



**ORIGINAL RESEARCH PAPER**

**Clinical Nutrition**

**PREBIOTIC ENRICHED BUTTERMILK AS A DIETARY INTERVENTION TO IMPROVE GUT HEALTH AND LOWER DEPRESSION**

**KEY WORDS:** Gut-brain axis, cortisol, depression

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**ABSTRACT**

Bioactive peptides released during fermentation of milk and prebiotics carries significant implications for research on gut brain axis. The study was designed to determine the relationship between consumption of prebiotic enriched buttermilk based fermented beverage and depression. Subjects from The MS University of Baroda who were willing to participate were screened using Beck's Depression Inventory. Gut microflora was determined with respect to Lactobacillus, Bifidobacteria and E.coli in fecal sample. Cortisol levels were determined using ROCHE machine. Supplementing 200 ml fermented buttermilk enriched with prebiotic for a period of 30 days to 35 subjects falling in the category of mild to moderate depression showed significant decrease in mean depression scores and cortisol values by 46.5% and 4.2 % respectively. The fecal log count of Lactobacillus and Bifidobacterium showed a significant increase by 10.2% and 36 % respectively and a reduction by 2.7% in E.coli.

**1. INTRODUCTION**

According to Global Health Estimates, an estimated 322 million people were affected by depression in 2015. Microbiota influence the development of brain regions involved in our response to stress, anxiety and depression. Microbes act upon dietary items pre-consumption, and in turn, these fermented dietary items influence our own microbiota. Fermentation may amplify the specific nutrient of foods, the ultimate value of which is associated with mental health (Selhub E. M. et al., 2014). Dairy products constitute a good source of bioactive compounds initially found in inactive form within the sequence of the precursor molecules alpha and beta-caseins, albumin and globulin. These bioactive molecules are released by fermentation of milk by the action of the microorganisms making the products directly beneficial without the need of live bacteria. These encrypted peptides are able to control nutrition and significantly reduce stress and cortisol levels. Prebiotics acts as good mood food (Korhonen and Pihlanto, 2003). Our brain contains billion of neurons, but we less often talk about the fact that trillions of "good" bacteria are alive in our intestinal tracts. Gut microbiota are important to healthy brain development. The gut-brain axis consists of bidirectional communication between the central and the enteric nervous system, linking emotional and cognitive centers of the brain with peripheral intestinal functions (Marilia Carabotti et al., 2015). Burnet and Schmidt studied the effect of prebiotics on emotional processing in humans and showed that subjects who were given prebiotics tended to be less attentive to negative stimuli. Combination of prebiotic added milk based fermented beverage which may have biogenic metabolites in sufficient amounts would trigger the colonization of good bacteria in the gut and thus activate the vagus nerves located in the intestine and bring about an improvement in the depression status of the individuals. The discovery that changing the microbiota in the gut and biogenic metabolites produced during fermentation can affect the brain carries significant implications for research that points the way toward dietary interventions to improve brain function. The study was designed with the Primary objective "How this ancient practice of consuming fermented beverage, modified by enriching with prebiotic associate between emotional psychiatry and gut microbiota".

**2. METHODS**

**2.1 Enrollment of subjects**

Subjects (291) aged 20-50 years from The MS University of Baroda who were willing to participate were screened using Beck's Depression Inventory (BDI). Enrollment of 30 subjects was done by systemized random sampling. Subjects suffering from any of the chronic diseases, taking any supplements and medicines and falling in category of severe depression were excluded from the

study.

**2.2 Determination of gut microbiota with respect to Lactobacillus, Bifidobacteria and E.coli in fecal sample.**

**a) Collection of the fecal sample**

The subjects were given uricols for the collection of stool. The samples were stored at -20° C.

**b) Sterilization of glassware**

Petri dishes were kept in hot air oven at 180° C for two hours. The micro tips were sterilized by autoclaving at 121°C for 20 minutes at 15lbs pressure. Other instruments were sterilized using 70% alcohol.

**c) Preparation and sterilization of dilution blank**

1 gm of peptone was dissolved in 1000ml of distilled water which was transferred in portion of 100 ml in 10 dilution bottles and autoclaved at 121° C for 20 minutes.

**d) Preparation of media**

The media used for enumeration of Bifidobacteria, Lactobacillus and E. coli was Bifidobacterium agar, MRS agar and VRB Agar. The prepared media was autoclaved at 121° C for 15 minutes.

e) Inoculation, incubation and enumeration of Lactobacillus, Bifidobacteria and E.coli One gram of fecal sample was mixed homogeneously in 99ml of 0.1% of peptone water. 1 ml of slurry was diluted serially in peptone water. Then 0.1 ml of dilution was added from each of the dilutions to the petri plates containing respective media inside laminar flow. The plates of Bifidobacterium were incubated at 37°C in the anaerobic jar in incubator with the gaspacks. Lactobacillus were placed in desiccators and E.coli were directly placed in the incubator. Readings for E.Coli were taken after 24 hours while Bifidobacteria and Lactobacillus readings were taken after 48 hours. Colonies were counted and which appeared in the range of 30-300 were converted into log counts (Ramona et al., 2001).

**2.3 Assessment of cortisol levels**

Serum cortisol levels were determined using Chemi Luminescent Immuno assay in ROCHE machine.

**2.4 Intervention trial**

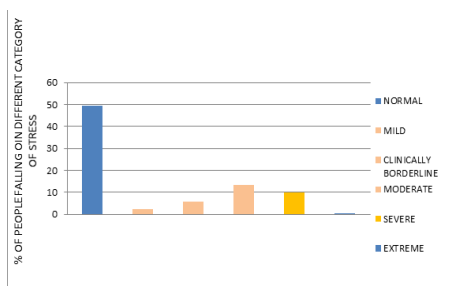
Using the pre post test quasi experimental study design 30 mild to moderately depressed subjects were supplemented with 200ml Fructooligosaccharide enriched rice and buttermilk based fermented beverage daily for a period of 30 days. Impact evaluation of supplementation was studied for the parameters similar to baseline.

**3. Results and Discussion**

**3.1 Stress scores**

Depression is on the rise among young people and is likely to become the second leading cause of death and disability in the world by 2020 (WHO 2012).

The BDI is developed to measure depth of depression. Internal consistency for the BDI ranges from 0.73 to 0.92 with a mean of 0.86 (Richter et al., 1998). Figure1 shows Percentage of people falling in different categories of depression, out of 291 people screened using BDI in present research. 49.5 % were normal, 2.4 % were in the category of mild depression, 5.6 % were on clinical borderline, 13.4 % were moderately depressed while 10.3 % showed severe depression and a case of extreme depression was also noted.



**Fig 1. Depression levels**

Another studies of prevalence of depression in adolescent students of a public school using BDI also showed about 18.4% students were depressed (Vivek Bansal et al., 2009 and Sahoo S et al., 2010). Psychologist says, "Since the last five years we have seen increase of around 75% in depression cases out of which, 60% also attempt suicide ranging from age 6 to 25." (Debarati, 2013). Detecting depressive symptoms in the college population is a critical preventive strategy, which can help in preventing disruption to the learning process.

**3.2 Mean values for cortisol levels and gut profile of subjects pre and post intervention.**

Cortisol is a physiologic measure of the hypothalamic pituitary adrenal axis activity which serves as the primary communication between the central nervous system and the endocrine system during times of stress as a physiological marker; altered cortisol indicates a stress response with immediate and long-term health consequences (M. A. Pico-Alfonso et al., 2006).

In the present study as shown in Table 1 the mean cortisol values for mild to moderately depressed individual was 11.14, as subjects were mild to moderately depressed so they showed high cortisol levels. Researchers have reported the association between type and number of gut microflora and depression. The microbiota has been suggested to be the key factor in the link between unhealthy diets and depression. The two most beneficial micro organisms found abundantly in gut are Lactobacillus and Bifidobacterium with typical bacterial counts of 108 -109 and 109- 1011 per gram of stool respectively in a healthy gut (Andrew L et al., 2008). In the present study the mean bacterial counts for Lactobacillus and Bifidobacterium were much lower than the healthy individual. The mean log values of CFU/g of stool samples of Lactobacillus, Bifidobacterium and E.coli were 5.5, 6.0 and 6.2 respectively. Supplementing the subjects with prebiotic enriched fermented buttermilk, increased the fecal log count of Lactobacillus and Bifidobacterium by 10.2% and 36 % respectively significant reduction by 2.7% in the fecal log counts of E.coli of depressed subjects after intervention and a decreased in mean stress score and cortisol levels was seen by 46.5% and 4.2% respectively.

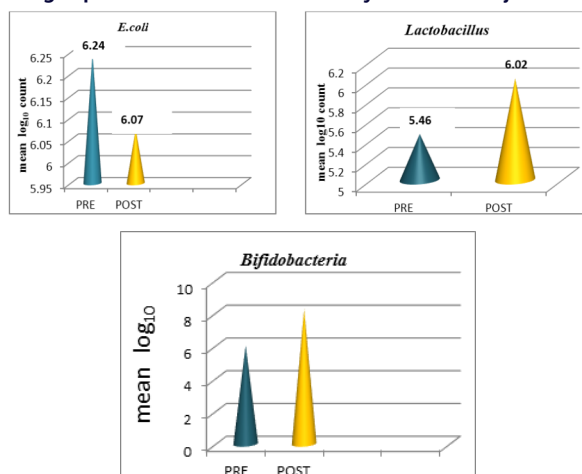
**Table 1. Mean values for stress score, cortisol and gut microflora of subjects (n=30) before and after intervention**

E coli	Pre intervention	6.248 ± 0.133
	Post intervention	6.074 ± 0.272
	Difference	0.174

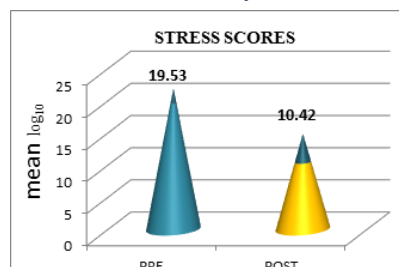
	% difference	2.78
	Paired 't'	4.148***
LAB	Pre intervention	5.462 ±0.244
	Post intervention	6.021±0.255
	Difference	0.559
	% difference	10.23
	Paired 't'	-16.1***
Biff	Pre intervention	6.027 ± 0.23
	Post intervention	8.217 ±0.185
	Difference	2.19
	% difference	36.33
	Paired 't'	-83***
Stress scores	Pre intervention	19.533 ± 5.204
	Post intervention	10.423 ± 4.932
	Difference	9.11
	% difference	46.5
	Paired 't'	7.015***
Serum cortisol	Pre intervention	11.148 ± 3.499
	Post intervention	10.684 ± 3.82
	Difference	0.464
	% difference	4.162
	Paired 't'	1.213 NS

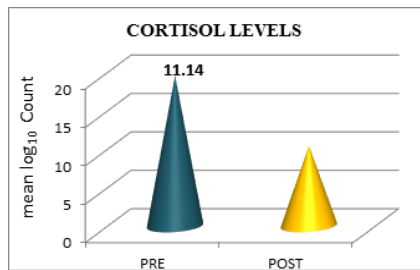
Note level of significance: \* p value < 0.05, \*\* p value < 0.01, \*\*\* p value < 0.001, NS = not significant

**Fig 2. Impact of prebiotic enriched fermented beverage on gut profile of mild to moderately stressed subjects**



**Fig 3. Impact of prebiotic enriched fermented beverage on stress scores and cortisol levels of mild to moderately stressed subjects.**





Similar trend is supported by researches which showed that the use of fermented foods in diets did confer gastrointestinal and cognitive benefits. (Selhub EM et al., 2014 and Kim et al., 2016) College undergraduates, who ate yogurt, were less likely to suffer from social anxiety, fear or depression (August 2015 issue of Psychiatry Research). Additional studies in recent years have shown that prebiotics confer similar anxiolytic and antidepressant effects as probiotics as they also diminish stress-induced changes to the colonic microbiota and create stabilized levels of Bifidobacteria and Lactobacilli population (Burokas A et al., 2017). Ample of studies have proved that FOS supplementation alter gut microbiome and consumption of fermented food improves mental status. The results of the present study supported the results demonstrated by other studies showing a positive relation between good gut environment and decrease in depression levels post intervention by FOS added buttermilk to mild to moderately depressed subjects.

**4. CONCLUSION**

From the above results and supporting studies, it can be concluded that there is a strong positive connection between gut microbiota of mild to moderately depressed subjects and consumption of prebiotic added fermented beverage. Thus, FOS added buttermilk can serve as a mean for reducing the levels of depression and may be safer than conventional medicines while providing additional health benefits.

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