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"Effects of Probiotic (Bifidobacteria) Yoghurt Consumption on the Serum Cholesterol levels in Hypercholestromic Cases"

Birupaksha Biswas[1], Damini Joshi[2], Arohi Singh[3], Angel Saluja[4], Dipti Kapadiya[5], Namrata Rathore[6]

[1]Parul Institute of public health (PIPH). MPH Scholar. Email: brishalibiswas22@gmail.com +917778019610 whatsapp. Journal Name: Frontiers in Health Informatics - FHI

[2]Assistant Professor,Parul Institute of Public Health (PIPH). Email: damini.joshi19835@paruluniversity.ac.in Journal Name: Frontiers in Health Informatics – FHI Whatsapp:+919099951459

[3]MPH scholar, Parul Institute Of Public Health. drarohisingh1@gmail.com Whatsapp: +91727539199 Journal Name: Frontiers in Health Informatics - FHI

[4]MPH Scholar,Parul Institute Of Public Health. Angel saluja8@gmail.com Whatsapp: +91816826062 Journal Name: Frontiers in Health Informatics - FHI

[5]MPH Scholar. Parul Institute Of Public Health Email : drdiptikapadiya95@gmail.com Whatsapp: +919265894019 Journal Name: Frontiers in Health Informatics - FHI

[6]MPH scholar,Parul Institute of Public Health Email: namratarathore0207@gmail.com Journal Name: Frontiers in Health Informatics – FHI Whatsapp: +916264619844

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Abstract: This study concentrated on assessing the goods of high- fat and probiotic diets on serum lipid lives and body weight in rats. A total of eight rats were divided into three salutary groups fat-rich diet (R1 – R4), control diet (C1 – C2), and probiotic diet (R5 – R8). The fat-rich diet was composed of vegetable fat (48), coconut oil painting oil (40), and win oil painting oil (6), while the probiotic yoghurt comported of liquid milk (95), skim milk cream (3), yoghurt culture (1), and probiotic culture (1). Compared to the control group, rats fed with the fat-rich diet demonstrated significant dyslipidemia Total cholesterol increased by 48(from 93.5 to 138.5 mg/ dL), Triglycerides(TG) rose by 299(from 45.0 to 179.5 mg/ dL), LDL(bad cholesterol) increased by 51(from 19.35 to 29.15 mg/ dL), HDL(good cholesterol) showed a mild 6 increase(from 53.5 to 56.9 mg/ dL). Following seven days of probiotic intervention, lipid lives in fat- fed rats improved significantly. Total cholesterol dropped by 30 (from 138.5 to 96.5 mg/ dL), TG situations dropped by 84 (from 179.5 to 28.75 mg/ dL), LDL dropped by 50 (from 29.15 to 14.53 mg/ dL), HDL increased by 22(from 52.9 to 64.5 mg/ dL). also, rats on the fat-rich diet gained farther weight than those on the probiotic diet. still, probiotic- fed rats endured healthier weight gain without associated lipid abnormalities. The cholesterol- lowering effect of probiotics is presumably due to cattiness tar hydrolase exertion, which promotes deconjugation and excretion of cattiness acids. This process increases cholesterol catabolism in the liver, reducing circulating lipid situations. Probiotic supplementation significantly bettered lipid lives and moderated weight gain in rats fed a high- fat diet. These findings suggest that probiotics may have remedial eventuality in the operation of hyperlipidemia and obesity. Probiotics are live microorganisms, and they have different positive goods on the host when consumed in

sufficient amount. According to several studies, probiotics have salutary goods on prevention and treatment of multitudinous conditions. The end of the study was to review beast and mortal studies on the part of probiotic in reducing serum cholesterol, their medium of action, and explanation of functional probiotic foods. The study was conducted to see the goods of probiotic yogurt on serum Cholesterol position. Hypercholesteremia is the most important trouble factor for cardiovascular complaint. In this study, we compared the effect of consuming probiotic yogurt with that of high fat diet on serum Cholesterol situations in mildly to fairly hypercholesterolemic subjects. Coemption of Probiotic yogurt containing Lactobacillus acidophilus and Bifidobacteria affect in the dropped total cholesterol and LDL and increased HDL. the important part of probiotics as a part of a healthy diet for humans as well as for brutes and may be an avenue to give a safe, cost effective, and 'natural' approach that adds a barricade against microbial infection.

Keywords: Hypercholestromic, probiotic, Bifidobacteria, Hypertension, Lipid Profile, Cardiovascular Disease, LDL Reducing.

Introduction

Hypercholesterolmia is the most common important threat for cardiovascular complaint. Salutary revision along with other life changes ply a profound effect on controlling Hypercholesterolmia.(1) High Cholesterol position is a major causative agent. Salutary and nutritive cares, diurnal balanced food constituents and avoiding redundant use of fats especially impregnated bones can help ward of the fat deposit in towel and blood vessel.(2) Probiotics are generally defined as microbial food supplement's with salutary goods on the consumers. utmost probiotic fall into the group of organism known as lactic acid producing bacteria and are typically consumed in the form of yoghurt.(3) Historically, the interest has centered on terrestrial organisms, and the term " probiotic " inescapably appertained to gram-positive bacteria associated with the rubric Lactobacillus.To humans and creatures, it can be assumed in monoculture that the intestinal microbiota does n't live as reality by itself but that there's a constant commerce with the terrain and the host functions. The rubrics present in the intestinal tract generally feel to be those from the terrain or the diet which can survive and multiply in the intestinal tract. The System has a much larger influence on the health status than with terrestrial creatures or humans.(4) The use of probiotics to enhance intestinal health has been proposed for numerous times. Probiotics are a feasible microorganism that have a salutary effect in the forestallment and treatment of specific pathologic conditions when they're ingested. It also used to help or treat intestinal diseases.(5) Probiotics are the lactic acid bacteria, particularly Lactobacillus sp. and Bifidobacterium sp. Probiotics have been examined for their effectiveness in the forestallment and treatment of a different diapason of gastrointestinal diseases similar as antibiotic-associated diarrhea, contagious bacterial and viral diarrhea and mortal immunodeficiency contagion, enteral feeding diarrhea, lactose dogmatism.(6) There are numerous mechanisms by which probiotics enhance intestinal health, including stimulation of impunity, competition for limited nutrients, inhibition of epithelial and mucosal adherence, inhibition of epithelial irruption and product of antimicrobial substances.(7) The principle of using inoffensive bacteria for conquering pathogens has been honored for numerous times. Probiotics – live microbial societies consumed for health benefits beyond furnishing introductory nutritive value. They cooperatively maintain a delicate balance between the GI tract and vulnerable system and encourage the growth and exertion of favorable intestinal bacteria are snappily gaining attention as functional foods.(8) Probiotics may offset the seditious process by stabilizing the microbial terrain and the permeability hedge of the intestine, and enhance the declination of entera antigens, altering their immunogenicity.(9) disquisition involving the part of probiotics in precluding the increased position of total cholesterol, LDL and balancing the

LDL and HDL situations and accordingly precluding and reducing the rate of CVD. Probiotics are the important microbes. The following are the positive donation of probiotics • Treatment of severe infections, diarrhea, especially in children • precluding microbial sanitarium acquired and trippers diarrheas and also antibiotic convinced diarrhea. • precluding the circumstance of atopic complaint • precluding the antipathetic condition • precluding post surgery infections • Lowering the blood Cholesterol position, balancing the LDL and HDL(10) The eventuality of yogurt and other fermented milk products to confer health benefits on the consumer by adding the number of lactic acid producing bacteria(LAB) similar as lactobacilli, bifidobacteria and streptococci in the intestinal tract is the subject of important current exploration in particular a possible part of salutary supplement of LAB in the forestallment of cancer of the colon.(11) Probiotic bacteria have been the focus of important scientific and marketable interest. Probiotics are retailed as Capsules, Maquillages, amended yogurts- yogurt like products Milks. illustration of Probiotics are Lactobacillus rhamnoses GG, Lactobacillus acidophilus and Bifidobacteria bifidum. Most importantly probiotics should have a salutary effect on mortal health. Several probiotics are claimed to stimulate the vulnerable system.(12) Bifidobacteria are Gram positive prokaryotes that naturally populate the mortal Gastrointestinal tract(GIT) and vagina.(13) Bifidobacteria are important ingredients of the intestinal microflora of mortal and creatures.(14) Bifidobacteria have been shown to ply several salutary goods on host health, including cholesterol reduction(15) perfecting lactose Application in mal absorbers(16) deconjugation of corrosiveness acids(17) and adding impunity in host creatures. conservation of a health intestinal microbiota and inhibiting pathogens are among multitudinous implicit probiotic goods associated with Bifidobacteria.(18) In addition, Bifidobacteria – containing formula has also been shown to suppress intestinal pathogens similar as Clostridium, Salmonella, Candida, Escherichia coli.(19) Bifidobacterium species are important in maintaining general health. Bifidobacteria belong to effective probiotics in precluding and reducing the inflexibility of some conditions by modulating the host vulnerable response. Each probiotic species is unique, and therefore its parcels and effect have to be assessed independently.(20) Lactobacillus and bifidobacteria are extremely rare causes of infection in mortal, as are probiotic grounded on these organism. strain used for new probiotic should be chosen from the marketable foliage of humans and should not carry natural resistance to antibiotic that would help treatment of a rare probiotic infection. Probiotic lactobacilli and bifidobacteria are suitable for child and children. Severel studies have shown that products that contain Lactobacilli and bifidobacteria are well permitted in this age group.(21) Bifidobacteria were first described in 1900 by Tissier and name by him Bacillus bifidus. They're rigorously anaerobic, fermentative rods, frequently Y- shaped. Bifidobacteria have been considered to be the most important organism in child. and lactobacilli are more multitudinous bacteria for children and grown-ups than bifidobacteria.(22) Lactic acid bacteria(LAB) species, including lactaobailli are indigenous members of the gastrointestinal microbiota of humans and enjoy a time honored character as health promoters. These bacteria have a generally regarded as safe(GRAS) status and are constantly use as probiotics.(23) In this disquisition i have been working the exertion of Bifidobacterium species in to the mortal body and the specis are

• B.bifidum
• B.lactis
• B.infantis

Bifidobacterium bifidum, docked B.bifidum is a fairly common probiotic bacteria in humans. It has some uncommon health benefits. B.bifidum is a lactic acid bacteria that also produces acetic acid, ethanol, and formic acid. B.bifidum are friendly microflora in the mortal digestive system which performs multitudinous functions vital to mortal health. Bbifidum are most generally made available in cream form in probiotic supplement. Presence of B.bifidum in the mortal body is responsible for numerous health benefits. They also enhance the

both vitamins as well as protein emulsion.(24) Probiotics represent a potentially significant remedial advance. In an trouble to drop reliance on antimicrobials, the time has fluently come to increase the exploration of the remedial operations of probiotics. There are too multitudinous reports describing the salutary goods of probiotics to dismiss this generality for preventing and treating a variety of intestinal conditions. Probiotics offer salutary means to support the balance of the intestinal leafage. They may be used to neutralize original immunological dysfunction, to stabilize the intestinal mucosal barricade function, to help contagious race of pathogenic microorganisms and to impact intestinal metabolism. still, any supposed benefit from consumption of probiotics or prebiotics should be accepted as fact only after extensive, multicenter testing in mortal clinical trials. There are still multitudinous undetermined issues that can be answered only by well- designed and well-controlled clinical trials. It's important to flash back that in vitro and beast studies are constantly not transmittable to humans. There are multitudinous implicit advantages to probiotics over conventional remedy, including fairly low cost, the fact that probiotics are doubtful to increase the frequence of antibiotic resistance, and the multiple mechanisms by which probiotics presumably inhibit pathogens, thereby abating the chances for development of resistance against the probiotic.(25) In the future, probiotics must be vanquished to the identical, rigorous scientific studies that are demanded of chemical drug realities, including randomized, placebo- controlled, double- visionless studies, pharmacokinetic studies(i.e., cure-dependent effectiveness, absorption, distribution, metabolism, excretion and duration of effect) and multicenter trials to establish reproducibility. In the history, formerly effectiveness had been demonstrated, numerous researchers have gone on to define a pharmacodynamic profile of the probiotic nor have extensive sweats been directed at relating mechanisms of action. The synbiotic generality must be tested more rigorously as must the use of multiple probiotic strain combinations. It will also be important to define more fluently the mechanisms of action of various probiotics. This will permit the scientific explanation for the selection of the swish species or strains to use against a particular pathogen. It may also permit the operation of heritable engineering to enhance the exertion of probiotics. It should be possible to bring together the capability to survive in the intestinal tract with the capability to produce metabolites that are responsible for the probiotic effect.(26) The part(s) of probiotics bacteria in dairy restlessness is to help in(i) the preservation of the milk by the generation of lactic acid and possibly antimicrobial mixes;(ii) the product of flavor mixes(e.g. acetaldehyde in yoghurt and rubbish) and other metabolites(e.g. extracellular polysaccharides) that will give a product with the organoleptic parcels asked by the consumer;(c) to meliorate the nutritional value of food, as in, for illustration, the release of free amino acids or the emulsion of vitamins; and(iv) the provision of special remedial or preventative parcels as cancer(27) and control of serum cholesterol situations.(28)

Table 1. Various special therapeutic or prophylactic properties of specific probiotics

Microflora	Associated actions	Reference
Bifidobacteria species	Reduced incidence of neonatal necrotizing enterocolitis	*Caplan, M.S. and Jilling, T. (2000) Neonatal necrotizing enterocolitis: possible role of probiotic supplementation. J Pediatr Gastroenterol Nutr 30, S18–S22.* [29]
Enterococcus faecium	Decreased duration of acute diarrhoea from gastroenteritis	*Marteau, P., De Vrese, M., Cellier, C.J. and Schrezenmeir, J.*

		(2001) Protection from gastrointestinal diseases with the use of probiotics. Am J Clin Nutr 73, 430S–436S. [30]
Lactobacillus acidophilus	Reduced incidence of diarrhoea in daycare centres when administered to only half of the children 337-346	*Tomas, M.S., Claudia Oter, M., Ocana, V. and Elena Nader-Macias, M. (2004) Production of antimicrobial substances by lactic acid bacteria I: determination of hydrogen peroxide. Methods Mol Biol 268, 337–346.*[31]

History

The conception of probiotics evolved around 1900, when Nobel Prize- winning Elie Metchnikoff hypothecated that the long, healthy lives of Bulgarian peasants were the result of their consumption of instigated milk products and latterly he was induced that yogurt contained the organisms necessary to cover the intestine from the dangerous goods of other dangerous bacteria. The first clinical trials were performed in the 1930s on the effect of probiotics on constipation. In the 1950s, a probiotic product was certified by the United States Department of Agriculture as a medicine for the treatment of comb(Escherichia coli infection) among gormandizers.(32) Over the last century, differentmicro-organisms have been used for their capability to help and cure conditions, leading to the coining of the term probiotics.(33) The discovery by Mann and Spoerig(1974) that people who drank yogurt instigated with wild strains of Lactobacillus sp. had veritably low values for blood serum cholesterol opened up a new area of study.(34) Harrison et al. 1975(35) reported that cells of Lactobacillus acidophilus added to child formula dropped situations of serum cholesterol, and Gilliland et al.(1985)(36) Buck and Gilliland(1994)(37) and Gilliland and Walker(1989)(38) showed control of serum cholesterol situations in adult mortal trials. In 1994, the World Health Organization supposed probiotics to be thenext-most important vulnerable defence system when generally specified antibiotics are rendered useless by antibiotic resistance.(39) The use of probiotics in antibiotic resistance is nominated as a microbial hindrance remedy.

Objective:

The main objective of this studies are:

1. To isolate and to identify the suitable Probiotic strains of *Bifidobacteria* and lactic acid Bacteria.
2. To prepare a Probiotic products like yogurt and its application to animal.
3. To study the effects of Probiotic for the reduction of serum cholesterol.
4. Subsequently, this could be used as therapeutic drugs for human.

Literature review

Historical overview:

Probiotics have been used for numerous times to prop in restoring and maintaining a health intestinal balance in favor of healthy bacteria which is essential to maintaining good health. (40) The term probiotics was introduced by Lilly and Stillwell in 1965 for growth promoting factors produced by microorganism. The word Probiotic is deduced from the Greek meaning for life and has had severel different meaning over the times. still, its use in this form did n't persist and it was latterly used by Sperti(1971) to describe towel excerpts which

stimulated microbial growth. Although Probiotic relating to food supplyment only dates from 1974, the history of live microbial feed supplyments goes back thousands of times. presumably the first foods that contained living microorganism were the fermented milk that are recorded in the old treatment.(41) Probiotic lactobacilli may ameliorate lactose digestion and reduce symptoms of lactose dogmatism. The effect of Probiotic on serum cholesterol is still inconclusive. A number of studies have examined the eventuality of probiotic products to reduce serum cholesterol.(42) Bifidobacteria were first discovered in child feces by Tissier in 1900. This bacterium had a characteristic ' Y' shape and was named Bacillus bifidus. It was anaerobic, gram positive and did n't develop gas during its growth.(43) From 1900 to 1957, many advances were mede in the knowledge of this bacteria. The Tissier strain was first replaced by Lactobacillus Bifidus.(44) The Actuality of multiple biotypes of Bifidobacterium caused the offer for a scheme for the isolation of these bacteria grounded on their carbohydrate turmoil patterns.(45) Seven species of Bifidobacterium were honored, in addition to the known B. bifidum.(46) The characteristics metabolic pathway of hexose turmoil in Bifidobacteria was clarified.(47) The crucial enzyme is fructose-6- phosphate phosphoketolase which splits the hexoes phosphate to erythrose-4- phosphate and acetyl phosphate. The DNA- RNA sludge hybridization procedure was developed in order to assess the validity of the Bifidobacteria 1 species preliminarily described and to fete new DNA homology groups among the strains being insulated in large number from divers ecological niches.(48) In the 8 th edition of Burgeys Manual of determinative Bacteriology.(49) Bifidobacteria were classified in the rubric Bifidobacterium using the same name originally espoused by Orla- jensen.(50) Bifidocin B is the only Bacteriocin produced by Bifidobacteria that has been purified, characterized and set up to inhibit growth of species of Listeria, Enterococcus, Bacillus, Lactobacillus and Pediococcus.(51)(52) There are presently about 30 known Bifidobacterium species. The Bifidobacterium species that inhabit the mortal intestinal tract are rather distinct from those that inhabit the bowel of creatures.(53) The representatives species of mortal origin include B. longum, B. breve, B. bifidum, B. adoltescentis and B. pseudocatenulatum. Representative of beast – deduced species include B. pseudolongum, B. thermophilus and B. animalis. Species of beast origin are noway insulated from the mortal intestinal tract and mortal origin species are nearly noway set up in beast bowel. The reason for this host- particularity is unknown, but is suspected to be due to difference among species in the capability to populate the host intestinal tracts. Among the Bifidobacterium Species used in colorful yogurts, B animalis is frequently linked.(54) This species is also not an tenant of the mortal intestinal tract. The use of mortal origin species as food supplyment seems to be the reasonable and correct choice. Bifidobacterium produce both lactic acid(L- isomer) and acetic acids from lactose.

Probiotic

The term probiotic was first introduced by Lilly and StillWell (1965) to describe growth promoting factors produced by the microorganism.[55] Probiotic is derived from Greek, means pro-life. Parker (1974) used the term probiotic for microorganism or substances , which contributes to intestinal microbial balance.[56] Crawford (1979) later defined probiotic as a culture of specific living micro organism (Primarilly Lactobacillus sp) . Which implants in the animal to which it is feed and ensures the effective establishment of the intestinal population of the beneficial organisms. A probiotic was designed to promote proliferation of the beneficial species of bacteria within the gut environment. [57] Fullar 1989 has defiend a probiotic as a life microbial feed supplyment. [58] Fuller have defined the probiotic as follows,

1. The probiotic must be capable of being prepared in a viable manner and on a large scale (for industrial pourpose)
2. During use and understorage the probiotic should remain viable
3. Stable

4. It should be able to servive in the intestinal ecosystem.
5. It has been Hypothisised that probiotic administrated in human may have positive effect in a number of biomedical test.[59]

Probiotic Concept:

Probiotics, a word derived from the Greek and meaning ' for life', are defined as live organisms that, when ingested in respectable amounts, ply a health benefit on the host. Lactobacilli, bifidobacteria andnon-pathogenic provocations, analogous as Saccharomyces boulardii, are among the most generally used and fully estimated probiotics.(60) While the forenamed description confines the use of the term probiotic to products that contain live organisms, the probiotic generality has, of late, and perhaps inaptly, been extended to include the use of killed organisms or indeed bacterial DNA. Prebiotics are defined asnon- digestible but fermentable products that beneficially affect the host by extensively stimulating the growth and exertion of one species, or a limited number of species, of bacteria, in the colon.(61) Compared with probiotics, which introduce exogenous bacteria into the mortal colon, prebiotics stimulate the preferential growth of a limited number of health- promoting commensal leafage formerly abiding in the colon, especially, but not simply, lactobacilli and bifidobacteria. The energy of this stimulant is, in part, dependent both on the birth density of the target probiotic leafage(bifidobacteria and/ or lactobacilli) and on the luminal pH. The oligosaccharides in mortal bone milk are considered the proteotypic prebiotic as they grease the preferential growth of bifidobacteria and lactobacilli, in the colon, in simply bone- fed babes. A combination of a probiotic and a prebiotic is appertained to as a synbiotic. collectively, these substances are sometimes appertained to as functional foods. For the purposes of this review, and given the deficiency of data on the use of prebiotics and other functional foods in IBS, we confine our commentary to probiotics.(62) The interpretation of the literature on probiotics, not to mind the multitudinous claims that are made in the lay press and other media, is fraught with difficulties, multitudinous related to factors natural to this area.(63) firstly, it's n't unusual for the benefits of a given species or organism to be touted predicated on the validation derived from the studies involving other organisms and species, despite the fact that detailed studies have demonstrated that, in terms of a probiotic property, be it vulnerable modulation or antibacterial exertion, there are tremendous differences between different lactobacilli and bifidobacteria, not to mention, for illustration, between lactobacilli and bifidobacteria.(64) No two probiotics are the same and extrapolations from one to another should be defied at all times.(65) Secondly, an existent who is about to consume a given probiotic drug should know exactly what he or she's about to take is it live, what is its attention, will the organism survive as it makes contact with acid, cattiness and digestive enzymes while coursing the gut, and what will be the factual attention of the organism at its asked point of action? numerous probiotic specifics have been characterized and formulated with sufficient rigour to allow the manufacturer to give answers to these critical questions.(66)(67) Of further concern, critical examinations of the factual constituents of commercially available probiotic specifics have revealed fussing diversions from those included in the product marker. ultimately, indeed lower still have been characterized in detail in terms of their microbiological parcels, immunological or physiological goods and only a sprinkle have been vanquished to clinical trial in humans.(68) The probiotic bacteria have been following characteristics

- Accurate taxonomic identification
- Normal tenant of the species targeted mortal origin for mortal Probiotic

- Non poisonous and non pathogenic
- Generally stable
- product of antimicrobial substances
- Resistant to acid
- Resistant to corrosiveness

Probiotic Preparations

The probiotic medications used in this- bacillus salivarius species salivarius UCC4331 and Bifidobac- terium infantis 35624, were firstly insulated from the ileoce- cal region of an adult human witnessing reconstructive surgery. These strains were named on the base of the follow- ing probiotic parcels being of mortal origin, nonpathogenic, and resistant to intestinal acid and corrosiveness; demonstrating an capability to cleave to mortal epithelial cells; and demonstrating an capability to temporarily populate and be metabolically active within the mortal gastrointestinal tract. 59,60 Further more, these organisms have been preliminarily shown in levy studies to survive conveyance through the gastrointestinal tract, to be free of side goods, and to demonstrateanti-inflammatory exertion in a number of models. 60,61 L salivarius UCC4331 was dressed in de Man/ Rogosa/ Sharp broth (Ox- oid, Basingstoke, United Kingdom) at 37 °C in an anaerobic terrain for 24 hours. B infantis 35624 was dressed in de Man/ Rogosa/ Sharp broth amended with cysteine at 37 °C in an anaerobic terrain for 48 hours.(69)

Composition of probiotic preparation:

The most commonly used microorganism in probiotic preparation are the lactc acid bacteria .These are found in large numbers in the gut of healthy animas and are in the words of the Ameican FDA ,Generally Regarded as safe.[70]

Table- The most commonly used species of lactic acid bacteria in probiotic preparation

Lactobacillus sp.	**Bifidobacterium sp.**	**Enterococcus sp.**	**Streptococcus sp.**
L acidophilus	B.bifidum	Ent faecalis	S. cremoris
L casei	B.adolescentis	Ent faecium	S. salivarius
L delbrueckii ssp.(bulgaricus)	B.animalis		S. diacetylactis
L cellobiosus	B,infantis		S. intermedius
L curvatus	B.thermophillum		
L fermentum	B.longum		
L lactis			
L plantarum			

HOW DO PROBIOTICS WORK

To understand how probiotics work, it's important to understand a little about the physiology, microbiology of GI tract and the digestive process.The digestive process begins as soon as food enters the mouth and to stomatch, the microbs present in GI tract have the eventuality to act in a favourable, nocuous or neutral manner. Certain intestinal microbes are known to produce vitamins, and they're nonpathogenic, theire metabolism is non- decay, and their presence is linked with a healthy intestinal herbage. The metabolic product of theire growth is organic acid that tends to lower pH of the intestinal contents. Probiotics may also impact other defensive functions of

the intestinal mucosa including conflation and hiding of antibacterial peptides. Normal microbial occupants of the GI tract also support the hedge function of the intestinal filling, abating translocation or passage of bacteria or antigens from the intestine into the blood conduit.(71) Probiotics modulate the composition of the intestinal microflora. The survival of ingested probiotics in different corridor of the gastrointestinal tract varies. This specific change may be seen for a multitudinous days after the launch of consumption of probiotic medicine, depending on the capacity and capsule. Probiotics must be ingested regularly for any health promoting parcels to persist.

Health benefit and therapeutic effects of probiotics

There are a variety of proposed beneficial health effects of probiotics; only a few have significant research to back up the claims and will be discussed in this paper. Clinical symptoms that have been reportedly treated or have the potential to be treated with probiotics include diarrhoea, gastroenteritis, irritable bowel syndrome, and inflammatory bowel disease (IBD; Crohn's disease and ulcerative colitis), cancer, depressed immune function, inadequate lactase digestion, infant allergies, failure-to-thrive, hyperlipidaemia, hepatic diseases, *Helicobacter pylori* infections, and others. The use of probiotics should be further investigated for its possible benefits and its side-effects if any.[72]

Probiotic used In children:

Several studies have examined the effect of probiotics on AAD in children [73] and found a significant difference in the incidence of AAD in children six to 36 months ages taking a probiotic formula consisting of Bifidobacterium lactis and Streptococcus thermophillus compared to placebo. (16% versus 31%).

The total efficiency of probiotic formula in preventing AAD was 47%. Probiotic did not significantly shorten the duration of diarrhea in the two groups although those patients talking the control formula had increased episode of AAD compared to those on the probiotic formula.

Criteria for classification as Probiotics

There is increasing evidence that probiotics that probiotics can benefit the human host by acting as a frist line of diseases against diseases-causing pathogens by improving the intestinal microflora. However, to have functional probiotic strains with predictable and measurable health benefits, a concerted effort for strain selection is required.

There is no one agreed set of selection criteria for classifying a viable bacterial strain as a probiotic. Common criteria used for isolating and defining probiotic bacteria and specific strains include the following:

- Genera of human origin
- Stability against bile acid, enzyme and oxygen
- Ability to adhere to intestinal mucosa
- Colonization potential in the human gastrointestinal tract
- Production of antimicrobial substances
- Demonstrable efficacy and safety

The parameters for screening micro-organisms for potentially valuble probiotic strains should include the fact that there is a necessity for the strain to be viable and metabolically active within the gastrointestinal tract and biologically active against the identified target. In addition, it is important that viability of the strain and stability of the desirable characteristics of the strain can be maintained during commercial production as well as in the final product. A combination of in vitro studies, including clinical trials, is required. Most current probiotics

have been selected using these criteria. However, in some cases the outcome of such selection has been questioned, especially the requirement for potential in the prophylactic management of gastrointestinal diseases (such as inhibition of pathogens, bowel cancer prevention) and more systematic effects (e.g. reduction in blood lipids, hormonal regulation). Before health claims for probiotics, probiotics and synbiotics can be made, more corroborative studies are required to associate changes in gut bacterial populations with physiological aspects in humans. In addition, a better understanding of how probiotics and probiotics cause changes in the microbial community structure is essential. [74]

Probiotic bacteria and Functional Food:

The growing understanding of the relationship between diet and health increased the demand for food with specific benefit beyond their basic nutrition such as improving the health and well being of human .This food is called Functional food .However,Functional food has defined as one, which provides a specific health benefit over and above its normal nutritional status.[75] It has been suggested that Food will use as functional when it has shown beneficial effect on one or more target in the body and that beside their nutritional effects such as well- being and health of the host[76] The old generation of Functional Foods indicates of using supplements to the food to increase their nutrition and health effects such as Vitamins and micronutrient. However the new concept b of functional foods there is more interest in the Gastrointestinal interactions.[77] Therefore , the use of Probiotic micro-flora is one of the most promising areas the for the development of functional foods as recent studies have been.[78] Bifidobacteria are the most dominated organism in the gastrointestinal tract and their validity and metabolic activity have shown beneficial effects on the health of the gastrointestinal tract [79] and that always related to the presence of suitable environment and nutrients which are very important for the validity and activity ,for Bifidobacteria to use it in the bowel as carbon and energy source , these compound were referred to as – Bifidogenic factors.[80] At present, Probiotic products and especially probiotics dairy foods are marketed successfully all over the world because of their acceptance of consumer and the awareness about their positive aspect for the health benefits.

Mechanism of probiotic action:

There are many different species of probiotics with a wide variety of mechanisms of action . The most commonly studied for use in the treatment of diarrhea are : *Lactobacillus. Bifidobacteriuym, Saccharomyces boulardii.* Other less studied ,but possibly effective ,agents are *Lactobacillus rhamnosus, Lactobacillus casei, L plantarum 299v, Entrococcus faecium, Lactococcus thermophilus and Saccharomyces cerevisiae*. Research suggests probiotics act through several different mechanism:[81]

- Protecting the intestinal epithelial cell and barrier function
- Preventing enterotoxin binding immunomodulation (enhance cellular and humoral immune response)
- Producing protective factors
- Improving the microbial balance of the intestinal tract
- Regulating intestinal function
- Inside the gut it fight against pathogenic ones to consume more nutrient ,so the pathogenic bacteria die duet lack of nutrient's
- It occupies the adhesion sites of the gut, so the pathogenic organism swept away by peristaltic action of food.
- Produce antimicrobial substances (acidolin,acidophilin,lactocidin)organic acids and decrease the pH of the gut and decrease the growth of pathogenic bacteria.

- It break down the polysaccharides, ferment the carbohydrates and produce folic acide,vitamin B1,B2,B3,B4,B5,B6,B12,A,K.
- It reduces serum cholesterol and TG, produces tryptophan.
- It increases immunity and prevents allergy.
- It contains lactase and decrease lactose intolerance.

Probiotic mechanism of action in Figure

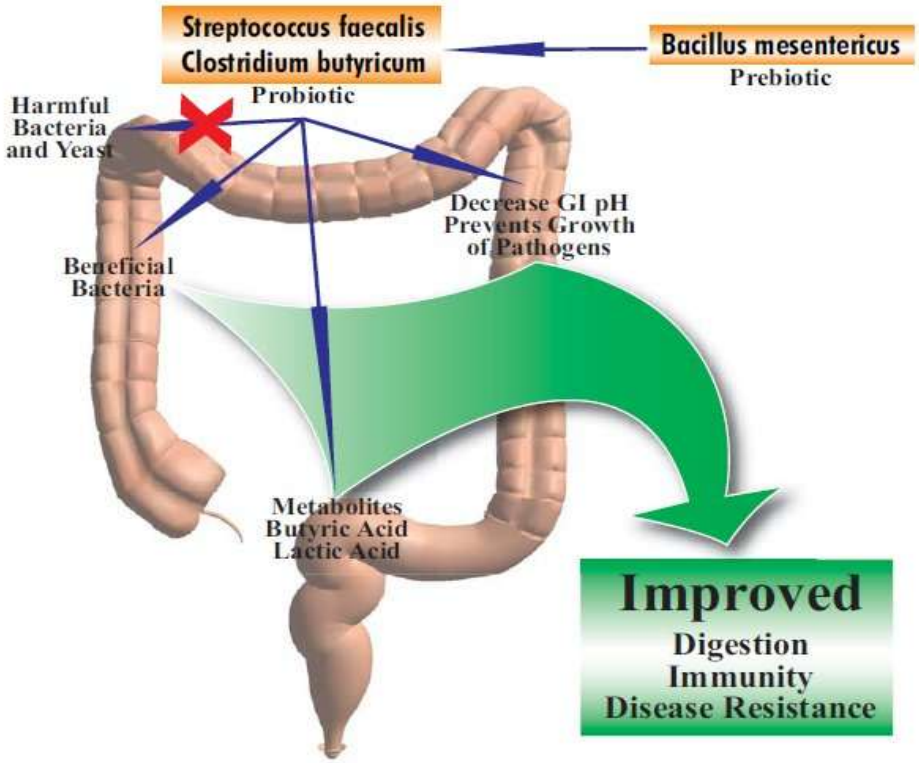
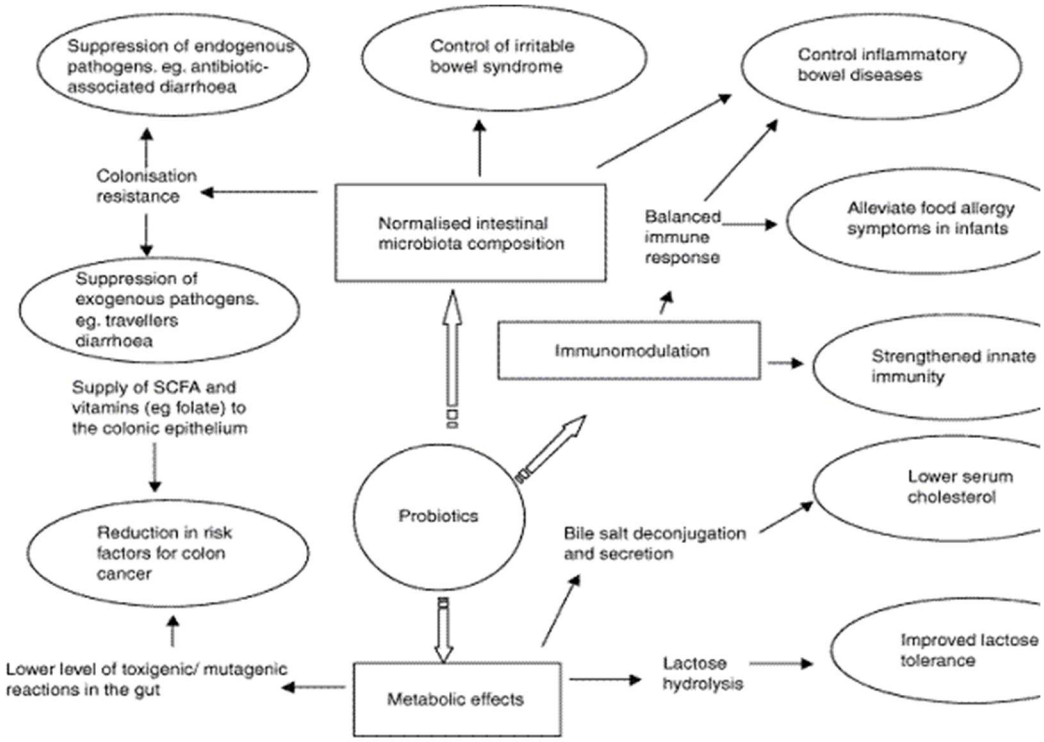


Figure: Probiotic mechanism of action

Each competes for nutrient which necessary for pathogen survival[82] as well as produce produce substances which inhibit or kill destructive pathogens and modulate toxin production.[83] They are thought to promote the secretion of antimicrobial substances and produce acetic acid , lactic acid , and hydrogen per-oxide . By restoring equilibrium to the altered gastrointestinal flora , Probiotic can protect against colonization by pathogens such as *C. difficile*. Probiotics are instrumental in adding in reestablishing or modifying intestinal microflora which has been damaged and disturbed.

Effects the probiotic on the immune system:

Probiotics are climed to stimulate the immune system. Their modes of action appear to be nonspecific ,resulting in increased immune responsiveness to a wide variety of antigens .



Classification Probiotic Bacteria :

The most important bacteria used as probiotic belong to the genera Lactobacillus (LAB) and Bifidobacterium(BB) .

Probiotic bateria

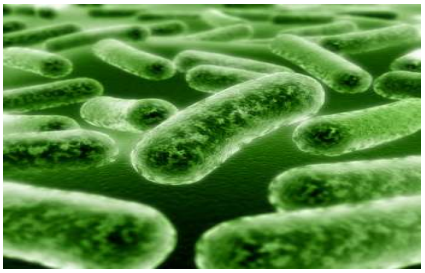


Figure: Bifidobacteria

Criteria for classification as Probiotics:

There is increasing evidence that probiotics that probiotics can benefit the human host by acting as a frist line of diseases against diseases-causing pathogens by improving the intestinal microflora. However, to have functional probiotic strains with predictable and measurable health benefits, a concerted effort for strain selection is required.

There is no one agreed set of selection criteria for classifying a viable bacterial strain as a probiotic. Common criteria used for isolating and defining probiotic bacteria and specific strains include the following:

- Genera of human origin
- Stability against bile acid, enzyme and oxygen
- Ability to adhere to intestinal mucosa
- Colonization potential in the human gastrointestinal tract
- Production of antimicrobial substances

- Demonstrable efficacy and safety

The parameters for screening micro-organisms for potentially valuble probiotic strains should include the fact that there is a necessity for the strain to be viable and metabolically active within the gastrointestinal tract and biologically active against the identified target. In addition, it is important that viability of the strain and stability of the desirable characteristics of the strain can be maintained during commercial production as well as in the final product. A combination of in vitro studies, including clinical trials, is required. Most current probiotics have been selected using these criteria. However, in some cases the outcome of such selection has been questioned, especially the requirement for potential in the prophylactic management of gastrointestinal diseases (such as inhibition of pathogens, bowel cancer prevention) and more systematic effects (e.g. reduction in blood lipids, hormonal regulation). Before health claims for probiotics, probiotics and synbiotics can be made, more corroborative studies are required to associate changes in gut bacterial populations with physiological aspects in humans. In addition, a better understanding of how probiotics and probiotics cause changes in the microbial community structure is essential. [84]

Bifidobacteria:

History of Bifidobacteria:

Bifidobacteria were first discovered in 1900 in the face of infants by Henry Tissier at Pasteur Institute, Paris, France who named it *Bacillus bifidus.*[85] They were gram positive lactic acid producing bacteria curved rods. They were found to be predominant in the intestinal lumen of breast-fed infants.

For taxonomical reasons, growth of the knowledge and uses of Bifidobacteria is divided into two distinct periods that is from 1899-1957 and from 1957 to present. During the first period: after the description and naming of Bifidobacteria studies were concernd with the growth promoting factors for Bifidobacterium spp.[86] The occurance of thise organisms in the human intestinal tract, their significance in the health of infants, and divising of culture media for isolation and maintainance of strain.[87]

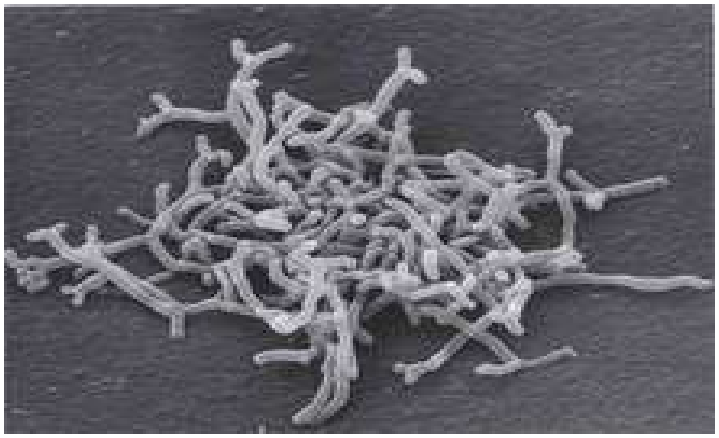


Figure: **Bifidobacterium** bifidum Restrain

Characteristics of Bifidobacteria species:

Biological:

Bifidobacteria are a group of microorganisms. They have important taxonomic characteristics, cell wall

constituents and Deoxyribo-nuclic acid (DNA) base composition. Mitsouka (1977) [88] reported that these organism are found in the intestinal tract of infants and adults, honeybase intestine, oralcavity, human vagina and in the elementary tract of various animals.

MorphologicaL:

Bifidobacteria are non-spore forming rods of variable appearance, spatulate or club shape& V shape. Their shape is convex to lense shape. A variation in cell morphology was found in some species, whicdh have distinctive cell shapes. Surface colonies grown on agar plate, incubated anaerobically, had variable shape, appearance, and size depending on nutritional condition and strain characteristics.[89]

Bifidogenic Factors:

Bifidobacteria are metabolically active in the lower gastrointestinal tract.[90] The production of lactic and acetic acid by Bifidobacteria depends on the metabolism of carbohydrates. Bifidogenic factors which comprise carbohydrates such as (FOS), (TOS) for supplymentation of human diet.[91]

Species of Bifidobacterium[92]

B.adolescentis	B.magnum
B.minimum	B.longum
B.pullorum	B. B.boum
B. indicum	B.subtile
B.saeculare	B.thermophilum
B.suis	B.animalis
B.angulatum	b.bifidum
B.asteriods	B.infatis
B.catenulatum	B.lactis
B.dentium	B.breve

Lactobacilli:

Bacteria of the genus Lactobacillus are gram positive, non-motile, non-sporing bacillivarying from slender, long fods to coccobacilli forms. There are species of Lactobacilli listed in the Bergys Manual of determinative bacteriology. The genus is a member of the family Lactobacillaceae and has been extensively studied.[93] Lactobacilli are Phenot ypically very heterogenus and are now being cclassified using 16S RNA sequence analysis .*[94]*

.Lactobacilli have complex nutritional requirement since in addition to carbohydrate as a sourch of energy and carbon , a variety of nucleotides , amino acids, peptides, fatty acids, salts and vitamins are also required for its growth.[95] By and large growth is optimum at pH 5.5 to 5.8 Fermentation of hexoes by Lactobacilli can occure by either the Embden- Meyerhop Paranas (glycolytic) pathway (homolactic fermentation) or the 6-Phosphogluconate fermentation (heterolactic fermentation) depending upon the species of Lactobacillus .Some species of Lactobacilli are stricts anaerobes e.g L. lactis ,L leichmanni and L. ruminus. While others are aerotolerent.[96] Plasmides have been foud to be relatively common in Lactobacilli including those isolated from the intestine.*[97]* Lactic acid bacteria are capable to producing and excreting inhibitory substances other than lactic and acitic acid . These substances are antagonistic to a wide spectrum of microorganism and play an important role in preservatine action. The produced small amount of lactic acid and acetic acid and includes formic acid, free fatty acid ammonia , ethanol, hydrogen peroxide ,bacteriolytic enzymes ,bacteriocins, and

antibiotics as well as several less well defined or completely undefined inhibitory substances .[98] [99][100][101] Some strains of lactobacilli colonise on the epithelial surface of the gut , forming a thick layer of adhering cells . Its colonization is host specific , forb example Lactobacilli from chicken crops epithelial cells will not adhere to pig gut epithelial cells and vice versa . Lactobacilli may also be present in the GI tract as transients from saliva and food .[102]

Most commonly used probiotic

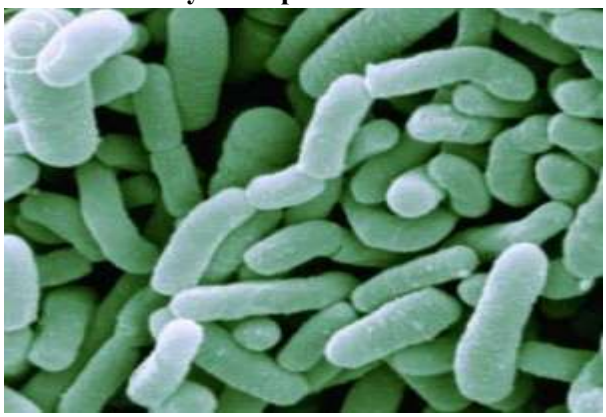


Figure: Lactobacillus Acidophillus

Lactic acid bacteria and bifidobacteria as probiotic

Traditionally ,the lactic acid bacteria are define by formation of lactic acid as a sole or main end-product n from carbohydrate metabolism . Lactic acid bacteria comprise a diverse group of Gram positive , non spore forming bacteria .They occurs as cocci or rod and are generally lacking catalase . They are Chemoorganotrophic and grow only in complex media . Fermentable carbohydrates are used as energy source. Hexox are degreded mainly to lactate (homofermantative) or to lactate and additional product such as acetate ,ethanol, CO_2, formate or succinate (heterofermentative).[103]

Theraputic Potential and health Benefits of Lactobacillus acidophilus and Bifidobacteria[104]

The main therapeutic and health benefits of L acidophilus and Bifidobacteria are:

Enhancement of immunity against intestinal infections.

Immune enhancement.

Prevention of diarrheal diseases.

Prevention of colon cancer.

Prevention of hypercholesterolaemia.

Improvement in lactose utilization.

Prevention of upper gastrointestinal tract diseases.

Stabilization of the gut mucosal barrier.

Prevention of intestinal infections Lactobacillus acidophilus and Bifidobacteria exert antagonistic effects on the

growth of pathogens such as Staphylococcus aureus Salmonella typhimurium, Yersinia enterocolitica and Clostridium perfringens.

Clinical benefits of Bifidobacteria[105]

- Possibly Bifidobacteria are effective for prevention of a type of colitis caused by bacteria (necrotizing enterocolitis).
- Prevention of diarrohea in infants, when used with other bacterium called Stretococcus thermophilus.
- Prevention of traveler's diarrohea[106]
- when used with other bacteria such as Lactobacillus acidophilus, Lactobacillus bulgaricus, or *Streptococcus thermophillus*
- Treating skin condition called atopic eczema.
- Inflammation of the gntestine in infants.
- Irritable bowel syndrome.
- Preveting a complication after surgery for ulcerative colitis called pouchitis.

Growth requirement of Bifidobacteria:

Bifidobacteria are anaerobes (because they are incapable of oxyzen respiration and growth under aerobic condition).[107]

studied the relationship between the oxyzen sensitivity and oxyzen metabolism of Bifidobacteria species .The Bifidobacteria species are reduced NAD -oxydase and NAD- peroxidase activities .

0_2+NADH H+⟶ H_2O_2+NAD $^+$

H_2O_2+ NADH+H_+⟶ $2H_2$ 0 + NAD $_+$

Human strains of Bifidobacteria grow at optimum temperature between 36 – 38^{0}C and Nutritional requirement depend on the species and strains. Some strains use ammonium salts as nitrogen whereas others require organic nitrogen.[108]

Optimum temperature, pH, and sensitivity of oxygen:

Human strains of Bifidobacteria grow at optimum temperature between 36 – 38^{0}C and Nutritional requirement depend on the species and strains. Some strains use ammonium salts as nitrogen whereas other require organic nitrogen.[109] Optimum temperature for growth is 37 -41 ^{o}C ,while no growth occure below 20^{0}C and above 46^{0}C.[110] Growth at 45^{0}C seems to discriminate between human and animal intestine.[111] The only exception is B.thermaci-dophilum able to growth at moderately thermophilic condition 49^{0}C.[112] Bifidobacteria are acid tolerance microorganism . The optimum pH is between 6,5 and 7,0. No growth is recorded at pH lower than 4,5 (only B.thermacidophilum has delayed ggrowth atPH 4) and higher than 8.5.Bifidobacteria are anaerobic however the sensitivity to oxygen change accordingly to the specis .

Nutritional requriremenrt of bifidobacteria :

Different nutrition aspect concerning the complex requirement of nitrogen, vitamin, growth factor and metal element by Bifidobacteria, have been extensively studied and reviewed. [113] N – acetylglucosamine and B-substitute disaccharide, N- acetyllactosamine are milk component and stimulate Bifidobacteria growth. When subcultured in media with the excess of N- acetylglucosamine ,Bifidobacteria cell have a more regular form .Probably cells assumes a blowing and branched form, for the liomiting concentration of N- acetyl-glucosamine which is an essential precursor for peptidologican biosynthesis .The important role of bifidigenic

factors was reviewed by Molder.[114] Lactulose was found to be an effective factor for Bifidobacterium growth and it is also applied in a wide varity of foods as bifidogenic factors .Lactose is readily metabolized by all the specis of Bifidobacterium.

Echology in the Human intestinal tract:

Before birth , the human fetus is gram- free and intestinal bacteria do not exist .From the time of birth ,bacteria begin to colonize the intestinal tract forming the intestinal microflora . At birth ,many bacterial species gain access in to the intestinal tract, but Bifidobacteria gradually become established as the main bacteria and predominate in the intestinal microflora during the neonatal period . This tendency is especially marked in the Brest-feed infants.[115] According to a study, Bifidobacteria constitute over 95% of the intestinal flora in breast feed infants.[116]

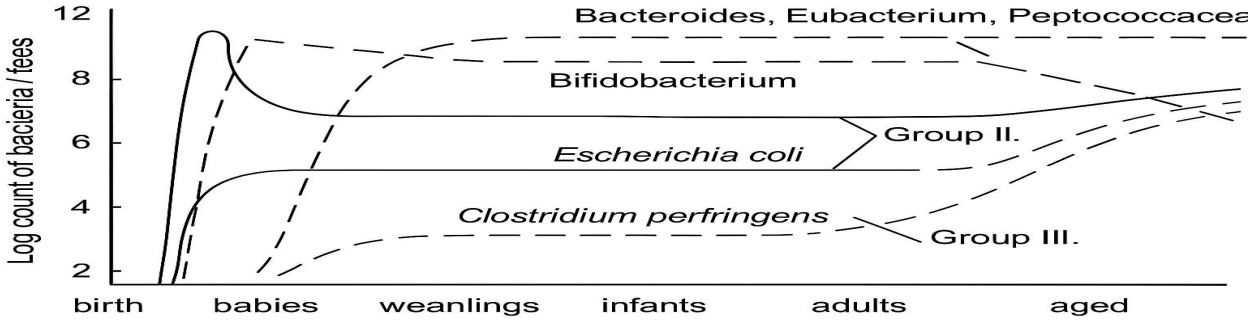


Figure : change of intestinal flora with age [117]

Table -1: Faecal Characteristics of Infant and Adult [118]

	Infant (3 months)	Adult(23 age)
Bifidobacteria (%)*	96%	19
pH	5.30	6.4
Ammonia(µ mol/g)	13.20	54.0
Indole (µ g/g)	0.76	56.2
P-cresol(µ g/g)	ND	68.7
Phenol (µ g/g)	0. 72	7.6
Urease**	22.50	911.0
Tryptophanase**	0.33	3.7
B-Glucronidase**	2.09	33.4

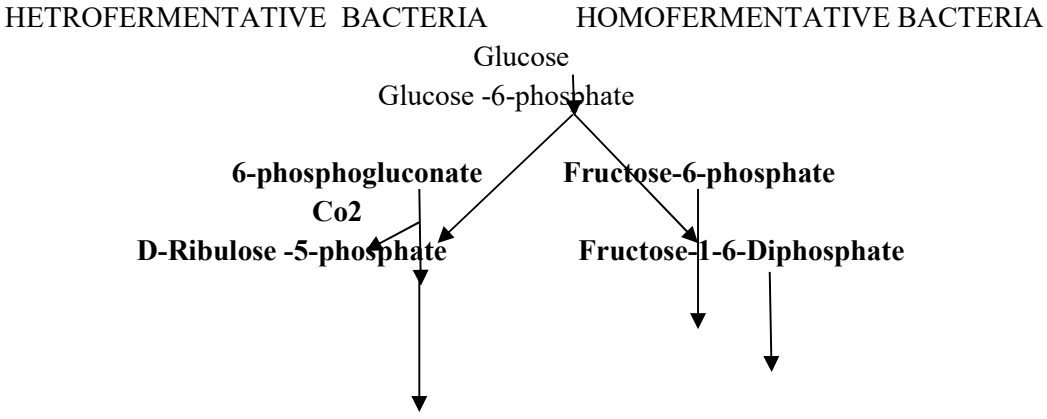
Survival of Bifidobacteria and Lactobacillus acidophilus:

Lactobacillus acidophilus and B.bifidum are inhabitants of the intestine of human as well as animal . Benificial effects of L.acidophillus and Bifidobacterium spp.can be expected only when viable cells of these

organisms are able to survive passage through the human stomatch and digestive system in buffering capacity (pH 3.72-7.74),[119] which may allow it to resist change in cytoplasmic pH and gain stability under acidic condition . Lactobacillus acidophilus is morte tolerant to acidic condition than B.bifidum and the growth of B.bifidum is significantly retraded below pH 5.0.[120]

However tolerance of Bifidobacterium to acidic stomatch condition has been reported to be strain specific.[121] B. longum shows better survival in acidic condition compared with B.infantis, B. adolescentis and B. bifidum.[122] Lankaputhra and Shah have studied survival of nine strains of Bifidobacterium spp. In acidic condition (pH 1.5-3.0); B.longum and B. pseudolongum shows the greatest survival.[123]

They have also reported that B.adolescentis and B.breve survive poorly at all pH levels (1.5,2.0,2.5, and 3.5) tested. Encapsulation of bacteria of bacteria in milk fat does not improve the survival of Bifidobacteria in high acid yogurt.[124] Gastrointestinal system have varying concentration of bile. The rate of secration of bile acid and its concentration depend on the type of food consumed.For example the fat content of the diet could influence the level of faecal bile acids and fatty acids may increase the inhibitory effect of bile acids towards Lactobacillus and Bifidobacterium spp.[125] Bile concentration range from 0.5 to 2.0% in the first hours of digestion and the level may decrease during the second hours.[126] Ibrahim and Bezkorovainy have reported that Biofidobacteria are able to survive physiological and higher bile salt levels.[127] They have reported that at 0.06-0.03% sodium glycolate , B. infantis shows the best survival ,followed by B. bifidum and B.breve , whereas B.longum shows least resistance to bile .HoweveClark and Martin reported that B.longum shows that best survival rate in 2% and 4% bile.81 survival numbers are approximately 107 CFU/ml after 12 h in 2% bile. Lankaputhra and Shah have reported that among six strain of lactobacilli ,two strain (L.acidophillus 2404 and 2415) shows the best tolerance to bile (1-1.5%) and among nine strains of Bifidobacterium spp.,B.longum , B.pseudolongum and B.infantis show the best tolerance to bile (1-1.5%).(%).[128]They have also pointed out that while one strain of B .longum (1941) is tolerant to bile , the other strain of B.longum (20097) does not survive well in bile .Both L .acidophilus and Bifidobacterium are able to survive and grow in soft-sever frozen yogurt after freezing and both are found to grow in up to 0.45% bile salts before and after freezing.[129]



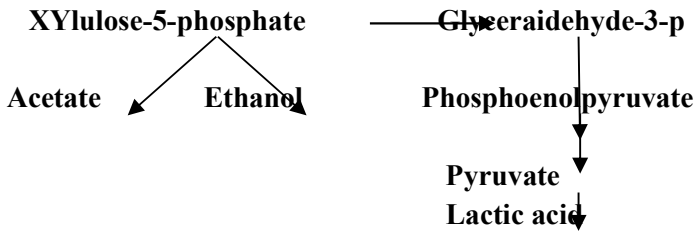


Figure: Brief summary of the Metabolic pathways products of hetro and Homofermentative bacteria and Bifidobacteria.[130]

Ways of increasing the survival of Bifidobacteria:

There are number of substances known to improve or promote the growth of probiotiv bacteria.

- Tomato juice and papaya pulp stimulate the growth of L.Acidophillus,resulting in higher viable count shorter generation time and and improve sugar utilization.[131] The stimulation could be attributed to greater availability of simple suger ,mainly glucose and fructose and minerals (i.e.Magnesium and manganese) which are growth promotes for L.Acidophillus.[132]
- Supplymentation of milk with combination of casitone ,casein hydrolysate and fructose result in a similar effects.[133] Growth of L.Acidophillus is enhanced by acetate.[134] Bifidobacteria show poor growth in milk.Vitamin,dextrose and maltose stimulate their growth but sucrose and iron salts have little effect.
- Oligosaccharides allow the preferential growth of probiotic organisms in the colon,because these substances are not use by other intestinal bacteria.[135] Manipulating the condition in the manufecture and storage of yogurt could increase the survival of Lactic acid bacteria and Bifidobacteria .The following methods have been reported to achive this objective:
- Terminating fermentation at a higher pH(>5; allows better survival of Bifidobacteria[136]
- Lowering the storage temperature to less than 3-4^0 C increase AB culture(L.Acidophillus and Bifidobacteria) survival.[137] Enrichment of Yogurt mix with whey protein concentration (Increase the buffering capacity of yogurt ,retards decrease in pH and prevent pH change during storage of yogurt).[138]
- Application of hydrostatic pressure (200-300 MPa for 10 min at room temperature) to yogurt prevent after acidification and hence maintain initial number of viable lactic acid bacteria .[139]
- Heat shock (58^0 C for 5 min) of the yogurt.[140]
- Lowering the incubation temperature to 3^{70} C favours growth of Bifidobacteria and increase incubation time.[141]

Different type of foods in which Bifidobacteria have been Successfully stabilized:

Now Food products have been formulated with the addition of probiotic cultures. Differents type of food matrices have been used such as various types of cheese, ice creams, milk based desserts, powdered milk.[142]

Dairy Products used as Probiotics:

Dairy products are especially considered as ideal vehicle for deliverting probiotic bacteria to the human

gastrointestinal tracts . The matrices used most frequently are cheese ,yogurt, ice cream and other dairy products.[143] Yogurt with high fat content showed inhibitory effects against probiotics cultures ,particularly B.bifidum BBI.

Bile Salt Tolerance:

The bile salt tolerance was determined on TPY broth containing bromocresol purple (0.04g/1) as a pH indicator,and supplymented with 0.3% bile salt. After autoclaving the bile salt media was inoculated with the strain of bacteria.After 24 hours incubation in Anaerobic condition cell growth was determined by measuring the optical density at 650 nm using a spectrophotometer .

How to Make Probiotic Yoghurt

Heat a quart of milk in the microwave for five minutes. Use a glass container for the milk.

Remove the scalded milk from the microwave and let cool to baby-warm.

Heat a couple quarts of water in a large pot on the stove. Get it as hot as you can and still put your hand in it. Turn off heat.

In a small cup, spoon a couple large soup spoons of plain yogurt (that you should have in the fridge leftover from the last time you made or bought yogurt) and break open two capsules of probiotic supplements into the yogurt.

starter for probiotic yogurt

Making the yogurt starter

Mix well. Add a little of the lukewarm milk to the “starter” mix and stir. Add this mixture back into the warm milk and stir well until all the starter mix is mixed in with the quart of warm milk.

Put your probiotic milk into a container and set inside the pot of hot water. Let it sit undisturbed for a couple hours. Do not stir the yogurt. Ever.

Remove the yogurt container after a couple hours and reheat the water. Place the yogurt back into the hot water. The culturing process will take five to six hours (you may have to re-heat the water one more time).The culturing process grows all the good bacteria back into a new batch of yogurt, saving you the need to buy expensive Probiotic supplements every month.

Materials and method

Research location:

The experiment was carried out at the Industrial Microbiology Laboratory under the Institute of Food Science and Technology (IFST) ,Bangladesh Council of Scientific and Industrial Research (BCSIR),Dr.Kudrat-I-Khuda Road , Dhanmondi, Dhaka- 1205.

Sampling site and collection of sample:

Desired Probiotic bacteria were collected from the YO-MIX, Yogurt Cultures 38360 Sasssenage, DANISCO FRANCE and HOWARU, Premium Probiotic Freeze drying cultures from Germany. According to the guideline as mentioned on the level for making mother cultures, 10 mg samples were measured from both the sachet and mixed into 100 ml of 45°C warm milk and incubated at 42°C for 6 hours. After preparation of mother cultures the sample was kept at 4°C for further analysis. After collection of sample were transported in a sterile box and brought to the laboratory where they were carefully preserved in the refrigerator at -10^{0}C for further microbiological analysis.

Microbiologycal Reagent and Media:

(1)De Man,Rogosa and Sharp (MRS) Medium

The MRS media consisting by peptone , 10(g/1); Meat extract, 10(g/1); Yeast extract, 5(g/1); D-(-)Glucose,20(g/1); Sodium acetate, 5(g/1); Dipotassium hydrogen phosphate, 0.2(g/1); Magnesium sulfate heptahydrate , 0.2(g/1); Magnesium sulfate tetrahydrate , 0.05(g/1); Agar, 10(g/1); Polysorbate 80(also known as Tween 80) 1(g/ml); pH 6.2-6.6.

(2) Trypticase phytone/peptone yeast (TPY) Medium

The probiotic sample were plated in to TPY consisting of (g/l): Tryptone , 10.0; Soy peptone , 5.0; Glucose, 5.0; Yeast extract, 2.5; Dipotassium hydrogen phosphate, 2.0; Cysteine hydrochloride, 0.5; Magnesium chloride hexahydrated , 0.5; Zinc sulphate heptahydrated , 0.25; Cqalcium chloride , 0.15; Agar, 10.0; Polysorbate ,80(also known as Tween 80) 1.0; Ferric chloride trace, 0.003(µg/l); pH-7 at 37^{0}C. Dissolved media was autoclaved at 15 Ibs pressure (121^{0}C) for 15 minutes .The Tpy medium was practically a selective medium for the isolation of Bifidobacteria.

Table -1: List of sampling date and sample collections

Sample name	Sampling date	Sampling place
Probiotic Yogurt P1	12-10-13	NFE LAB ,DIU
P2	12-10-13	NFE LAB ,DIU
P3	12-10-13	NFE LAB ,DIU
P4	12-10-13	NFE LAB ,DIU
P5	12-10-13	NFE LAB ,DIU
P6	12-10-13	NFE LAB ,DIU
P7	12-10-13	NFE LAB ,DIU
P8	12-10-13	NFE LAB ,DIU
P9	12-10-13	NFE LAB ,DIU
P10	12-10-13	NFE LAB ,DIU
P11	12-10-13	NFE LAB ,DIU
P12	12-10-13	NFE LAB ,DIU

P13	12-10-13	NFE LAB ,DIU
P14	12-10-13	NFE LAB ,DIU
P15	12-10-13	NFE LAB ,DIU
P16	12-10-13	NFE LAB ,DIU
P17	12-10-13	NFE LAB ,DIU
P18	12-10-13	NFE LAB ,DIU
P19	12-10-13	NFE LAB ,DIU
P20	12-10-13	NFE LAB ,DIU
P21	12-10-13	NFE LAB ,DIU
P22	12-10-13	NFE LAB ,DIU
P23	12-10-13	NFE LAB ,DIU

(3)Trypticase phytone yeast (TPY) Broth

The sample were inoculated into TPY broth consisting of (gm/l): Tryptone , 10.0; Soy peptone, 5.0; Yeast extract, 2.5; Dipotassium hydrogen phosphate , 2.0; Cystein hydrochloride , 0.5; Glucose ,5.0; Magnessium chloride hexahydrated , 0.5; Zinc sulphate heptahydrated , 0.25; Calcium chloride , 0.15; Polysorbate 80(also known as Tween 80) 1.0; Ferric chloride trace , 0.003 (µg/1); pH-7 at 37^0C . Dissolved media was autoclaved at 15 Ibs pressure (121^0C) for 15 minutes .

(4)Nutrient agar media :

Nutrient agar media consisting of : Peptic digest of animal tissue 5g, Sodium chloride 5g, Beef extract 1.5g, Yeast extract 1.5g, Agar 15.g and water 1 L was used for the growth of microorganisms. Dissolved was autoclaved at 15 Ibs pressure (121^oC) for 15 minutes and the final pH (at 25^0C) was 7.4 ± 0.2 .

(5)Buffer peptone water :

Preparation of Buffer peptone water required distilled water , peptone and L- cystine .Dissolved media was autoclaved at 15 Ibs pressure (121^0C) for 15 minutes and the final pH(at 25^0C) was 7.4±0.2.

(6)Normal saline solution

Normal saline solution was made by 0.85g NACL dissolve in 100 ml water for dissolving different test microorganisms. Dissolved media was autoclaced AT 15 Ibs pressure (121^0C) for 15 minutes and the final pH(at25^0C) was 7.4±0.2.

(7)Technique for sample isolation

Twenty-two freswh facial samples were obtained from newborn infants (aged 2 to 4 months). For each sample, about 1g of freshly feces was transferred in to McCartney bottles containing 9ml of pre-reduced salt solution (0.9% NACL) and (0.2% cysteine – HCl and suspension was homogenized for 2 min.Serial dilution were made, and 0.1 ml of the suspension was inoculated in TPY agar medium. All plates were incubated anaerobically at 37^0C for two days in anaerobic gas jars Capable of produching hydrogen and carbon dioxide. Bifidobacteria was ednumerated using MRS or TPY solid medium. Fifteen colonies from the highest dilution of each sample were picked at random and inoculated into TPY agar medium. At last colonies picked from

countable plates were selected for gram reaction , morphology and biochemical test .

Figure of Anaerobic jar
This photo has taken inside the IFST during our Research

Identification of Bifidobacteria :

(1)Morphology based identification :

The isolated bacteria were subjected to a range of morphology based tests in order to help in their identification .

(2) Colony morphology

Bifidobacteria grown on MRS agar and TPY agar . Colonies were observed for morphology.

(3) Gram staining

A smear of bacteria was prepared on a clean glass slide using a sterile inoculating loop.The smear was fixed with heat and then treated with ammonium oxalate crystal violate solution forf 30 seconds . This was gently rinsed off and iodine solution was applied for 30 seconds .This was drained off and 95% ethanol was then applied for 20 seconds as decolorizing agent .Finally a counter sterain, safranine was added for 10 seconds. Then the slide was gently rinsed off with water and dried .The slide was viewed under 100 X magnification under microscope . The result was recorded as gram positive or negative .

Biochemical Test for Identification of Bifidobacteria

The following biochemical studies were carried out in order to characterize the isolated bacteria .

(1)Catalase test

- A clean glass slide was divided into two sections with CD marker . One was labeled as testr and other as control.
- A small drop of saline was placed on each area
- With a sterilized and cooled inoculating loop, a small amount of culture from the TPY slant was picked up .
- One or two colonies were emulsified on each drop to make a smooth suspension . The smear was about size of a pea.
- With a Pasteur pipette , one drop of hydrogen peroxide was placed over the test smear .

- Nothing was put in the other drop that's serves as control
- The fluid over the smear was observed for the appearance of gas bubbles.

Arginine test

The arginine test were determined on TPY broth containing bromocresol purple (0.04g/l) as a pH indicator , and supplemented with 0.5% of L-arginine. After autoclaving the media was inoculated with the strains of bacteria .After 24 hours incubation in anaerobic condition cell growth was determined by measuring the optical density at 650 nm using a spectrophotometer .

Nitrate test

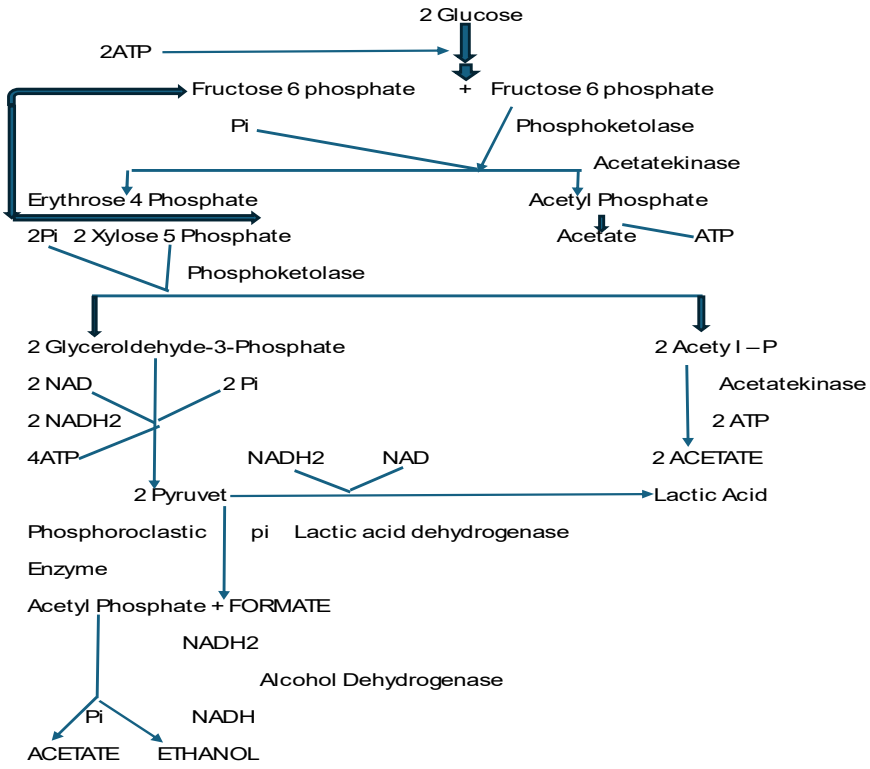
Nitrate broth was prepared and inoculated with the isolated bacteria and incubated at 37^0C for 24 hours . The broth was checked for nitrogen gas before adding the reagents. 6-8 drops of nitrite reagent **A** were then added to the broth.This was followed with 6-8 drops of nitrite reagent **B**. The color of the broth was recorded after a few minutes .

Indole test

10ml Tryptophan medium was poured in to McCartney bottles and the media was autoclaved (for 15 minutes in 15 Ibs pressure at 121^0C) to sterilize it .The sterile Tryptophan media was then inoculated with the isolated bacteria and incubated for 24 hours at 37^0C .*E.coli* ATCC 8739 was used as positive control and a lab isolate of *Pseudomonas aeruginosa* was used as a negative control. Kovacs reagent was added to all of the tubes and the result were recorded.

Fermentation of Carbohydrate profile :

Bifidobacteria fermets glucose and produced acetic acid and lactic acid , generally in a ratio of 3:2, although some frormic acid and succinic acid may also be produced.[144] Fermentation involves a pathway unique to Bifidobacteria , known as the 'bifid shunt'. The key enzyme of this pathway is a fructose -6-phosphate phosphoketolase (F6PPK) which spilts fructose -6- phosphate into erythrose-4-phosphet and acetylphosphete .[145] Bidobacteria are also unique in that all the lactic acid produced is in the L(+)form [146] The pathway of carbohydrates by Bifidobacterium speies differ from that of both Homo-and heterofermentative bacteria.



Metabolic pathway of Bifidobacteria[147]

Experimental Frame work for Animal trail

Three weeks old Drausely Rates were collected from the Animal House in Institute of Food Science and Technology (IFST), BCSIR, Dhaka . The whole rates were divided into two groups one is control and another is experimental ,One group contains eight rates which marked R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 another two samples selected for control which is marked as C_1, C_2,C_3,C_4. R_1, R_2(Tail mark), R_3, R_4(Right front), R_5, R_6(Left back), R_7, R_8(Unmarked) and the control C_1, C_2, C_3, C_4 Initial mean body weight of first group rate (R_1-R_8) were

Identification	Body weight
R_1(Tail mark ,Yellow color)	159gm
R_2(Tail mark, Red color)	157gm
R_3(Right front, Yellow color)	152gm
R_4(Right fron,Red color)	153gm
R_5(Left back,Yellow color)	152gm

R_6(Left back,Red color)	157gm
R_7(Unmark,Yellow color)	151gm
R_8(Unmark, Red color)	159 gm

and the weight of the control group rat(C_1,C_2,C_3,C_4) were

Identification	Body weight
C_1(Tail mark,)	162gm
C_2(Right front,)	165gm
C_3(Left back)	172gm
C_4(Unmark)	181gm

The experimental rats were given high fat diet mice pellets (15gm/mice/day) and sterile water for drinking .The mice were individually housed in lean dry cages and their daily body weight were recorded .After seven days the weight of the mices were

Identification	Body weight
R_1(Tail mark ,Yellow color)	159gm
R_2(Tail mark, Red color)	157gm
R_3(Right front,Yellow color)	133gm
R_4(Right fron,Red color)	158gm
R_5(Left back,Yellow color)	154gm
R_6(Left back,Red color)	157gm
R_7(Unmark,Yellow color)	153gm
R_8(Unmark, Red color)	156gm

One the other hand control mice were given normal diet . After seven days the weight of the control rats were

Identification	Body weight
C_1(Tail mark,)	165gm
C_2(Right front,)	169gm
C_3(Left back)	175gm
C_4(Unmark)	185gm

Now the blood sample were collected from two Experimental rats and analysis the Lipid profile .Test report were(Lipid Profile) bellow

Table 1:Animal feeding report,(fat diet) Biochemical Analysis for different Rat

Number of Sample	**Serum Lipid Profile**			
(1)R_1 (Tail mark, Yellow)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	137.0 [<200]	203.0 [<200]	50.0 [>40]	27.5[<150]

Number of Sample	**Serum Lipid Profile**			
(2)R_2 (Tail Mark,	T Cholesterol	TG	HDL	LDL

Red)	(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)
	147.0 [<200]	130.0 [<200]	62.0 [>40]	33.3 [<150]

Number of Sample	**Serum Lipid Profile**			
(3)R_3 (Right Front,Yellow)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	130.0 [<200]	190.0 [<200]	48.0 [>40]	25.5[<150]

Number of Sample	**Serum Lipid Profile**			
(4)R_4 (Right front ,Red)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	140.0 [<200]	195.0 [<200]	49.5 [>40]	30.3 [<150]

On the other hand blood sample were collected from two Control rats and analysis the Lipid profile .Test report were(Lipid Profile) bellow

Table 2: Animal feeding report,(control diet)and their Biochemical Analysis

Number of Sample	**Serum Lipid Profile**			
(1) C_1(Tail mark,)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	89.0 [<200]	40.0 [<200]	58.0 [>40]	20.1[<150]

Number of Sample	**Serum Lipid Profile**			
(2) C_2(Right front)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	98.0 [<200]	50.0 [<200]	49.0 [>40]	18.6 [<150]

Each mice was supplied these diets once daily to increase cholesterol level .After seven days we supplied Probiotic diet . These Probiotic diet was given once a day .After seven days we measured the weight of the rats , which were following

Identification	Body weight
R_5(Left back,Yellow color)	135gm
R_6(Left back,Red color)	160gm
R_7(Unmark,Yellow color)	145gm
R_8(Unmark, Red color)	142gm

Then we collect the blood sample and analysis the Lipid profile.

Table 3: Animal feeding report,(Probiotic diet) Biochemical Analysi for different Rats

Number of Sample	**Serum Lipid Profile**	

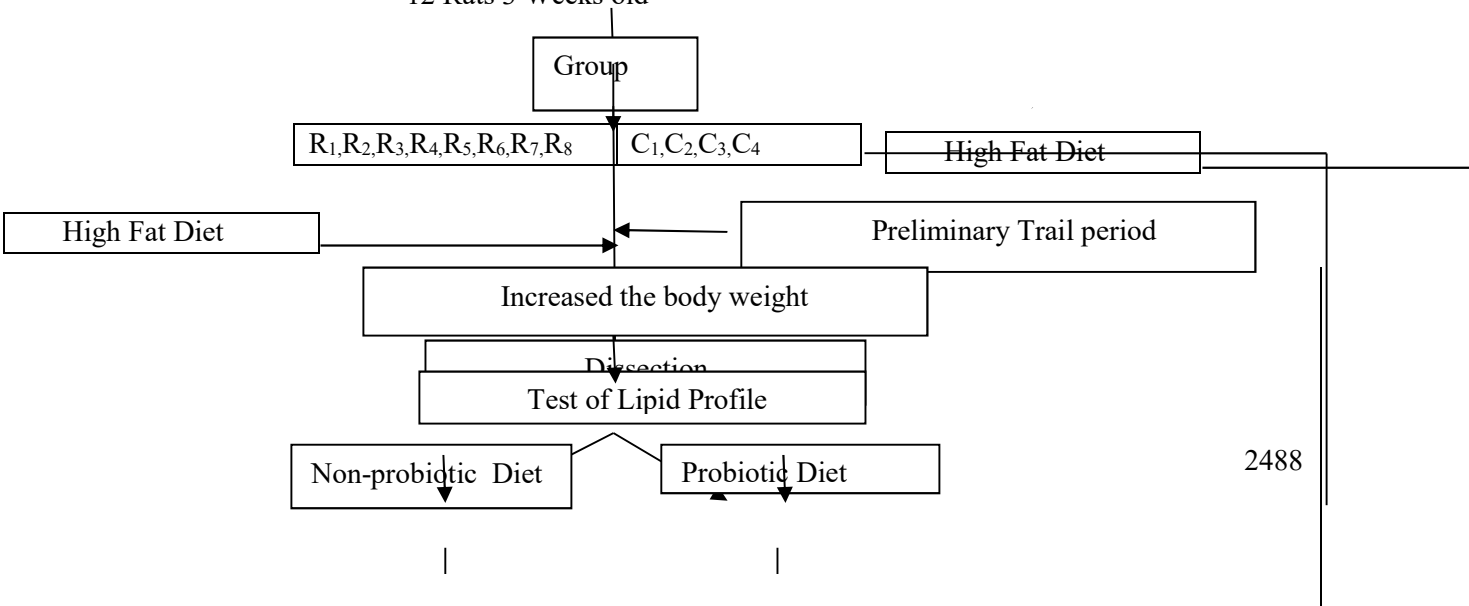
(5) R_5(Left back, Yellow)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	84.0 [<200]	37.0 [<200]	62.0 [>40]	14.6[<150]

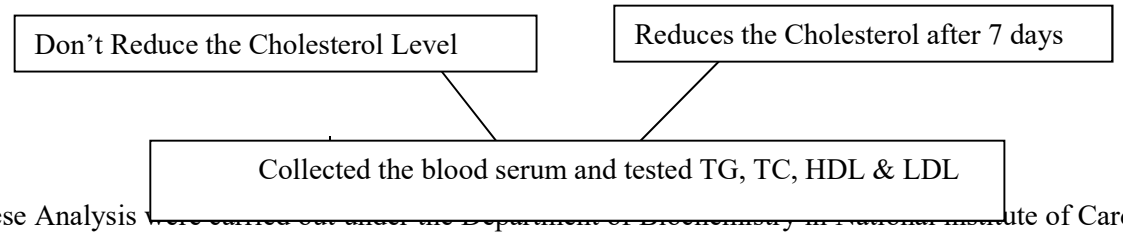
Number of Sample	**Serum Lipid Profile**			
(6) R_6 (Left back, Red)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	101.0 [<200]	26.0 [<200]	81.0 [>40]	14.8 [<150]

Number of Sample	**Serum Lipid Profile**			
(7) R_7 (Unmark, Yellow)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	100.0 [<200]	25.0 [<200]	60.0 [>40]	14.5[<150]

Number of Sample	**Serum Lipid Profile**			
(8) R_8 (Unmark, Red)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	101.0 [<200]	27.0 [<200]	55.0 [>40]	14.2 [<150]

Flow Chart for the Animal Feeding Trial





These Analysis were carried out under the Department of Biochemistry in National Institute of Cardiovascular Disease (NICVD). Sher-e- Bangla Nagar , Dhaka-1207.

Preparation of Probiotic diet(Yoghurt):

One litre raw milk was taken into SS pan and boiled up to 90C for 50 minutes .Ten percent Skim milk powder (SMP) was added into milk and mixed vigorously . When temperature reduced to 50C then added Probiotic culture (Danisco Probiotic Yoghurt culture from Denmark) The samples poured into different cups.The milk samples incubated at 42C for Eight hours . The sample was taken from the incubator and kept in a Refrigerator for further analysis .This part was carried out at Nutrition Food Engineering Microbiological Lab of DIU.

Feeding of Probiotic and non Probiotic diet to animal:

20 gm probiotic yoghurt were mixed individually with 20 gm palatalized diets in a sterile beaker .The probiotics mixed pallets were kept in a drier at 37C for drying slightly. 50 gm of Probiotic pallets was supplied twice each rat . This feeding protocol for each rat was continued 10 days. On the other hand the control rat was given normal pallets as routine diets for 10 days .

Dissection:

From each set of rat when the body weight regain up to a minimum levels (143gm). They were Anesthetized by Chloroform and dislocated by cervical vertebrate by sterile surgical knife .The blood was collected into a sterile tube and kept at Ambient temperature for one hour. The blood tubes were centrifuged at 4000rpm and the supernatant was collected into Appendox tube and kept at -20C for further analysis. The Blood samples was tested for lipid profile such as LDL,HDL,TG, Cholesterol and the results shown in table.

Number of Sample	**Serum Lipid Profile**			
(1)P-1 (Unmarked)	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	137.0 [<200]	203.0 [<200]	50.0 [<40]	27.5[<150]

Number of Sample	**Serum Lipid Profile**			
(1)P-2 (Left back	T Cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
	147.0 [<200]	130.0 [<200]	62.0 [<40]	33.3 [<150]

Results and Discussion:

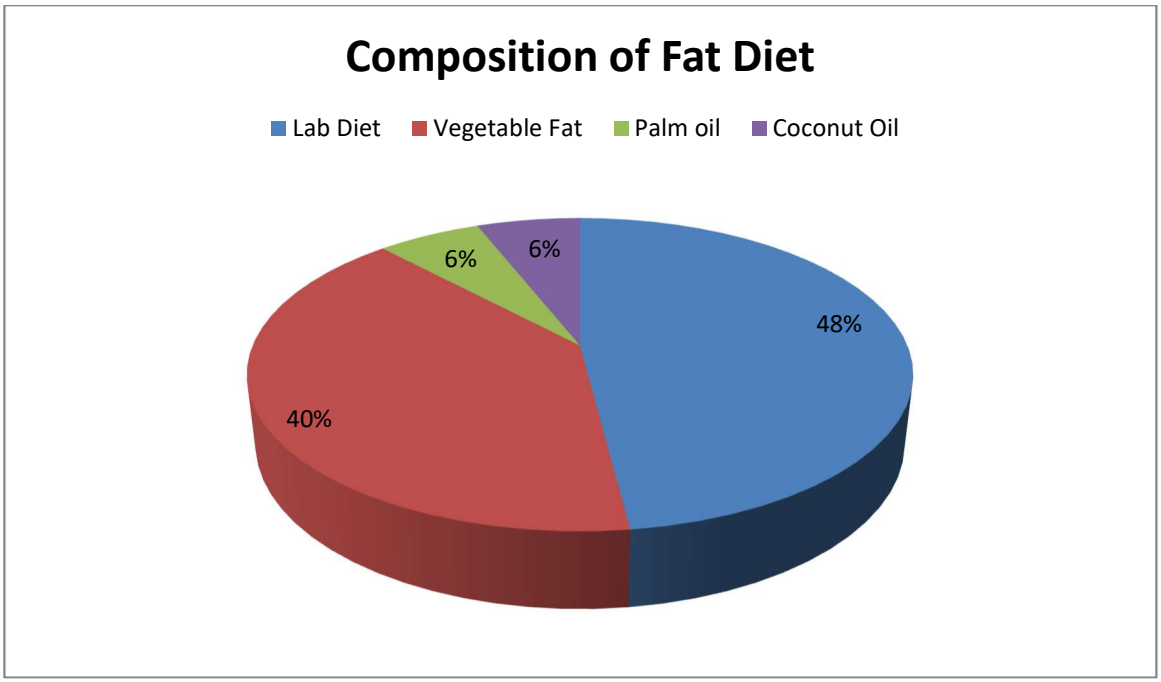


Fig 1: Composition of Fat diet

Figure 1 show that the composition of fat diet was prepared in the Animal House of IFST lab. The following ingredients were mixed together as per animal house diet feeding standard. The ingredients are Vegetable fat, coconut oil and palm oil, were used as 48%, 40%, 6% & 6% respectively. All the ingredients were mixed as dough and kept into Electric drier for 30 hours at 60°C to reduced the moisture level up to 12%. The diet was kept into sterile poly bag and kept at ambient temperature for further feeding analysis.

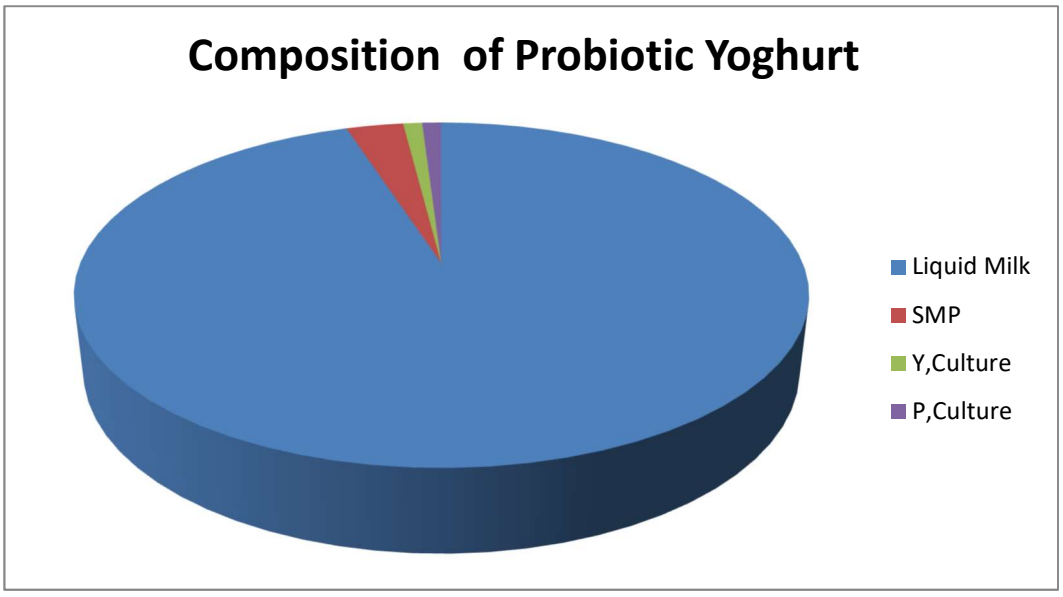


Fig 2: Probiotic Yoghurt

Fig 2 shows that the composition of Probiotic yoghurt are liquid milk=95%, SMP=3%, Yoghurt culture=1%, Probiotic culture=1% respectively.

Average body weight of different rats during feeding period

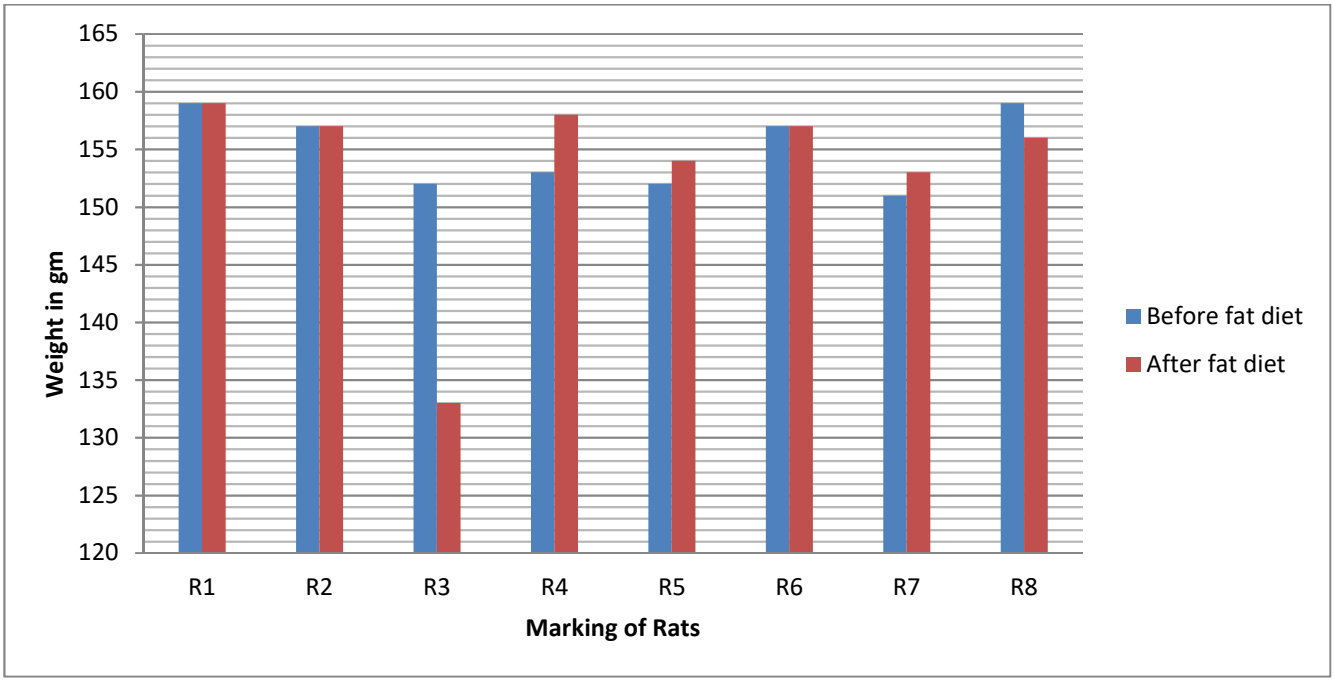


Figure: 3 Comparison of rats' body weight before and after fat diet

Figure 3 shows that different rat at the initial stage were given normal control diet. After two hours all the rats fed with high fat diet for seven days .It was observed from the study that the total body weight after feeding of high fat diet abruptly increased except the Rats 3. We investigated this reason for rat 3 and found that the Rats was looking little pale and week. It was found that Rat 3 regular food intake was less comparative to other 3 Rats .The reason was not knone.Beside this other seven Rats food intake were normal .from this study it can be estimated that feeding of high fat diet to 7 Rats increase their body weight abraoghly

Average body weight of rats

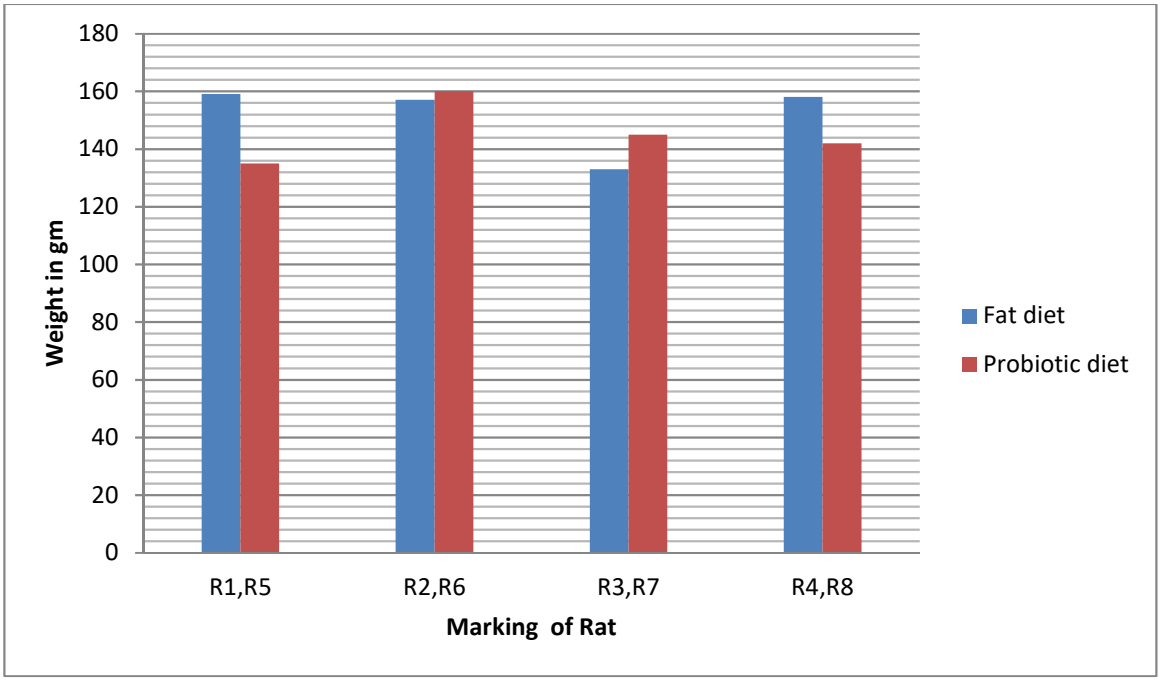


Fig 4: Body Weight of different rats for fat diet and probiotic diet

Figure4: shows that R1=Rat1 (Fat diet), R2=Rat 2(Fat diet) ,R3=Rat 3(Fat diet),R4=Rat4(Fatdiet),R5=Rat5(Probioticdiet),R6=Rat6(Probioticdiet),R7=Rat7(Probioticdiet),R8=Rat8(Probiotic diet).

From this study showed that 4 Rats were given high fat diet blended with Vegetable oil,palm oiland coconut oil mixed together and late on injested to the 4 different Rats.It was found that feeding of high fat diet R1,R2,R3,and R4 increase their body weight simultaneously. On the other hand effect of feeding probiotic diet to R5,R6,R7and R8 also increased their body weight which was less than that of high fat diet Rats .But incase of R2 and R6 their body weight significantly different each other. R6 160 gm and R2 158.

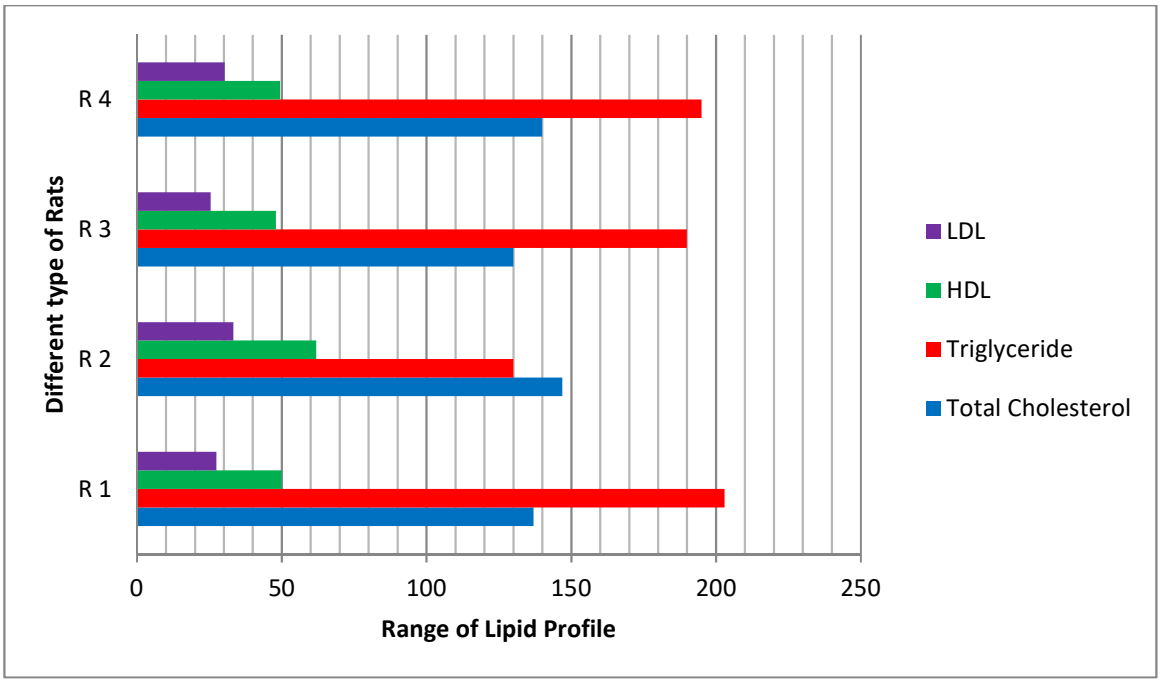


Figure 5: Comparison of lipid profile in different rats

Figure 5 shows that comparison of lipid profile for four different rats such as R1,R2,R3and R4 after given fat diet were investigated . From this result it shows that the amount of TG found in Rat 1 was higher such as (210mg/dl), but R2,R3,R4 were 140mg/dl ,190mg/dl and 195mg/dl respectively . On the other hand total LDL content for 4 different Rats tremendously reduce to (25mg/dl) for Rat 3 . Accordingly the HDL level for R1,R2,R3 and R4 increased as compare to the control sample.Similarly total cholesterol level for R1,R2.R3,R4 abroughli reduced to 25 mg/dl,32mg/dl,25 mg/dl and 30 mg/dl respectively. From this study it was ensured that probiotic feeding diet had good effect to reduce the LDL,TG,HD and Cholesterol level for high fat diet Rats.

Physiological study of Average lipid profile of different rats

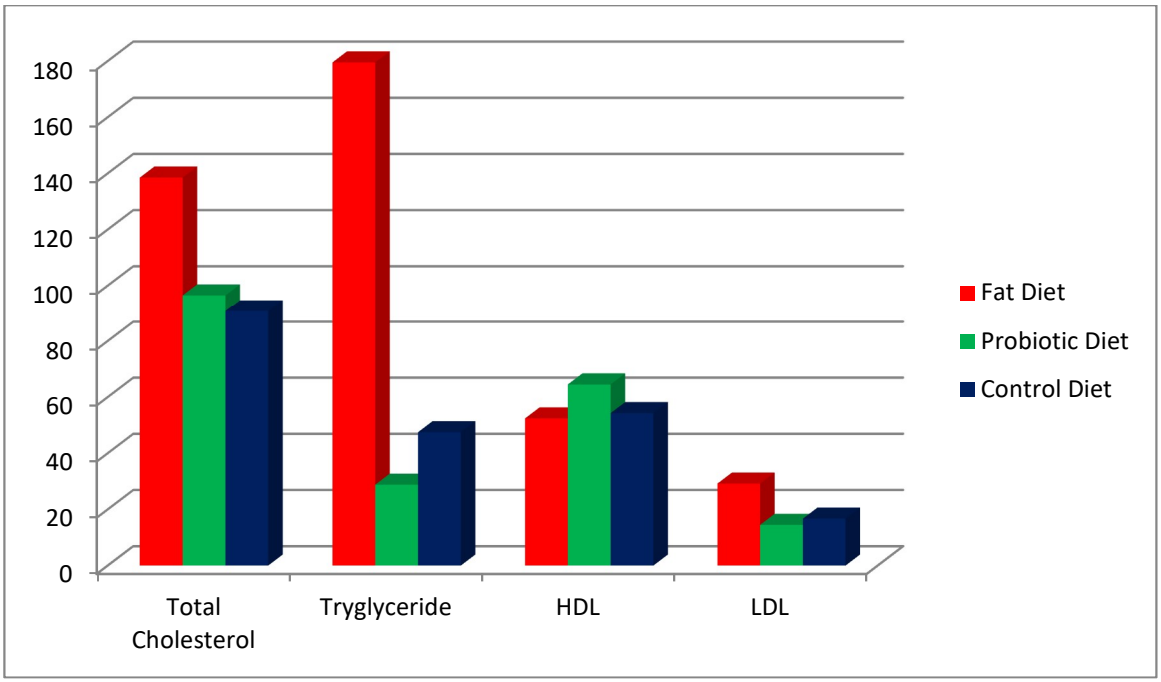


Figure 6: Comparison of Lipid Profile of Different rat after feeding of Probiotic Diet

Figure 6 indicate that that the average Lipid profile of different Rats after given 3 different diet (Fat, Probiotic , Coconut).It is surprisly found that the TG of fat diet Rats (170 mg/dl) more than eight time higher that the probiotic diet (20 mg/dl) defined that the LDL is reduced to (15mg/dl) which is twice lower than fat diet (25mg/dl). Its shows from this results that Total cholesterol was(90 mg/dl) for control diet ,(95 mg/dl) for probiotic diet and (140 mg/dl) for fat diet.Similarly the TG level for high fat diet shows that 170 mg/dl,Probiotic diet (20 mg/dl), and control diet (42 mg/dl).On the other hand HDL level for fat diet 45 mg/dl,Probiotic diet 60 mg/dl, and control sample 45 mg/dl. Baside this ,the LDL level for fat diet shows that 23 mg/dl,Probiotic diet 10 mh/dl and control diet 12 mg/dl respectively.

Mechanisms of Cholesterol-Lowering Effects

Past *in vitro* studies have evaluated a number of mechanisms proposed for the cholesterol-lowering effects of probiotics. One of the purported mechanisms includes enzymatic deconjugation of bile acids by bile-salt hydrolase of probiotics. Bile, a water-soluble end product of cholesterol in the liver, is stored and concentrated in the gallbladder, and released into the duodenum upon ingestion of food.[148] It consists of cholesterol, phospholipids, conjugated bile acids, bile pigments and electrolytes. Once deconjugated, bile acids are less soluble and absorbed by the intestines, leading to their elimination in the feces. Cholesterol is used to synthesize new bile acids in a homeostatic response, resulting in lowering of serum cholesterol.[149]

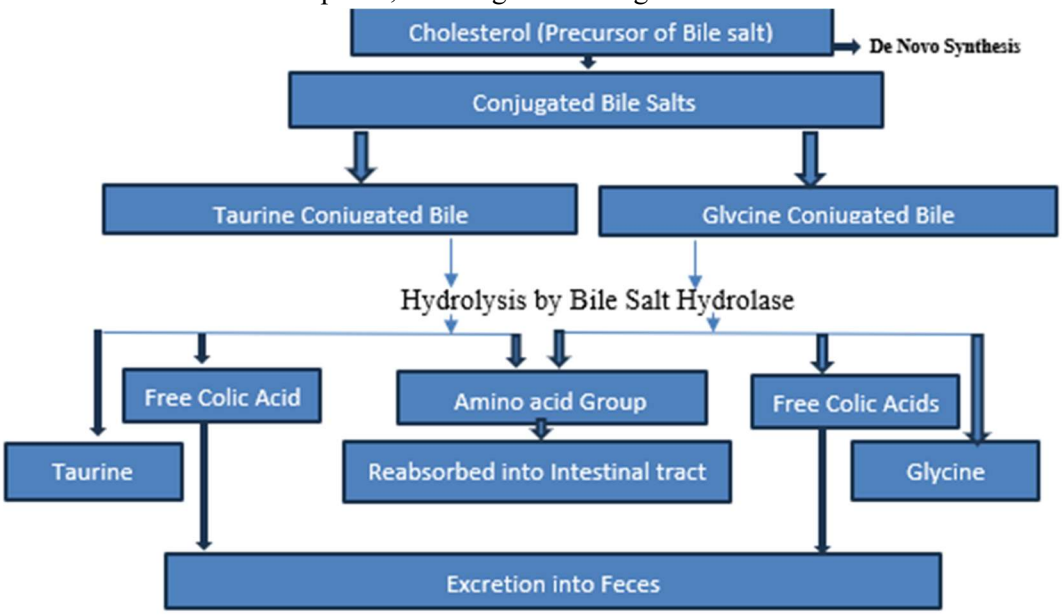


Figure: Cholesterol as the precursor for the synthesis of new bile acids and the hypocholesterolemic role of bile salt hydrolase.

In an *in vitro* study, Jones *et al.*[150] evaluated the role of bile salt hydrolase in cholesterol-lowering using *Lactobacillus plantarum* 80 (pCBH1). Bile salt hydrolase (BSH) is the enzyme responsible for bile salt deconjugation in the enterohepatic circulation. It has been detected in probiotics indigenous to the gastrointestinal tract. The authors found that BSH activity was able to hydrolyze conjugated glycodeoxycholic acid and taurodeoxycholic acid, leading to the deconjugation of glyco- and tauro-bile acids. The hypocholesterolemic effect of the probiotics has also been attributed to their ability to bind cholesterol in the small intestines . Usman and Hosono.[151] previously reported that strains of *Lactobacillus gasseri* could remove cholesterol from laboratory media via binding onto cellular surfaces. The ability of cholesterol-binding appeared to be growth and strain specific. Kimoto *et al.*[152] later strengthened such a hypothesis by evaluating the removal of cholesterol by probiotics cells during different growth conditions. Live and growing cells were compared to those that were non-growing (live but suspended in phosphate buffer) and dead (heat-killed). The authors found that although growing cells removed more cholesterol than dead cells, the heat-killed cells could still remove cholesterol from media, indicating that some cholesterol was bound to the cellular surface.

Discussion:

Probiotics are live microbes that can be formulated into many different type of products.

Preparation of probiotic yoghurt is a totally new concept. In our country a huge number of people have suffering with high Cholesterol problem and ultimate it is converted to the CVD (Cardio Vascular Disease).The main reason is due to the adulterated food.So overcome this type of problem Preparation and consumption of Probiotic yoghurt will have potential impact.

In the Microbiological analysis this probiotic strains was analyzed .These Probiotic strain and probiotic yoghurt culture was collected from Danisco Denmark. The probiotic yoghurt was prepared in the NFE Lab and Animal feeding trail was conducted at animal house of IFST,BCSIR Lab Dhaka. High fat diet blended with 40% Vegetable fat,48% Lab diet, Coconut oil 6% and Palm oil 6%(Figure-1) mixed together and ingected to the animal. It was found that after 7 days feeding of high fat diet the total body weight increased rapidly as compared to the control normal diet (Figure-3). Similarly Probiotic Diet was prepared mixed with Liquid milk, Skim milk, Yoghurt culture and Probiotic culture(Figure-2).As per Experimental design 8 Rats fed with Probiotic diet for 7 days .It was found from the Serum report that effects of Probiotic diet had significant effects on Lipid profile(Figure-5). It means the total LDL content, TG and Total cholesterol reduced on the other HDL increased. According to *Lay- Gaik Ooi* probiotics are effective in improving lipid profiles, including the reduction of serum/plasma total cholesterol, LDL-cholesterol and triglycerides or increment of HDL-cholesterol. However, other past studies have also shown that probiotics and had insignificant effects on lipid profiles, disputing the hypocholesterolemic claim.

Similarly (figure-6) shows that total Cholesterol for seven Rats were analyzed it was found that total cholesterol for probiotic diet was 90 mg/dL whereas for control diet was 82 mg/dL. The TG level for high fat diet 170 mg/dl and Probiotic diet was 30 mg/dL and control diet 40 mg/dL respectively. Accordingly the LDL 25 mg/dL, Probiotic diet 10 mg/dl and control diet 12 mg/dL.Beside this the total HDL content for high fat diet was 45 mg/dL probiotic diet 60 mg/dL and control diet 46 mg/dL.

Conclusion:

Probiotic have been widely assessed for their effects on lipid profiles such as total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. However, not all trials have yielded conclusive results. Certain strains of probiotic have demonstrated cholesterol-lowering property while others did not. In order to justify the varying cholesterol-lowering effect exhibited by various strains of probiotics, researchers have endeavored to reveal the mechanisms of probiotics on hypocholesterolemic effect through *in vitro* and *in vivo* studies. Many of the proposed mechanisms and experimental evidence specifically targeting cholesterol-lowering effects remain controversial. Thus, more properly-designed *in vivo* trials may disclose additional understanding and knowledge to eliminate the controversies, to better understand the underlying mechanisms and for better safety assessment prior to consumption.

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