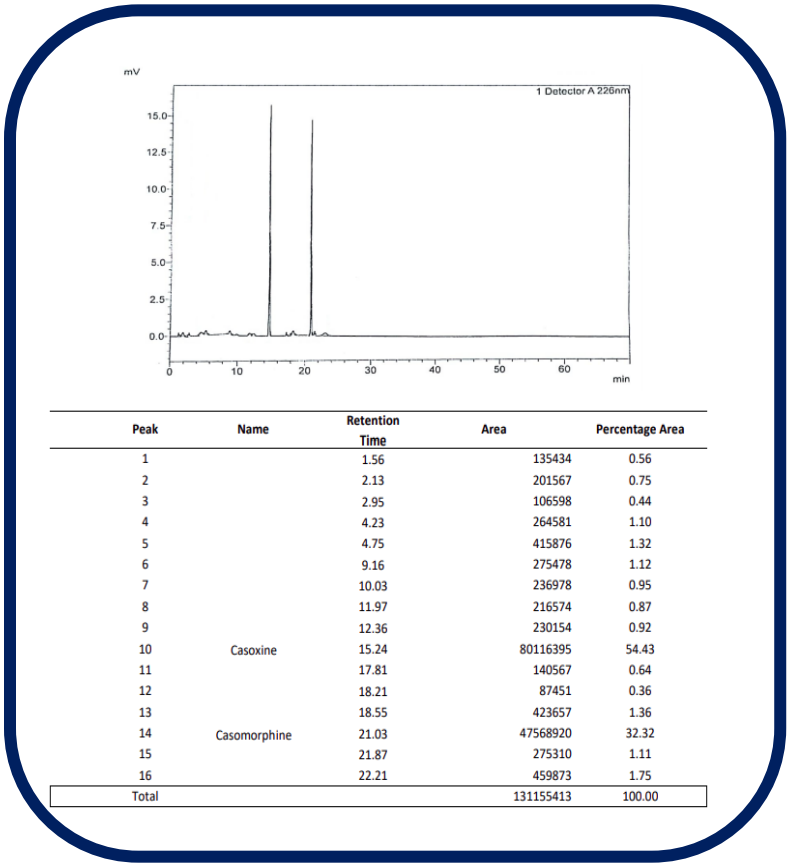
PHASE -VI: Impact evaluation of intervention trials with tetrapacked buttermilk on the fecal microbial counts (Lactobacillus, Bifidobacteria and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.

PHASE -VII: Evaluating which intervention had highest impact in reversing depression scores, cortisol levels and modulating gut microbiota composition.

PHASE -VIII: Developing an audio-visual animated film as Information Education Communication material to make general people aware about increasing trend of depression and how to cope up with it.

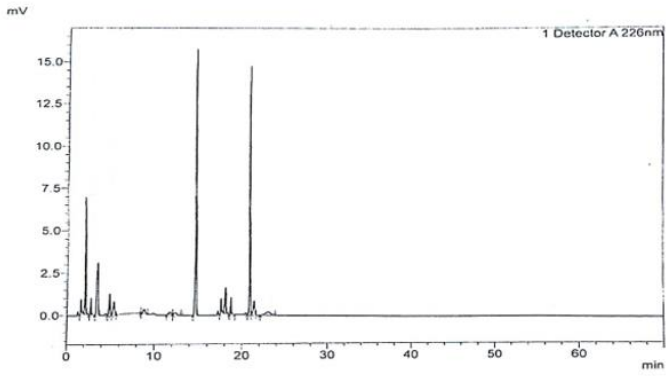
PHASE-I: Quantification of Biogenic metabolites (ß Casomorphin and Casoxin c) in ambil using High Performance Liquid Chromatography.

Biologically active peptides in milk are initially present in inactive form within the sequence of the precursor molecules which releases on fermentation. *Ambil* is an indigenous natural cereal buttermilk based fermented beverage, enriching it with prebiotic FOS has enhanced its potential which is becoming apparent in increasing the bacterial bionomics. In this study biogenic metabolites viz *Casoxin C* and ß *Casomorphin* from this fermented beverage were calculated using High Performance Liquid Chromatography (HPLC). The retention time of biogenic metabolites *Casoxin C* and ß *Casomorphin* standards and samples as projected on HPLC graph (Figure 5.1.1 and Figure 5.1.2) was 15.24 and 21.03 respectively. Percentage area covered by standards of *Casoxin C* and ß *Casomorphin* was 54.43% and 32.32% respectively. Percentage area covered by these biogenic metabolites in analysed fermented beverage was 27.96 % and 17.60 % respectively.



Peak	Name	Retention Time	Area	Percentage Area
1		1.56	135434	0.56
2		2.13	201567	0.75
3		2.95	106598	0.44
4		4.23	264581	1.10
5		4.75	415876	1.32
6		9.16	275478	1.12
7		10.03	236978	0.95
8		11.97	216574	0.87
9		12.36	230154	0.92
10	Casoxine	15.24	80116395	54.43
11		17.81	140567	0.64
12		18.21	87451	0.36
13		18.55	423657	1.36
14	Casomorphine	21.03	47568920	32.32
15		21.87	275310	1.11
16		22.21	459873	1.75
Total			131155413	100.00

Figure 5.1.1 : Retention time and peak of standards



Peak	Name	Retention Time	Area	Percentage Area
1		2.15	6997504	2.76
2		2.79	29333738	11.57
3		3.05	5907313	2.33
4		3.75	27761835	10.95
5		4.35	9355358	3.69
6		4.67	5653780	2.23
7		9.56	5476307	2.16
8		11.89	4031171	1.59
9		12.33	3726931	1.47
10	Casoxine c	15.24	70887753	27.96
11		17.89	3676225	1.45
12		18.11	6465085	2.55
13		19.01	8442640	3.33
14	ß casomorphine	21.03	44626407	17.60
15		21.69	8797586	3.47
16		22.21	12397751	4.89

Figure 5.1.2 : Retention time and peak of ambil

Percentage of Casoxin C and ß Casomorphin in ambil was calculated using the formula: Weight of standard/ weight of sample x peak area of sample/ peak area of standard x factor

% Casoxin C	**102.25/102.35 x 70887753/80116395 x 4.82 = 4.26 X 5= 21.3 mg /kg**
% ß Casomorphin	**102.20/102.35 x 44626407/47568920 x 1.32 = 1.24x5 = 6.2mg/kg**

Result Highlights of Phase I

- Retention time of biogenic metabolites Casoxin C and ß Casomorphin standards and samples as projected on HPLC graph was 15.24 and 21.03 respectively.
- Percentage area covered by standards of Casoxin C and ß Casomorphin was 54.43% and 32.32% respectively.
- Percentage area covered by Casoxin C and ß Casomorphin in analysed fermented beverage was 27.96 % and 17.60 % respectively.
- Prebiotic enriched buttermilk based fermented beverage (ambil) reported the presence of 21.3 mg /kg of Casoxin C and 6.2mg/kg of ß Casomorphin.

PHASE II: Snap -Shoting the presence of mild to moderate depression among the subjects in the age group of 19-30 in the Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda, Vadodara.

Depression is on the rise and more prevalent among youth, affecting the life of almost seventh individual in India (Sagar et al., 2020). In view of the current scenario, Phase II of the study was planned to study the dominant status of depression among the subjects aged 19-30 years. Attempt was made to determine the role of various contributory factors which included general characteristics, medical history, dietary patterns, defecation profile, food and exercise habits. Recent studies have verified intestinal microbiome environment directly affects one's mental status. To further validate, the association between gut flora (*Bifidobacteria, LAB* and enteric pathogen) and brain correlation was observed.

For achieving the desired objectives, a total of 683 subjects were screened from the Faculty of Family and Community Sciences, The M.S University Vadodara. The methodology to collect the above-mentioned information is elaborated in Chapter 4, Methods and Material chapter and results are presented under the sections 5.2.1 to 5.2.5.

Results of Phase -II are discussed under following section

Section 5.2.1: Screening of the subjects from the Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda for mild to moderate depression using Beck's Depression Inventory.

Section 5.2.2: Collection of baseline data regarding general information.

Section 5.2.3: Macro and micro nutrient intake of normal, mild to moderate and severe depressed subjects as per 24 dietary recalls.

Section 5.2.4: Medical history, defecation profile, personal habits of normal, mild to moderate and severe depressed subjects

Section 5.2.5: Correlation observed among various parameters of mild to moderately depressed subjects.

5.2.1: Screening subjects from various departments of Faculty of Family and Community Sciences the Maharaja Sayajirao University of Baroda for their depression levels.

A total of 683 subjects participated in the study to whom Beck's Depression Inventory was administered. Based on inventory's score subjects were categorized into normal, mild to moderate and severe depression category.

As shown in Table 5.2.1 and graphically represented in Figure 5.2.1, high percentage (53.8%) of university students scored between 11-30 on Beck's depression Inventory and were categorized as mild to moderately depressed. Around 32 students (4.68 %) obtained scores between 31-40 which categorized them as severely depressed, and less than 50% out of total screened i.e., 283(41.43%) scored normal score (0-10).

Table 5.2.1: Distribution of screened subjects according to various depression categories

Category	Number of Subjects (n= 683)	Percent Subjects (%)
Normal	283	41.43
Mild -Moderate	368	53.8
Severe	32	4.68

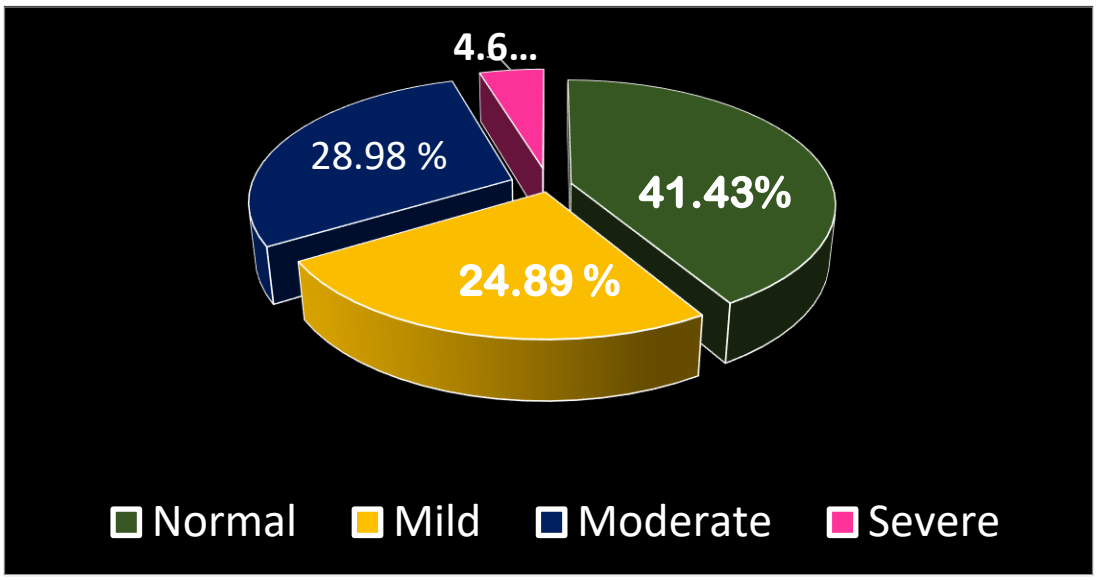


Figure 5.2.1: Percentage presence of depression levels in screened subjects according to Beck's Depression Inventory

5.2.2: Baseline data of enrolled subjects regarding their general information.

As seen in Table 5.2.2, most subjects (94.87%) were in the age group of 19-25 and were females of which 53.5% reported to be mild to moderately depressed. The status of depression between the age group studied was significant (p value < 0.05). Highly significant difference (p value< 0.001) was observed in the category of depression with regard to major religion studied. High percentage of Muslim population (60.88%) were mild to moderately depressed followed by Christians and Hindus. Education didn't make any significant difference in terms of depression severity. Around 54% students in mild to moderate depression category were pursuing graduation. Significant difference (p value< 0.001) was observed between depression severity and family structure. People belonging to nuclear family (74.37%) reported to be more depressed than those who dwell in joint and extended joint families. Monthly per capita family income showed highly significant association with the depression status, (p value < 0.001).

Table 5.2.2: General characteristics of normal, mild to moderate and severely depressed subjects

Parameter	Normal n= 283	Mild- Moderate n= 368	Severe n= 32	Total n= 683	X^2
Age					
19-25	267 (41.20%)	349 (53.85%)	32(4.93%)	648 (94.87%)	7.704* p value 0.021
>25	16 (45.71%)	19 (54.28%)	-	35 (5.12%)	
Gender					
Female	281 (41.69%)	361 (53.56%)	32	674 (98.68%)	2.206 NS p value 0.332
Male	2	7		9	
Religion					
Hindu	202 (45.59%)	222 (50.11%)	19 (4.28%)	443 (64.86%)	23.868*** p value 0.000
Muslim	78 (34.66%)	137 (60.88%)	10 (4.4%)	225(32.94%)	
Christian	3 (20%)	9 (60%)	3 (20%)	15(2.19%)	
Education					
Pursuing graduation	240 (40.54%)	321 (54.22%)	31 (5.23%)	592(86.67%)	3.837 NS p value 0.147
Graduate and above	43 (47.25%)	47 (51.64%)	1	91(13.32%)	
Type of family					
Joint	182 (65.23%)	94(33.69%)	3(1%)	279(40.84%)	181.61*** p value 0.000
Nuclear	64(17.82%)	267(74.37%)	28(7.79%)	359 (52.56%)	
Extended	37(82.22%)	7(15.55%)	1	45(6.58%)	
Monthly per capita family income					
<20,000	17 (34.69%)	30 (61.22%)	2 (4%)	49	46.23*** p value 0.000
20,000-60,000	157 (55.67%)	121(42.9%)	4 (1.41%)	282	
>60,000	109 (30.96%)	217 (61.64%)	26 (7.38%)	352	

NOTE: * significant at pvalue<0.001; * significant at pvalue<0.05; NS=notsignificant**

5.2.3 Macro and micro nutrient intake of normal, mild to moderately and severely depressed subjects as per 24 dietary recalls.

As depicted in Table 5.2.3 the dietary profile of the subjects showed significant difference
(p value ≤ 0.001) between severity of depression with respect to energy and macronutrient consumption. Nutrient intake of subjects was compared to RDA for macronutrients and micronutrients for Indian Women, ICMR NIN 2020 [Appendix VI]. Mean energy, carbohydrate and protein consumption of mild to moderate and severely depressed subjects was less than RDA by 18.99%, 21.66%, and 8.26%; and 27.87%, 26.33%, and 15.21% respectively. Significant difference (p value ≤ 0.001) was observed between fat and depression status. However, fat consumption was relatively higher than RDA in all the subjects irrespective of depression severity.

Consumption of the micronutrients assessed found to be lower than RDA in all the subjects irrespective of their depression status. Moreover, less consumption of micronutrients was observed in subjects experiencing severe depression when compared to normal. Statistically significant association between consumption of calcium (p value ≤ 0.05), magnesium (p value ≤ 0.05) and zinc (p value ≤ 0.001) and non-significant association with iron, omega 3 and omega 6 were observed with degree of depression. Mean intake of omega 3 and omega 6 was lower in severely depressed subjects (0.5 gm and 1.49 gm) than mild to moderately depressed
(0.51gm and 1.53gm) and normal (0.71 and 2.12).

Table 5.2.3: Macro and micro nutrient intake of normal, mild to moderate and severe depressed subjects as per 24 hours dietary recall

Nutrients	Normal		Mild- Moderate		Severe		F – Test	
	Mean	% Difference from RDA	Mean	% Difference from RDA	Mean	% Difference from RDA		RDA
Energy (Kcal)	1920.75 ± 229.72	9.85 ↓	1725.44 ± 228.98	18.99 ↓	1536.34 ± 157.80	27.87 ↓	82.10***	2130
CHO (g)	264.25 ± 55.71	12 ↓	235.73 ± 52.31	21.66 ↓	221.09 ± 38.41	26.33 ↓	26.89***	300
Protein (g)	53.08 ± 6.21	15.21 ↑	42.26 ± 5.53	8.26 ↓	39.03 ± 4.02	15.21 ↓	159.01***	46
Fat (g)	68.14 ± 6.44	63.25 ↑	62.55 ± 9.67	68 ↑	51.90 ± 5.44	51.83 ↑	73.91***	25
Calcium (mg)	599.72 ± 67.88	40.02 ↓	588.61 ± 74.84	41.14 ↓	565.12 ± 67.85	43.49 ↓	4.29*	1000
Iron (mg)	8.04 ± 1.85	70.02 ↓	8.07 ± 2.10	70.11 ↓	7.71± 1.52	71.4 ↓	0.50^{NS}	27
Magnesium (mg)	304.85 ± 160.06	30.71 ↓	296.61 ± 52.49	32.59 ↓	244.78 ± 26.79	44.36 ↓	4.30*	440
Zinc (mg)	5.82 ± 1.67	59.86 ↓	4.57 ± 1.80	68.48 ↓	4.47 ± 1.43	69.17 ↓	43.76***	14.5
Omega-3 (g)	0.71 ± 3.35		0.51 ± 0.05		0.50 ± 0.05		0.77^{NS}	
Omega-6 (g)	2.12 ± 9.47		1.53 ± 0.08		1.49 ± 0.05		0.80^{NS}	

NOTE: * significant at pvalue<0.001; * significant at pvalue<0.05; NS=notsignificant**
RDA is with respect to Indian women: ICMR, NIN 2020.

5.2.4: Frequency of consumption of probiotics and prebiotic supplements among mild to moderately depressed subjects.

Subjects falling in the mild to moderate depression category were assessed for the frequency of probiotic consumption, the foods studied were curd, buttermilk, yogurt, shrikhand, matho and lassi. It was also taken into account whether pre/probiotic supplementations in any form of medication was part of their daily diet. Amongst the probiotic rich foods, buttermilk was consumed most frequently followed by curd. Around 63% subjects (Figure 5.2.2) reported consumption of pre/probiotics less frequently. Subjects who reported frequent consumption were not included for any of the intervention phases.

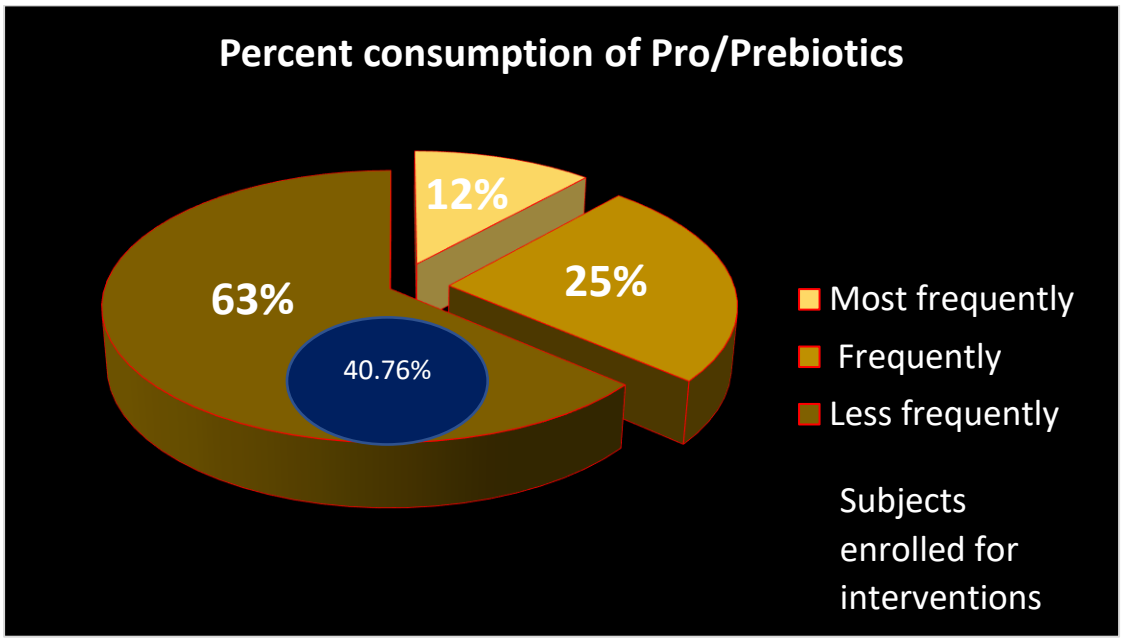


Figure 5.2.2: Frequency of consumption of probiotics and prebiotic foods

5.2.4.1: Medical history of normal, mild to moderately and severely depressed subjects.

Medical history of screened subjects was noted through previously available health records and questionnaire (Table 5.2.4, Figure 5.2.3). Statistically significant association (p value ≤ 0.05) between hypothyroidism disorder and degree of depression was seen. High percentage of people (6.25%) suffering from severe depression reported hypothyroidism disorder.

A non-significant difference was seen between Diabetes Melitus and degree of depression.

Gastrointestinal disorders, abdominal pain and flatulence have also shown to be significantly related with depression severity (p value ≤ 0.001). A non-significant difference was seen between hypertension and degree of depression.

Though, higher percentage of people reported UTI in normal category compared to mild to moderately depressed, differences observed were significant at p value 0.01. Dermatological changes, specially change in hair/ nail were reported by more than 50% of mild to moderately depressed population. Association observed with severity of depression were statistically significant (p value ≤ 0.001).

As severity of depression increased level of confusion as a symptom also increased and this was found to be statistically significant (p value ≤ 0.001). A female reported she had panic attack a week before questionnaire was administered, while another one reported to be epileptic.

Table 5.2.4: Percent prevalence of medical complications among normal, mild to moderately depressed and severely depressed subjects

Medical History	Normal n= 283	Mild-Moderate n=368	Severe n=32	X^2
Endocrine/glandular				
Had Hypothyroidism disorder	4 (1.41%)	17 (4.61%)	2 (6.25%)	5.91 0.05*
Didn't have Hypothyroidism disorder	279	351	30	
Had Diabetes Melitus	2 (0.7%)	6 (1.63%)	-	0.289 0.246^{NS}
Didn't have Diabetes Melitus	281	362	-	
Gastrointestinal Disorders				
Had Acidity	33 (11.6%)	26 (7.06%)	4 (12.5%)	4.465 0.10^{NS}
Didn't have Acidity	250	342	28	
Had Abdominal Pain	26 (9.18%)	12 (3.26%)	7 (21.87%)	16.76 0.000***
Didn't have Abdominal Pain	257	356	25	
Had Flatulence	14 (4.94%)	17 (4.61%)	6 (18.75%)	17.033 0.000***
Didn't have Flatulence	269	351	26	

Cardiovascular				
Had Hypertension	9 (3.18%)	13 (3.53%)	-	0.805 0.493^{NS}
Didn't have Hypertension	274	355		
Kidney /Urinary				
Had Kidney stones	13 (4.59%)	6 (1.63%)	-	4.958 0.026*
Didn't have Kidney stones	270	356		
Had Urinary tract Infection	24 (8.48%)	11 (2.98%)	2 (6.25%)	
Didn't have Urinary tract Infection	259	357		9.46 0.009**
Dermatological/Dental				
Had Skin trouble/rashes	13 (4.59%)	12 (3.26%)	8 (25%)	30.31 0.000***
Didn't have Skin trouble/rashes	270	356	24	
Had Change in hair or nail	67 (23.67%)	217 (58.96%)	9 (28.12%)	99.31 0.000***
Didn't have Change in hair or nail	216	151	23	
Neurological				
Had Confusion	77 (27.2%)	172 (46.73%)	21 (65.62%)	35.09 0.000***
Didn't have Confusion	206	196	11	
Panic attack		1	1	
Epilepsy		1		

NOTE: : * significant at pvalue<0.001; ** significant at pvalue<0.01; * significant at pvalue<0.05; NS=notsignificant**

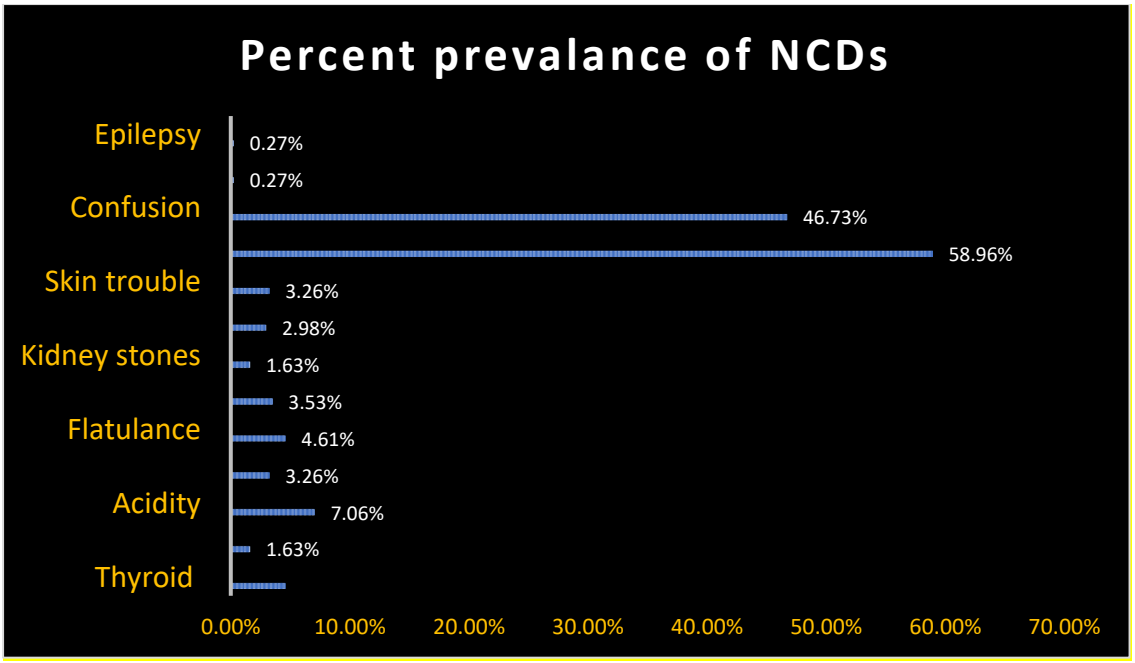


Figure 5.2.3: Percent prevalence of medical complications among normal, mild to moderately depressed

5.2.5. Food and Exercise habit of normal, mild to moderately depressed and severely depressed subjects.

Table 5.2.4 depicts food and exercise habit of normal, mild to moderately depressed and severely depressed subjects. Type of diet has shown highly significant relation (p value ≤ 0.001) with depression severity. Vegetarians reported to be more severely depressed (43.75%) than ovo- lacto vegetarians (37.5%) and non-vegetarians (18.75%). This was found to be statistically significant at p value < 0.001. Frequency of consumption of tea/ coffee didn't show any significant relation with depression. People falling in the category of severe depression reported frequent consumption of aerated beverage (25%) compared to mild to moderately depressed (14.94%) this association was found to be significant (p value < 0.001). Physical activities like yoga, breathing exercise, walking and jogging practices have shown significant association with severity of depression (p value ≤ 0.001). Of severely depressed and mild to moderately depressed people nearly 65% and 56% never practiced yoga or breathing exercises.

Table 5.2.5: Percent prevalence of food and exercise habit of normal, mild to moderately depressed and severely depressed subjects

Category	Normal	Mild-Moderate	Severe	X^2
Type of diet				
Vegetarian	209 (73.85%)	281 (76.3 %)	14 (43.75%)	19.928*** 0.001
Ovo- Lacto Vegetarian	41 (14.48%)	61 (16.57 %)	12(37.5%)	
Non- Vegetarian	33 (11.66%)	26 (7%)	6 (18.75%)	
Number of tea or coffee/day				
1 cup	61 (21.55%)	53 (14.4%)	5 (15.62%)	11.63 NS 0.20
2-3 cups	127(44.87%)	211(57.3%)	19 (59.37%)	
>3 cups	95(33.56%)	104 (28.26%)	8 (25%)	
Consumption of aerated beverages				
Frequently	64(22.61%)	55 (14.94%)	8 (25%)	185.14*** 0.000
Moderately	207 (73.14%)	296 (80.43%)	21 (65.62%)	
Never	12 (4.24%)	17 (4.61%)	3 (9.375%)	
Practicing Yoga or Breathing exercise				
Frequently	84 (29.68%)	52 (14.1%)	4 (12.75%)	35.421*** 0.000
Moderately	41(14.48%)	107 (32.88%)	7 (21.87%)	
Never	158(55.83%)	209 (56.79%)	21 (65.62%)	
Walking/Jogging				
Frequently	87 (30.74%)	83 (22.55%)	4 (12.5%)	51.874*** 0.000
Moderately	146(51.59%)	201 (54.6%)	5 (15.62%)	
Never	50 (17.66%)	84 (22.82%)	23 (71.87%)	

NOTE: * significant at pvalue < 0.001; NS = not significant**

5.2.6: Defecation pattern

As seen in Table 5.2.5 and Figure 5.2.4 severe constipation was reported by subjects falling in severe depression category (18.75%) as compared to mild and moderately depressed (12.5 %). Only 2% people among normal population report ed stool frequency < 2 times/week. The association observed between stool frequency and depression severity was highly significant (p value < 0.001).

Table 5.2.6: Defecation pattern according to score analysis in normal, mild to moderately depresses and severely depressed subjects

Degree of Depression	More than normal (>14stools/week)	Normal (7-14 stools/week)	Mild Constipation (5-6 stools/week)	Moderate Constipation (3-5 stools/week)	Severe Constipation (< 2 stools/week)	X^2
Normal N=283	13 (4.5%)	208 (73.49%)	32 (11.30 %)	24 (8.48%)	6 (2.12%)	91.147***
Mild-Moderate N=368	8 (2.17%)	179 (48.64%)	61 (16.57)	74 (20.10%)	46 (12.5%)	
Severe N=32	2 (6.25%)	3 (9.37%)	13 (40.62%)	8 (25%)	6 (18.75%)	

NOTE: * significant at pvalue<0.001**

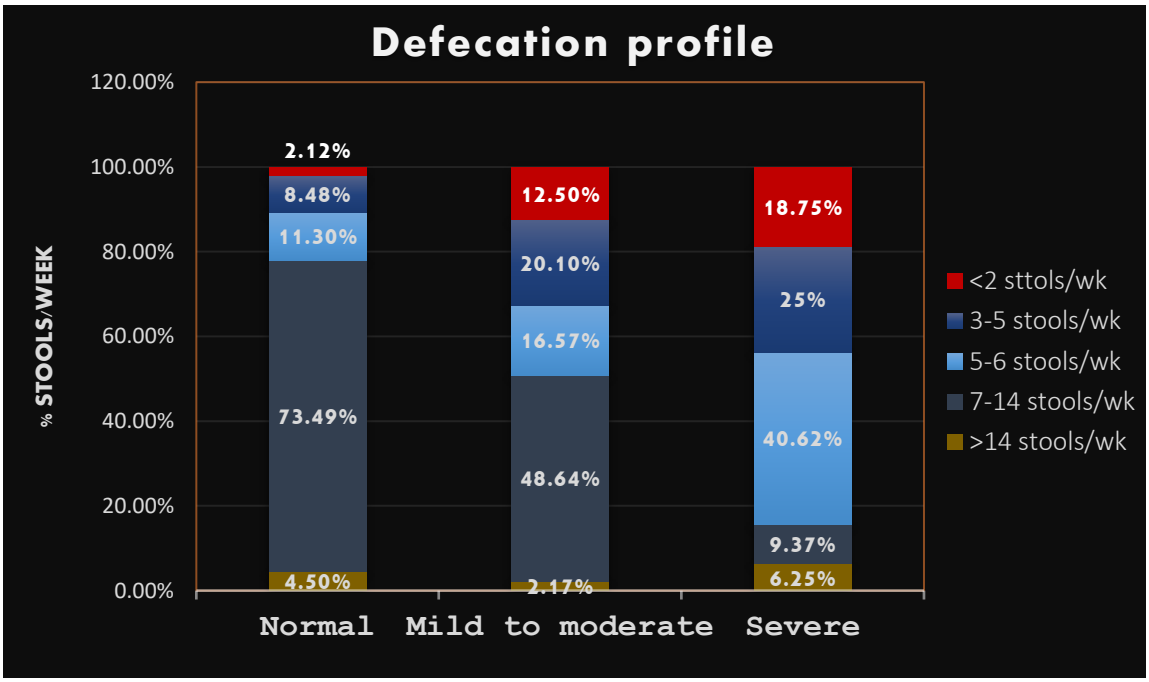


Figure 5.2.4: Defecation pattern according to score analysis in normal, mild to moderate and severely depressed subjects

5.2.7: Pearson's Correlation observed among various parameters of mild to moderately depressed subjects

Table 5.2.7 shows correlation observed among log count of *Lactobacillius*, *Biffidobacterium*, *E. coli*, serum cortisol, constipation, depression and nutrients intake. Statistically significant negative corelation of depression was seen with log count of *Lactobacillius* (p value<0.001). *Biffidobacterium* and serum cortisol showed non-significant negative corelation with depression. Statistically significant positive corelation of depression was seen with *E. coli* (p value<0.001), and constipation (p value<0.001). Serum cortisol showed non-significant negative corelation with log count of *Lactobacillius* and, positive correlation with log count of *E. coli* and constipation.

Fecal log count of *Lactobacillus* showed significant negative correlation with log count of *E. coli* (p value<0.001) and constipation (p value<0.001). A non-significant positive correlation was observed with log count of *Biffidobacterium.*

Log count of *Biffidobacterium* showed non-significant negative correlation with log count of *E. coli* and constipation. Log count of *E. coli* also showed to be positively associated with constipation (p value<0.001).

With respect to the correlation observed among different nutrient consumptions and parameters assessed, protein showed positive significant association with log count of *Biffidobacterium* (p value<0.01). Carbohydrate showed positive correlation with depression (p value<0.05). Zinc also showed to be positively associated with constipation (p value<0.05). Omega 6 has shown to be positively associated with log count of *Lactobacillus* (p value<0.05).

Result Highlights of Phase II

† As per the scores obtained through Beck's Depression Inventory, high percentage (53.8%) of screened subjects were suffering from mild to moderate depression. Less than fifty percent (41.43%) fell under normal category while 4.68% reported to be suffering from severe depression.

† High percentage of muslim population (60.88%) were mild to moderately depressed followed by christians and hindus.

† Education level of the subject didn't make any significant difference in terms of depression severity.

† Significant difference (p value< 0.001) was observed between depression severity and family structure. People belonging to nuclear family (74.37%) reported to be more depressed than those who dwell in joint and extended joint families.

† The dietary profile of the subjects showed significant difference (p value ≤ 0.001) between severity of depression with respect to energy and macronutrient consumption. Mean energy, carbohydrate and protein consumption of mild to moderate and severely depressed subjects was less than RDA by 18.99%, 21.66%, and 8.26%; and 27.87%, 26.33%, and 15.21% respectively. Significant difference (p value ≤ 0.001) was observed between fat and depression status. However, fat consumption was relatively higher than RDA in all the subjects irrespective of depression severity.

† Consumption of the micronutrients assessed found to be lower than RDA in all the subjects irrespective of their depression status. Statistically significant difference between consumption of calcium (p value ≤ 0.05), magnesium (p value ≤ 0.05) and zinc (p value ≤ 0.001) were observed with degree of depression.

† Subjects falling in the mild to moderate depression category were assessed for the frequency of milk based pre/probiotics. Frequent consumption was reported by 25% of the subjects while 63% subjects reported less consumption.

† Type of diet has shown highly significant relation (p value ≤ 0.001) with depression severity. Vegetarians reported to be more severely depressed (43.75%) than ovo- lacto vegetarians (37.5%) and non-vegetarians (18.75%).

- Frequency of consumption of tea/ coffee didn't show any significant relation with depression. People falling in the category of severe depression reported frequent consumption of aerated beverage (25%) compared to mild to moderately depressed (14.94%).

- Physical activities like yoga, breathing exercise, walking and jogging practices have shown significant association with severity of depression (p value ≤ 0.001). Of severely depressed and mild to moderately depressed people nearly 65% and 56% never practiced yoga or breathing exercises.

- A highly significant positive association (p value < 0.001) was observed between constipation and depression.

- Statistically significant negative corelation of depression was seen with log count of *Lactobacillius* (p value<0.001). *Biffidobacterium* and serum cortisol showed non-significant negative corelation with depression. Statistically significant positive corelation of depression was seen with E. coli (p value<0.001), and constipation (p value<0.001). Serum cortisol showed non-significant negative corelation with log count of *Lactobacillius* and, positive correlation with log count of *E. coli* and constipation.

- Fecal log count of *Lactobacillus* showed significant negative correlation with log count of E. coli (p value<0.001) and constipation (p value<0.001). A non-significant positive correlation was observed with log count of *Biffidobacterium*.

- Log count of *Biffidobacterium* showed non-significant negative correlation with log count of *E. coli* and constipation. Log count of *E. coli* also showed to be positively associated with constipation (p value<0.001).

PHASE -III: Impact evaluation of intervention trials with ambil on the fecal microbial counts (*Lactobacillus, Bifidobacteria* and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.

In this phase of the research, mean values for depression score, serum cortisol, log count of gut microbiota (*Lactobacillus, Bifidobacteria* and *E. coli*) and defecation profile of mild to moderately depressed subjects were assessed pre and post 45 days of 200 ml ambil supplementation.

As shown in Table 5.3.1 and depicted in Figures 5.3.1- 5.3.3, a significant decrease in mean depression scores (p value ≤ 0.001) was observed post intervention (46.45%). The fecal log count of *Lactobacillus* and *Bifidobacterium* showed a significant increase (p value ≤ 0.001) by 10.05% and 36.15% respectively and a significant reduction of 2.88% (p value ≤ 0.001) in *E. coli* was observed. Improvement in defecation profile (p value ≤ 0.001) in terms of stool frequency was seen by 0.28%.

Table 5.3.1: Mean values for depression score, serum cortisol and log count of gut microbiota and defecation profile of mild to moderate depressed subjects before and after ambil supplementation

Parameters	Intervention Phase	Experimental Group	Control Group	Student 't'
Depression score	**Pre intervention**	19.46 ± 5.43	23.20 ± 6.39	1.92 NS
	Post intervention	10.42± 4.93	22.80 ± 5.88	7.21***
	Paired 't' test	7.8***	0.39 NS	
	% Difference	46.45 ↓	1.72 ↓	
Serum Cortisol	**Pre intervention**	11.43 ± 3.58	10.36 ± 5.43	0.50 *
	Post intervention	10.68 ± 3.82	10.72 ± 4.24	0.28 NS
	Paired 't' test	1.21 NS	0.55 NS	
	% Difference	6.56 ↓	3.47 ↑	
Lactobacillus* (*log*$_{10}$ *CFU//g)	**Pre intervention**	5.47 ± 0.22	5.78 ± 0.30	3.59*
	Post intervention	6.02 ± 0. .25	5.95 ± 0.24	1.9*
	Paired 't' test	16.11***	1.61 NS	
	% Difference	10.05 ↑	2.94 ↑	
***Bifidobacterium* (*log10 CFU*/g)**	**Pre intervention**	6.03 ± 0.24	5.89 ± 0.23	1.75 NS
	Post intervention	8.21 ± 0.18	5.84 ± 0.26	33.91***
	Paired 't' test	83.42***	1.81 NS	
	% Difference	36.15 ↑	0.84 ↓	
***E. coli* (*log10 CFU//*g)**	**Pre intervention**	6.25 ± 0.13	6.26 ± 0.12	1.55 NS
	Post intervention	6.07 ± 0.27	6.27 ± 0.09	3.356**
	Paired 't' test	4.14***	0.63NS	
	% Difference	2.88 ↓	0.15 ↑	
Defecation profile (constipation)	**Pre intervention**	3.38 ± 0.85	3.60 ±1.01	0.088 NS
	Post intervention	2.96 ± 0.87	3.66± 1.07	1.93*
	Paired 't' test	4.282***	0.46 NS	
	% Difference	0.287 ↓	0.01 ↑	

NOTE: * significant at pvalue<0.001; ** significant at pvalue<0.01; * significant at pvalue<0.05; NS=notsignificant**

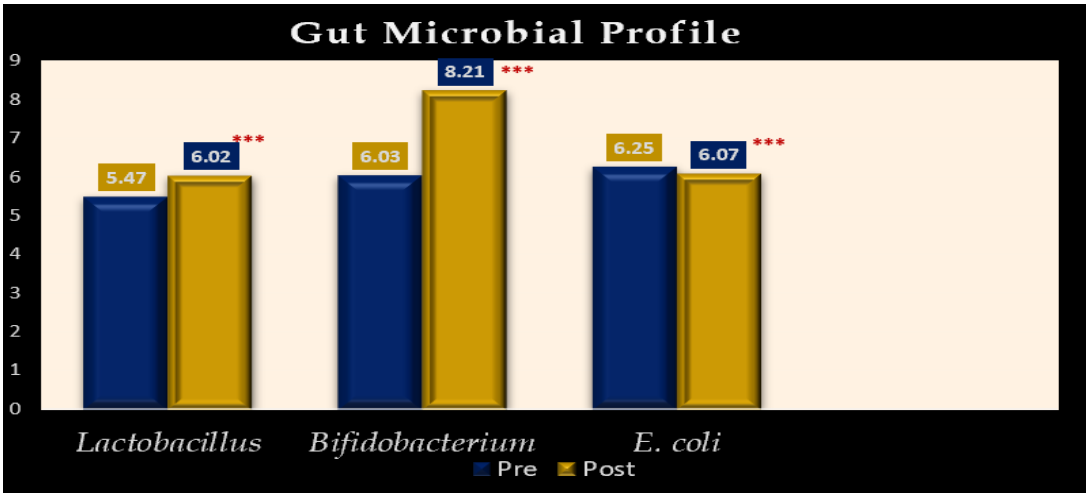


Figure 5.3.1: Log count of gut microbiota *Lactobacillus, Bifidobacterium and E.coli* before and after ambil supplementation

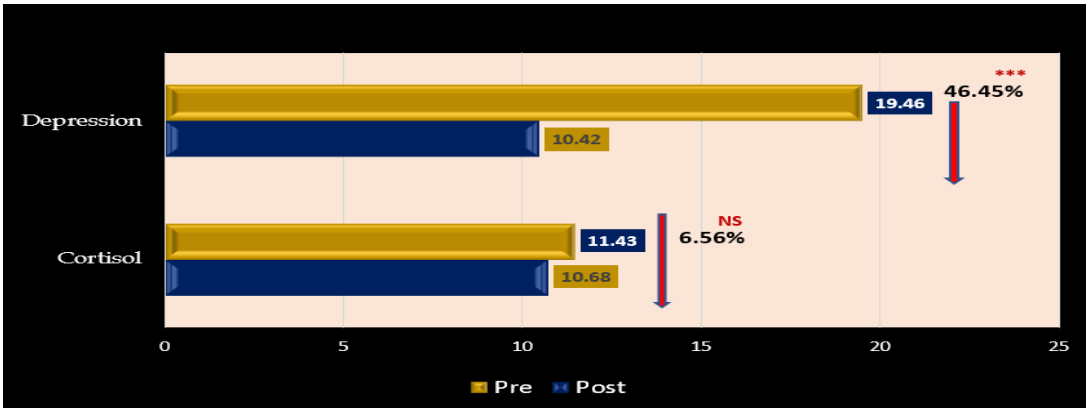


Figure 5.3.2: Changes in mean values of depression scores and blood serum cortisol post ambil supplementation

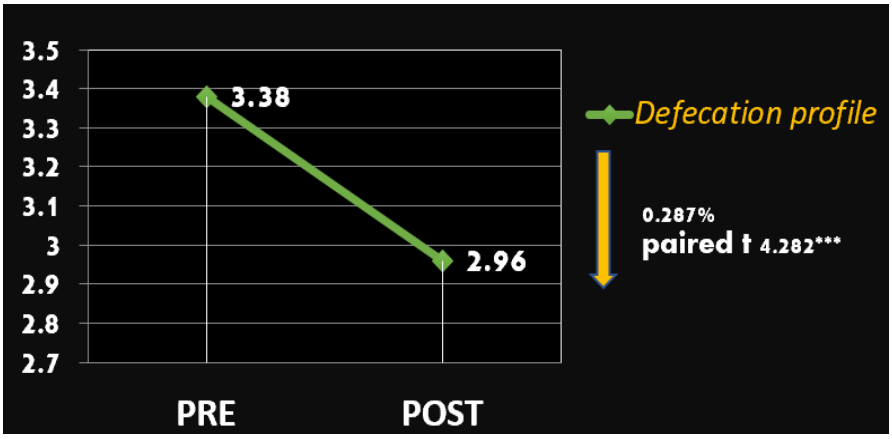


Figure 5.3.3: Percentage improvement in constipation post ambil supplementation

Result Highlights of Phase III

† A significant decrease in mean depression scores (p value ≤ 0.001) was observed post intervention (46.45%) with ambil.

† The fecal log count of *Lactobacillus* and *Bifidobacterium* showed a significant increase (p value ≤ 0.001) by 10.05% and 36.15% respectively and a significant reduction of 2.88% in *E. coli* was observed (p value ≤ 0.001).

† Statistically significant improvement in defecation profile (p value ≤ 0.001) in terms of stool frequency was seen by 0.28%.

† A non-significant reduction was seen in the levels of serum cortisol in the experimental group.

PHASE IV: Impact evaluation of intervention trials with fructooligosaccharide on the fecal microbial counts (*Lactobacillus, Bifidobacteria* and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.

In phase IV of the study, intervention group was supplemented with 10 ml of prebiotic-fructooligosaccharide for 45 days. Comparative Pre and post data analysis was carried out within the experimental group and with control group with regard to the microbial composition of *Lactobacillus, Bifidobacterium* and *E. coli.*, blood serum cortisol, defecation profile and depression levels.

As represented in Table 5.4.1 and Figures 5.4.1 -5.4.3, no significant difference was observed post supplementation in any of the parameters assessed in the control group. However, intervention with FOS showed significant decrease (p value < 0.001) in percentage of depression and log count of *E. coli* by 18.69% and 3.72% respectively. Experiential Increase in colonization of *Lactobacillus and Bifidobacterium* was also seen by 6.8% and 6.13% which was highly significant *(*p value < 0.001).

Table 5.4.1: Mean values for depression score, serum cortisol and log count of gut microbiota and defecation profile of mild to moderate depressed subjects before and after fructooligosaccharide supplementation

Parameters	Intervention Phase	Experimental Group	Control Group	Student 't'
Depression score	**Pre intervention**	19.26 ± 5.00	23.20 ± 6.39	0.758 ^{NS}
	Post intervention	15.66 ± 7.07	22.80 ± 5.88	3.578***
	Paired 't' test	3.78***	0.39 ^{NS}	
	% Difference	18.69 ↓	1.72 ↓	
Serum Cortisol	**Pre intervention**	9.72 ± 6.74	10.36 ± 5.43	0.317 ^{NS}
	Post intervention	9.55 ± 3.50	10.72 ± 4.24	0.917 ^{NS}
	Paired 't' test	0.13 ^{NS}	0.55 ^{NS}	
	% Difference	1.7 ↓	3.47 ↑	
Lactobacillus (log10 CFU//g)	**Pre intervention**	5.73 ± 0.44	5.78 ± 0.30	0.38^{NS}
	Post intervention	6.14 ± 0.31	5.95 ± 0.24	2.10*
	Paired 't' test	4.53***	1.61NS	
	% Difference	6.80 ↑	2.94 ↑	
Bifidobacterium (log10 CFU/g)	**Pre intervention**	5.87 ± 0.49	5.89 ± 0.23	0.17 ^{NS}
	Post intervention	6.23 ± 0.43	5.84 ± 0.26	3.71 ***
	Paired 't' test	5.59***	1.81 ^{NS}	
	% Difference	6.13 ↑	0.84 ↓	
E. coli (log10 CFU//g)	**Pre intervention**	6.17 ± 0.14	6.26 ± 0.12	1.93 ^{NS}
	Post intervention	5.94 ± 0.39	6.27 ± 0.09	4.29***
	Paired 't' test	3.13*	0.63^{NS}	
	% Difference	3.72 ↓	0.15 ↑	
Defecation profile (constipation)	**Pre intervention**	3.50 ± 1.13	3.60 ± 1.01	0.19 ^{NS}
	Post intervention	2.83 ± 1.10	3.66 ± 1.07	2.57**
	Paired 't' test	2.021*	0.46 ^{NS}	
	% Difference	0.190 ↓	0.01 ↑	

NOTE: * significant at pvalue<0.001; ** significant at pvalue<0.01; * significant at pvalue<0.05; NS=notsignificant**

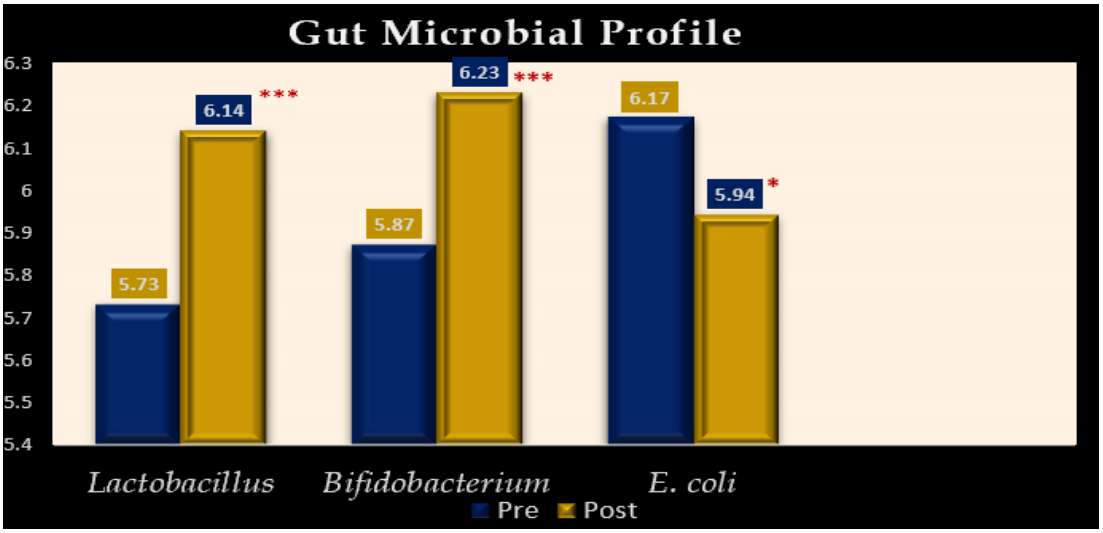


Figure 5.4.1: Log count of gut microbiota *Lactobacillus, Bifidobacterium* and *E.coli* before and after fructooligosaccharide supplementation

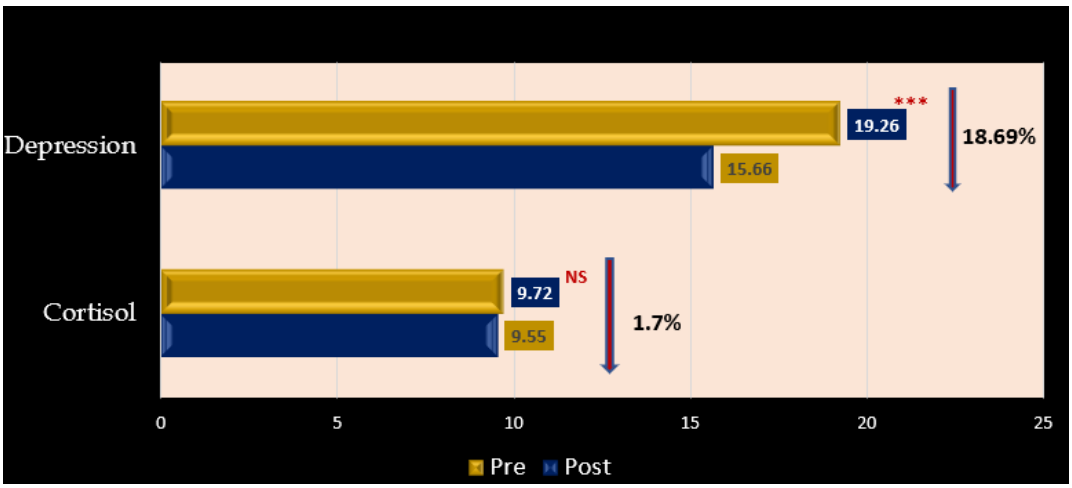


Figure 5.4.2: Changes in mean values of depression scores and blood serum cortisol post fructooligosaccharide supplementation

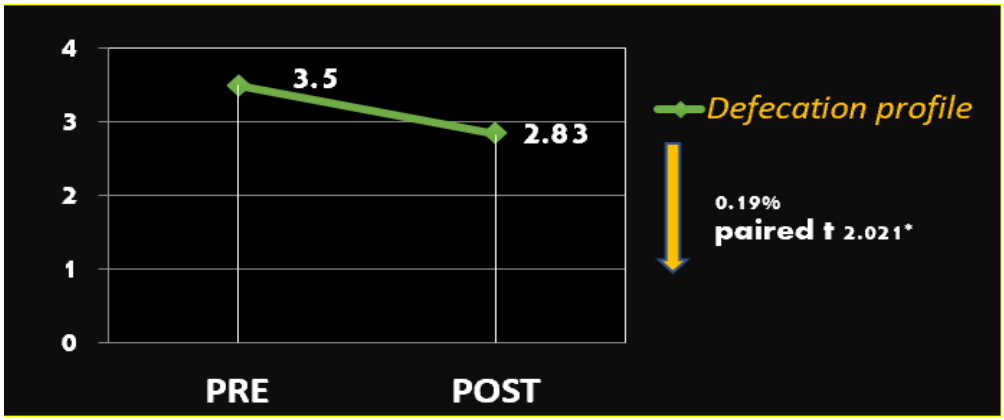


Figure 5.4.3: Percentage improvement in stool frequency post fructooligosaccharide supplementation

Result Highlights of Phase IV

† Impact evaluation of fructooligosaccharide supplementation showed significant decrease (p value < 0.001) in percentage of depression and log count of E. coli by 18.69% and 3.72% respectively.

† Experiential Increase in colonization of *Lactobacillus and Bifidobacterium* was seen by 6.8% and 6.13% which was highly significant (p value < 0.001).

† Statistically significant reduction in constipation (p value ≤ 0.05) in terms of stool frequency was seen by 0.19%.

† A non-significant difference was observed post supplementation in the parameters assessed in the control group.

Phase V: Impact evaluation of intervention trials with fresh buttermilk on the fecal microbial counts (*Lactobacillus, Bifidobacteria* and Enteric pathogens), depression status, serum cortisol and defecation profile of mild to moderately depressed subjects.

In Phase V, we supplemented the subjects with fresh buttermilk. Assessment of gut microbial profile (*Lactobacillus, Bifidobacteria* and *E. coli*), serum cortisol and depression scores were done pre and post supplementation and comparatively with control group.

As seen in Table 5.5.1 and Figures 5.5.1-5.5.3, supplementation with fresh buttermilk, bought about significant diminution ($p\,value < 0.001$) in the depression scores and log count of *E. coli* by 14.21 % and 2.71%. Gut health improved with significant increase ($p\,value < 0.001$) in colonization of *Lactobacillus* and *Bifidobacterium* by 5.28% and 4.51%. No significant changes were seen in control group with respect to any of the parameters pre and post intervention.

Table 5.5.1: Mean values for depression score, serum cortisol and log count of gut microbiota and defecation profile of mild to moderate depressed subjects before and after fresh buttermilk supplementation

Parameters	Intervention Phase	Experimental Group	Control Group	Student 't'
Depression score	**Pre intervention**	21.60 ± 4.18	23.20 ± 6.39	8.11 ^{NS}
	Post intervention	18.53 ± 3.71	22.80 ± 5.88	2.374*
	Paired 't' test	3.43***	0.39 ^{NS}	
	% Difference	14.21 ↓	1.72 ↓	
Serum Cortisol	**Pre intervention**	14.47 ± 5.16	10.36 ± 5.43	2.12 ^{NS}
	Post intervention	13.82 ± 4.817	10.72 ± 4.24	1.87 ^{NS}
	Paired 't' test	0.72 ^{NS}	0.55 ^{NS}	
	% Difference	4.49 ↓	3.47 ↑	
Lactobacillus (log10 CFU//g)	**Pre intervention**	5.92 ± 0.24	5.78± 0.30	1.45 ^{NS}
	Post intervention	6.25 ± 0.19	5.95 ± 0.24	4.12***
	Paired 't' test	6.31***	0.82 ^{NS}	
	% Difference	5.28 ↑	2.94 ↑	
Bifidobacterium (log10 CFU/g)	**Pre intervention**	5.98 ± 0.20	5.89 ± 0.23	1.07 ^{NS}
	Post intervention	6.25 ± 0.09	5.84 ± 0.26	5.78***
	Paired 't' test	6.53***	1.81 ^{NS}	
	% Difference	4.51 ↑	0.84 ↓	
E. coli (log10 CFU//g)	**Pre intervention**	6.27 ± 0.13	6.26 ± 0.12	0.22 ^{NS}
	Post intervention	6.10 ± 0.15	6.27 ± 0.09	3.57***
	Paired 't' test	8.19***	0.63 ^{NS}	
	% Difference	2.71 ↓	0.15 ↑	
Defecation profile (constipation)	**Pre intervention**	3.6 ± 1.03	3.60 ± 1.01	1.632 ^{NS}
	Post intervention	3.166 ± 0.98	3.66 ± 1.07	1.63 ^{NS}
	Paired 't' test	4.176 ***	0.46 ^{NS}	
	% Difference	0.12 ↓	0.01 ↑	

NOTE: * significant at pvalue<0.001; * significant at pvalue<0.05; NS=notsignificant**

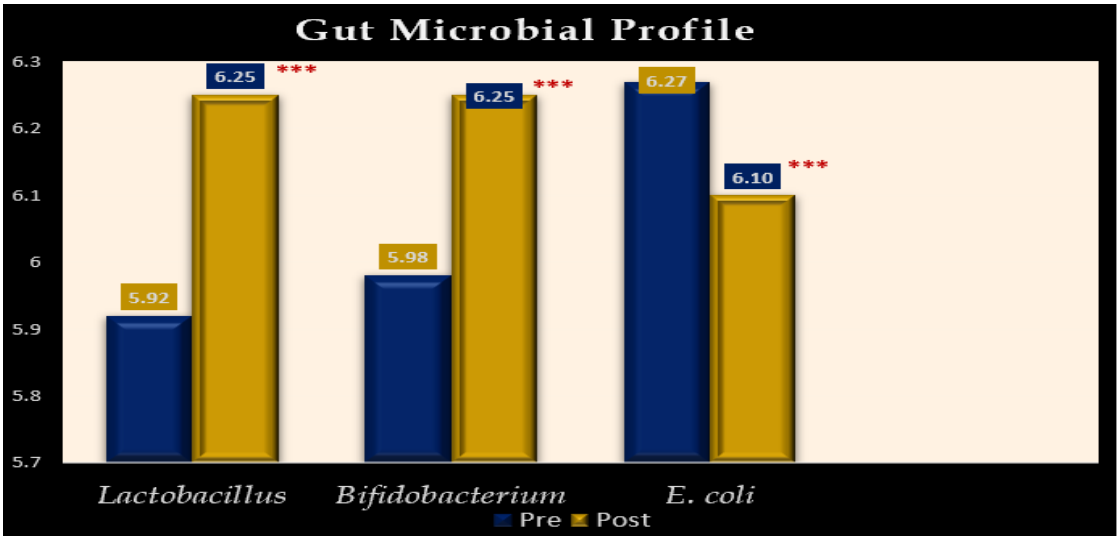


Figure 5.5.1: Log count of gut microbiota *Lactobacillus, Bifidobacterium* and *E. coli* before and after fresh buttermilk supplementation

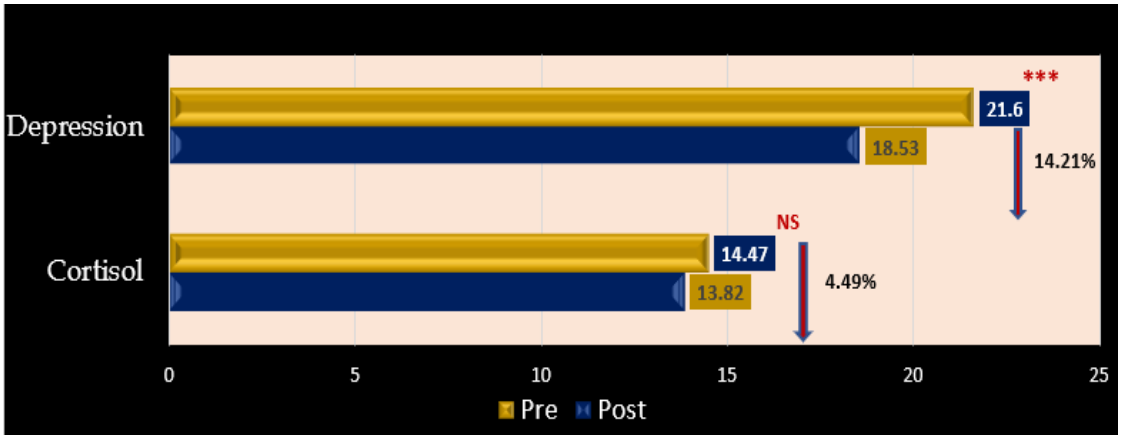


Figure 5.5.2: Changes in mean values of depression scores and blood serum cortisol post fresh buttermilk supplementation

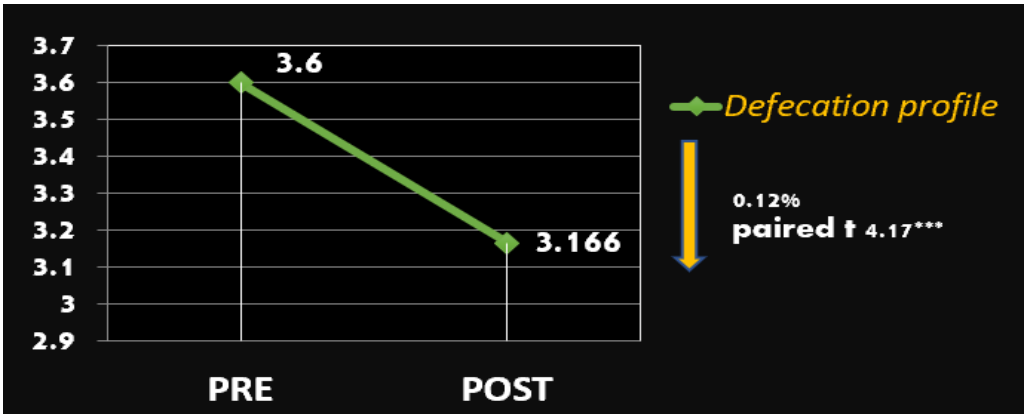


Figure 5.5.3: Percentage improvement in stool frequency post fresh buttermilk supplementation

Result Highlights of Phase V

- Supplementation with fresh buttermilk, bought significant diminution (p value < 0.001) in the depression scores and log count of *E. coli* by 14.21 % and 2.71%.
- Gut health improved with significant increase (p value < 0.001) in colonization of *Lactobacillus* and *Bifidobacterium* by 5.28% and 4.51%.
- No significant changes were seen in control group with respect to any of the parameters pre and post intervention.
- A non-significant reduction in the levels of serum cortisol was seen upon supplementation and comparative analysis with controls.

PHASE VI: Impact evaluation of intervention trials with tetrapacked buttermilk on the fecal microbial counts (*Lactobacillus, Bifidobacteria* and Enteric pathogens), depression status, serum cortisol and defecation *profile of mild to moderately depressed subjects.*

Phase VI of the study involved supplementation with 200 ml tetra packed buttermilk. Examination on the differences observed post intervention was carried out with respect to the depression scores, cortisol levels, gut macrofloral count *(Lactobacillus, Bifidobacteria and E. coli)* and defecation pattern. For impact analysis of the supplementation, the data was compared with the control group to which no intervention was prearranged.

As observed in Table 5.6.1 and Figures 5.6.1- 5.6.3, supplementation bought significant decrease in the depression scores ($p\ value < 0.005$) and log count of *E. coli* ($p\ value < 0.001$) by 13.43% and 1.58%. Gut health improved ($p\ value < 0.001$) with significant increase in the colonization of *Lactobacillus* and *Bifidobacterium* by 5.47% and 5.68%. A not significant decrease in the levels of serum cortisol was marked by 7.38%. No significant changes were seen in the control group with respect to any of the parameters post intervention.

Table 5.6.1: Mean values for depression score, serum cortisol and log count of gut microbiota and defecation profile of mild to moderate depressed subjects before and after buttermilk tetra packed supplementation

Parameters	Intervention Phase	Experimental Group	Control Group	Student 't'
Depression score	**Pre intervention**	19.06± 6.31	23.20 ± 6.39	1.92*
	Post intervention	16.5 ± 5.93	22.80 ± 5.88	3.36**
	Paired 't' test	3.40*	0.39 NS	
	% Difference	13.43 ↓	1.72 ↓	
Serum Cortisol	**Pre intervention**	11.38 ± 3.79	10.36 ± 5.43	0.65 NS
	Post intervention	10.54 ± 3.43	10.72 ± 4.24	0.14 NS
	Paired 't' test	1.22 NS	0.55 NS	
	% Difference	7.38 ↓	3.47 ↑	
Lactobacillus (log10 CFU//g)	**Pre intervention**	5.83 ± 0.18	5.78 ± 0.30	0.78NS
	Post intervention	6.15 ± 0.14	5.95± 0.24	3.46**
	Paired 't' test	9.35***	1.61NS	
	% Difference	5.4 ↑	2.94 ↑	
Bifidobacterium (log10 CFU/g)	**Pre intervention**	5.98 ± 0.40	5.89 ± 0.23	0.74 NS
	Post intervention	6.32 ± 0.08	5.84 ± 0.26	6.87***
	Paired 't' test	4.88***	1.81 NS	
	% Difference	5.68 ↑	0.84 ↓	
E. coli (log10 CFU//g)	**Pre intervention**	6.32 ± 0.12	6.26 ± 0.12	0.92 NS
	Post intervention	6.22 ± 0.22	6.27 ± 0.09	0.11 NS
	Paired 't' test	2.78**	0.63 NS	
	% Difference	1.58 ↓	0.15 ↑	
Defecation profile (constipation)	**Pre intervention**	3.6 ± 1.00	3.60 ± 1.01	0.86 NS
	Post intervention	3.26 ± 1.1	3.66 ± 1.07	1.23 NS
	Paired 't' test	3.80***	0.46 NS	
	% Difference	0.09 ↓	0.01 ↑	

NOTE: * significant at pvalue<0.001; ** significant at pvalue<0.01; * significant at pvalue<0.05; NS=notsignificant**

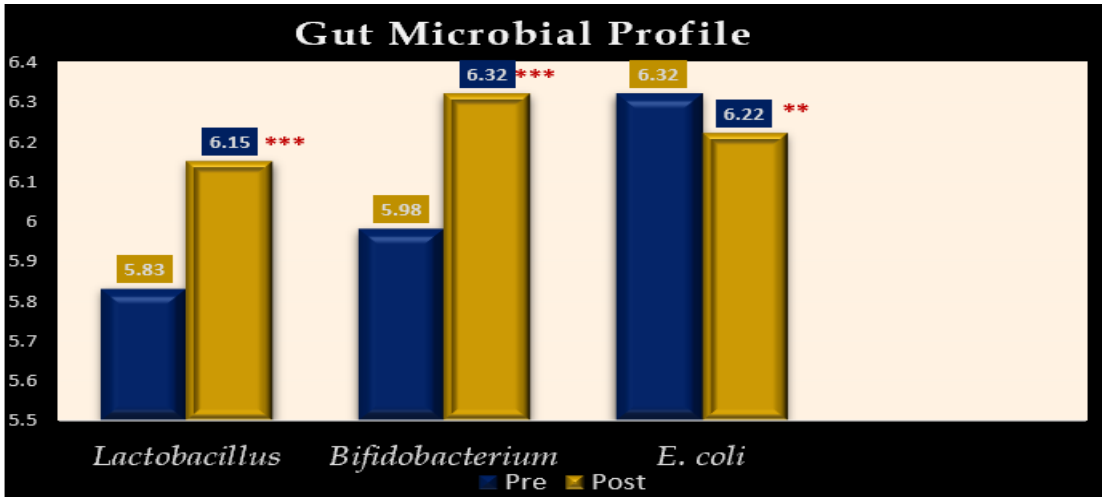


Figure 5.6.1: Log count of gut microbiota *Lactobacillus, Bifidobacterium and E. coli* before and after buttermilk tetra packed supplementation

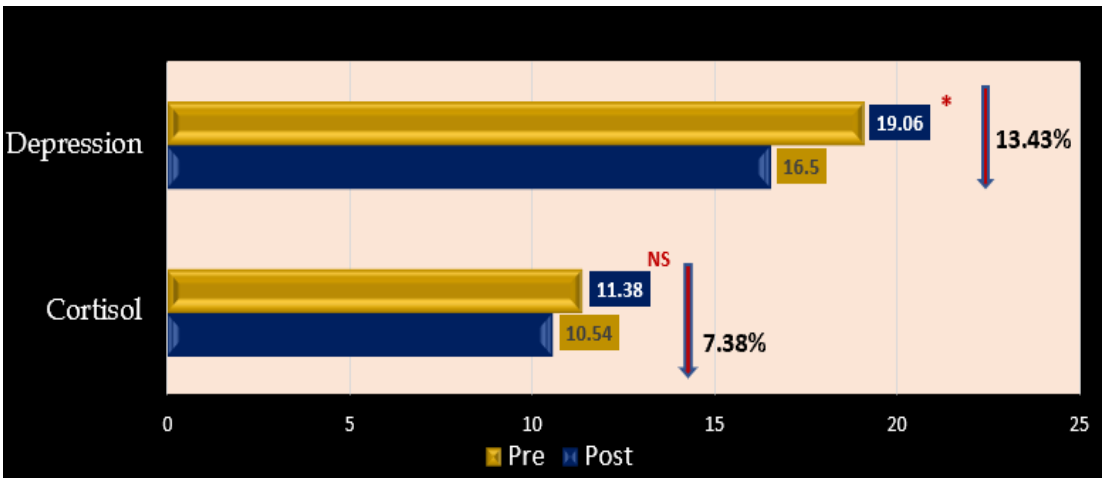


Figure 5.6.2: Changes in mean values of depression scores and blood serum cortisol post buttermilk tetra packed supplementation

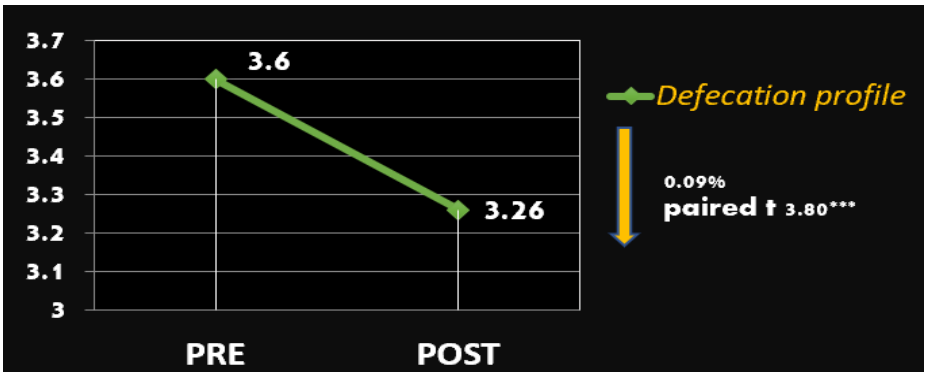


Figure 5.6.3: Percentage improvement in stool frequency post buttermilk tetra packed supplementation

Result Highlights of Phase VI

† Tetra packed buttermilk supplementation bought significant decrease in the depression scores (p value < 0.05) and log count of *E. coli* (p value < 0.01) by 13.43% and 1.58% respectively.

† Gut health improved (p value < 0.001) with significant increase in the colonization of *Lactobacillus* and *Bifidobacterium* by 5.47% and 5.68%.

† A not significant decrease in the levels of serum cortisol was marked by 7.38%. No significant changes were seen in the control group with respect to any of the parameters post intervention.

† Comparative analysis with control group revealed, significant difference in depression scores and *Lactobacillus* count (p value < 0.001) and highly significant difference in the mean log count of *Bifidobacterium (*p value < 0.001).

PHASE VII: Evaluating which intervention had highest impact in reversing depression, cortisol levels and modulating selected gut microbiota composition.

In Phase VII of this study post hoc test was used to determine the differences in mean scores of depressions, serum cortisol levels and gut *Lactobacillus*, *Bifidobacteria* and *E. coli* post supplementation with ambil, FOS, fresh buttermilk, and tetra packed buttermilk. The analysis was made to figure out which supplementation proved out to be effective in reversing depression scores, cortisol levels and setting the gut microbial modulation to a positive balance. Table 5.7.1 portrays pre and post assessment of different parameters in mild to moderately depressed subjects after intervention with FOS and fermented beverages.

Table 5.7.1: Pre and post assessment of depression, blood serum cortisol, and log count with respect to *Lactobacillus, Bifidobacteria* and *E. coli* in mild to moderately depressed subjects after intervention with FOS and fermented beverages.

Parameters	Intervention Phase	Ambil Group	FOS Group	Buttermilk Group (Fresh)	Buttermilk Group (Tetra packed)	Control Group	Post- hoc test
Depression score	**Pre intervention**	19.46 ±5.43	19.26 ±5.00	21.60 ± 4.18	19.06± 6.31	23.20 ± 6.39	F value 8.02***
	Post intervention	10.42±4.93	15.66 ± 7.07	18.53 ± 3.71	16.5 ± 5.93	22.80 ± 5.88	P value 0.00
	Paired 't' test	7.8***	3.78***	3.43***	3.40*	0.39 NS	
	% Difference	46.45	18.69	14.21	13.43		
Serum Cortisol	**Pre intervention**	11.43 ± 3.58	9.72 ± 6.74	14.47 ± 5.16	11.38 ± 3.79	10.36 ± 5.43	F value 4.32 NS
	Post intervention	10.68 ± 3.82	9.55 ± 3.50	13.82 ± 4.817	10.54 ± 3.43	10.72 ± 4.24	P value 0.07
	Paired 't' test	1.21 NS	0.13 NS	0.72 NS	1.22 NS	0.55 NS	
	% Difference	6.56	1.7	4.49	7.38		
Lactobacillus* (log_{10} *CFU//g)	**Pre intervention**	5.47 ± 0.22	5.73 ± 0.44	5.92 ± 0.24	5.83 ± 0.18	5.78 ± 0.30	F value 3.26**
	Post intervention	6.02 ± 0.25	6.14 ± 0.31	6.25 ± 0.19	6.15 ± 0.14	5.95 ± 0.24	P value 0.02
	Paired 't' test	16.11***	4.53***	6.31***	9.35***	1.61 NS	
	% Difference	10.05	6.80	5.28	5.4		
Bifidobacterium (log10 CFU/g)	**Pre intervention**	6.03 ± 0.24	5.87 ± 0.49	5.98 ± 0.20	5.98 ± 0.40	5.89 ± 0.23	F value 353.63***
	Post intervention	8.21 ± 0.18	6.23 ± 0.43	6.25 ± 0.09	6.32 ± 0.08	5.84 ± 0.26	P value 0.00
	Paired 't' test	83.42***	5.59***	6.53***	4.88***	1.81 NS	
	% Difference	36.15	6.13	4.51	5.68		
E. coli (log10 CFU//g)	**Pre intervention**	6.25 ± 0.13	6.17 ± 0.14	6.27 ± 0.13	6.32 ± 0.12	6.26 ± 0.12	F value 4.68*
	Post intervention	6.07 ± 0.27	5.94 ± 0.39	6.10 ± 0.15	6.22 ± 0.22	6.27 ± 0.09	P value 0.04
	Paired 't' test	4.14***	3.13*	8.19***	2.78**	0.63NS	
	% Difference	2.88	3.72	2.71	1.58		

NOTE: * significant at pvalue<0.001; ** significant at pvalue<0.01; * significant at pvalue<0.05; NS=notsignificant**

Ambil was most effective in lowering depression followed by FOS, fresh buttermilk, and tetra packed buttermilk (Figure 5.7.7 and Figure 5.7.8). Mean square difference within the groups and in between the group for depression status was highly significant (p value < 0.001).

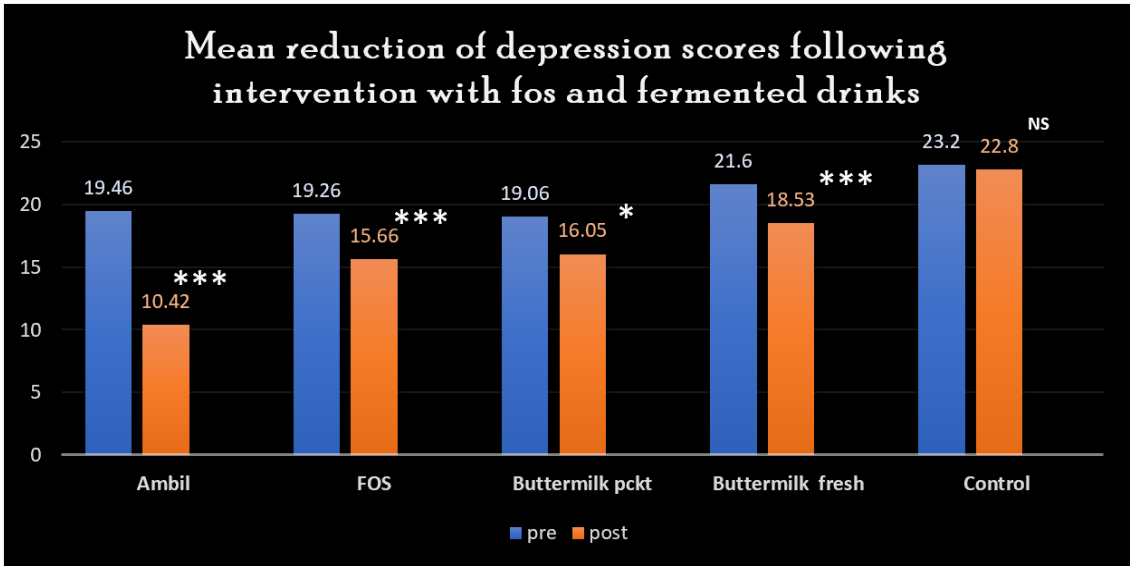


Figure 5.7.1: Range wise effectiveness of different supplementation products on mean depression scores

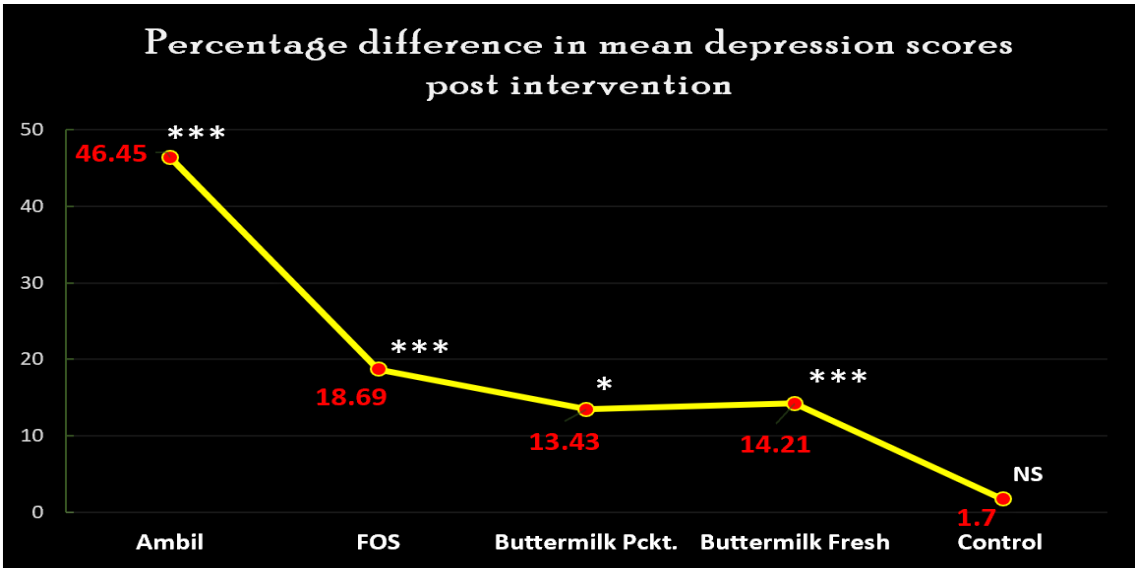


Figure 5.7.2: Percentage difference in mean depression scores post intervention With different supplementation

Tetra packed buttermilk proved out to be most effective intervention in reducing cortisol levels tailed by ambil, fresh buttermilk and FOS (Figure 5.7.9 and Figure 5.7.10). These differences were non-significant within and in between the experimental groups as interpreted by post hoc test.

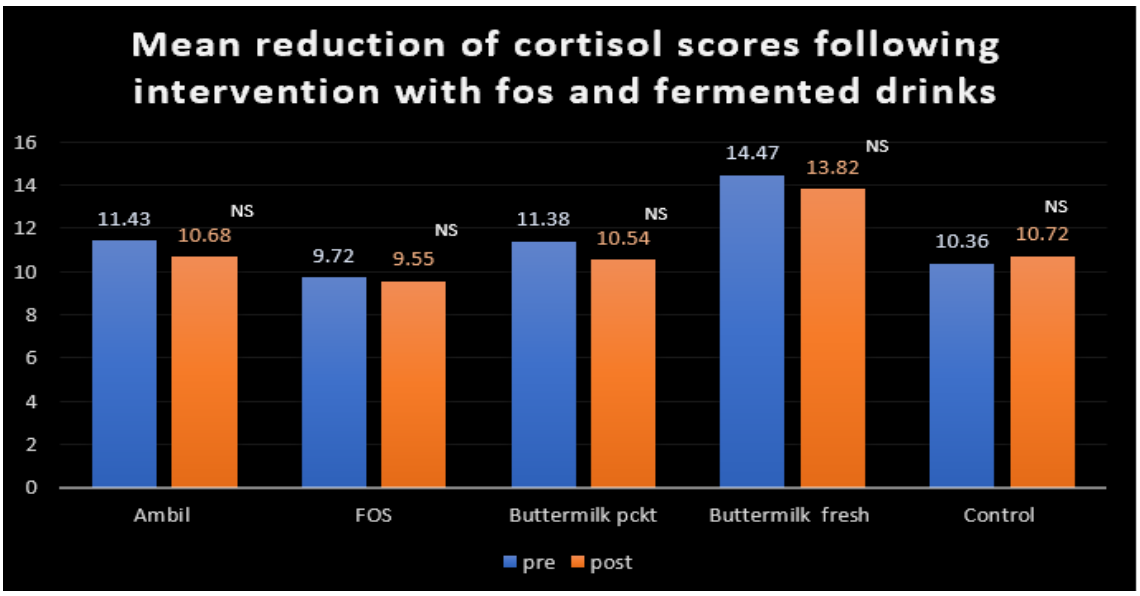


Figure 5.7.3: Range wise effectiveness of different supplementation products on mean blood serum cortisol

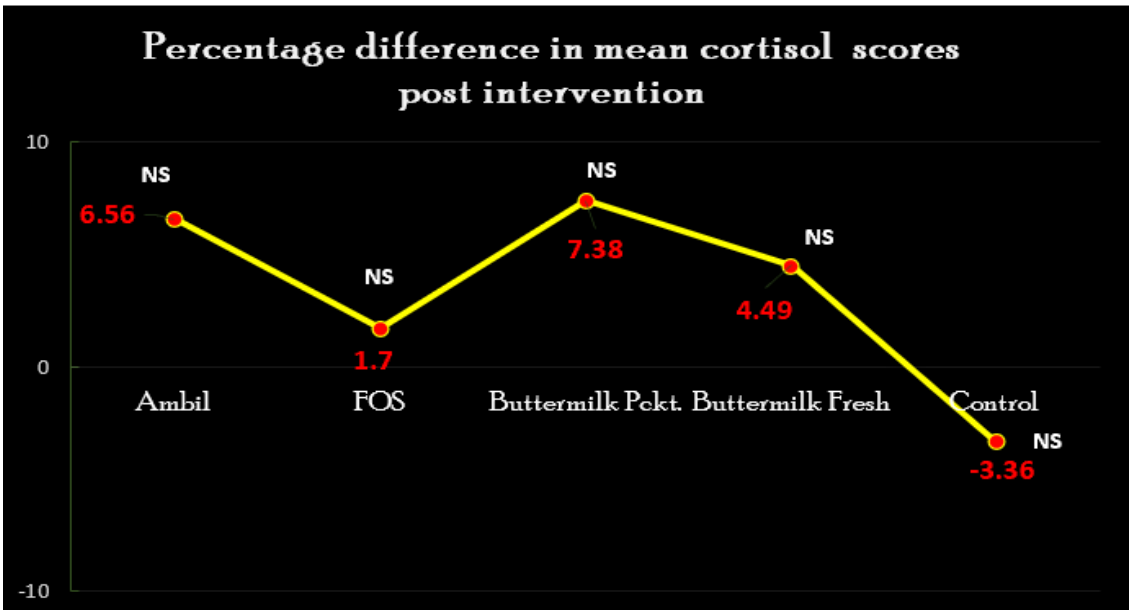


Figure 5.7.4: Percentage difference in mean blood serum cortisol post intervention with different supplementation

Log count of *Lactic acid bacteria* increased highest in the group supplemented with ambil followed up by FOS, tetra packed buttermilk and fresh buttermilk (Figure 5.7.1 and Figure 5.7.2). Mean square difference between groups and within groups was significant at F value 3.26 and p value< 0.01.

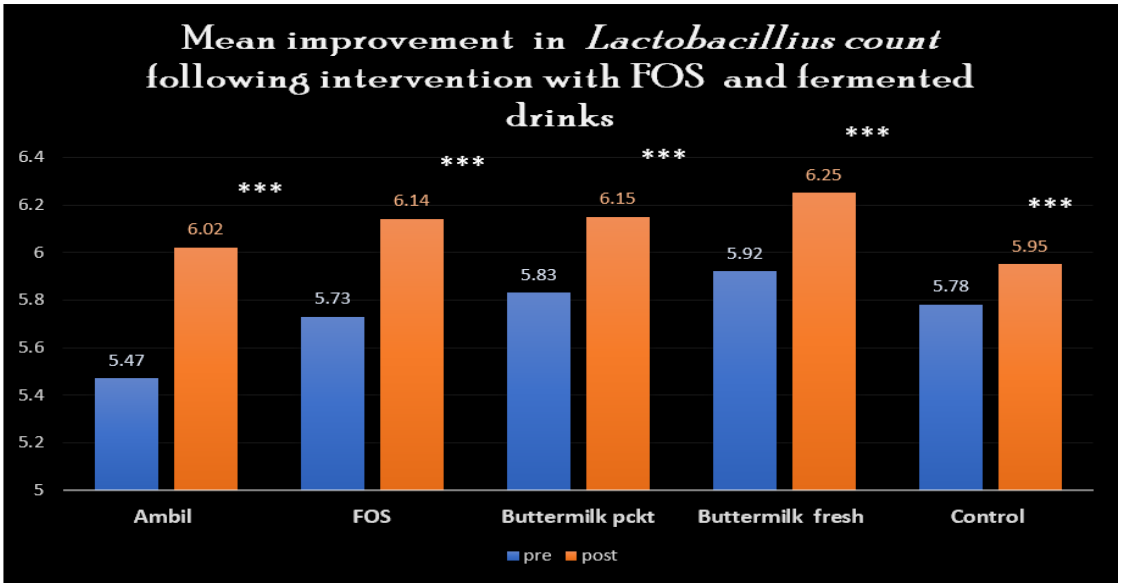


Figure 5.7.5: Range wise effectiveness of different supplementation products on mean log count of fecal *Lactobacillius*

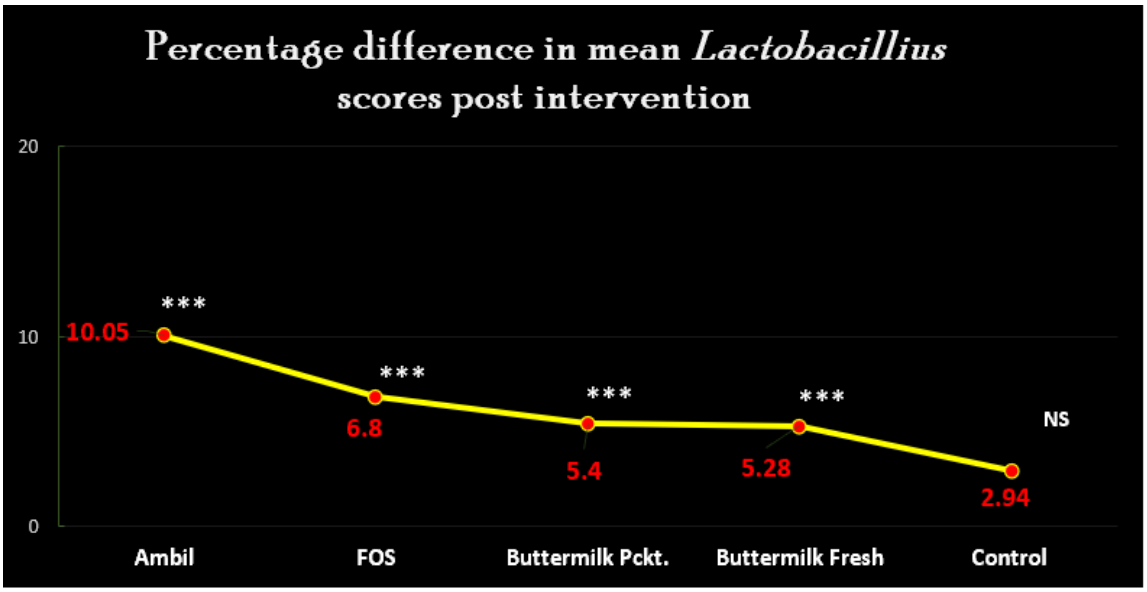


Figure 5.7.6: Percentage difference in mean *Lactobacillus* count post intervention with different supplementation

Ambil proved out to be most effective supplementation measure in increasing the colonization of *Bifidobacterium* followed by FOS, tetra packed buttermilk and fresh buttermilk (Figure 5.7.3 and Figure 5.7.4). The mean difference between the intervention groups and within groups showed high significance (p value < 0.001).

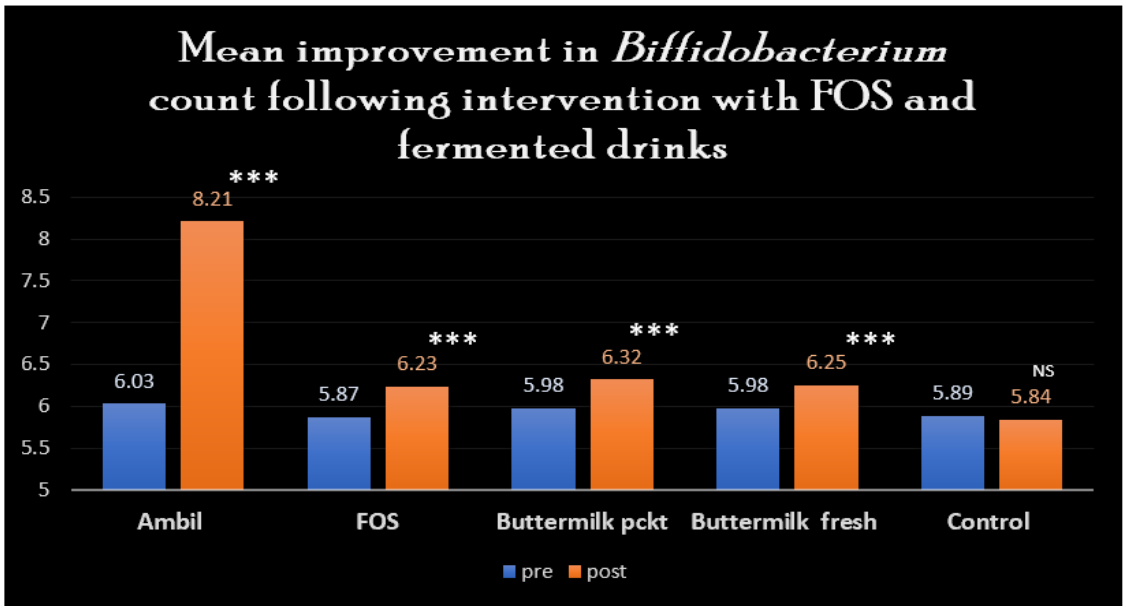


Figure 5.7.7: Range wise effectiveness of different supplementation products on mean log count of fecal *Bifidobacteria*

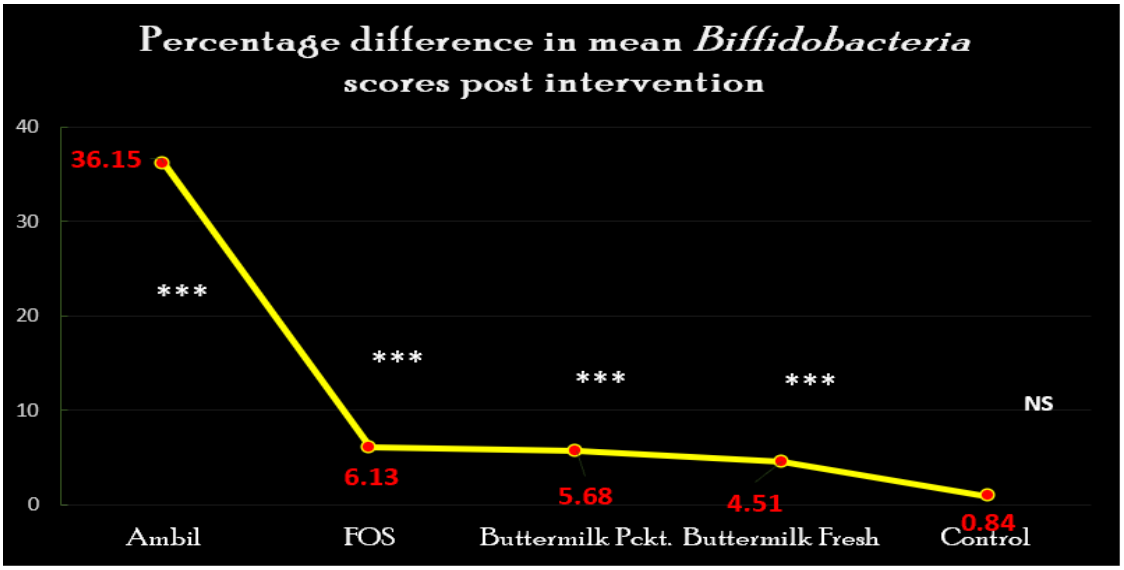


Figure 5.7.8: Percentage difference in mean *Bifidobacteria* count post intervention with different supplementation

Statistically significant difference (p value <0.05) was observed in reduction of *E. coli* upon supplementation with various psychobiotics. Highest reduction in the count of pathogenic bacteria *E. coli* was reported in group on FOS supplementation followed by ambil, fresh buttermilk and tetra packed buttermilk supplementation (Figure 5.7.5 and Figure 5.7.6).

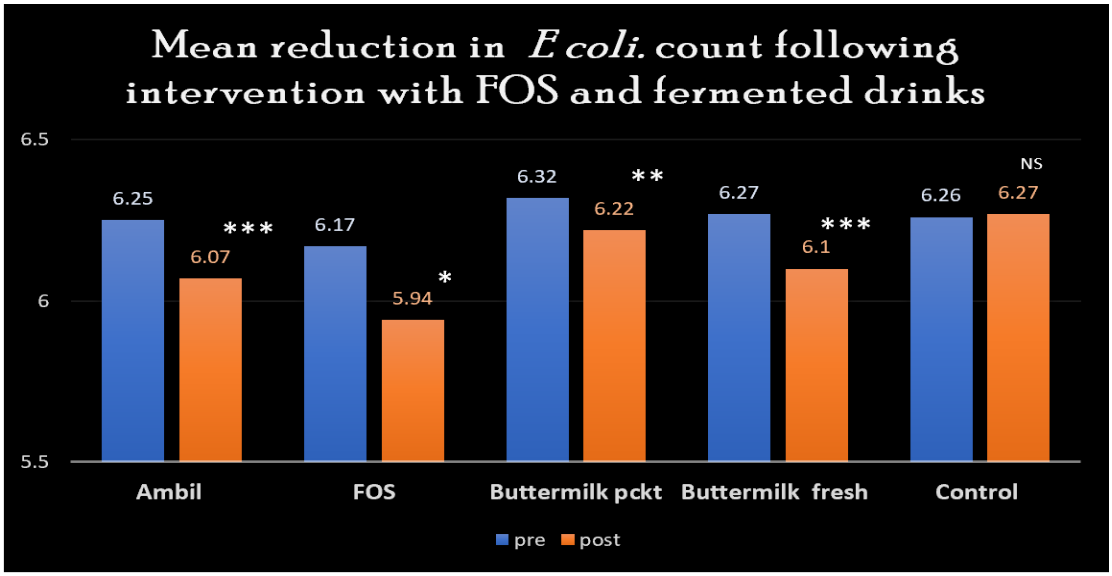


Figure 5.7.9: Range wise effectiveness of different supplementation products on mean log count of fecal *E.coli*

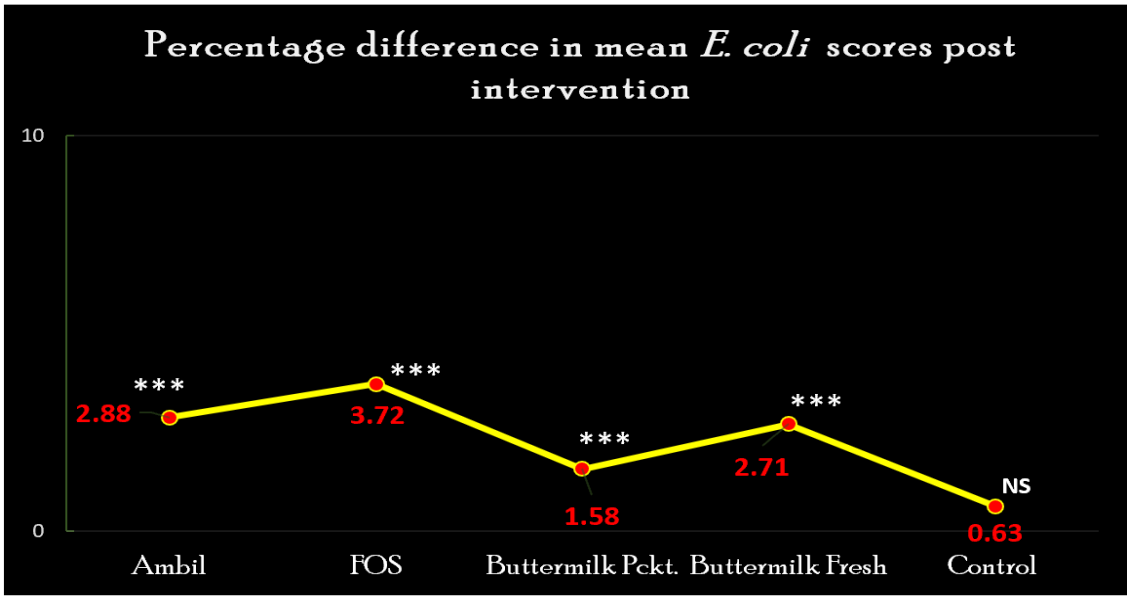


Figure 5.7.10: Percentage difference in mean *E.coli* count post intervention with different supplementation

Intervention trials with all the four food products viz. ambil, FOS, tetra packed buttermilk and fresh buttermilk shifted the gut microbial colonization to positive balance and bought about significant changes in depression status. Plate 5.7.1 shows typical growth of *Bifidobacteria, E. coli,* and *Lactic acid bacteria.*

Result Highlights of Phase VII

† Intervention trials with all the four food products viz. Ambil, FOS, tetra packed buttermilk and fresh buttermilk shifted the gut microbial colonization to positive balance and bought about significant changes in depression status.

† Ambil was most effective in lowering depression followed by FOS, fresh buttermilk, and tetra packed buttermilk. Mean square difference within the groups and in between the group for depression status was highly significant (p value < 0.001).

† Tetra packed buttermilk proved out to be most effective intervention in reducing cortisol levels tailed by ambil, fresh buttermilk and FOS. These differences were non-significant within and in between the experimental groups as interpreted by post hoc test.

† Log count of *Lactic acid bacteria* increased highest in the group supplemented with ambil followed up by FOS, tetra packed buttermilk and fresh buttermilk. Mean square difference between groups and within groups was significant at F value 3.26 and p value< 0.01.

† Ambil proved out to be most effective supplementation measure in increasing the colonization of *Bifidobacterium* followed by FOS, tetra packed buttermilk and fresh buttermilk. The mean difference between the intervention groups and within groups showed high significance (p value < 0.001).

† Statistically significant difference (p value <0.05) was observed in reduction of *E. coli* upon supplementation with various psychobiotics. Highest reduction in the count of pathogenic bacteria E. coli was reported in group on FOS supplementation followed by ambil, fresh buttermilk and tetra packed buttermilk supplementation.

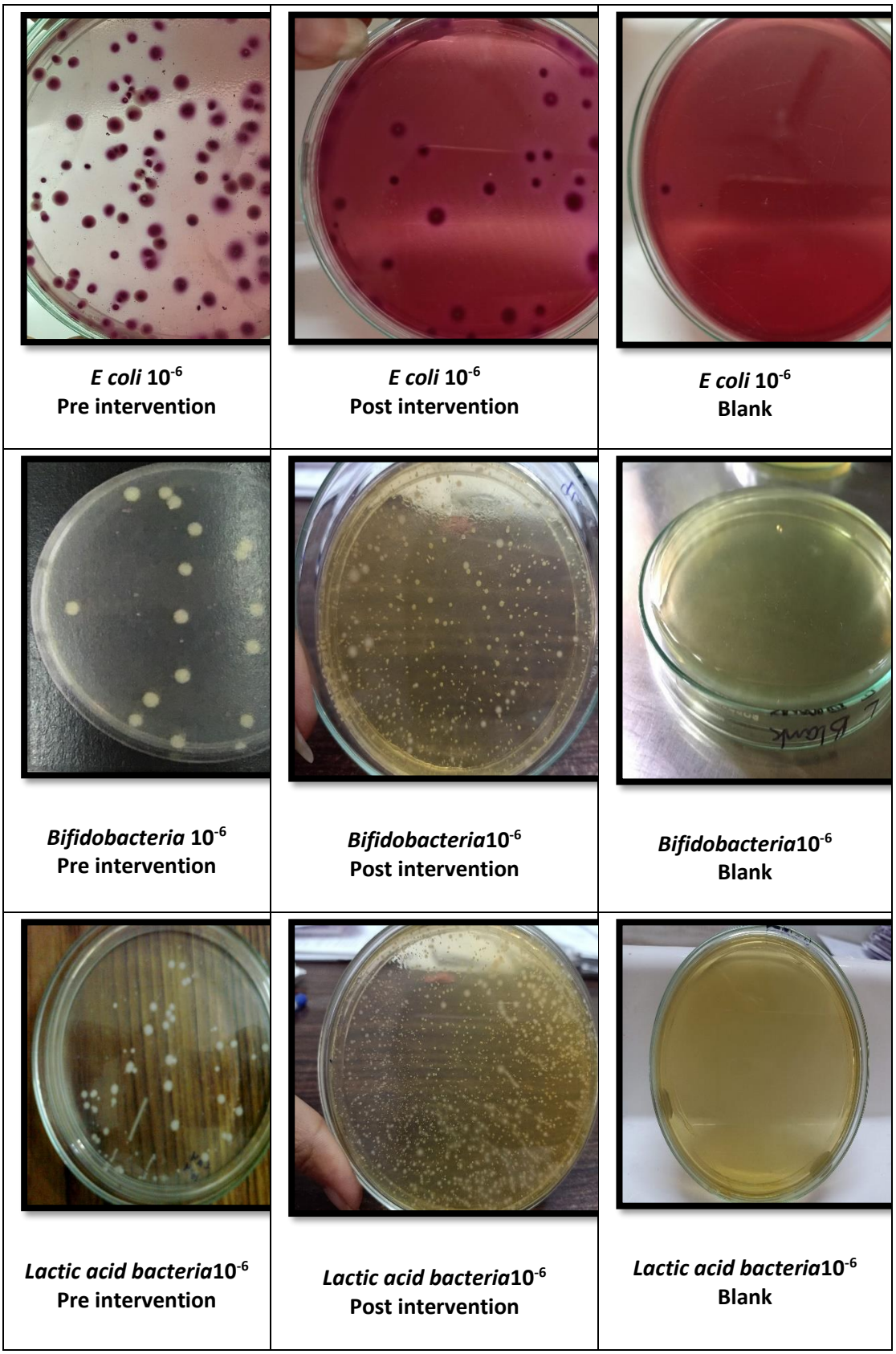


Plate 5.1: Typical growth of *Bifidobacteria, E. coli, and Lactic acid bacteria*

PHASE VIII: Developing an audio-visual animated film as Information Education Communication material to make general people aware about increasing trend of depression and how to cope up with it.

Animated movie was developed using the software ***Animaker*.** To make general people aware we tried to cover every aspect of depression including symptoms, need to communicate with subject expert, the role of nutrition and exercise, and how to formulate new habits for the betterment of self. The movie is named 'RESTART' which aims to ring the wake-up call to restart with new hope, new strength and new thought to overcome depression. Glimpse of the scenes from movie made for IEC is depicted in plates 5.8.1 (a)-(e).

Plate 5.8 (a)

Plate 5.8 (b)

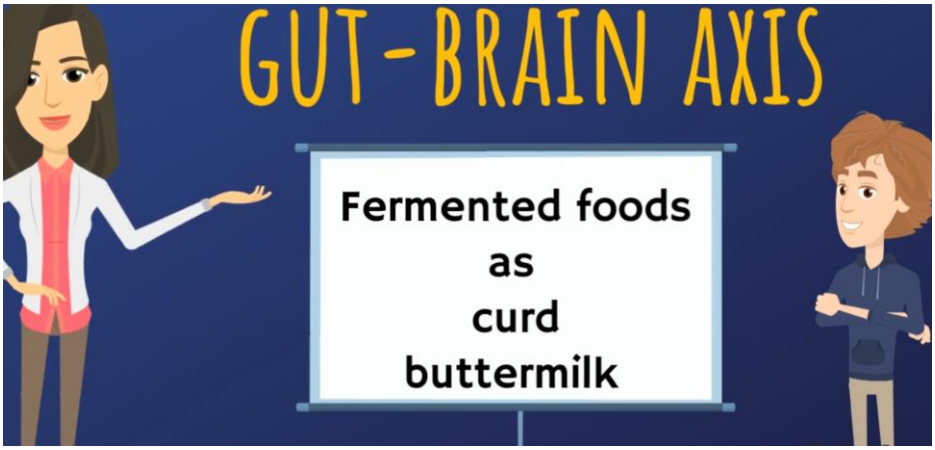


Plate 5.8 (c)

Plate 5.8 (d)

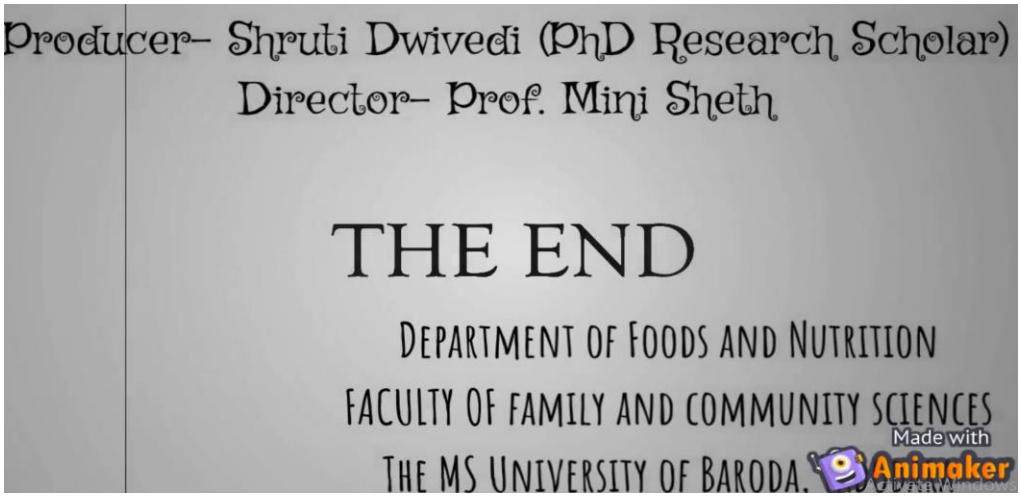


Plate 5.8 (e)

Plate 5.8.1: Glimpse of the scenes from movie made for IEC