

## CHAPTER 5

# RESULTS AND DISCUSSION

Undernutrition giving deeper dents to the development of India. Age old policies failed to bring any substantial difference and undernutrition is persistently growing its roots and seriously wounding the future of this country. As the fact of the matter, infections are the underlined causes of undernutrition and gut microbiome are the quintessential route for one's immunity. Therefore, present research focuses on curbing undernutrition via supplementing prebiotic (FOS) in the diet of undernourished children, build favorable gut microbiome and thereby reduce the rates of infection and ultimately supporting the well-being of children.

This chapter deals with the findings, statistical analysis, interpretation and discussion of the data, obtained during the course of research. Based on the objectives, results of the present research are divided into following three phases:

### **Phase I: Screening of nutritional status and survey for the background information of school going children.**

*5.1.1. Class, gender and age wise distribution of children under study*

*5.1.2. Nutritional status of primary school going children*

*5.1.2.1. Age wise trend in nutritional status of primary school going children*

*5.1.2.2. Gender wise trend in nutritional status of primary school going children*

*5.1.3. Background information of the parents of primary school going children*

5.1.4. *Baseline Morbidity profile of the children under study*

5.1.5. *Appetite profile of the children*

5.1.6. *Exposure/Outcome Ratios (OR) between Socioeconomic and Health variables*

5.1.7. *Association between nutritional status of children and their various health parameters*

**Phase II: Scenario of various parameters *viz.* morbidity profile, gut microflora; serum IgA levels and dietary intake of Nourished and Undernourished children.**

5.2.1. *Morbidity profile of nourished and undernourished school going children*

5.2.2. *Gut-Microbiota of Nourished and Undernourished Children*

5.2.3. *Serum IgA levels of Nourished and Undernourished Children*

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5.2.6.1. *Correlation between the gut health and morbidity profile*

5.2.6.2. *Linear Regression for BMI for age and gender*

### **Phase III: Impact of Fructooligosaccharide supplementation on morbidity profile, gut microflora and serum IgA levels of undernourished children**

- 5.3.1. *Impact of FOS supplementation on Gut microbiota of the undernourished school going children*
- 5.3.2. *Impact of FOS supplementation on morbidity profile of the undernourished school going children*
- 5.3.3. *Impact of FOS supplementation on nutritional status of the undernourished school going children*
- 5.3.4. *Impact of FOS supplementation on serum IgA levels of the undernourished school going children*

### **Phase IV: Development of a bilingual booklet entitled “Prebiotic: Our Gut Guardians”**

- *Book was written in two languages :Hindi and English and includes:*
  - *Twenty one recipes*
  - *Four categories of foods namely breakfast and snack item; Main course item; Dessert and Beverages*
  - *Importance of Prebiotics and its health benefits*
  - *Addresses and other details of Retailers of Prebiotics*

### **Phase I: Screening of nutritional status and survey for the background information of school going children**

Phase I was carried out to see the prevalent status of nutrition among the studied group of children of class I to V. Further, trend of Undernutrition was also analyzed across age and gender of the children. Parents/guardians of the children were interviewed for various aspects such as socio-economic status and educational status of the parents, practice of exclusive breastfeeding and immunization status of children.

#### ***5.1.1. Class, gender, and age wise distribution of children under study***

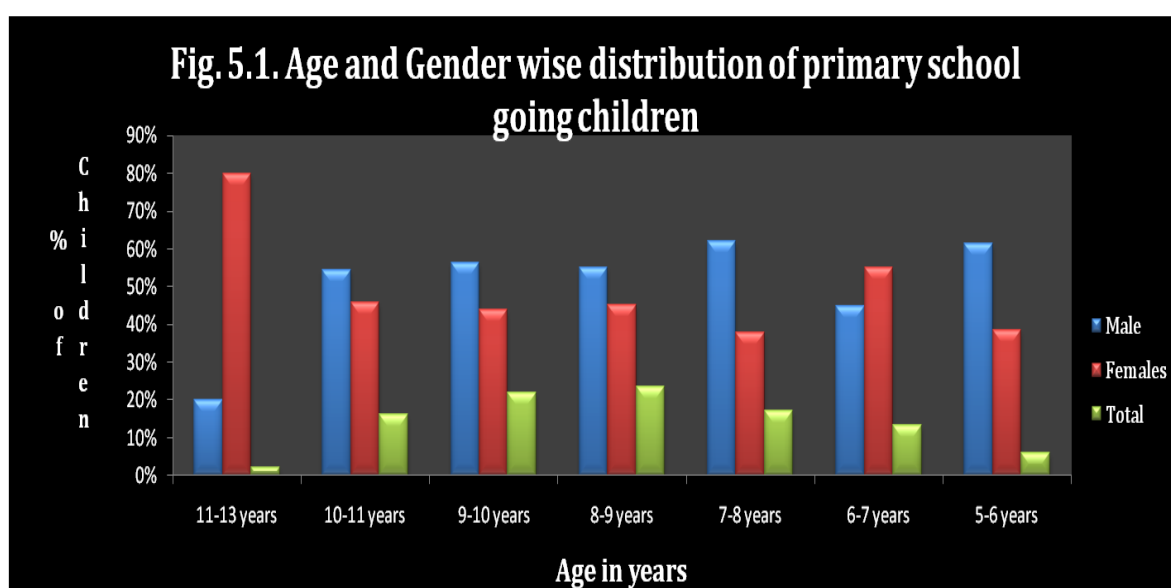
Table 5.1 reveals that, out of 218 primary school children, 99 (45.41%) were girls and 119 (54.59%) were boys. The overall gender ratio of girls to boys was observed to be 0.45:0.54. Maximum numbers of girls were in class-V (29%) whereas maximum numbers of boys were studying in class IV (28%).

Chi square test revealed that over-all gender distribution across all the classes was statistically non significant.

Table 5.1: Class and Gender wise distribution of primary school children

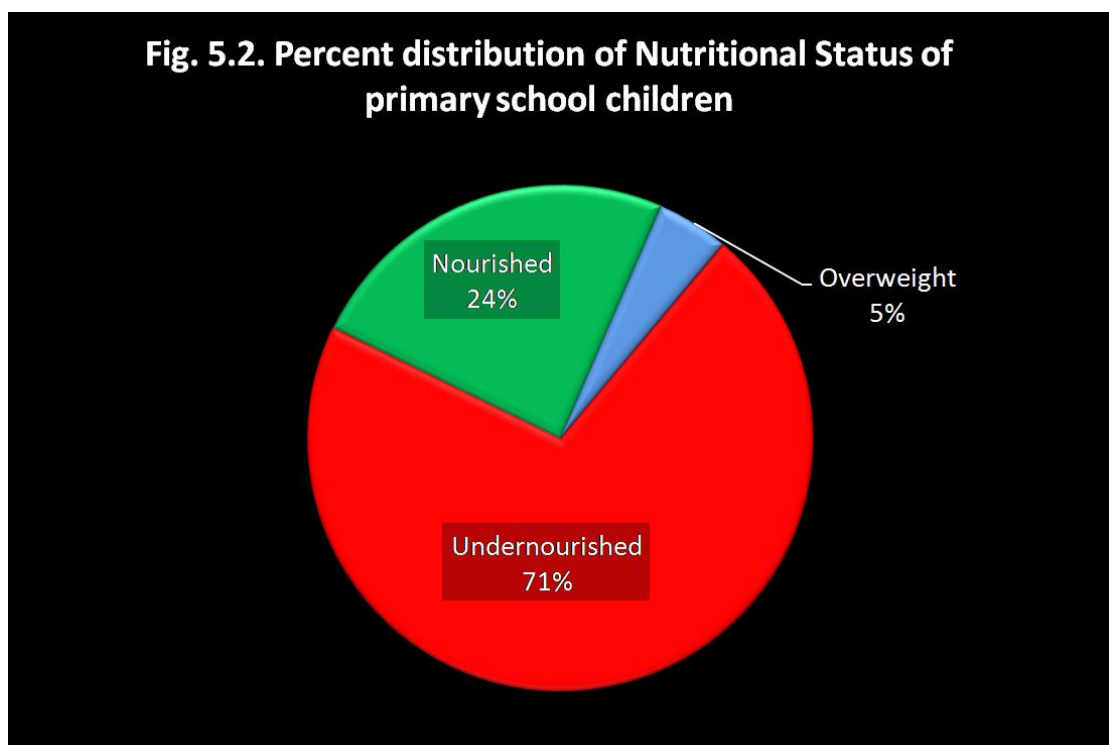
Categories	Males		Females		Total		$\chi^2$ [p value]
	No	%	No	%	No	%	
Class- I	20	16.81	12	12.12	32	14.76	5.61 [0.23] NS
Class- II	18	15.13	17	17.17	35	16.12	
Class-III	22	18.49	24	24.24	46	21.19	
Class-IV	33	27.73	17	17.17	50	23.06	
Class-V	26	21.85	29	29.29	55	25.33	
<b>Total</b>	<b>119</b>	<b>54.59</b>	<b>99</b>	<b>45.41</b>	<b>218</b>	<b>100</b>	

NS= Statistically Non Significant



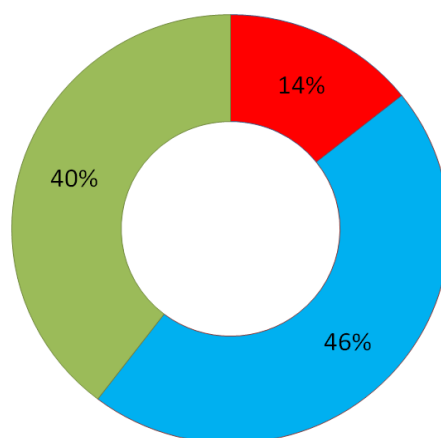
### 5.1.2. Nutritional status of primary school going children

Undernutrition was prevalent in 71% (n=155) of school children, only 24% (n=53) of the children were found to be nourished and 5% (n=10) children were overweight (Fig. 5.2). As seen in fig. 5.3, distribution of undernutrition status revealed that out of 71% of undernourished children, 14 % were severely undernourished (grade III, n=22), 46% were moderately undernourished (grade II, n=71) where as 40% of the children were mildly undernourished (grade I, n=62).



**Fig. 5.3. Percent distribution of undernourished school going children**

■ Grade III UN ■ Grade II UN ■ Grade I UN



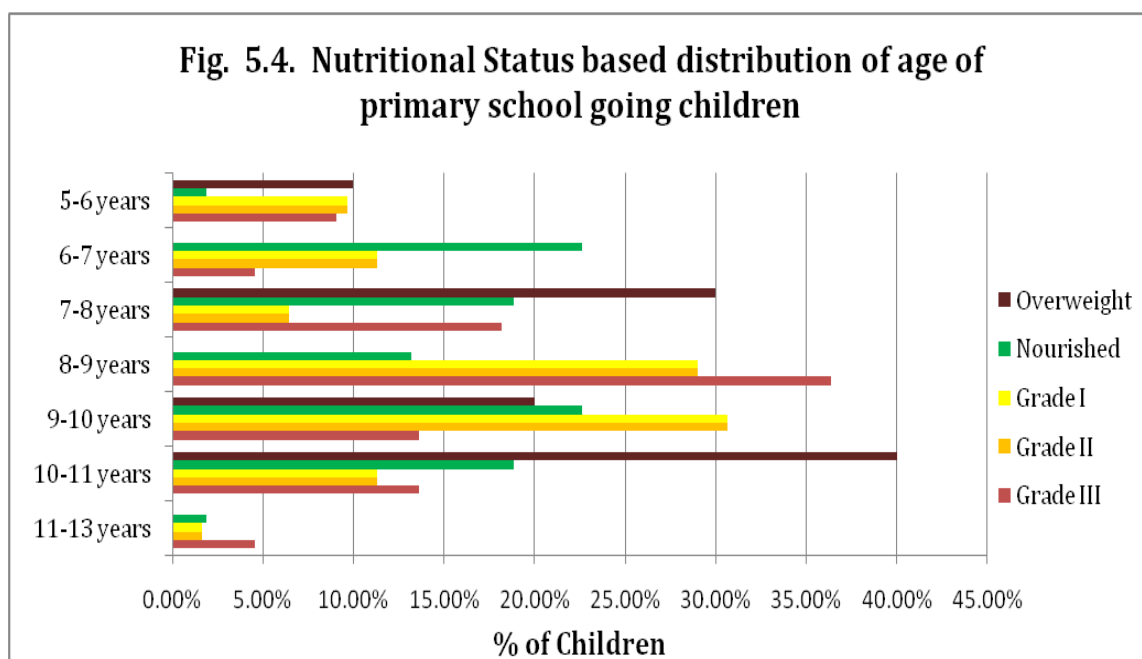
**5.1.2.1. Age wise trend in nutritional status of primary school going children**

Certain level of polarization was observed in terms of age based nutritional status as evident in Table 5.2 and Fig 5.4. Maximum number of severely undernourished (36%) and moderately undernourished (25%) children was seen in the age group of 8-9 years. Mild undernutrition was highest in the age group of 9-10 years. Age group of 9-10 and 6-7 years had maximum number of nourished children.

**Table 5.2. Age wise trend of Nutritional Status in school going children**

Age	Grade III Undernutrition		Grade II Undernutrition		Grade I Undernutrition		Nourished		Overweight		Total
	No	%	No	%	No	%	No	%	No	%	No
11-13 years	1	4.5	2	2.81	1	1.61	1	1.88	0	0	5
10-11 years	3	13.6	11	15.4	7	11.29	10	18.86	4	40	35
9-10 years	3	13.6	12	16.9	19	30.64	12	22.64	2	20	48
8-9 years	8	36.0	18	25.35	18	29.03	7	13.20	0	0	51
7-8 years	4	18.1	16	22.53	4	6.45	10	18.86	3	30	37
6-7 years	1	4.5	9	12.67	7	11.29	12	22.64	0	0	29
5-6 years	2	9.0	3	4.22	6	9.67	1	1.88	1	10	13
<b>Total</b>	<b>22</b>	<b>100</b>	<b>71</b>	<b>100</b>	<b>62</b>	<b>100</b>	<b>53</b>	<b>100</b>	<b>10</b>	<b>100</b>	<b>218</b>





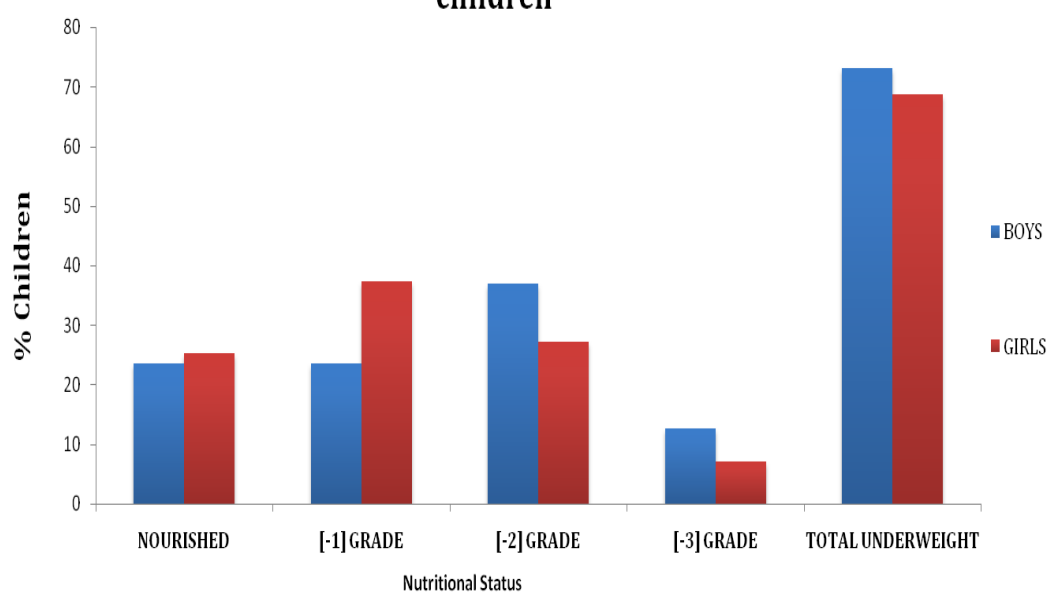
#### 5.1.2.2. Gender wise trend in nutritional status of primary school going children

Table 5.3 reveals that of the total 119 boys 87 were undernourished (73%) whereas 69% girls were found to be undernourished. However, nourished children were present in almost similar numbers in both the genders as 24% boys and 25 % girls were found to be nourished. Overweight Girls (6%) were observed more in numbers compared to boys (3%).

Gender based analysis of undernutrition status depicts that 13% of the total boys were severely undernourished compared to only 7% girls (Fig. 5.5). Moderate undernutrition also found to be higher in boys (37%) compared to girls (27%), whereas a contrary picture was seen in the case of mild undernutrition wherein girls (37%) outnumbered boys (24%).

**Table 5.3. Gender based distribution of Nutritional Status of primary school going children**

Nutritional Status	Males		Females	
	No.	%	No.	%
Nourished	28	23.53	25	25.25
Undernourished	87	73.11	68	68.69
<b>[-3] Grade</b>	15	12.61	7	7.07
<b>[-2] Grade</b>	44	36.97	27	27.27
<b>[-1] Grade</b>	28	23.53	37	37.37
Overweight	4	3.36	6	6.06
TOTAL	119		99	

**Fig.5.5. Gender wise scenario of Nutritional Status of school going children**

### ***5.1.3. Background information of the parents of primary school going children***

Background information of the subjects prior to selecting them for intervention trial was obtained through the interview of both or either of the parents of school going children. As seen in Table 5.4 sixty percent of the children belonged to either joint or extended family and only 40% of the children were from nuclear family. Fifty eight (57.76%) percent of parents of the children had an income above Rs. 5000 per month while 42% of them were earning below Rs. 5000 per month.

Literacy status of parents revealed that 21.5% mothers and 14% fathers of the children were illiterate. Primary and secondary education was attained by 63.5% mothers and 65% fathers of the children. Completion of higher secondary classes or above was seen in very less number of mothers (15%) whereas 21% fathers were found to be qualified up to higher secondary class or above.

Occupational status of the parents of children showed that 69% mothers were housewives. Majority (60%) of fathers were occupied in service or small businesses whereas 40% were labourers.

Fifty seven percent children were exclusively breast-fed with more girls (61%) than boys (51%) and complete immunization was reported in 83% children.

**Table 5.4. Background information of the children under study**

<b>Categories</b>		<b>No.</b>	<b>%</b>
<b>Gender(n=218)</b>	Females	99	45.41
	Males	119	54.59
<b>Exclusive breast feeding</b>	Male	48	51
	Female	44	66
<b>Complete Immunization</b>	Male	76	81
	Female	57	85
<b>Type of family (n=161)</b>	Joint/ Extended	96	59.62
	Nuclear	65	40.37
<b>Income per month (Rs.) (n=161)</b>	<5000	93	57.76
	5000-1500	68	42.23
<b>Educational status of Fathers (n=161)</b>	Illiterate	23	14.3
	Primary/Secondary	104	64.7
	Higher secondary or above	34	20.9
<b>Educational status of Mothers (n=161)</b>	Illiterate	35	21.5
	Primary/Secondary	102	63.3
	Higher secondary or above	24	15
<b>Occupational status of fathers (n=161)</b>	Job/ Small Business	97	60
	Labourers	64	39.8
<b>Occupational status of mothers (n=161)</b>	Housewife	111	69.2
	Working	50	30.7

#### 5.1.4. Baseline Morbidity profile of the children under study

Past one month history of morbidity profile reveals that 33% of children suffered with diarrhea, 85% complained about stomach ache and 65 % had common colds in the past month (Table 5.5).

<b>Table 5.5. Morbidity profile of school going children(n=153)</b>				
<b>Parameters</b>	<b>Present</b>		<b>Absent</b>	
	<b>No</b>	<b>%</b>	<b>No</b>	<b>%</b>
Diarrhoea	51	33	102	67
Stomach ache	85	56	68	44
Flatulence	28	18	125	82
Constipation	33	22	120	78
Common colds	100	65	53	35

#### 5.1.5. Appetite profile of the children

Table 5.6 revealed significant differences in the appetite profile of the school going children as reported by their parents. Majority (63%) of severely undernourished children had “very low appetite” contrarily to which majority (70%) of nourished children reportedly had “normal appetite”.

Table 5.6. Appetite Profile Of Primary School Children(n=153)									
	Grade III		Grade II		Grade I		Nourished		$\chi^2$
	No.	%	No.	%	No.	%	No.	%	
Normal	2	13%	27	54%	21	42%	26	70%	38.24*** (p=0.000)
Low	4	25%	10	20%	14	28%	11	30%	
Very low	10	63%	13	26%	15	30%	0	0%	
<b>Total</b>	<b>16</b>		<b>50</b>		<b>50</b>		<b>37</b>		

\*\*\*=significant at 99.99%

#### 5.1.6. Exposure/Outcome Ratios (OR) between Socioeconomic and Health variables

Univariate analysis was used to evaluate the extent to which various socioeconomic factors and other variables have affected the nutritional status, incidence of diarrhea and common cold in children as presented in table 5.7, 5.8 and 5.9 respectively.

Gender wise analysis of the data revealed that odds of boys getting undernourished (OR=1.49), having diarrhea (OR= 1.21) and common colds (OR=1.21) were greater than girls as depicted in table 5.7, 5.8 and 5.9.

Data on type of family showed that children belonging to joint family had higher risk of developing undernutrition (OR=1.536) and common colds (OR=1.02) whereas children belonging to nuclear families were at greater risk of diarrhea (OR=1.246).

Parity level below two turned out to be a risk factor for undernutrition, diarrhea and common colds in the present study, as children born out of mothers with lower parity were seen at a greater risk of developing undernutrition (OR=2.910) and diarrhea (OR=1.913) and common colds (OR=1.12).

Exclusive breast feeding also appeared as an significant indicator for diarrhea and undernutrition as the odds of occurring diarrhea in children who did not had breast milk was significantly higher (OR=2.8) compared to those who had. Similarly undernutrition was more prevalent in children whose mother did not practiced exclusive breast feeding (OR=1.45).

Educational status of parents had a significantly thumping impact on the health status of children, as children belonging to illiterate father had significantly greater risk of undernutrition (OR=5.305), diarrhea (OR= 1.319) and common colds (1.04). More undernourished children belonged to illiterate mothers (OR=1.27) and also had significantly higher incidence of diarrhea (OR=3.160) and common colds (OR=1.528).

Occupational status of parents were also seen to be associated with well-being of children as, risk of undernutrition (OR=1.557) and diarrhea (OR=1.242) was found to be significantly higher in children belonging to father in service and business sectors than those belonging to father who were laborers whereas on a contrary, odds of occurrence of common colds (OR=1.29) was higher in children whose fathers were laborers. Children belonging to non-working mothers were

at greater risk of undernutrition (OR=1.808), diarrhea (OR=1.05) and common cold (OR= 1.01).

Total family income was found to be a significant determinant of undernutrition as, children belonging to families with total income up to Rs. 5000 per month were more undernourished (OR=2.541), had higher incidences of diarrhea (OR=1.11) and common colds (OR=2.207).

Immunization was also came out to be a determinant of health status of children as odds of getting undernourished (OR=1.63), having diarrhea (OR=1.86) and common colds (OR=1.66) were significantly greater in children who had nil or incomplete immunization status.

In the present research, children born out of mother with Normal Delivery were seen to be at higher risk of undernutrition (OR=1.179) and diarrhea (OR=1.869) and common colds (OR=1.27).



<b>Table 5.7. Exposure/Outcome Ratios (OR) between Socioeconomic and other variables and Under nutrition</b>				
<b>Variable</b>	<b>OR</b>	<b>95% CI</b>	<b><math>\chi^2</math></b>	<b>'p'Value</b>
Gender of child				
Male	<b>1.4947</b>	0.70-3.14	1.1249	0.288
Female	0.669	0.31-1.40	1.1249	0.288
Type of family				
Nuclear	0.6111	0.28-1.29	1.657	0.198
Joint/ Extended	<b>1.5364</b>	0.77-3.47	1.657	0.198
Birth order				
<2	<b>2.91</b>	1.22-6.93	6.1303	0.0132
> 2	0.0792	0.03-0.18	40.0293	0
Exclusive breastfed				
Yes	0.6676	0.23-1.90	0.573	0.4487
No	<b>1.4511</b>	0.50-4.15	0.484	0.486
Mother's education				
literate	0.7826	0.29-2.10	0.2368	0.6265
illiterate	<b>1.2778</b>	0.47-3.43	0.2368	0.62651
Father's education				
literate	0.1885	0.02-1.48	3.0818	0.0791
illiterate	<b>5.3053</b>	0.67-41.82	3.0818	0.0791
Mother's occupation				
Nonworking	<b>1.808</b>	0.82-3.93	2.2527	0.133
Working	0.5531	0.25-1.20	2.2527	0.133
Father's occupation				
Service/business	<b>1.5568</b>	0.72-3.35	1.28	0.25
Labourers	0.6424	0.29-1.38	1.28	0.25
Type of Delivery				
Normal	<b>1.1787</b>	0.47-2.92	0.1255	0.723
Caesarean	0.8484	0.34-2.10	0.1255	0.723
Total Family Income				
up to 5000	<b>2.541</b>	1.16-5.53	5.7103	0.016
5000-10000	0.393	0.18-0.85	5.7103	0.016
Immunization				
Complete	0.6124	0.19-1.93	0.7109	0.3991
Partial/Nil	<b>1.6328</b>	0.51-5.14	0.7109	0.3991

<b>Table 5.8. Exposure/Outcome Ratios (OR) between Socioeconomic and other variables and Diarrhoea</b>				
<b>Variable</b>	<b>OR</b>	<b>95% CI</b>	<b><math>\chi^2</math></b>	<b>'p'Value</b>
GENDER of child				
Male	<b>1.2129</b>	0.61-2.38	0.3143	0.575
Female	0.8245	0.41-1.61	0.3143	0.575
Type of family				
Nuclear	<b>1.2463</b>	0.6376-2.4361	0.4152	0.519
Joint/ Extended	0.8024	0.4105	0.4152	0.519
Birth order				
<2	<b>1.9133</b>	0.7678-4.767	1.984	0.15
> 2	0.5227	0.2098-1.3024	1.984	0.15
Exclusive breastfed				
Yes	0.3486	0.15-0.80	6.43	0.011
No	<b>2.869</b>	1.24-6.61	6.43	0.011
Mother's education				
literate	0.3165	0.1397-0.7168	8.0523	045
illiterate	<b>3.1597</b>	1.39-7.1563	8.0523	045
Father's education				
literate	0.7582	0.2544-2.26	0.2478	0.618
illiterate	<b>1.3188</b>	0.442-3.93	0.2478	0.618
Mother's occupation				
Nonworking	<b>1.0553</b>	0.5126-2.1725	0.0213	0.8839
Working	0.948	0.4603-1.9509	0.0213	0.8839
Father's occupation				
Service/business	<b>1.2418</b>	0.6133-2.5147	0.3626	0.547
Labourers	0.8053	0.3977-0.16306	0.3626	0.547
Type of Delivery				
Normal	<b>1.8688</b>	0.7504-4.6539	1.8427	0.1746
Caesarean	0.5816	0.2319-1.4591	1.8427	0.1746

Total Family Income				
up to 5000	<b>1.11</b>	0.56-2.16	0.0944	0.7586
5000-10000	0.9	0.46-1.75	0.0944	0.7586
Immunization				
Complete	0.5376	0.23-1.25	2.1124	0.146
Partial/Nil	<b>1.86</b>	0.79-4.32	2.1124	0.146

<b>Table5.9. Exposure/Outcome Ratios (OR) between Socioeconomic and other variables and Common Colds</b>				
<b>Variable</b>	<b>OR</b>	<b>95% CI</b>	<b><math>\chi^2</math></b>	<b>'p'Value</b>
GENDER of child				
Male	<b>1.1533</b>	0.59-2.21	0.183	0.668
Female	0.8671	0.45-1.66	0.183	0.668
Type of family				
Nuclear	0.9719	0.50-1.88	07	0.932
Joint/ Extended	<b>1.028</b>	0.53-1.99	07	0.932
Birth order				
<2	<b>1.12</b>	0.5022-2.4978	0.0767	0.781
> 2	0.8929	0.4004-1.9912	0.0767	0.781
Exclusive breastfed				
Yes	<b>1.0165</b>	0.43-2.38	014	0.9698
No	0.9837	0.42-2.30	014	0.9698
Mother's education				
literate	0.6545	0.2695-1.5899	0.8842	0.347
illiterate	<b>1.5278</b>	0.6290-3.7110	0.8842	0.347
Father's education				
literate	0.9574	0.3093-2.9639	057	0.9398
illiterate	<b>1.0444</b>	0.3374-3.2332	057	0.9398
Mother's occupation				
Nonworking	<b>1.0194</b>	0.5012-2.0733	028	0.957
Working	0.9809	0.4823-1.9950	028	0.957
Father's occupation				
Service/business	0.7705	0.3810-1.5579	0.5281	0.4674
Labourers	<b>1.2979</b>	0.6419-2.6244	0.5281	0.4674
Type of Delivery				
Normal	<b>1.2741</b>	0.5642-2.8772	0.3406	0.5594
Caesarean	0.7849	0.3476-1.7725	0.3406	0.5594
Total Family Income				
up to 5000	<b>2.207</b>	1.11-4.38	5.198	0.0226
5000-10000	0.453	0.22-0.90	5.198	0.0226
Immunization				
Complete	0.6	0.23-1.52	1.1756	0.278
Partial/Nil	<b>1.6616</b>	0.65-4.22	1.1756	0.278

### ***5.1.7. Association between nutritional status of children and their various health parameters***

To obtain the scenario of relationship between the grades of nutritional status and scores given to past occurrence of diseases like diarrhea, common colds, stomach ache, flatulence, constipation; educational status of parents of the children; type of delivery of the children; past breast feeding practice and immunization of the children, Spearman's correlation was exercised in order to identify best correlated variables.

The scores given to various parameters are mentioned in table 5.10; all the variables were scored accordingly and were then analyzed for *Spearman's rho*.

As seen in table 5.11 nutritional status showed strong positive correlation with total family income ( $p < 0.01$ ) and significantly positive correlation with child's appetite ( $p < 0.01$ ) also a negative correlation with incidence of common colds ( $p < 0.05$ ) indicating children with poor nutritional grade had poor appetite profile and higher incidences of common colds.

Total income was also found to be correlated with the incidence of common colds depicting that children from the families with better income had lesser occurrence of common colds. This variable was also significantly positively correlated with mother's education and occupation.

Low child's appetite as reported by the parents of the children showed significant correlations with the history of gastro intestinal complications.

Children with better appetite had lesser episodes of diarrhea, constipation, stomach ache and flatulence.

Past history of diarrhea showed significant correlations with child's appetite and exclusive breast feeding. It also showed significant relationships with the other morbidities viz. common colds, stomach ache and flatulence depicting the significance of complex morbidity. In the similar manner, incidence of stomach ache was found to be correlated with flatulence.

Cesarean delivery was found to be significantly correlated with higher educational status of parents.

**Table 5.10. Scores/grades given to various parameters for Spearman's correlation**

Parameters		Scores/Grades
Nutritional Status	Nourished	4
	Grade I Undernutrition -	3
	Grade II Undernutrition-	2
	Grade III Undernutrition-	1
Morbidity Profile[Diarrhoea, common cold, stomach-ache, flatulence and constipation]	Yes	1
	No	2
Educational status of parents	Illiterate and primary education	1
	Secondary and above	2
Immunization	In Complete	1
	Complete	2
Exclusive breast feeding	Not practiced	1
	Completed	2
Total family Income	<5000	1
	>5000	2
Child's appetite	Poor	1
	Moderate	2
	Good	3
Type of delivery	Normal	1
	Caesarean	2
Occupational status of Parents:		
Mothers	Housewife	1
	Working	2
Fathers	Labour and small business	1
	Job	2

**Table 5.11. Spearman's correlations between grades of nutrition and scores of various socio-economic and health parameters**

Parameters	Spearman's rho	Nutritional Status	Total Income	Child Appetite	Diarrhoea Y/N	Common Cold Y/N	Constipation Y/N	Flatulence Y/N	Stomach ache Y/N	Exclusive Breastfeeding Y/N	Immunization Complete/Incomplete	Type of Delivery	Father's Occupation	Father's Education	Mother's Occupation	Mother's Education	Type of Family Joint/Extended
Nutritional Status	r	1.0	<b>0.246**</b>	<b>0.289*</b>	0.154	<b>0.163*</b>	-0.023	0.085	0.148	0.028	0.034	0.117	-0.017	0.074	0.114	0.079	0.108
Total Income	r	<b>0.246**</b>	1.0	0.087	0.022	<b>0.248**</b>	0.104	0.088	0.017	0.017	0.057	0.087	0.113	0.021	<b>-0.201*</b>	<b>-0.197*</b>	0.117
Child Appetite	r	<b>0.289**</b>	0.087	1.0	<b>0.226**</b>	0.070	<b>0.186*</b>	0.194*	<b>0.167*</b>	0.017	0.146	-0.041	0.105	<b>0.186*</b>	0.107	0.061	-0.028
Diarrhoea Y/N	r	0.154	0.022	<b>0.226*</b>	1.0	<b>0.165*</b>	-0.133	<b>0.218**</b>	<b>0.272*</b>	<b>0.187*</b>	0.087	0.105	<b>-0.162*</b>	0.122	0.050	0.155	-0.028
Common Cold Y/N	r	<b>0.163*</b>	<b>0.248**</b>	0.070	<b>0.165*</b>	1.0	0.146	0.021	0.075	0.021	0.143	0.056	-0.121	0.018	-0.038	0.039	0.005
Constipation Y/N	r	-0.023	0.104	<b>0.186*</b>	-0.133	0.146	1.0	0.144	0.113	-0.037	<b>0.172*</b>	-0.023	0.074	0.014	-0.007	-0.094	-0.009
Flatulence Y/N	r	0.085	0.088	<b>0.194*</b>	<b>0.218**</b>	0.021	0.144	1.0	<b>0.370*</b>	0.102	0.059	0.025	0.136	0.072	0.044	0.049	-0.047
Stomach ache Y/N	r	0.148	0.017	<b>0.167*</b>	<b>0.272**</b>	0.075	0.113	<b>0.370**</b>	1.0	0.048	0.083	-0.024	0.028	0.011	0.110	0.011	0.068



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Exclusive Breastfeeding Y/N	r	0.028	0.017	0.017	<b>0.187*</b>	0.021	-0.037	0.102	0.048	10	0.149	0.102	-0.038	08	00	-0.037	0.048
Immunization Complete/Incomplete	r	0.034	0.057	0.146	0.087	0.143	<b>0.172*</b>	0.059	0.083	0.149	10	-0.087	-0.012	0.129	0.051	-0.028	-0.017
Type of Delivery	r	0.117	0.087	-0.041	0.105	0.056	-0.023	0.025	-0.024	0.102	-0.087	1.0	0.065	-0.079	0.035	<b>0.187*</b>	0.022
Father's Occupation	r	-0.017	0.113	0.105	<b>-0.162*</b>	-0.121	0.074	0.136	0.028	-0.038	-0.012	0.065	1.0	<b>0.188*</b>	-0.145	0.121	0.099
Father's Education	r	0.074	0.021	<b>0.186*</b>	0.122	0.018	0.014	0.072	0.011	0.008	0.129	-0.079	<b>0.188*</b>	1.0	0.067	<b>0.344**</b>	-0.014
Mother's Occupation	r	0.114	- <b>0.201*</b>	0.107	0.050	-0.038	-0.007	0.044	0.110	0.00	0.051	0.035	-0.145	0.067	1.0	0.088	<b>0.180*</b>
Mother's Education	r	0.079	- <b>0.197*</b>	0.061	0.155	0.039	-0.094	0.049	0.011	-0.037	-0.028	<b>0.187*</b>	0.121	<b>0.344**</b>	0.088	1.0	-0.144
Type of Family Joint/Extended	r	0.108	0.117	-0.028	-0.028	0.005	-09	-0.047	0.068	0.048	-0.017	0.022	0.099	-0.014	<b>0.180*</b>	-0.144	1.0

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*. Correlation is significant at the 0.01 level (2-tailed).

*Note: higher scores were allotted for absence of diarrhea, common colds, constipation, flatulence, and stomach ache*

### *Result Highlights of Phase I*

- ❖ 71% of the studied population was undernourished with 14%, 46% and 40% severe, moderate and mild undernutrition.
- ❖ More number of boys (73%) were undernourished than girls (69%).
- ❖ Following statistically significant contributors for undernutrition (mild, moderate, severe) were observed:
  - ✓ Joint/extended family (OR=1.53)
  - ✓ Not practicing exclusive breast feeding (OR=1.45)
  - ✓ Partial/Nil immunization status of child (OR=1.63)
  - ✓ Illiterate mother (OR=1.27)
  - ✓ Illiterate father (OR=5.30)
  - ✓ Low family income (OR=2.57)
- ❖ 33% of the children had diarrhea in the last month and the Statistically significant contributors for Diarrhea were:
  - ✓ Not practicing exclusive breast feeding (OR= 2.86)
  - ✓ Partial/Nil immunization status of child (OR= 1.86)
  - ✓ Illiterate mother (OR= 3.15)
  - ✓ Illiterate father (OR= 1.31)
  - ✓ Low family income (OR=1.11)
- ❖ 65% of the children suffered with Common colds and the statistically significant contributors for Common colds were:
  - ✓ Partial/Nil immunization status of child (OR= 1.66)
  - ✓ Illiterate mother (OR=1.52)
  - ✓ Low family income (OR= 2.20)
- ❖ Appetite had a greater impact on nutritional status and morbidity profile.

### **Phase II: Comparative analysis between undernourished and nourished school going children for their morbidity profile, gut microflora; serum IgA levels and dietary intake**

This phase of the study was carried out in order to explicit the differences of various parameters among nourished and undernourished children along with the distinctions present within the various grades of undernutrition.

#### ***5.2.1. Morbidity profile of nourished and undernourished school going children***

Table 5.12 reveals past 1 month medical history reported by the parents at the time of interview. Seventy Percent undernourished children suffered from common colds when compared with the nourished ones ( $p < 0.04$ ). With regards to stomach ache, 59.4% undernourished children reported to suffer compared to 43 % nourished children however, this difference was not statistically significant. Past one month morbidity history of severely undernourished children revealed higher incidences of Diarrhea (63%), Common colds (81%), Stomach ache (81%) and flatulence (25%) when compared to moderately and mildly undernourished children (Table 5.13).

Chi square test showed a statistically significant difference in the diarrheal episodes amongst the three grades of undernourished children with higher

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number of severely undernourished children who suffered from diarrhea. Contrary to this, almost double number of mildly undernourished children (36%) suffered with constipation compared to the other two grades of undernutrition ( $p < 0.03$ ).

<b>Table 5.12. Difference in morbidity profile of nourished and undernourished school going children</b>			
<b>Incidence of morbidity</b>	<b>Nourished (n=37)</b>	<b>Undernourished (n=116)</b>	<b><math>\chi^2</math>[p]</b>
	<b>Present No. (%)</b>	<b>Present No. (%)</b>	
Diarrhoea	12 (32)	39 (34)	0.02 [0.89]
Common Cold	19 (51)	81 (70)	<b>4.20* [0.04]</b>
Stomach ache	16 (43)	69 (59.4)	2.98 [0.08]
Flatulence	6 (16)	22 (19)	0.14 [0.70]
Constipation	5 (13.5)	28 (24)	1.86 [0.17]

\*= significant at 95%    \*\*=significant at 99%    \*\*\*=significant at 99.99%

<b>Table 5.13. Difference in morbidity profile of school going children with various grades of undernutrition</b>				
<b>Incidence of morbidity</b>	<b>Severe Undernutrition/Grade III [n= 16]</b>	<b>Moderate Undernutrition /Grade II [n= 50]</b>	<b>Mild Undernutrition/Grade I [n= 50]</b>	<b><math>\chi^2</math> [p]</b>
	<b>No. (%)</b>	<b>No. (%)</b>	<b>No. (%)</b>	
Diarrhoea	10 (62.5)	18 (36)	11 (22)	<b>9.13** [0.01]</b>
Common Cold	13 (81.25)	34 (68)	34 (68)	1.15 [0.56]
Stomach ache	13 (81.25)	27 (54)	29 (58)	3.81 [0.15]
Flatulence	4 (25)	11 (22)	7 (14)	1.48 [0.47]
Constipation	3 (18.75)	7 (14)	18 (36)	<b>6.90* [0.03]</b>

\*= significant at 95%    \*\*=significant at 99%    \*\*\*=significant at 99.99%

### 5.2.2. Gut-Microbiota of Nourished and Undernourished Children

In this section of study the researcher tried to look for the various distinctions, if present, in the gut microbiota of nourished and undernourished children in terms of pathogenic bacteria *i.e. E.coli* and favorable bacteria *i.e. Lactic acid bacteria* and *Bifidobacteria*.

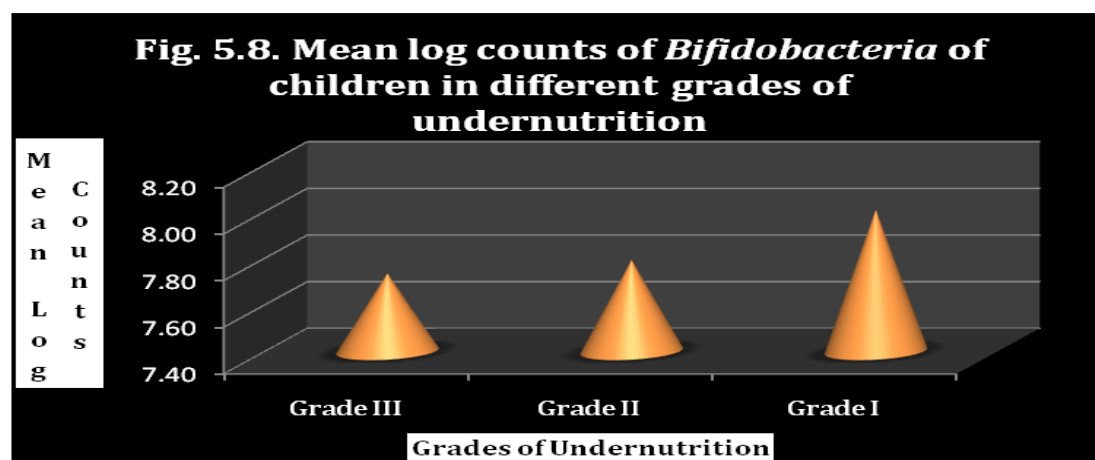
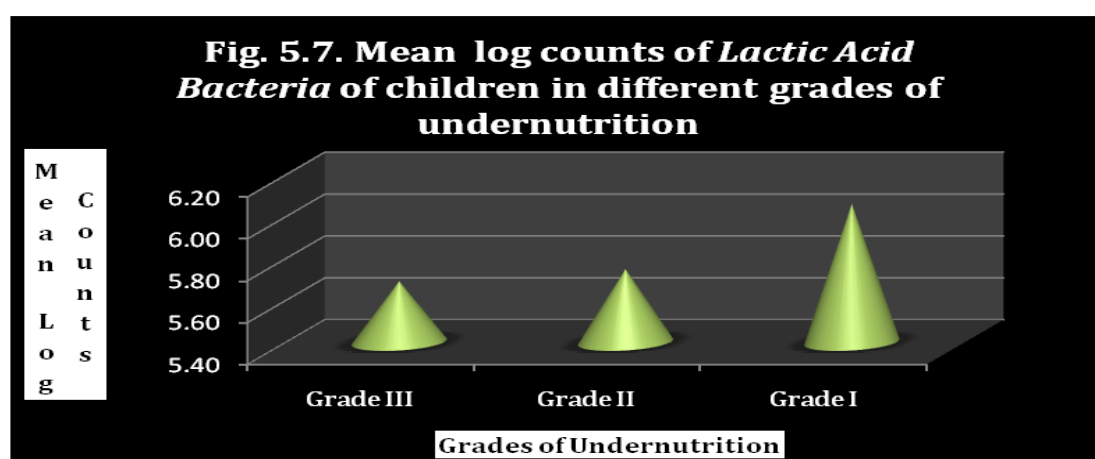
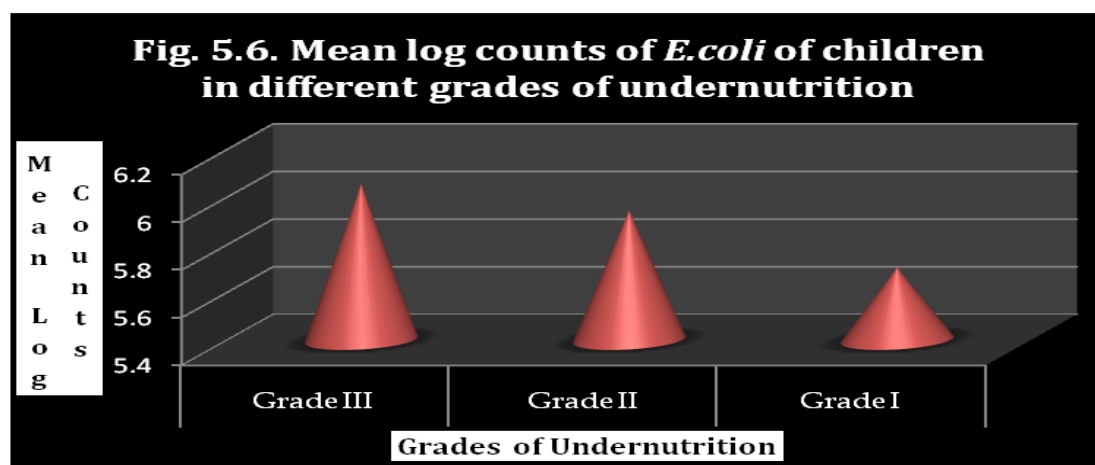
Student's 't' test, as presented in the table 5.14 determined that the gut of the nourished children colonized significantly higher number of favorable bacteria ( $p=0.000$ ) and significantly lower number of pathogenic bacteria ( $p=0.000$ ) when compared to undernourished children.

Table 5.14. Baseline gut microflora in nourished and undernourished school going children				
Gut flora	Category Mean values $\log_{10}$ CFU/g		Student's 't' test	'p' value
	Nourished (n= 30)	Undernourished (n= 80)		
<i>Lactic acid bacteria</i>	6.02 $\pm$ 0.21	5.83 $\pm$ 0.25	3.78***	0.000
<i>Bifidobacteria</i>	8.12 $\pm$ 0.13	7.84 $\pm$ 0.19	7.45***	0.000
<i>E.coli</i>	5.73 $\pm$ 0.23	5.89 $\pm$ 0.221	3.36**	0.001

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%



Further, analysis of variance in the mean log counts of gut microflora in different grades of undernutrition (table 5.15) revealed that all the three grades of

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undernutrition were significantly different with each other ( $p=0.000$ ) in terms of all the analyzed parameters of gut microbiota (Fig. 5.6; 5.7; 5.8).

Table 5.15. Analysis of variance in mean log counts (CFU/g) of gut microflora in different grades of undernutrition						
Parameters		n	Mean	Std. Deviation	F	Sig.
E.coli	Grade 1 Undernutrition	25	5.70	0.133	23.304***	0.000
	Grade 2 Undernutrition	41	5.94	0.199		
	Grade 3 Undernutrition	16	6.05	0.164		
<i>Lactic acid bacteria</i>	Grade 1 Undernutrition	25	6.06	0.253	20.941***	0.000
	Grade 2 Undernutrition	41	5.75	0.188		
	Grade 3 Undernutrition	16	5.69	0.192		
<i>Bifidobacteria</i>	Grade 1 Undernutrition	25	8.00	0.205	16.577***	0.000
	Grade 2 Undernutrition	41	7.79	0.119		
	Grade 3 Undernutrition	16	7.73	0.212		

\*\*\*=significant at 99.99%

Post-hoc (LSD) test (table 5.16) revealed, as the grade of nutrition deteriorates, colonization of pathogenic bacteria in the gut increases from 2% to 6% which was statistically significant ( $p=0.000$ ).

There was statistically significant linear decline in the counts of *Lactic acid bacteria* (6%-1%) and *Bifidobacteria* (3% -0.7%) from grade I to grade III undernutrition

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from, however these distinctions between grade II and grade III were statistically insignificant.

Table 5.16. Post Hoc Multiple Comparisons of Gut Microflora in different Grades of Undernutrition			
Parameters	Grade 1 Vs. 2	Grade 1 Vs. 3	Grade 2 Vs. 3
	% Difference [p]	% Difference [p]	% Difference [p]
<i>E.coli</i>	-4 [0.000***]	-6.1 [0.000***]	-1.85 [0.000***]
<i>Lactic acid bacteria</i>	5.12 [0.000***]	6.10 [0.000***]	1.04 [0.300]
<i>Bifidobacteria</i>	2.62 [0.000***]	3.37 [0.000***]	0.77 [0.200]

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%



### 5.2.3. Serum IgA levels of Nourished and Undernourished Children

High serum IgA level is said to be an important biochemical indicator of one's poor immunity standards. Therefore, Serum IgA levels of nourished and undernourished school going children, followed by trend analysis of serum IgA in different nutritional status, profile of children having abnormal IgA levels, were determined and statistically interpreted in this section of results chapter.

#### 5.2.3.1. Difference in mean serum IgA levels based on Nutritional status

Table 5.17 represents the mean serum IgA levels of school going children, Student's 't' test showed an insignificant difference in nourished and undernourished children across all the reference ranges. However, serum levels of IgA of undernourished children between the age of 5-7 years and 7-10 years were higher than the nourished children; but this difference was not statistically significant.

Table 5.17. Mean serum IgA levels (mg/dl) of nourished and undernourished school going children (5-12 y)				
Age Range	Reference Range	Nourished (n=32)	Undernourished (n=80)	"t"
5-7 y	29- 256 mg/ dl	98.9±36.31 [n=11]	112.16±45.77 [n=18]	0.86 NS
7-10 y	34-274 mg/ dl	134.89±43.58 [n=19]	138.76±72.52 [n=52]	0.27 NS
10-12 y	42-295 mg/ dl	192±19.79 [n=2]	163.2±70.20 [n=10]	1.09 NS

NS= Statistically Non-significant

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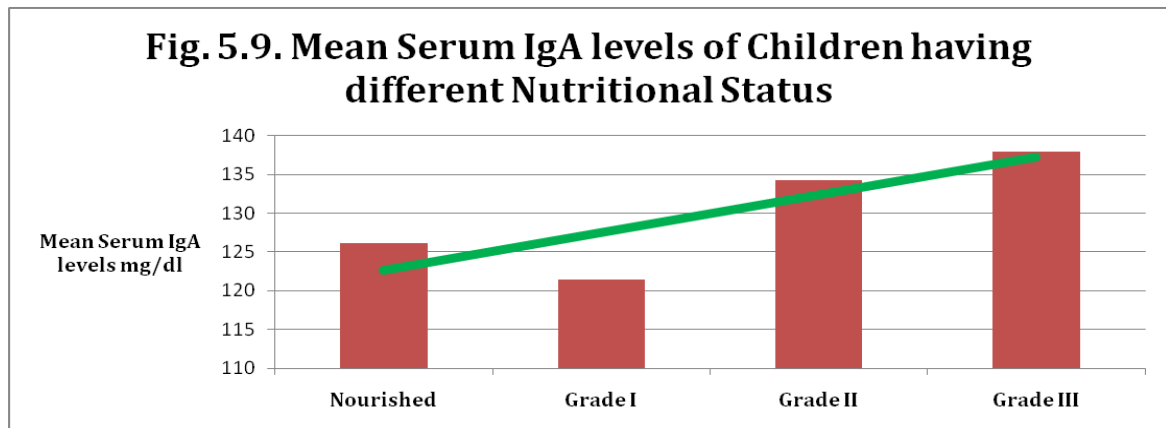
Serum IgA levels were further tested for analysis of variance (Table 5.18) among the various grades of nutritional status. Insignificant 'F' statistic revealed that mean serum levels of IgA across all the grades of nutritional status remained indifferent including severely undernourished children.

Table 5.18. Analysis of variance in mean serum IgA levels in different grades of nutritional status					
Nutritional Status	n	Mean	SD	F	Significance
Nourished	32	126.09	46.17	0.48 NS	0.70
-1 Undernutrition	15	121.40	36.06		
-2 Undernutrition	38	134.34	51.45		
-3 Undernutrition	15	138.00	50.24		

*NS= Statistically Non-significant*

### ***5.2.3.2. Trend of serum IgA levels in various grades of nutritional status***

Trend analysis of mean serum IgA levels showed a hike with corresponding deterioration of nutritional status except for mild undernutrition (Fig 5.9).



#### ***5.2.3.2. Profile of school going children with abnormal serum IgA levels***

Out of 112 (Undernourished=80 and Nourished=32) children whose serum levels were analyzed for IgA, six children found to be having abnormal IgA levels when compared with the normal range (29-295mg/dl) of serum IgA.

Abnormally low IgA in the cases studied, had higher incidences of diarrhea and common colds compared to cases with abnormally higher levels of serum IgA (Table 5.19 and 5.20).

Table. 5.19. Profile of Children having Abnormally Low Serum IgA Levels (mg/dl) [n= 3]						
Mean Nutritional Status	Mean Serum IgA Levels	Mean Diarrheal Episodes	Mean Common Cold Episodes	Mean Log counts of <i>E.coli</i>	Mean Log counts of <i>Lactic acid bacteria</i>	Mean Log counts of <i>Bifidobacteria</i>
-1.33 ± 0.58	8.33 ± 11.85	1.33 ± 1.15	1.33 ± 1.15	5.76 ± 0.13	5.81 ± 0.14	8.03 ± 0.32

Table. 5.20. Profile of Children having Abnormally High Serum IgA Levels (mg/dl) [n=3]						
Mean Nutritional Status	Mean Serum IgA Levels	Mean Diarrheal Episodes	Mean Common Cold Episodes	Mean Log counts of <i>E.coli</i>	Mean Log counts of <i>Lactic acid bacteria</i>	Mean Log counts of <i>Bifidobacteria</i>
-2 ± 1.0	352 ± 62.95	0.00 ± 0.00	0.66 ± 1.1	5.76 ± 0.13	5.65 ± 0.19	7.88 ± 0.11

However, this interpretation needs to be confirmed using a larger sample size.

### 5.2.4. Quartile based analysis of serum IgA levels

Due to the wide variations in the reference range of serum IgA, the values were analyzed based on the quartiles. Over-all profiles of children were assessed and predictors of BMI were determined based on the quartiles of serum IgA levels of school going children. Extreme values were excluded from the analysis.

Table 5.21 depicts the analysis of variance in different quartiles of serum IgA. Results showed that mean counts of pathogenic bacteria significantly differed among the quartiles. Frequency of diarrhea in the past month also found to be relevantly different in the children belonging to various quartiles of serum IgA levels.

Table 5.21. Analysis of variance in different parameters based on quartiles of serum IgA levels (mg/dl)					
Parameters	Quartiles	Range	Mean $\pm$ S.D	F	Significance
IgA	1st (n=26)	56-95 mg/dl	78.15 $\pm$ 12.6	150.09***	0.000
	2nd (n=25)	96-130 mg/dl	111.24 $\pm$ 10.91		
	3rd (n= 26)	131-159 mg/dl	143.07 $\pm$ 9.8		
	4th (n=23)	160-273 mg/dl	195.56 $\pm$ 36.34		
<i>E.coli</i>	1st (n=26)	56-95 mg/dl	5.79 $\pm$ 0.19	3.79**	0.01
	2nd (n=25)	96-130 mg/dl	5.78 $\pm$ 0.22		
	3rd (n= 26)	131-159 mg/dl	5.95 $\pm$ 0.26		
	4th (n=23)	160-273 mg/dl	5.92 $\pm$ 0.22		
<i>Lactic acid</i>	1st (n=26)	56-95 mg/dl	5.85 $\pm$ 0.27	0.2	0.89

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<i>bacteria</i>	2nd (n=25)	96-130 mg/dl	5.90 ± 0.27		
	3rd (n= 26)	131-159 mg/dl	5.90 ± 0.23		
	4th (n=23)	160-273 mg/dl	5.86 ± 0.26		
<i>Bifidobacteri a</i>	1st (n=26)	56-95 mg/dl	7.88 ± 0.24	0.38	0.76
	2nd (n=25)	96-130 mg/dl	7.90 ± 0.23		
	3rd (n= 26)	131-159 mg/dl	7.92 ± 0.19		
	4th (n=23)	160-273 mg/dl	7.94 ± 0.21		
Body mass index (for age and gender, WHO, 2007)	1st (n=26)	56-95 mg/dl	14.16 ± 1.28	1.14	0.33
	2nd (n=25)	96-130 mg/dl	14.23 ± 1.28		
	3rd (n= 26)	131-159 mg/dl	13.68 ± 0.90		
	4th (n=23)	160-273 mg/dl	14.25 ± 1.53		
Frequency of Diarrhea	1st (n=26)	56-95 mg/dl	0.62 ± 1.20	16.59**	0.00
	2nd (n=25)	96-130 mg/dl	0.80 ± 1.15		
	3rd (n= 26)	131-159 mg/dl	2.42 ± 0.75		
	4th (n=23)	160-273 mg/dl	0.74 ± 1.09		
Frequency of Common Colds	1st (n=26)	56-95 mg/dl	1.65 ± 1.41	0.49	0.68
	2nd (n=25)	96-130 mg/dl	1.80 ± 1.78		
	3rd (n= 26)	131-159 mg/dl	1.77 ± 2.26		
	4th (n=23)	160-273 mg/dl	2.30 ± 2.42		

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%

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Post hoc LSD test (Table 5.22) revealed that the mean log counts of *E.coli* were significantly different between 2<sup>nd</sup> and 3<sup>rd</sup> and 2<sup>nd</sup> and 4<sup>th</sup> quartiles whereas frequency of diarrhea differed between 1<sup>st</sup> and 3<sup>rd</sup>; 2<sup>nd</sup> and 3<sup>rd</sup> and 3<sup>rd</sup> and 4<sup>th</sup> quartile of serum IgA.

Table 5.22. Post-Hoc test for different parameters based on quartiles of serum IgA levels (mg/dl)						
Parameters/ Quartiles	Quart 1 vs. 2	Quart 1 vs. 3	Quart 1 vs. 4	Quart 2 vs. 3	Quart 2 vs. 4	Quart 3 vs. 4
Serum IgA levels (mg/ dl)	0.000	0.000	0.000	0.000	0.000	0.000
<i>E.coli</i>	0.92	<b>0.01**</b>	0.05	<b>0.008**</b>	<b>0.04*</b>	0.57
<i>Lactic acid bacteria</i>	0.57	0.53	0.95	0.95	0.63	0.59
<i>Bifidobacteria</i>	0.77	0.48	0.32	0.68	0.47	0.74
Body mass index (for age and gender, WHO, 2007)	0.84	0.17	0.81	0.12	0.96	0.12
Frequency of Diarrhea	0.53	<b>0.000***</b>	0.68	<b>0.000***</b>	0.34	<b>0.000***</b>
Frequency of Common Colds	0.79	0.89	0.25	0.95	0.38	0.35

\*= significant at 95%    \*\*=significant at 99%    \*\*\*=significant at 99.99%

### 5.2.4.1. First quartile of serum IgA levels of school going children

BMI of the children falling in the first quartile of serum IgA showed a significantly negative correlation with frequency of common colds and mean log counts of *E.coli* also, a significant positive correlation was seen between BMI and mean log counts of *Lactic acid bacteria* and *Bifidobacteria* (Table 5.23). This

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indicates higher the BMI lower incidences of common colds and lower counts of pathogenic bacteria and higher counts of favorable bacteria.

Table 5.23. Pearson's Correlations among various parameters based on 1st Quartile of Serum IgA								
Parameters		BMI	Frequency of Diarrhoea	Frequency of Common Colds	Serum IgA	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
BMI	'r'	1	0.15	<b>-0.444*</b>	-0.031	<b>-0.438*</b>	<b>0.469*</b>	<b>0.610**</b>
Frequency of Diarrhoea	'r'	0.159	1	0.248	-0.035	-0.246	0.047	-0.016
Frequency of Common Colds	'r'	<b>-0.444*</b>	0.248	1	0.052	0.049	-0.078	-0.309
Serum IgA	'r'	-0.031	-0.035	0.052	1	-0.067	-0.110	0.126
<i>E.coli</i>	'r'	<b>-0.438*</b>	-0.246	0.049	-0.067	1	-0.310	-0.205
<i>Lactic acid bacteria</i>	'r'	<b>0.469*</b>	0.047	-0.078	-0.110	-0.310	1	<b>0.417*</b>
<i>Bifidobacteria</i>	'r'	<b>0.610**</b>	-0.016	-0.309	0.126	-0.205	<b>0.417*</b>	1
*Correlation is significant at the 0.05 level (2-tailed).								
** Correlation is significant at the 0.01 level (2-tailed).								

Graphical representation of linear regression for predicting the regressors for BMI presented in Fig 5.10 reveals that *Bifidobacteria* positively regressed the BMI of the school going children whereas *E.coli* showed a contrary role (Table 5.24).

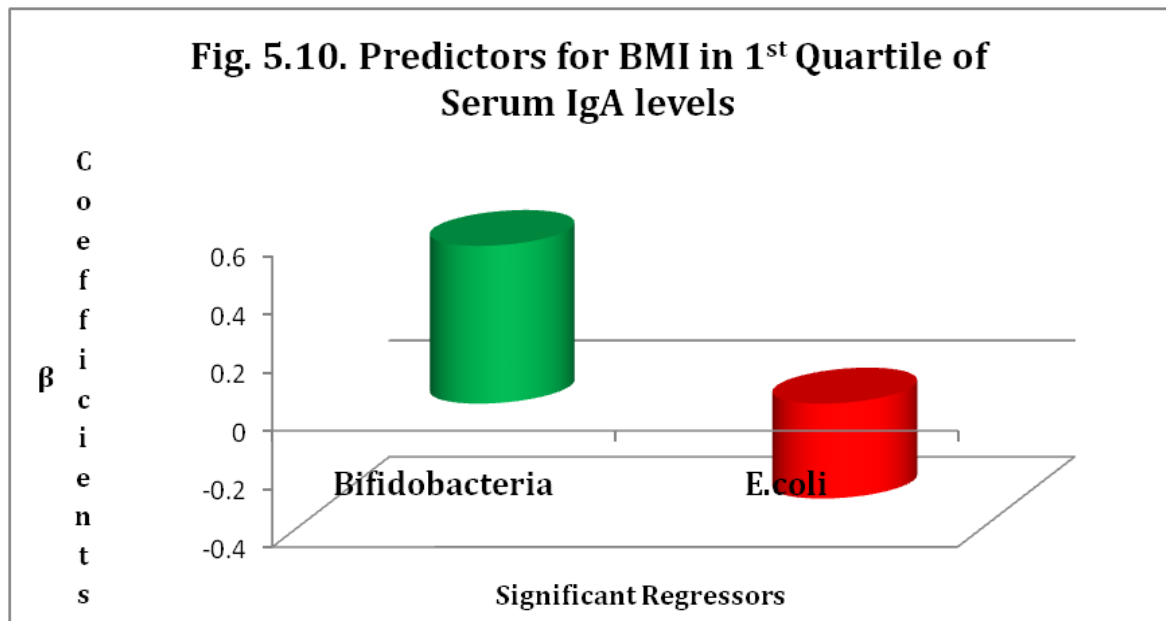


Table 5.24. Predictors for BMI in 1 <sup>st</sup> Quartile of Serum IgA levels				
Variables Entered	Adjusted R <sup>2</sup>	Significant Regressors		
		Name	Standardized $\beta$ coefficient	Significance
<i>Bifidobacteria</i>	42.9	<i>Bifidobacteria</i>	0.543	0.000***
Serum IgA		<i>E.coli</i>	-0.327	0.000***
<i>E.coli</i>				
<i>Lactic acid bacteria</i>				
Frequency of Diarrhoea				
Frequency of Common colds				

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%



#### 5.2.4.2. Second quartile of serum IgA levels of school going children

A significant negative correlation was seen between mean counts of *Bifidobacteria* and past incidence of diarrhea whereas significant positive correlation was found

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between frequency of common colds and mean log counts of *E.coli* negatively significant correlation was also seen between counts of *E.coli* and *Bifidobacteria*. All other significant correlations were same as of first quartile (Table 5.25).

Table 5.25. Pearson's Correlations among various parameters based on 2nd Quartile of Serum IgA								
Parameters		BMI	Frequency of Diarrhoea	Frequency of Common Colds	Serum IgA	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
BMI	'r'	1	-0.295	<b>-0.552**</b>	-0.272	<b>-0.632**</b>	<b>0.507**</b>	<b>0.689**</b>
Frequency of Diarrhoea	'r'	-0.295	1	0.365	0.116	0.211	-0.369	<b>-0.498*</b>
Frequency of Common Colds	'r'	<b>-0.552**</b>	0.365	1	0.183	<b>0.445*</b>	-0.060	-0.327
Serum IgA	'r'	-0.272	0.116	0.183	1	0.113	-0.015	-0.206
<i>E.coli</i>	'r'	<b>-0.632**</b>	0.211	<b>0.445*</b>	0.113	1	-0.320	-0.386
<i>Lactic acid bacteria</i>	'r'	<b>0.507**</b>	-0.369	-0.060	-0.015	-0.320	1	<b>0.503*</b>
<i>Bifidobacteria</i>	'r'	<b>0.689**</b>	<b>-0.498*</b>	-0.327	-0.206	-0.386	<b>0.503*</b>	1
** Correlation is significant at the 0.01 level (2-tailed).								
* Correlation is significant at the 0.05 level (2-tailed).								

Linear regression of second quartile showed 43% of prediction level ( $R^2 = 42.92$ ). Mean log counts of *E.coli* and *Bifidobacteria* appeared as significant regressors, even in the second quartile of serum IgA (Table 5.26, Fig. 11).

**Table 5.26. Predictors for BMI in 2<sup>nd</sup> Quartile of Serum IgA levels**

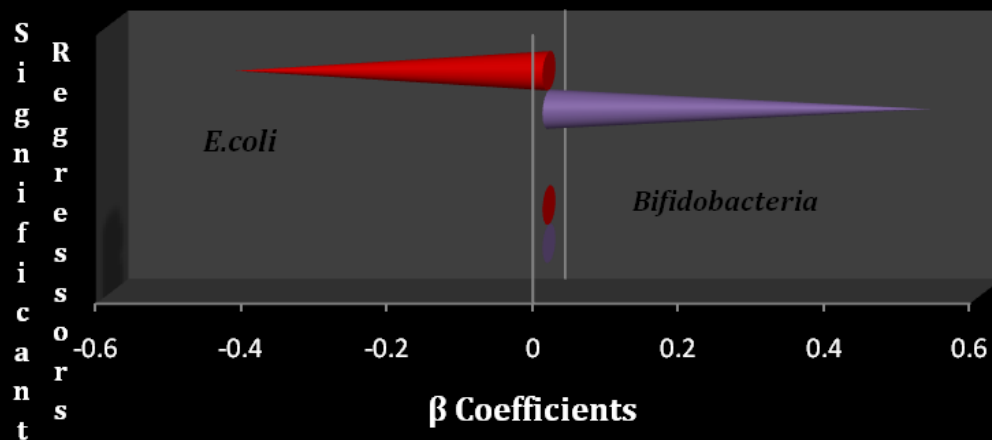
Variables Entered	Adjusted R <sup>2</sup>	Significant Regressors		
		Name	Standardized $\beta$ coefficient	Significance
<i>Bifidobacteria</i>	42.9	<i>Bifidobacteria</i>	0.523	<b>0.000***</b>
Serum IgA		<i>E.coli</i>	-0.43	<b>0.000***</b>
<i>E.coli</i>				
<i>Lactic acid bacteria</i>				
Frequency of Diarrhoea				
Frequency of Common colds				

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%

**Fig. 5.11. Predictors for BMI in 2<sup>nd</sup> Quartile of Serum IgA levels**



### 5.2.4.3. Third quartile of serum IgA levels of school going children

As seen in table 5.27 Children suffering with common colds had lower counts of *Lactic acid bacteria* in the third quartile of serum IgA unlike the previous two quarts. Relevantly negative relationship was also seen in this quart between mean log counts of *E.coli* and *Lactic acid bacteria*.

Table 5.27. Pearson's Correlations among various parameters based on 3rd Quartile of Serum IgA								
Parameters		BMI	Frequency of Diarrhoea	Frequency of Common Colds	Serum IgA	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
BMI	'r'	1	-0.065	-0.338	0.007	<b>-0.397*</b>	<b>0.468*</b>	<b>0.573**</b>
Frequency of Diarrhoea	'r'	-0.065	1	-0.177	-0.160	0.211	-0.013	-0.189
Frequency of Common Colds	'r'	-0.338	-0.177	1	-0.119	-0.131	<b>-0.540**</b>	-0.073
Serum IgA	'r'	0.007	-0.160	-0.119	1	-0.041	0.056	-0.130
<i>E.coli</i>	'r'	<b>-0.397*</b>	0.211	-0.131	-0.041	1	-0.110	-0.216
<i>Lactic acid bacteria</i>	'r'	<b>0.468*</b>	-0.013	<b>-0.540**</b>	0.056	-0.110	1	0.275
<i>Bifidobacteria</i>	'r'	<b>0.573**</b>	-0.189	-0.073	-0.130	-0.216	0.275	1
* Correlation is significant at the 0.05 level (2-tailed).								
** Correlation is significant at the 0.01 level (2-tailed).								

Stepwise Linear regression in the third quartiles (Table 5.27) for the predictors of BMI showed only *Bifidobacteria* count as a significant regressor (Fig. 5.12).

**Table 5.28. Predictors for BMI in 3<sup>rd</sup> Quartile of Serum IgA levels**

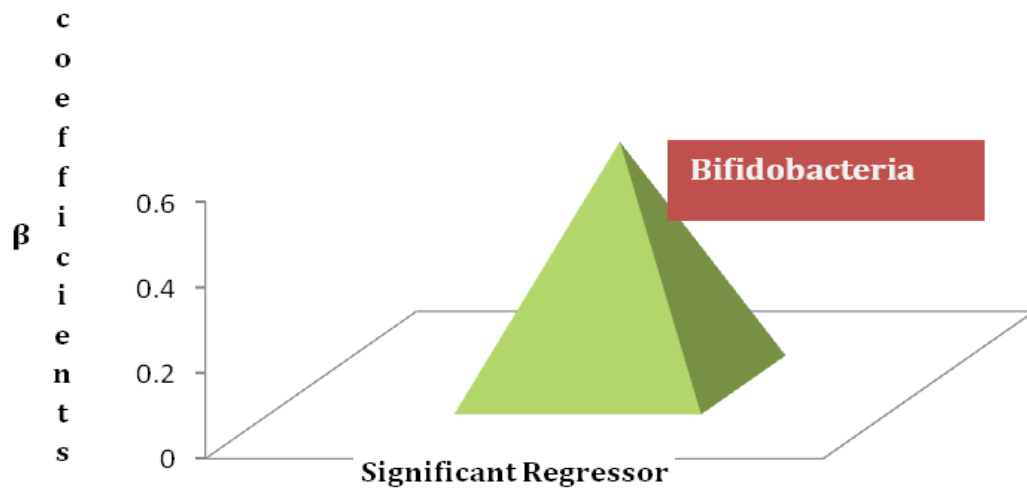
Variables Entered	Adjusted R <sup>2</sup>	Significant Regressors		Significance
		Name	Standardized $\beta$ coefficients	
<i>Bifidobacteria</i>	30.00	<i>Bifidobacteria</i>	0.57	<b>0.002**</b>
Serum IgA				
<i>E.coli</i>				
<i>Lactic acid bacteria</i>				
Frequency of Diarrhoea				
Frequency of Common colds				

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%

**Fig. 5.12. Predictor for BMI in 3<sup>rd</sup> Quartile of Serum IgA levels**



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### 5.2.4.4. Fourth quartile of serum IgA levels of school going children

Significant relationship between mean log counts of *E.coli* and serum, IgA levels was observed in the fourth quartile (Table 5.29) which was not present in all the previous analyzed quarts. *E.coli* showed relevantly negative relationship with both the favorable bacteria viz. *Lactic acid bacteria* and *Bifidobacteria*.

Table 5.29. Pearson's Correlations among various parameters based on 4th Quartile of Serum IgA								
Parameters		BMI	Frequency of Diarrhoea	Frequency of Common Colds	Serum IgA	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
BMI	'r'	1	-0.247	0.255	-0.261	-0.503*	0.627**	0.797**
Frequency of Diarrhea	'r'	-0.247	1	0.052	0.320	0.397	-0.182	-0.245
Frequency of Common Colds	'r'	0.255	0.052	1	-0.330	-0.292	0.090	0.099
Serum IgA	'r'	-0.261	0.320	-0.330	1	0.463*	-0.124	-0.242
<i>E.coli</i>	'r'	-0.503*	0.397	-0.292	0.463*	1	-0.444*	-0.391
<i>Lactic acid bacteria</i>	'r'	0.627**	-0.182	0.090	-0.124	-0.444*	1	0.499*
<i>Bifidobacteria</i>	'r'	0.797**	-0.245	0.099	-0.242	-0.391	0.499*	1
* Correlation is significant at the 0.05 level (2-tailed).								
** Correlation is significant at the 0.01 level (2-tailed).								

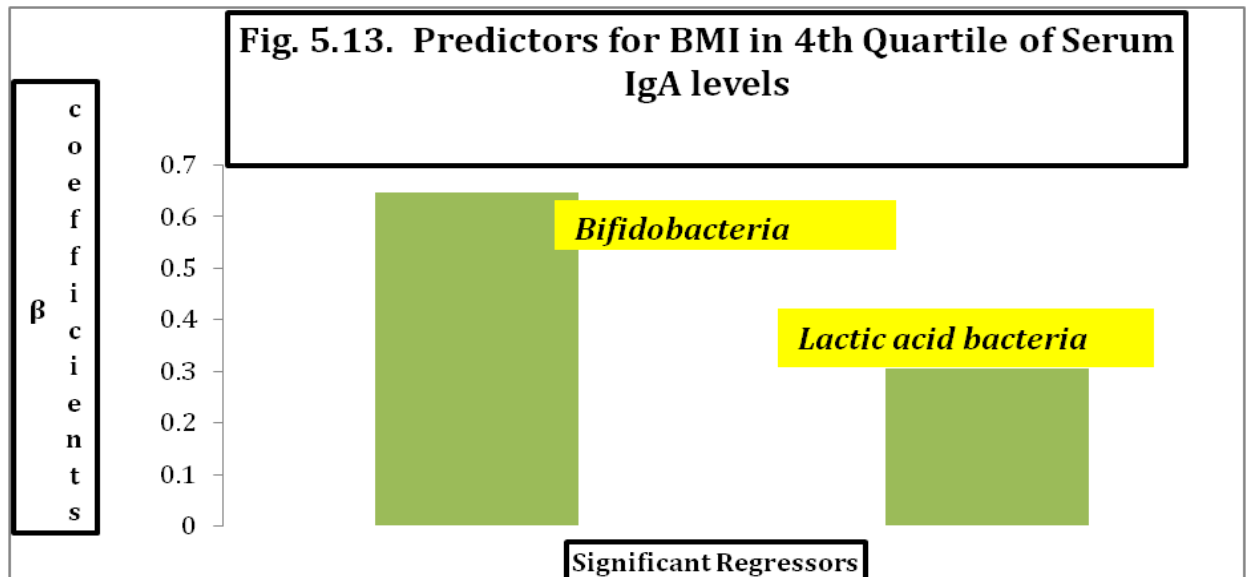
Regression model with prediction level of almost 68% ( $R^2 = 67.6$ ) showed *Bifidobacteria* and *Lactic acid bacteria* as significant predictors of BMI, this observation unlike in previous quarts (Table 5.30, Fig. 5.13).

Table 5.30. Predictors for BMI in 4 <sup>th</sup> Quartile of Serum IgA levels				
Variables Entered	Adjusted R <sup>2</sup>	Significant Regressors		Significance
		Name	Standardized $\beta$ coefficients	
<i>Bifidobacteria</i>	67.60	<i>Bifidobacteria</i>	0.645	<b>0.000***</b>
Serum IgA		<i>Lactic acid bacteria</i>	0.305	<b>0.042*</b>
<i>E.coli</i>				
<i>Lactic acid bacteria</i>				
Frequency of Diarrhea				
Frequency of Common colds				

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%



### 5.2.5. Dietary Intake of Nourished and Undernourished Children

As seen in table 5.31 mean intake of energy; macro nutrients *viz.* carbohydrates, protein, fat, micronutrient *viz.* iron, calcium, zinc; vitamin C and total dietary fiber of nourished children was significantly higher than undernourished children ( $p=0.000$ ).

<b>Table 5.31. Difference in the mean nutrient of nourished and undernourished children under study and its comparison with the recommended dietary allowances</b>					
Nutrients	#Average RDA (5-12 years)	Undernourished (n = 80) Mean $\pm$ SD	Nourished (n = 30 ) Mean $\pm$ SD	% Difference	"t" value (p Value)
Energy (Kcal/d)	1810 $\pm$ 370	1457 $\pm$ 426	2215 $\pm$ 961	34.22	7.13*** (0.000)
Carbohydrates (g/d)	NA	196 $\pm$ 55	288 $\pm$ 129.3	31.94	6.45*** (0.000)
Protein (g/d)	32 $\pm$ 10	37 $\pm$ 12.89	58 $\pm$ 25.96	36.20	7.3*** (0.000)
\$Total Fat (g/d)	57 $\pm$ 4	56 $\pm$ 24.00	89 $\pm$ 47.18	37	6.04*** (0.000)
Total dietary fibre (g/d)	NA	8.80 $\pm$ 5.17	19.80 $\pm$ 21.77	55.55	4.62*** (0.000)
Iron (mg/d)	19 $\pm$ 6	10.60 $\pm$ 4.48	17.06 $\pm$ 11.44	37.86	5.12*** (0.000)
Calcium (mg/d)	700 $\pm$ 115	495 $\pm$ 403	872.42 $\pm$ 597.7	43.26	5.49*** (0.000)
Zinc (mg/d)	8.25 $\pm$ 0.95	3.89 $\pm$ 1.5	5.85 $\pm$ 2.95	33.50	5.93*** (0.000)
Vitamin C (mg/d)	40 $\pm$ 0	48 $\pm$ 39.32	98.07 $\pm$ 81.41	51.05	5.46*** (0.000)

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%

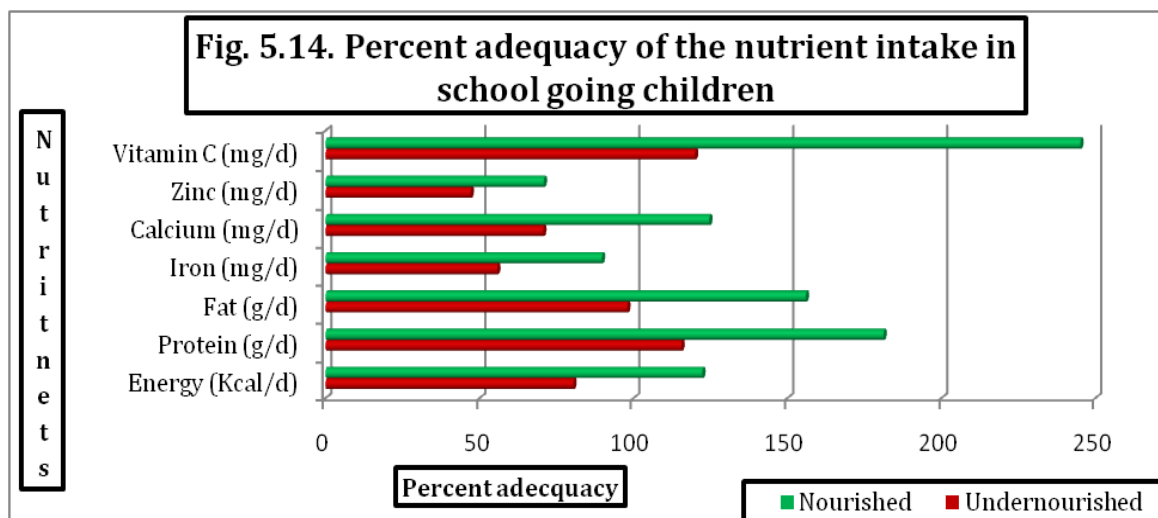
#- mean of RDA for the age group of 4-12 years

\$- Average consumption of total fat was calculated as 25 g (+ 32g visible fat) per child including milk fat.



### 5.2.5.1. Percent adequacy of the diet of nourished and undernourished school going children

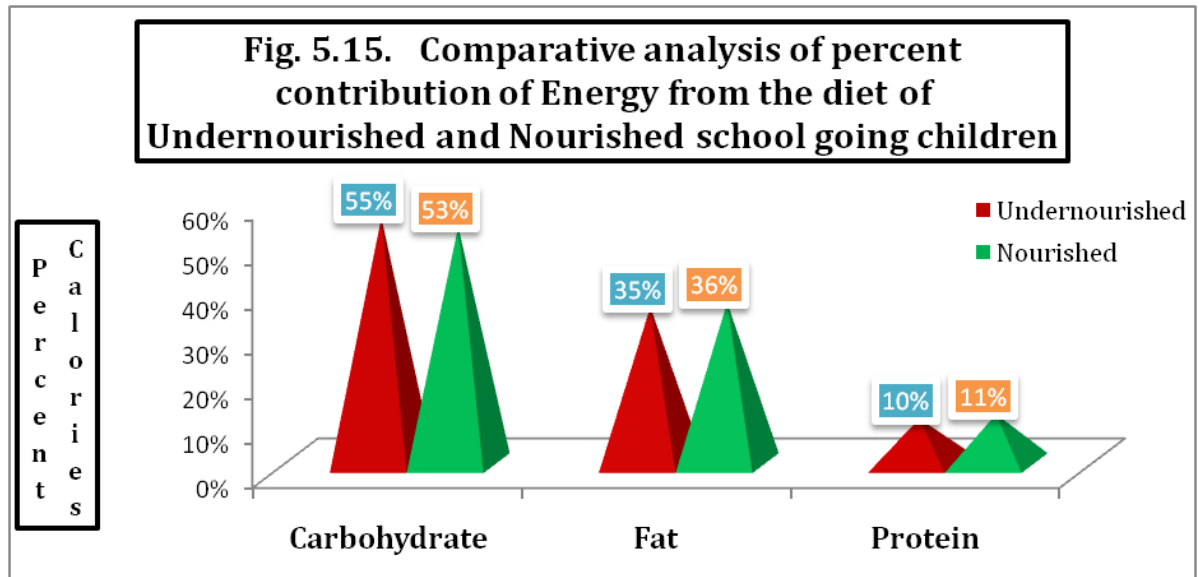
When compared with the average recommended dietary allowances for the age group of 4-12 years, undernourished children observed to be consuming lesser calories, iron, calcium and zinc however, their intake of protein, fat and vitamin C was higher than the recommended values. Nourished children reported to be consuming higher amounts of almost all the nutrients except zinc and iron than the recommended allowance (Fig. 5.14).



### 5.2.5.2. Calorific distribution of the diet of nourished and undernourished children

Calories derived from various macronutrients did not differ in the nourished and undernourished children. Carbohydrate contributed 55% and 53%; protein

contributed 10% and 11%; and fat contributed 35% and 36% in the diet of undernourished and nourished children respectively (Fig. 5.15).



### 5.2.5.3 Associations between various dietary components, nutritional status, morbidity profile and biochemical parameters of school going children

In order study the impact of dietary intake on various health variables, Pearson's correlation was exercised. As seen in table 5.32 to 5.41, intake of all the calculated nutrients was strongly correlated with the body mass index and grades of nutritional status.

Children who consumed higher amount of energy, protein, fat, carbohydrates, calcium, iron, and zinc had higher log counts of *Bifidobacteria*. Total dietary fiber

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and zinc intake was inversely proportional to the log count of pathogenic bacteria i.e. *E.coli*.

Higher consumption of calcium significantly associated with higher levels of serum IgA (Table 5.41).

**Table. 5.32. Association between Energy intake and nutritional status, morbidity profile and gut microflora of school going children**

Parameters	Energy [Kcal]	BMI	Nutritional Status	Diarrhoea Incidence	Common cold Incidence	Stomach ache Incidence	Flatulence Incidence	Constipation Incidence	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
Energy [Kcal]	"r" 1	<b>.530**</b>	<b>.527**</b>	.009	-.140	-.009	-.001	-.057	-.166	.164	.310**
BMI	"r" <b>.530**</b>	1	<b>.721**</b>	<b>-.221*</b>	<b>-.268*</b>	<b>-.247*</b>	-.111	-.065	<b>-.484**</b>	<b>.484**</b>	<b>.672**</b>
Nutritional Status	"r" <b>.527**</b>	.721**	1	-.159	<b>-.226*</b>	-.213	-.138	-.202	<b>-.314**</b>	<b>.284**</b>	<b>.597**</b>
Diarrhoea Incidence	"r" .009	<b>-.221*</b>	-.159	1	.001	<b>.262*</b>	<b>.222*</b>	-.134	.250*	-.138	-.210
Common cold Incidence	"r" -.140	<b>-.268*</b>	<b>-.226*</b>	.001	1	.021	.098	.185	.094	<b>-.307**</b>	<b>-.218*</b>
Stomach ache Incidence	"r" -.009	<b>-.247*</b>	-.213	<b>.262*</b>	.021	1	<b>.371**</b>	.062	<b>.229*</b>	-.062	-.164
Flatulence Incidence	"r" -.001	-.111	-.138	<b>.222*</b>	.098	<b>.371**</b>	1	<b>.251*</b>	-.059	.084	-.188
Constipation Incidence	"r" -.057	-.065	-.202	-.134	.185	.062	<b>.251*</b>	1	.033	.015	-.009
<i>E.coli</i>	"r" -.166	<b>-.484**</b>	<b>-.314**</b>	<b>.250*</b>	.094	.229*	-.059	.033	1	<b>-.282**</b>	<b>-.333**</b>
<i>Lactic acid bacteria</i>	"r" .164	<b>.484**</b>	<b>.284**</b>	-.138	<b>-.307**</b>	-.062	.084	.015	<b>-.282**</b>	1	<b>.370**</b>
<i>Bifidobacteria</i>	"r" <b>.310**</b>	<b>.672**</b>	<b>.597**</b>	-.210	<b>-.218*</b>	-.164	-.188	-.009	<b>-.333**</b>	<b>.370**</b>	1

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

**Table. 5.33. Association between Carbohydrates intake and nutritional status, morbidity profile and gut microflora of school going children**

Parameters		Carbohydrates [gms]	BMI	Nutritional Status	Diarrhoea Incidence	Common cold Incidence	Stomach ache Incidence	Flatulence Incidence	Constipation Incidence	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
Carbohydrates [gms]	Pearson Correlation	1	<b>.506**</b>	<b>.494**</b>	-.049	-.165	-.016	.040	-.061	-.207	.150	<b>.291**</b>
BMI	Pearson Correlation	<b>.506**</b>	1	<b>.721**</b>	<b>-.221*</b>	<b>-.268*</b>	<b>-.247*</b>	-.111	-.065	<b>-.484**</b>	<b>.484**</b>	<b>.672**</b>
Nutritional Status	Pearson Correlation	<b>.494**</b>	<b>.721**</b>	1	-.159	<b>-.226*</b>	-.213	-.138	-.202	<b>-.314**</b>	<b>.284**</b>	<b>.597**</b>
Diarrhoea Incidence	Pearson Correlation	-.049	<b>-.221*</b>	-.159	1	.001	<b>.262*</b>	<b>.222*</b>	-.134	<b>.250*</b>	-.138	-.210
Common cold Incidence	Pearson Correlation	-.165	<b>-.268*</b>	<b>-.226*</b>	.001	1	.021	.098	.185	.094	<b>-.307**</b>	<b>-.218*</b>
Stomach ache Incidence	Pearson Correlation	-.016	<b>-.247*</b>	-.213	<b>.262*</b>	.021	1	<b>.371**</b>	.062	<b>.229*</b>	-.062	-.164
Flatulence Incidence	Pearson Correlation	.040	-.111	-.138	<b>.222*</b>	.098	<b>.371**</b>	1	<b>.251*</b>	-.059	.084	-.188
Constipation Incidence	Pearson Correlation	-.061	-.065	-.202	-.134	.185	.062	<b>.251*</b>	1	.033	.015	-.009
<i>E.coli</i>	Pearson Correlation	-.207	<b>-.484**</b>	<b>-.314**</b>	<b>.250*</b>	.094	<b>.229*</b>	-.059	.033	1	<b>-.282**</b>	<b>-.333**</b>
<i>Lactic acid bacteria</i>	Pearson Correlation	.150	<b>.484**</b>	<b>.284**</b>	-.138	<b>-.307**</b>	-.062	.084	.015	<b>-.282**</b>	1	<b>.370**</b>
<i>Bifidobacteria</i>	Pearson Correlation	<b>.291**</b>	<b>.672**</b>	<b>.597**</b>	-.210	<b>-.218*</b>	-.164	-.188	-.009	<b>-.333**</b>	<b>.370**</b>	1

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

**Table. 5.34. Association between Protein intake and nutritional status, morbidity profile and gut microflora of school going children**

Parameters		Protein [gms]	BMI	Nutritional Status	Diarrhoea Incidence	Common cold Incidence	Stomach ache Incidence	Flatulence Incidence	Constipation Incidence	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
Protein [gms]	'r'	1	<b>.529**</b>	<b>.549**</b>	.025	-.107	-.011	-.035	-.064	-.186	.157	<b>.353**</b>
BMI	'r'	<b>.529**</b>	1	<b>.721**</b>	<b>-.221*</b>	<b>-.268*</b>	<b>-.247*</b>	-.111	-.065	<b>-.484**</b>	<b>.484**</b>	<b>.672**</b>
Nutritional Status	'r'	<b>.549**</b>	<b>.721**</b>	1	-.159	<b>-.226*</b>	-.213	-.138	-.202	<b>-.314**</b>	<b>.284**</b>	<b>.597**</b>
Diarrhoea Incidence	'r'	.025	<b>-.221*</b>	-.159	1	.001	<b>.262*</b>	<b>.222*</b>	-.134	<b>.250*</b>	-.138	-.210
Common cold Incidence	'r'	-.107	<b>-.268*</b>	<b>-.226*</b>	.001	1	.021	.098	.185	.094	<b>-.307**</b>	<b>-.218*</b>
Stomach ache Incidence	'r'	-.011	<b>-.247*</b>	-.213	<b>.262*</b>	.021	1	<b>.371**</b>	.062	<b>.229*</b>	-.062	-.164
Flatulence Incidence	'r'	-.035	-.111	-.138	<b>.222*</b>	.098	<b>.371**</b>	1	<b>.251*</b>	-.059	.084	-.188
Constipation Incidence	'r'	-.064	-.065	-.202	-.134	.185	.062	<b>.251*</b>	1	.033	.015	-.009
<i>E.coli</i>	'r'	-.186	<b>-.484**</b>	<b>-.314**</b>	<b>.250*</b>	.094	<b>.229*</b>	-.059	.033	1	<b>-.282**</b>	<b>-.333**</b>
<i>Lactic acid bacteria</i>	'r'	.157	<b>.484**</b>	<b>.284**</b>	-.138	<b>-.307**</b>	-.062	.084	.015	<b>-.282**</b>	1	<b>.370**</b>
<i>Bifidobacteria</i>	'r'	<b>.353**</b>	<b>.672**</b>	<b>.597**</b>	-.210	<b>-.218*</b>	-.164	-.188	-.009	<b>-.333**</b>	<b>.370**</b>	1

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

**Table. 5.35. Association between Fat intake and nutritional status, morbidity profile and gut microflora of school going children**

Parameters		Fat [gms]	BMI	Nutritional Status	Diarrhoea Incidence	Common cold Incidence	Stomach ache Incidence	Flatulence Incidence	Constipation Incidence	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
Fat [gms]	"r"	1	<b>.461**</b>	<b>.467**</b>	.083	-.106	.014	-.027	-.063	-.098	.156	<b>.254*</b>
BMI	"r"	<b>.461**</b>	1	<b>.721**</b>	<b>-.221*</b>	<b>-.268*</b>	<b>-.247*</b>	-.111	-.065	<b>-.484**</b>	<b>.484**</b>	<b>.672**</b>
Nutritional Status	"r"	<b>.467**</b>	<b>.721**</b>	1	-.159	<b>-.226*</b>	-.213	-.138	-.202	<b>-.314**</b>	<b>.284**</b>	<b>.597**</b>
Diarrhoea Incidence	"r"	.083	<b>-.221*</b>	-.159	1	.001	<b>.262*</b>	<b>.222*</b>	-.134	.250*	-.138	-.210
Common cold Incidence	"r"	-.106	<b>-.268*</b>	<b>-.226*</b>	.001	1	.021	.098	.185	.094	<b>-.307**</b>	<b>-.218*</b>
Stomach ache Incidence	"r"	.014	<b>-.247*</b>	-.213	<b>.262*</b>	.021	1	<b>.371**</b>	.062	<b>.229*</b>	-.062	-.164
Flatulence Incidence	"r"	-.027	-.111	-.138	<b>.222*</b>	.098	<b>.371**</b>	1	<b>.251*</b>	-.059	.084	-.188
Constipation Incidence	"r"	-.063	-.065	-.202	-.134	.185	.062	<b>.251*</b>	1	.033	.015	-.009
<i>E.coli</i>	"r"	-.098	<b>-.484**</b>	<b>-.314**</b>	<b>.250*</b>	.094	<b>.229*</b>	-.059	.033	1	<b>-.282**</b>	<b>-.333**</b>
<i>Lactic acid bacteria</i>	"r"	.156	<b>.484**</b>	<b>.284**</b>	-.138	<b>-.307**</b>	-.062	.084	.015	<b>-.282**</b>	1	<b>.370**</b>
<i>Bifidobacteria</i>	"r"	<b>.254*</b>	<b>.672**</b>	<b>.597**</b>	-.210	<b>-.218*</b>	-.164	-.188	-.009	<b>-.333**</b>	<b>.370**</b>	1

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

**Table. 5.36. Association between Total dietary fibre intake and nutritional status, morbidity profile and gut microflora of school going children**

Parameters		Total Dietary Fibre [gms]	BMI	Nutritional Status	Diarrhoea Incidence	Common cold Incidence	Stomach ache Incidence	Flatulence Incidence	Constipation Incidence	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
Total Dietary Fibre [gms]	"r"	1	<b>.391**</b>	<b>.435**</b>	-.031	-.142	.165	<b>.283**</b>	-.107	<b>-.215*</b>	.198	.179
BMI	"r"	<b>.391**</b>	1	<b>.721**</b>	<b>-.221*</b>	<b>-.268*</b>	<b>-.247*</b>	-.111	-.065	<b>-.484**</b>	<b>.484**</b>	<b>.672**</b>
Nutritional Status	"r"	<b>.435**</b>	<b>.721**</b>	1	-.159	<b>-.226*</b>	-.213	-.138	-.202	<b>-.314**</b>	<b>.284**</b>	<b>.597**</b>
Diarrhoea Incidence	"r"	-.031	<b>-.221*</b>	-.159	1	.001	<b>.262*</b>	<b>.222*</b>	-.134	<b>.250*</b>	-.138	-.210
Common cold Incidence	"r"	-.142	<b>-.268*</b>	<b>-.226*</b>	.001	1	.021	.098	.185	.094	<b>-.307**</b>	<b>-.218*</b>
Stomach ache Incidence	"r"	.165	<b>-.247*</b>	-.213	<b>.262*</b>	.021	1	<b>.371**</b>	.062	<b>.229*</b>	-.062	-.164
Flatulence Incidence	"r"	<b>.283**</b>	-.111	-.138	<b>.222*</b>	.098	<b>.371**</b>	1	<b>.251*</b>	-.059	.084	-.188
Constipation Incidence	"r"	-.107	-.065	-.202	-.134	.185	.062	<b>.251*</b>	1	.033	.015	-.009
<i>E.coli</i>	"r"	<b>-.215*</b>	<b>-.484**</b>	<b>-.314**</b>	<b>.250*</b>	.094	<b>.229*</b>	-.059	.033	1	<b>-.282**</b>	<b>-.333**</b>
<i>Lactic acid bacteria</i>	"r"	.198	<b>.484**</b>	<b>.284**</b>	-.138	<b>-.307**</b>	-.062	.084	.015	<b>-.282**</b>	1	<b>.370**</b>
<i>Bifidobacteria</i>	"r"	.179	<b>.672**</b>	<b>.597**</b>	-.210	<b>-.218*</b>	-.164	-.188	-.009	<b>-.333**</b>	<b>.370**</b>	1

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).



**Table. 5.37. Association between Iron intake and nutritional status, morbidity profile and gut microflora of school going children**

Parameters		Iron [mgs]	BMI	Nutritional Status	Diarrhoea Incidence	Common cold Incidence	Stomach ache Incidence	Flatulence Incidence	Constipation Incidence	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
Iron [mgs]	"r"	1	<b>.417**</b>	<b>.424**</b>	.019	-.149	.079	.158	-.087	-.181	.141	<b>.323**</b>
BMI	"r"	<b>.417**</b>	1	<b>.721**</b>	<b>-.221*</b>	<b>-.268*</b>	<b>-.247*</b>	-.111	-.065	<b>-.484**</b>	<b>.484**</b>	<b>.672**</b>
Nutritional Status	"r"	<b>.424**</b>	<b>.721**</b>	1	-.159	<b>-.226*</b>	-.213	-.138	-.202	<b>-.314**</b>	<b>.284**</b>	<b>.597**</b>
Diarrhoea Incidence	"r"	.019	<b>-.221*</b>	-.159	1	.001	<b>.262*</b>	<b>.222*</b>	-.134	<b>.250*</b>	-.138	-.210
Common cold Incidence	"r"	-.149	<b>-.268*</b>	<b>-.226*</b>	.001	1	.021	.098	.185	.094	<b>-.307**</b>	<b>-.218*</b>
Stomach ache Incidence	"r"	.079	<b>-.247*</b>	-.213	<b>.262*</b>	.021	1	<b>.371**</b>	.062	<b>.229*</b>	-.062	-.164
Flatulence Incidence	"r"	.158	-.111	-.138	<b>.222*</b>	.098	<b>.371**</b>	1	<b>.251*</b>	-.059	.084	-.188
Constipation Incidence	"r"	-.087	-.065	-.202	-.134	.185	.062	<b>.251*</b>	1	.033	.015	-.009
<i>E.coli</i>	"r"	-.181	<b>-.484**</b>	<b>-.314**</b>	<b>.250*</b>	.094	<b>.229*</b>	-.059	.033	1	<b>-.282**</b>	<b>-.333**</b>
<i>Lactic acid bacteria</i>	"r"	.141	<b>.484**</b>	<b>.284**</b>	-.138	<b>-.307**</b>	-.062	.084	.015	<b>-.282**</b>	1	<b>.370**</b>
<i>Bifidobacteria</i>	"r"	<b>.323**</b>	<b>.672**</b>	<b>.597**</b>	-.210	<b>-.218*</b>	-.164	-.188	-.009	<b>-.333**</b>	<b>.370**</b>	1
** Correlation is significant at the 0.01 level (2-tailed).												
* Correlation is significant at the 0.05 level (2-tailed).												

Table. 5.38. Association between Calcium intake and nutritional status, morbidity profile and gut microflora of school going children												
Parameters		Calcium [mgs]	BMI	Nutritional Status	Diarrhea Incidence	Common cold Incidence	Stomach ache Incidence	Flatulence Incidence	Constipation Incidence	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
Calcium [mgs]	"r"	1	<b>.405**</b>	<b>.372**</b>	-.106	-.027	-.084	.010	.090	-.036	.176	<b>.262*</b>
BMI	"r"	<b>.405**</b>	1	<b>.721**</b>	<b>-.221*</b>	<b>-.268*</b>	<b>-.247*</b>	-.111	-.065	<b>-.484**</b>	<b>.484**</b>	<b>.672**</b>
Nutritional Status	"r"	<b>.372**</b>	<b>.721**</b>	1	-.159	<b>-.226*</b>	-.213	-.138	-.202	<b>-.314**</b>	<b>.284**</b>	<b>.597**</b>
Diarrhoea Incidence	"r"	-.106	<b>-.221*</b>	-.159	1	.001	<b>.262*</b>	<b>.222*</b>	-.134	<b>.250*</b>	-.138	-.210
Common cold Incidence	"r"	-.027	<b>-.268*</b>	<b>-.226*</b>	.001	1	.021	.098	.185	.094	<b>-.307**</b>	<b>-.218*</b>
Stomach ache Incidence	"r"	-.084	<b>-.247*</b>	-.213	<b>.262*</b>	.021	1	<b>.371**</b>	.062	<b>.229*</b>	-.062	-.164
Flatulence Incidence	"r"	.010	-.111	-.138	<b>.222*</b>	.098	<b>.371**</b>	1	<b>.251*</b>	-.059	.084	-.188
Constipation Incidence	"r"	.090	-.065	-.202	-.134	.185	.062	<b>.251*</b>	1	.033	.015	-.009
<i>E.coli</i>	"r"	-.036	<b>-.484**</b>	<b>-.314**</b>	<b>.250*</b>	.094	<b>.229*</b>	-.059	.033	1	<b>-.282**</b>	<b>-.333**</b>
<i>Lactic acid bacteria</i>	"r"	.176	<b>.484**</b>	<b>.284**</b>	-.138	<b>-.307**</b>	-.062	.084	.015	<b>-.282**</b>	1	<b>.370**</b>
<i>Bifidobacteria</i>	"r"	<b>.262*</b>	<b>.672**</b>	<b>.597**</b>	-.210	<b>-.218*</b>	-.164	-.188	-.009	<b>-.333**</b>	<b>.370**</b>	1
** Correlation is significant at the 0.01 level (2-tailed).												
* Correlation is significant at the 0.05 level (2-tailed).												

**Table. 5.39. Association between Zinc intake and nutritional status, morbidity profile and gut microflora of school going children**

Parameters		Zinc [mgs]	BMI	Nutritional Status	Diarrhea Incidence	Common cold Incidence	Stomach ache Incidence	Flatulence Incidence	Constipation Incidence	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
Zinc [mgs]	"r"	1	<b>.457**</b>	<b>.448**</b>	.003	-.113	.015	.104	-.049	<b>-.258*</b>	.211	<b>.218*</b>
BMI	"r"	<b>.457**</b>	1	<b>.721**</b>	<b>-.221*</b>	<b>-.268*</b>	<b>-.247*</b>	-.111	-.065	<b>-.484**</b>	<b>.484**</b>	<b>.672**</b>
Nutritional Status	"r"	<b>.448**</b>	<b>.721**</b>	1	-.159	<b>-.226*</b>	-.213	-.138	-.202	<b>-.314**</b>	<b>.284**</b>	<b>.597**</b>
Diarrhea Incidence	"r"	.003	<b>-.221*</b>	-.159	1	.001	<b>.262*</b>	<b>.222*</b>	-.134	<b>.250*</b>	-.138	-.210
Common cold Incidence	"r"	-.113	<b>-.268*</b>	<b>-.226*</b>	.001	1	.021	.098	.185	<b>.094</b>	<b>-.307**</b>	<b>-.218*</b>
Stomach ache Incidence	"r"	.015	<b>-.247*</b>	-.213	<b>.262*</b>	.021	1	<b>.371**</b>	.062	<b>.229*</b>	-.062	-.164
Flatulence Incidence	"r"	.104	-.111	-.138	<b>.222*</b>	.098	<b>.371**</b>	1	<b>.251*</b>	-.059	.084	-.188
Constipation Incidence	"r"	-.049	-.065	-.202	-.134	.185	.062	<b>.251*</b>	1	.033	.015	-.009
<i>E.coli</i>	"r"	<b>-.258*</b>	<b>-.484**</b>	<b>-.314**</b>	<b>.250*</b>	.094	<b>.229*</b>	-.059	.033	1	<b>-.282**</b>	<b>-.333**</b>
<i>Lactic acid bacteria</i>	"r"	.211	<b>.484**</b>	<b>.284**</b>	-.138	<b>-.307**</b>	-.062	.084	.015	<b>-.282**</b>	1	<b>.370**</b>
<i>Bifidobacteria</i>	"r"	<b>.218*</b>	<b>.672**</b>	<b>.597**</b>	-.210	<b>-.218*</b>	-.164	-.188	-.009	<b>-.333**</b>	<b>.370**</b>	1

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

Table. 5.40. Association between Vitamin C intake and nutritional status, morbidity profile and gut microflora of school going children												
Parameters		Vitamin C [mgs]	BMI	Nutritional Status	Diarrhea Incidence	Common cold Incidence	Stomach ache Incidence	Flatulence Incidence	Constipation Incidence	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
Vitamin C [mgs]	"r"	1	<b>.299**</b>	<b>.375**</b>	.083	-.023	-.113	-.098	-.088	-.146	-.027	.114
BMI	"r"	<b>.299**</b>	1	<b>.721**</b>	<b>-.221*</b>	<b>-.268*</b>	<b>-.247*</b>	-.111	-.065	<b>-.484**</b>	<b>.484**</b>	<b>.672**</b>
Nutritional Status	"r"	<b>.375**</b>	<b>.721**</b>	1	-.159	<b>-.226*</b>	-.213	-.138	-.202	<b>-.314**</b>	<b>.284**</b>	<b>.597**</b>
Diarrhea Incidence	"r"	.083	<b>-.221*</b>	-.159	1	.001	<b>.262*</b>	<b>.222*</b>	-.134	.250*	-.138	-.210
Common cold Incidence	"r"	-.023	<b>-.268*</b>	<b>-.226*</b>	.001	1	.021	.098	.185	.094	<b>-.307**</b>	<b>-.218*</b>
Stomach ache Incidence	"r"	-.113	<b>-.247*</b>	-.213	<b>.262*</b>	.021	1	<b>.371**</b>	.062	.229*	-.062	-.164
Flatulence Incidence	"r"	-.098	-.111	-.138	<b>.222*</b>	.098	<b>.371**</b>	1	<b>.251*</b>	-.059	.084	-.188
Constipation Incidence	"r"	-.088	-.065	-.202	-.134	.185	.062	<b>.251*</b>	1	.033	.015	-.009
<i>E.coli</i>	"r"	-.146	<b>-.484**</b>	<b>-.314**</b>	<b>.250*</b>	.094	<b>.229*</b>	-.059	.033	1	<b>-.282**</b>	<b>-.333**</b>
<i>Lactic acid bacteria</i>	"r"	-.027	<b>.484**</b>	<b>.284**</b>	-.138	<b>-.307**</b>	-.062	.084	.015	<b>-.282**</b>	1	<b>.370**</b>
<i>Bifidobacteria</i>	"r"	.114	<b>.672**</b>	<b>.597**</b>	-.210	<b>-.218*</b>	-.164	-.188	-.009	<b>-.333**</b>	<b>.370**</b>	1
** Correlation is significant at the 0.01 level (2-tailed).												
* Correlation is significant at the 0.05 level (2-tailed).												

**Table. 5.41. Association between Serum IgA levels and dietary intake of primary school going children**

Parameters		IgA_mg/dl	Protein [gms]	Fat [gms]	Carbohydrates [gms]	Calcium [mgs]	Iron [mgs]	Energy [Kcal]	Vitamin C [mgs]	Zinc [mgs]	Total Dietary Fibre [gms]
IgA_mg/dl	"r"	1	.099	.090	.004	.262*	.036	.061	-.023	.100	.163
Protein [gms]	"r"	.099	1	.859**	.890**	.715**	.792**	.947**	.299*	.827**	.658**
Fat [gms]	"r"	.090	.859**	1	.768**	.662**	.639**	.930**	.260*	.716**	.544**
Carbohydrates [gms]	"r"	.004	.890**	.768**	1	.650**	.879**	.947**	.339**	.814**	.675**
Calcium [mgs]	"r"	.262*	.715**	.662**	.650**	1	.613**	.724**	.176	.616**	.593**
Iron [mgs]	"r"	.036	.792**	.639**	.879**	.613**	1	.817**	.244*	.761**	.771**
Energy [Kcal]	"r"	.061	.947**	.930**	.947**	.724**	.817**	1	.316*	.819**	.657**
Vitamin C [mgs]	"r"	-.023	.299*	.260*	.339**	.176	.244*	.316*	1	.492**	.395**
Zinc [mgs]	"r"	.100	.827**	.716**	.814**	.616**	.761**	.819**	.492**	1	.783**
Total Dietary Fibre [gms]	"r"	.163	.658**	.544**	.675**	.593**	.771**	.657**	.395**	.783**	1
** Correlation is significant at the 0.01 level (2-tailed).											
* Correlation is significant at the 0.05 level (2-tailed).											

### ***5.2.6. Relationship between BMI for age and gender and other biochemical parameters of school going children***

To obtain the significance of linear relationships between body mass index for age and gender and the morbidity profile and biochemical parameters of the school going children, Pearson's correlation was exercised. Further, same variables were also put in linear regression model in order to identify the significant regressors for BMI using best fit model.

#### ***5.2.6.1. Pearson's correlations between BMI, gut microflora, and morbidity profile of school going children***

As depicted in table 5.42 body mass index of the school going children found to negatively correlated with frequency of common colds; stomachache and mean log counts of *E.coli* reflecting higher incidences of common colds and stomach ache and higher amounts of pathogenic bacteria (*E.coli*) in undernourished children.

Higher height weight proportion of school going children found to be significantly associated with higher mean log counts of healthy bacteria (*Lactic acid bacteria* and *Bifidobacteria*).

A significantly negative relationship was observed in the mean log counts of *Bifidobacteria* and *Lactic acid bacteria* with frequency of diarrhea and common colds respectively, revealing that children who experienced higher episodes of diarrhea and common colds also had poor amount of healthy gut bacteria.

## Results and Discussions

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Co-existence of stomach ache and flatulence along with diarrhea was also determined in this set of analysis as the frequency of above said trio significantly correlated.

Children with higher counts of *E.coli* also reported to have higher incidences of stomach ache, showed the significant positive correlation among the two. Episodes of flatulence showed the significant positive correlation with the frequency of common colds and constipation.

Serum IgA levels of the school going children showed a significantly positive relationship with the mean log counts of *E.coli* indicating that higher serum IgA levels may also lead to higher amounts of pathogenic bacteria.

Amongst the various gut microbes studied, mean log counts of *E.coli* showed a significantly negative relationship with *Bifidobacteria* and *Lactic acid bacteria*, while mean log counts of *Bifidobacteria* and *Lactic acid bacteria* were highly correlated with each other.

Based on the significant positive or negative relationships of above mentioned variables, the mean log counts of *E.coli*, *Bifidobacteria* and *Lactic acid bacteria* therefore can be stated as an important indicators of not only child's nutritional status but also for his/her morbidity scenario.

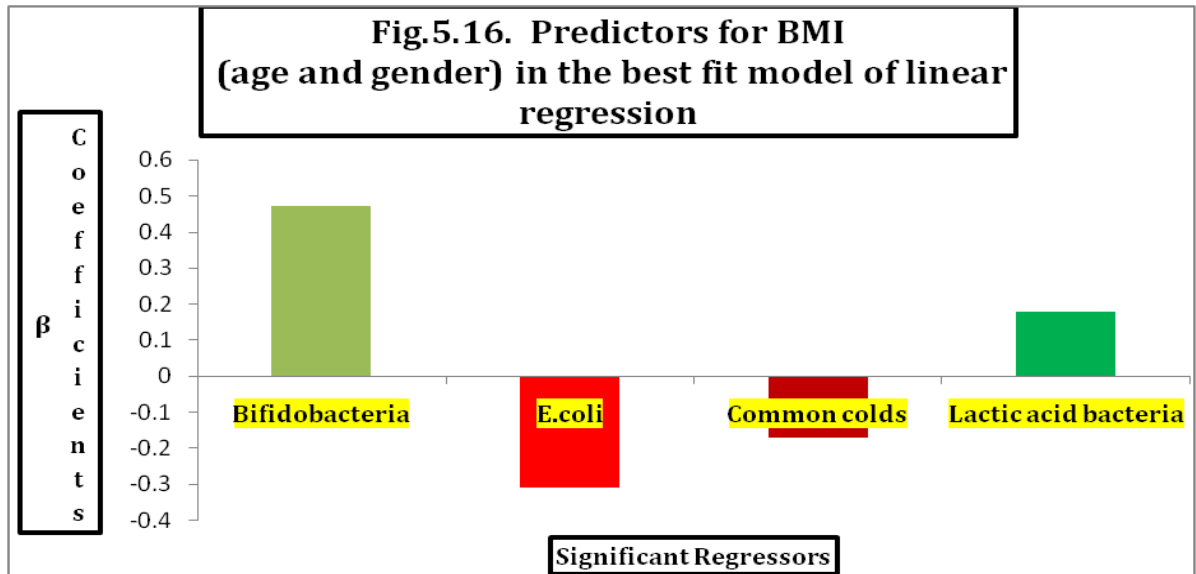
<b>Table 5.42. Associations between BMI for age and gender and morbidity and biochemical parameters of school going children</b>											
Parameters		BMI	Frequency of Diarrhea	Frequency of Stomach ache	Frequency of Flatulence	Frequency of Constipation	Frequency of Common colds	IgA mg/dl	<i>E.coli</i>	<i>Lactic acid bacteria</i>	<i>Bifidobacteria</i>
BMI	r	1	-0.122	<b>-0.256*</b>	-0.162	-0.025	<b>-0.323**</b>	-0.082	<b>-0.488**</b>	<b>0.499**</b>	<b>0.650**</b>
Frequency of Diarrhea	r	-0.122	1	<b>0.262**</b>	<b>0.232*</b>	-0.14	0.014	0.087	0.164	-0.113	<b>-0.220*</b>
Frequency of Stomach ache	r	<b>-0.256*</b>	<b>0.262**</b>	1	<b>0.530**</b>	0.104	0.127	-0.071	<b>0.210*</b>	0.015	-0.117
Frequency of Flatulence	r	-0.162	<b>0.232*</b>	<b>0.530**</b>	1	<b>0.198*</b>	<b>0.264**</b>	0.013	0.033	0.088	-0.13
Frequency of Constipation	r	-0.025	-0.14	0.104	<b>0.198*</b>	1	0.183	-0.007	0.028	0.086	0.051
Frequency of Common colds	r	<b>-0.323**</b>	0.014	0.127	<b>0.264**</b>	0.183	1	0.097	0.105	<b>-0.232*</b>	-0.168
IgA mg/dl	r	-0.082	0.087	-0.071	0.013	-0.007	0.097	1	<b>0.303**</b>	-0.026	0.048
<i>E.coli</i>	r	<b>-0.488**</b>	0.164	<b>0.210*</b>	0.033	0.028	0.105	<b>0.303**</b>	1	<b>-0.263**</b>	<b>-0.246*</b>
<i>Lactic acid bacteria</i>	r	<b>0.499**</b>	-0.113	0.015	0.088	0.086	<b>-0.232*</b>	-0.026	<b>-0.263**</b>	1	<b>0.428**</b>
<i>Bifidobacteria</i>	r	<b>0.650**</b>	<b>-0.220*</b>	-0.117	-0.13	0.051	-0.168	0.048	<b>-0.246*</b>	<b>0.428**</b>	1
*. Correlation is significant at the 0.05 level (2-tailed).											
**. Correlation is significant at the 0.01 level (2-tailed).											



### 5.2.6.2. Linear Regression for BMI for age and gender of primary school going children

Various regression models were exercised in order to reach the best fit model ( $R^2 = 58.3$ ) for the identification of substantial regressors for the BMI of the school going children. As seen in table 5.43, stepwise linear regression was used and out of all the variables entered in the regression equation, *Bifidobacteria* and *Lactic acid bacteria* came out to be positively impacting the body mass index of the school going children. On the negative mode, frequency of common colds and mean log counts of *E.coli* significantly regressed the BMI of the children.

Table 5.43. Stepwise Linear Regression for predicting the BMI (for age and gender) of school going primary school children				
Variables Entered	Adjusted $R^2$	Significant Regresses		
		Name	Standardized $\beta$ coefficients	Significance
<i>Bifidobacteria</i>	58.3	<i>Bifidobacteria</i>	0.47	0.000
Serum IgA		<i>E.coli</i>	-0.308	0.000
<i>E.coli</i>		Frequency of Common colds	-0.171	0.12
<i>Lactic acid bacteria</i>		<i>Lactic acid bacteria</i>	0.177	0.19
Frequency of Diarrhea				
Frequency of Common colds				
Frequency of Stomach ache				
Frequency of Constipation				
Frequency of Flatulence				



### ***Result Highlights of Phase II***

- ❖ Mean counts of *E.coli* were significantly associated with lower BMI of the children whereas higher counts of *Bifidobacteria* and *Lactic acid bacteria* associated significantly with better nutritional status.
- ❖ Morbidity profile was significantly associated with the gut microflora. Children with lower counts of *Bifidobacteria* ( $r = -0.220$ ) and *Lactic acid bacteria* ( $r = -0.232$ ) experienced significantly higher episodes of diarrhea and common colds respectively.
- ❖ Counts of favorable bacteria (*Lactic acid bacteria*  $r = -0.263$ ; *Bifidobacteria*  $r = -0.246$ ) were inversely proportional to pathogenic bacteria (*E.coli*).
- ❖ Incidence of common cold was 19% ( $p < 0.04$ ) higher in undernourished children compared to nourished ones.
- ❖ Severely undernourished children had significantly higher ( $p < 0.01$ ) incidence (62.5%) of diarrhea compared to moderate (36%) and mildly (22%) undernourished children.
- ❖ Counts of pathogenic bacteria i.e. *E.coli* were 2.75% higher in the gut of undernourished children compared to nourished ones ( $p < 0.001$ ).

### ***Result Highlights of Phase II (Contd.)***

- ❖ Counts of favorable bacteria i.e. *Bifidobacteria* (3.50%) and *Lactic acid bacteria* (3.20%) were significantly higher ( $p=0.000$ ) in the gut of nourished children compared to undernourished children.
- ❖ Gut microflora profile showed significant deviations ( $p=0.000$ ) across all the three grades of undernutrition:
  - ✓ Severely undernourished children had 6.1% and 1.85% higher counts of *E.coli* compared to mild and moderate ( $p=0.000$ ) whereas moderately undernourished children had 4.10% higher counts compared to mildly undernourished ( $p=0.000$ ).
  - ✓ Counts of *Bifidobacteria* were 3.37% and 2.62% higher in mildly and moderately undernourished compared to severely undernourished children respectively ( $p=0.000$ ).
  - ✓ Moderate and mildly undernourished children had 6.10% and 5.12% higher counts of *Lactic acid bacteria* compared to severely undernourished ones ( $p=0.000$ ).
- ❖ Serum IgA levels showed an increasing trend with decreasing status of nutrition, indicating higher rates of infection in undernourished children.

### ***Result Highlights of Phase II (Contd.)***

- ❖ Quartile analysis showed that higher ranges of serum IgA had a significantly positive correlation with colonization of *E.coli* ( $r= 0.463$ ).
- ❖ Gut microflora especially *Bifidobacteria* ( $\beta= 0.543-0.645$ ) came out to be significant positive regressors for BMI in almost all the quartiles of serum IgA.
- ❖ Analysis of variance indicated significant differences in the counts of pathogenic bacteria ( $p<0.01$ ) and incidence of diarrhea ( $p<0.00$ ) across the quartiles of serum IgA levels (56-273 mg/ dl).
- ❖ Intake of energy (34.22%), carbohydrate (31.94%), protein (36%), fat (36%), dietary fibre (55.55%), iron (37.86%), calcium (43.26%), zinc (33.50%), and vitamin C (51.05%) were significantly higher ( $p=0.000$ ) in the nourished children compared to undernourished children.
- ❖ *Bifidobacteria* counts showed significant positive correlation with energy, carbohydrate, protein, fat, iron, calcium and zinc intakes.
- ❖ Low consumption of total dietary fibre and zinc impacted higher log counts of *E.coli*.

### **Phase III: Impact of Fructooligosaccharide supplementation on morbidity profile, gut microflora and serum IgA levels of undernourished children.**

This phase of the study was conducted in order to ascertain the role of FOS supplementation in improving the gut of undernourished children and thereby impacting their morbidity and nutritional status.

Based on the grade of undernutrition children were stratified into two groups i.e. placebo and experimental. Experimental group received FOS incorporated ice-cream (95% sugar as FOS) and placebo group received regular vanilla ice-cream for 30 days over a period of 45 days.

Post data was collected for children with maximum compliance (80-95%) and impact analysis was exercised for all the baseline parameters at the end of intervention.

#### ***5.3.1. Impact of FOS supplementation on Gut microbiota of the undernourished school going children***

As seen in table 5.44 consumption of ice cream with FOS for thirty days showed significant improvements in the mean log counts of beneficial gut microbiota i.e. *Bifidobacteria* and *Lactic acid bacteria* by 29 % ( $p=0.000$ ) and 2.56 % ( $p<0.04$ ) respectively, along with 2% reduction in *E.coli* ( $p<0.001$ ) (Table 5.44, Fig. 5.17-5.19).

**Table 5.44. Impact of FOS incorporated ice-cream on gut-microflora establishment of undernourished children**

Parameters		Control Group (25)	Experiment Group (30)	Student 't' Test	'p' value
<i>E.coli</i>	Pre Intervention	5.91 ± 0.20	5.86 ± 0.22	1.04	0.30 NS
	Post intervention	5.92± 0.11	5.72 ± 0.20	4.5***	0.000
	Paired "t" Test ["p" value]	0.034 [0.972 NS]	1.787 [0.001**]		
	% increase/decrease	-0.16%	-2.38%		
<i>Bifidobacteria</i>	Pre Intervention	7.86 ± 0.22	7.82 ± 0.20	1.01	0.30 NS
	Post intervention	7.83 ± 2.40	10.06 ± 0.19	38.03***	0.000
	Paired "t" Test ["p" value]	0.059 [0.953 NS]	37.493 [0.000***]		
	% increase/decrease	-0.38%	+28.64%		
<i>Lactic acid bacteria</i>	Pre Intervention	5.82 ± 0.22	5.84 ± 0.28	0.37	0.71NS
	Post intervention	5.85 ± 0.25	5.99± 0.21	1.80*	0.04
	Paired "t" Test ["p" value]	0.678 [ 0.504 NS]	2.56 [0.024**]		
	% increase/decrease	+0.51%	+2.56%		

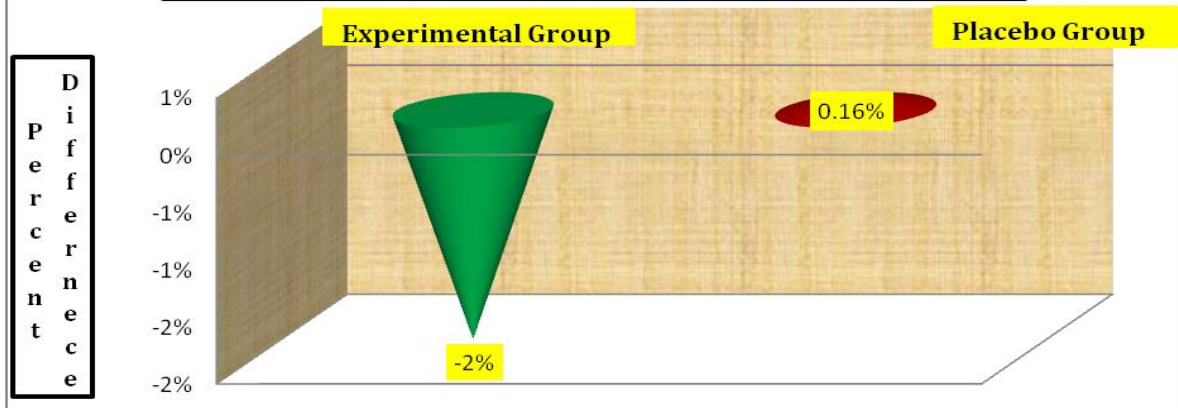
\* = significant at 95%

\*\* = significant at 99%

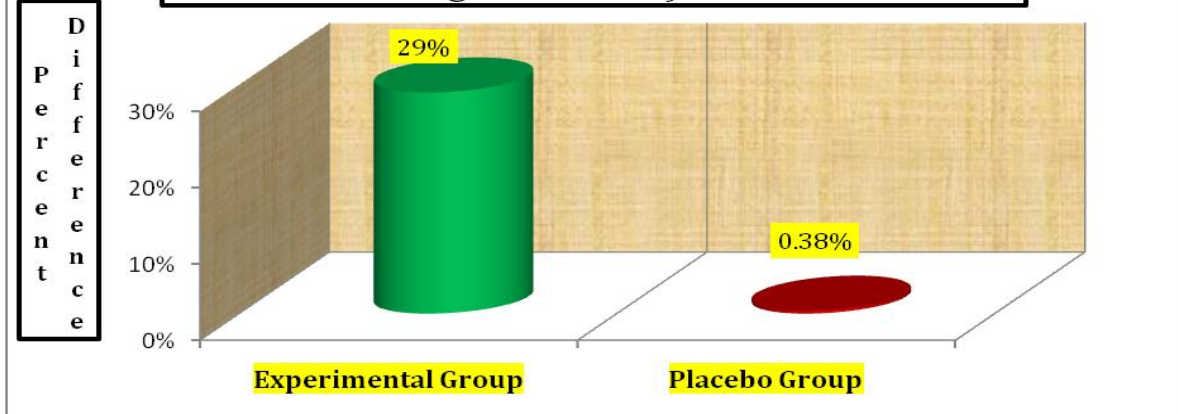
\*\*\* = significant at 99.99%

NS = statistically non significant

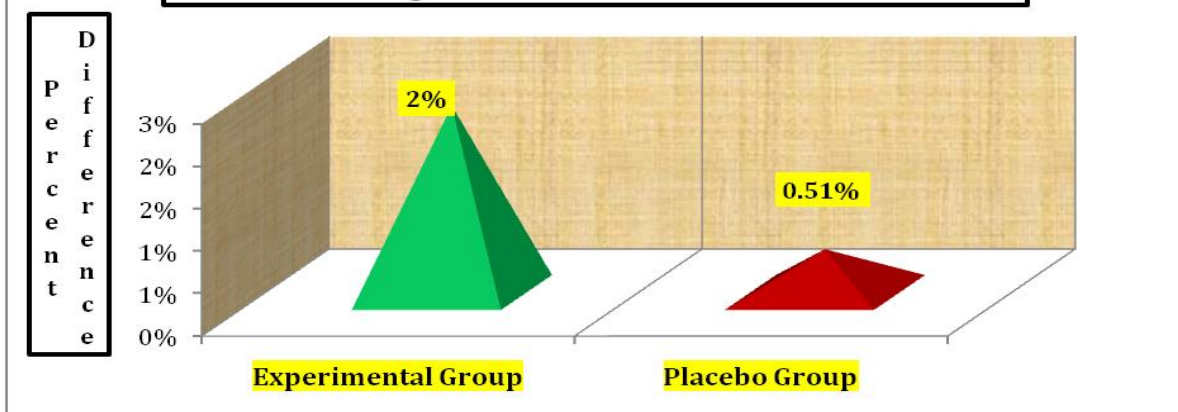
**Fig. 5.17. Per Cent Difference after Intervention in mean log counts of *E.coli***



**Fig. 5.18. Per Cent Difference after Intervention in mean log counts of *Bifidobacteria***



**Fig. 5.19. Per Cent Difference after Intervention in mean log counts of *Lactic acid bacteria***





### 5.3.2. Impact of FOS supplementation on morbidity profile of the undernourished school going children

Calendars were given to parents at the time interview (Phase I) and they were asked to indicate the incidence of any morbidity during the course of intervention, along with the regular monitoring by the researcher before serving ice-cream to the children.

Post intervention, a significant reduction in the episodes of diarrhea and common cold was observed by 79.6% and 82.3% respectively in the experimental group (Fig 5.20). Although a significant reduction in the incidence of common cold was also noticed in placebo group, however it was 24.4% lower than that seen experimental group (Table 5.45).

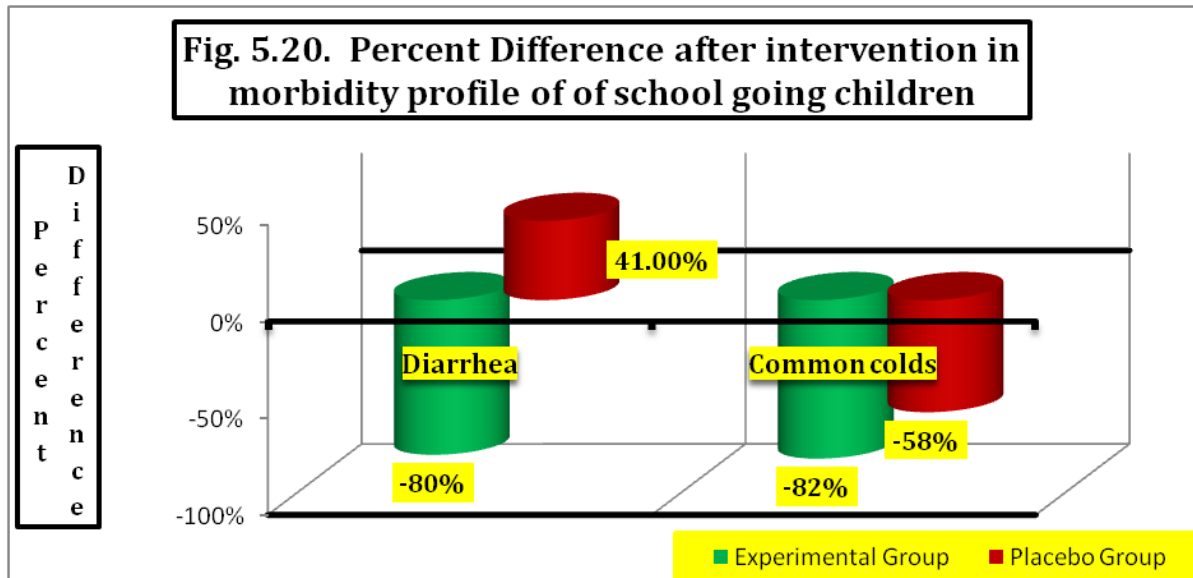
Table. 5.45. Impact of FOS incorporated ice-cream on morbidity status of undernourished children					
Parameters		Control Group (30)	Experiment Group (30)	Student 't' Test	'p' value
Common Cold	Pre Intervention	1.97±2.3	2.10 ± 1.90	0.24 NS	0.8
	Post intervention	0.83 ± 1.17	0.37 ± 0.80	1.78 NS	0.07
	Paired "t" Test	2.89**	5.166***		
	% increase/decrease	-57.86	-82.38		
Diarrhoea	Pre Intervention	0.80 ± 1.24	1.13 ± 1.16	1.07 NS	0.28
	Post intervention	1.13 ± 1.22	0.23 ± 0.7	3.4***	0.001
	Paired "t" Test	0.07 NS	3.4**		
	% increase/decrease	+41.25	-79.6		

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%

NS= statistically non significant



After considering the reduction in common colds episodes of placebo group (57.86%), the net impact of FOS supplementation on common colds was came out to be 24.52% and hence the prebiotic effect of FOS played an important role in reducing common colds and diarrhea.

## 5.3.3. Impact of FOS supplementation on nutritional status of the undernourished school going children

Statistically significant increments were observed after the intervention in the mean weight and height of school going children of both the interventional group (Table 5.46). However, exclusive impact of FOS on anthropometric measurement was insignificant.

Table. 5.46. Impact of FOS incorporated ice-cream on anthropometric measurements of undernourished children					
Parameters		Control Group (30)	Experiment Group (30)	Student 't' Test	'p' value
Weight (kg)	Pre Intervention	19.52 ±3.69	19.69 ± 3.76	0.09 NS	0.90
	Post intervention	20.02 ± 3.84	19.98 ± 3.90	0.04 NS	0.96
	Paired "t" Test	4.73*** [0.000]	3.69*** [0.000]		
	% increase/decrease	+2.56	+1.47		
Height (cms)	Pre Intervention	119.53 ± 8.96	121.33 ± 10.4	0.71 NS	0.40
	Post Intervention	120.92 ±8.89	122.63 ± 10.65	0.67 NS	0.50
	Paired "t" Test	5.78*** [0.000]	9.44*** [0.000]		
	% increase/decrease	+1.16	+1.07		
BMI	Pre Intervention	13.53 ± 0.84	13.18 ± 0.61	1.83 NS	
	Post Intervention	13.57 ± 1.02	13.15 ± 0.70	1.87 NS	
	Paired "t" Test	0.40 [NS]	0.59 [NS]		
	% increase/decrease	+0.29	-0.22		

\*= significant at 95%

\*\*=significant at 99%

\*\*\*=significant at 99.99%

NS= statistically non significant

### 5.3.4. Impact of FOS supplementation on serum IgA levels of the undernourished school going children

FOS intervention did increased 1% serum IgA levels in the experimental group which was statistically non significant. However, placebo group showed a 9% reduction in the serum levels of IgA after supplementation (Table 5.47).

Table.5. 47. Impact of FOS incorporated ice-cream on serum IgA levels of undernourished children					
Parameters		Control Group (30)	Experiment Group (30)	Student 't' Test	'p' value
Serum IgA Levels	Pre Intervention	126.8±46.10	133.06±70.26	0.40 NS	0.68
	Post intervention	115.73±38.53	134.43±57.84	1.47 NS	0.14
	Paired "t" Test ["p" value]	1.77 NS [0.08]	0.20 NS [0.83]		
	% increase/decrease	-8.73	+1.02		

NS= statistically non significant

### ***Result Highlights of Phase III***

- ❖ 30 days of FOS supplementation over a period of 45 days beneficially impacted the undernourished children by:
  - ✓ Increase in 28.6% *Bifidobacteria*( $p=0.000$ ) and 2.56% of *Lactic acid bacteria* colonization ( $p<0.00$ )
  - ✓ Decrease in colonization of *E.coli* by 2.38% ( $p<0.00$ )
  - ✓ Reduction in diarrheal episodes by 79.6% ( $p<0.00$ ) and in common colds episodes by 82% ( $p=0.000$ ).

\*After considering the reduction in common colds episodes of placebo group (57.86%), the net impact of FOS supplementation on common colds was came out to be 24.52% and hence the prebiotic effect of FOS played an important role in reducing common colds and diarrhea.

- ❖ Exclusive impact of FOS incorporated ice cream did not reveal a significant improvement in the nutritional status of undernourished children as both the groups reported gain in weight.

### **Phase IV: Development of a bilingual booklet entitled “Prebiotic: Our Gut Guardians”**

In this phase of the study, a compilation of recipes incorporated with prebiotic *viz.* inulin and FOS, in a form of bilingual booklet was undertaken, in order to bring out a ready reckoner of prebiotic rich foods (Appendix 8). These recipes were standardized and developed by the various researchers working in the field of prebiotics at the department of Foods and Nutrition, Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda. The recipes were computed for an appropriate portion size considering the maximum allowance of prebiotic that would result in most acceptable products.

## DISCUSSION

This study attempts to give insight into the concepts of intriguingly prevalent undernutrition in Indian children. Present chapter dialogues the various aspects attached with undernutrition which were yet not explored in spite of having the potential to substantiate the interventional strategies. It also intends to open an argument that why we should not ignore the complected relationship of nutrition and infections while planning the preventional strategies for undernourished Indian children.

Chronic under-nutrition is considered to be the primary cause of ill health and premature mortality among children in developing countries [Nandy *et al.*, 2005]. Under-nutrition among children is prevalent in almost all the states in India [Som *et al.*, 2006]. Child malnutrition has risen in recent years in India [Chaterjee, 2007]. Nutritional deprivation is rampant in children of school age particularly primary school children ranging in magnitude from 20-80%. Since deficient physical growth is naturally reflected in their suboptimal mental achievement, the assessment of nutritional status of this segment of population is essential for making progress towards improving overall health of the school age children [Fazili *et al.*, 2012].

Seventy one per cent of the school going children of urban Vadodara were found to be undernourished in the present set of research which is almost comparable to data on undernourished children of government school of

Mumbai [Madan *et al.*, 2014]. Contrarily, Masry (2013) identified the prevalence of malnutrition (under) among Egyptian children involving 1365 children, in the age ranged from 6 -11 years, as 3.7% for stunting (Z-scores for height for- age [HAZ] <-2), 0.7% for wasting (Z-scores for weight-for-height [WHZ] <-2) and 0.0% for underweight (Z-scores for weight-forage [WAZ] <-2). Although, in case of India the scenario is contra positive as “it has been estimated that approximately 70% of the world’s malnourished children live in Asia, giving that region the highest concentration of worldwide childhood malnutrition and the occurrence of undernutrition is highest among Indian children [Thakur and Gautam, 2014] .

Rejecting the popular belief that girls are more undernourished than boys, anthropometric assessments in the present study provided a contrary picture. Unlike, Medhi *et al.*, (2006a) who studied tea garden children and revealed that boys were heavier and taller than girls till the age of 10 years, after which, the mean height and weight of girls exceeded the mean height and weight of boys. The study by Medhi *et al.*, (2007) and Manna *et al.*, (2011) showed that the mean height of girls is higher at the ages 10, 11, 12 than boys, which is also true in case of present study.

One of the significant outcome of the present research as indicated by odds ratio analysis, that lower literacy levels of parents was an essential risk factor for undernutrition and high morbidity levels in the school going children. Bain (2014) also said that improving the educational status of parents,



especially of mothers, on nutrition, sanitation and common disease prevention strategies should logically reduce the malnutrition related mortality and morbidity. He also quoted that the way to the child's stomach is through the mind of the mother and the quality of food taken, choices, and quantity are all at the discretion of the mother or care giver.

Children who were not breastfed were more undernourished compared to those who were and undernourished children had poor number of beneficial gut flora. Available evidence suggests that breastfeeding may have long-term benefits [WHO, 2007]. This algorithm can be explained by the review published in Immunology letters by Kaetzel (2014) which states that Polymeric IgA (pIgA) is synthesized by local plasma cells in the lamina propria of lactating mammary glands, and transported across alveolar epithelial cells into milk by pIgR (polymeric IgA receptor). The pIgR-derived SC (secretory component) moiety protects SIgA (secretory IgA) from degradation during transit through the gastrointestinal tract of the suckling infant. Newborn mammals do not produce endogenous SIgA in the intestine, and are reliant on breast milk-derived SIgA for antibody-mediated protection in the intestinal lumen. The pace of development of endogenous SIgA production in human infants varies widely, largely depending on environmental factors such as microbial load in the intestine, and this may take several years to achieve adult levels. Further, researches suggest that prebiotics such as galactooligosaccharides (GOSs) found in human breast milk which stimulate the growth and metabolic activity of beneficial bacteria in

the gut flora, which may also produce a direct immunological effect [Boehm and Stahl, 2007; Schley and Field, 2002].

Undernourished children have insufficient resistance to infection; they are more likely to die from common childhood ailments like diarrheal diseases and respiratory infections; and for those who survive, frequent illness saps their nutritional status, locking them into a vicious cycle of recurring sickness, faltering growth, and diminished learning ability [Joshi *et al.*, 2011]. It is generally accepted that undernutrition causes increased susceptibility to infection and infection causes increased susceptibility to undernutrition [Hart *et al.*, 2008]. A clear reflection of this theory is noticeable in the present research, as incidence of common colds were significantly higher in undernourished children when compared with nourished and incidence of diarrhea was significantly higher in severely undernourished children compared to the other two grades of undernutrition. There are multiple mechanisms of action in the relationship between undernutrition and susceptibility to infectious diseases. Significant negative correlations were observed between past month morbidities and BMI of the children in the present study. As explained by, Rundles *et al.*, (2005), stimulation of an immune response by infection increases the demand for metabolically derived anabolic energy, leading to a synergistic vicious cycle of adverse nutritional status and increased susceptibility to infection. Infection itself can cause a loss of critical body stores of protein, energy, minerals, and vitamins. During an immune response, energy expenditure increases at the same time the

infected host experiences a decrease in nutrient intake. Prospective observation of infants in an urban slum demonstrated that diarrheal diseases were associated with the development of malnutrition that was in turn linked to intestinal barrier disruption and that diarrhea was more severe in already malnourished children [Mondal, 2012].

Even with the vigorous functioning of Integrated Child Development Scheme (ICDS) since 1975, no substantial change is reflected in the statistics of undernutrition in India. Authors of the present study therefore intended to study the gut flora modulation in undernourished children. "The gut is important in medical research, not just for problems pertaining to the digestive system but also problems pertaining to the rest of the body," says Pankaj J. Pasricha, chief of the division of gastroenterology and hepatology at Stanford University School of Medicine [Wang, 2012]. The different compartments of the gastrointestinal tract are inhabited by populations of micro-organisms. By far the most important predominant populations are in the colon where a true symbiosis with the host exists that is a key for well-being and health. For such a microbiota, 'normobiosis' characterizes a composition of the gut 'ecosystem' in which micro-organisms with potential health benefits predominate in number over potentially harmful ones, in contrast to 'dysbiosis', in which one or a few potentially harmful micro-organisms are dominant, thus creating a disease-prone situation [Roberfroid *et al.*, 2010]. There is an increasing awareness of the interplay between the microbiota and gastrointestinal structure and functions and its

implications for health and disease [Martorell, 2000; Beisel, 1982; Chandra, 1980]. Several studies suggest *Bifidobacterium* spp. and *Lactobacillus* spp. as sensitive indicators of host well-being [Gibson and Roberfroid, 1995]. Recent research has documented that the gut of healthy children comprise greater amount of *Lactobacillales* and *Bifidobacteriales* [Gupta *et al.*, 2011]. Present study also manifested similar findings as, higher number of *Lactic acid bacteria* and *Bifidobacteria* were found in the gut flora of nourished children compare to undernourished ones, with significantly higher number of *E.coli* in the gut flora of undernourished children.

Incidences of diarrhea were significantly correlated with lower counts of *Bifidobacteria* in undernourished children of the present research. Also, substantiated by Knolet *et al.*, (2005) who stated that stimulation of *Bifidobacteria* reduces the presence of clinically relevant pathogens in the faecal flora, indicating that prebiotic substances might have the capacity to protect against enteric infections. Stool samples from children with diarrhea attending the General Hospital Minna, Nigeria were analyzed for the presence of different types of bacteria revealed that *Escherichia coli* accounted for most cases of infantile diarrhea [Galadima and Kolo, 2014].

*Lactic acid bacteria* may play an important role in protection against infectious agents [Martin *et al.*, 2003; Martin *et al.*, 2005]. Needless to explain that why children under present study, who suffered with common colds in the past

month colonized significantly lesser number of *Lactic acid bacteria*, it again confirms the inter-relation between gut microbiome and morbidity profile.

Undisputedly IgA is an inevitable aspect of immune system. It is a predominant immunoglobulin in mucosal secretions and serves as the first line of humoral defense at all mucosal surfaces: binding of IgA antibodies to microorganisms reduces their motility and adhesive properties within the mucosal lumen and its surface [Kamata *et al.*, 2000]. Increasing trend of serum IgA was seen in grade III and grade II undernourished children when compared to nourished children which could be indicative of higher rates of infection in undernourished children. Present research exhibited significantly positive relationship between highest quartile of serum IgA levels with higher colonization of *E.coli*, which was also negatively correlated with nutritional status indicating the role of systemic immunity in the severe grades of undernutrition. This observation corresponds with the general finding, that serum immunoglobulin levels are often elevated in malnourished children with infections [Bell *et al.*, 1976; Jose and Welch, 1970]. Induction of a secretory immune response is often associated with elevation of corresponding serum IgG and IgA (in humans mainly monomeric) antibodies. These antibodies can reach external secretions by passive paracellular diffusion and may thus contribute to immune exclusion. As a consequence, it is often difficult if not impossible to distinguish between the roles of secretory versus systemic immunity in local defense [Johansen, 1999]. However, there is dearth of data on this aspect and

more studies need to be undertaken to establish this fact along with determination of other immune components which may provide precise picture of a relationship between immune components and gut health of undernourished children.

Nutritional status, gut microbiome and immunity are inevitably affected by individual's dietary intake. Nutrient, like Zinc is an essential component and its deficiency causes malnutrition and results in defects in innate and acquired immune responses. Also, zinc is important for highly proliferating cells, especially in the immune system and influences both innate and acquired immune functions [Baba *et al.*, 2015]. It may be the probable reason for significant reciprocal association of zinc with unfavorable gut microbiome (*E.coli*) and significant non-reciprocal relationship with favorable gut microbiome (*Bifidobacteria*) in the present study. As, Crane *et al.*, (2014) also demonstrated that zinc's ability to protect against enteric bacterial pathogens may be the result of its combined effects on host tissues as well as inhibition of virulence in some pathogens.

Prebiotic effects may influence the immune system directly or indirectly as a result of intestinal fermentation and promotion of growth of certain members of the gut microbiota [Roberfroid *et al.*, 2010]. Abundance of enteric pathogens in the gut of undernourished children is known to cause intestinal inflammation resulting in malabsorption of nutrients [Gupta *et al.*, 2011]. This, was also apparent in the present research, as children who were consuming lesser

## Results and Discussions

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amounts of total dietary fiber had significantly higher counts of pathogenic bacteria (*E.coli*), which contemplates the algorithm of relationship between gut health, undernutrition and nutrient intake.

Fructooligosaccharides (FOS) a known prebiotic, which is defined as non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health [Gibson *et al.*, 2010]. Intervening school going children with 10g of FOS in the present randomized placebo control trial resulted in significant improvements in the colonization of *Bifidobacteria* and *Lactic acid bacteria* likewise, Simakachorn *et al.*, (2011) treated ninety-four patients between 1 and 3 years old under mechanical ventilation requiring enteral feeding, patients were randomized to receive either a test formula containing a synbiotic blend (composed of 2 probiotic strains (*Lactobacillus paracasei* NCC 2461 and *Bifidobacterium longum* (NCC 3001), fructooligosaccharides (FOS, inulin, and Acacia gum), or a control formula, their trial resulted in higher faecal *Bifidobacteria* and *Lactobacilli* and diminished counts of *Enterobacteria* in the test group. Also corroborated by Rhoades (2006) and Rivero (2001), FOS have been used with increasing frequency as food additives, with possible benefits including support of beneficial gastrointestinal flora and reduction of infectious diarrhea.

Augmented establishment of favorable gut microbiome (*Bifidobacteria* and *Lactic acid bacteria*) through FOS supplementation, postulated for alleviating 80%

diarrheal and 82% (24% higher than placebo) common cold incidences in the present trial. De Vrese *et al.*, (2006) also reported that use of probiotic bacteria significantly shortened the mean duration of common cold episodes by about 2 days and reduced the severity of symptoms, the reduction in the severity and duration may be due to immune stimulatory effects of living or dead probiotic bacteria during gastrointestinal passage. The first evidence that prebiotic strains could prevent respiratory tract infections was shown when mice were successfully protected against influenza through the administration of *Bifidobacterium breve* (*B. breve*) YIT4064 augmented anti-influenza IgG [Yasui *et al.*, 1999]. Jose *et al.* (2011) also stated that administration of a follow-on formula with *L. fermentum* CECT5716 may be useful for the prevention of community-acquired gastrointestinal and upper respiratory infection.

Finnish researchers conducted studies amongst children in daycare centers who were given milk containing *Lactobacillus rhamnosus* (*L.rhamnosus*) GG (ATCC 53103) during winter reported similar results [Hatakka *et al.*, 2001]. Maura *et al.*, (2006) have also reported that functional oligosaccharides play a role in ameliorating diarrhea, especially when it is associated with intestinal infections. According to Chi, Chen, Wang, Xiong, and Li (2008), this may be directly related to the possible inhibitory effect of *Bifidobacteria* both on Gram+ and Gram- bacteria. In infants, scGOS/lcFOS also increased resistance to pathogenic infections and attenuated allergy development [Moro *et al.*, 2006; Van *et al.*, 2009; Arslanoglu *et al.*, 2007; Arslanoglu *et al.*, 2008; and Boehm and Moro, 2008].



However, one study [Hatakka *et al.*, 2007] showed that the prebiotics did not have any effect on upper respiratory infections after the intervention and a trial conducted in Bangladesh [Nakamura, 2006] concluded that, daily intake of FOS was associated neither with the children's growth nor with the number of diarrhea episodes, but a significant reduction in the duration of diarrhea days was observed.

Statistical difference in the objective biochemical parameter of immunity i.e. serum IgA was not visible after supplementation in the present trial, but it significantly impacted the subjective parameters i.e. morbidity profile. Because, immune responses that start in the gut have the potential to affect immune responses at other mucosal surfaces [Brownawell *et al.*, 2012]. As, gut associated lymphoid tissue (GALT) represents the largest mass of lymphoid tissue in the body, approximately 25% of the intestinal mucosa consists of lymphoid tissue [Kagnoff, 1987]. About 60% of the total immunoglobulins produced daily are secreted into the GI tract [Gibson *et al.*, 2010]). Accumulating evidence shows that IgA-producing cells are generated by multiple pathways, in organized and non-organized follicular structures, by T-dependent and T-independent mechanisms. However, the functional differences between IgAs generated in different anatomical sites by different mechanisms are still unclear. Also unknown are the mechanisms by which the antigen-specific IgAs regulate the amount and composition of the microbial community or how exactly the innate IgA inhibits host-bacteria interactions in the gut [Suzuki and Nakajima, 2014]. Vulvelic (2013)

commented that it can be challenging to demonstrate specific effects of the commensal microbiota on IgA responses in humans, a recent study demonstrated that treatment of adult volunteers with prebiotic oligosaccharides altered the composition of the gut microbiota and was correlated with an increase in fecal SIgA levels. Ghaneiet *al.*, (2015) treated the children of 7-24 months of age with inulin + fructooligosaccharide, revealed that the treated group had reduced serum IgE levels and significant improvement in the total scores of atopic dermatitis compared to control group.

Exclusive impact of daily supplementation of 10g FOS for 30 days was not noticeable in the nutritional status of the children as both the groups (experimental and placebo) showed significant rise in their weight with placebo group showing a better picture. A plausible reason for un-impacted anthropometric measurements was the striking calorific difference in placebo and FOS ice-creams. As FOS also behaves like fat replacer, the final composition of FOS ice cream had lesser fat (13%) and SNF (36%), compared to regular ice-cream which had 14% of fat and 40 % of SNF, which caused the concomitant difference of 85 kilo calories per cup in the final compositions leading to a total extra addition of 2,550 kilo calories in the placebo group. Though, Das Gracias *et al.*, (2014) also conducted a trial using yacon in order to provide 0.14g of FOS/kg bodyweight to 2-5 years of children resulted in improved intestinal immune response but demonstrated no effect on the nutritional status of iron and zinc in preschool children.

Overall aims of the present trial were therefore to balance the gut microbiota composition with prebiotic supplementation and consequently improve children's well-being and reduce their disease risk. Hence, FOS can be recommended as a potent tool to modulate the nutritional status in a "long run" not merely in terms of increasing weight but via reducing incidence of infection in young school going children through improved colonization of beneficial gut microbiota viz. *Lactic acid bacteria* and *Bifidobacteria*.