CHAPTER 1

1.0 INTRODUCTION

The use of lactic acid bacteria starter cultures in the production of fermented foods ensures rapid growth of the bacteria with the resultant reduction in pH to below 4, which is critical for controlling pathogens (Gadaga *et al*., 2004). The lacticacid bacteria (LAB) are Gram-positive, acid-tolerant, generally non-sporulating, non-respiring rod or cocci that are associated by their common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and lactic products, produce lactic acid as the major metabolic end-product of carbohydrate fermentation. Those lactic acid bacteria with scientifically supported health claims are known as probiotics(Fijan, 2014).

Probiotics are live microorganisms in foodstuffs which, when consumed at certain levels in nutrition stabilizes the gastrointestinal tract microflora thereby conferring health benefits on the consumer. Members of the genera *Lactobacillus* and *Bifidobacterium* are mainly used as human probiotics and they are considered safe for human use when administered in food.Other organisms applied are members of the genera *Lactococcus* and *Enterococcus*(Vandenplas*etal*., 2007).

Lactobacillus fermentum has been identified as a potential probiotic (Mikelsaar, 2009). It is a normal inhabitant of the human intestinal tract. Some of the commercialized strains used as probiotics are PCC and ME-3.*L. fermentum* demonstrates a significant pH and bile tolerance in terms of its consideration as a probiotic. Testing of *Lactobacillus fermentum* against different pH concentration solutions revealed that it has a strong pH tolerance by its ability to grow and survive a few hours after being incubated in a pH 3 solution. Strains of *Lactobacillus fermentum* have also been tested in different bile concentrations and demonstrated to have good bile tolerance when incubated with 3 g/L of bile salt. *Lactobacillus fermentum* has been found to survive in these conditions further supporting the idea that it can act as a probiotic (Pan *et al.,* 2011; Srinu *et al.,* 2013).

One of the ways in which *Lactobacillus fermentum* has been seen as a probiotic is by its ability to reduce cholesterol levels. Tests conducted using several strains of *Lactobacillus* and cholesterol broths demonstrated that *Lactobacillus fermentum* had the largest removal

of cholesterol. One of the mechanisms by which *L. fermentum* may remove cholesterol *in vivo* is by the absorption of cholesterol, which as a result accelerates cholesterol metabolism. Another method is by the incorporation of cholesterol in the host body into its cell membrane or walls. This would also increase resistance of the bacterial cell membranes to environmental challenge. A third mechanism is by causing the body to consume more cholesterol. *L. fermentum* would interfere with the recycling of bile salt and facilitate its elimination, which as a result would increase the demand for bile salt made from cholesterol. In order for *L. fermentum* to be considered as a potential probiotic, it must also not contain any transferable resistant genes so as not to lessen the effect of the use of antibiotics (Zeng *et al.*, 2010).

L. fermentum has also been established to reduce total number of days with respiratory illness in endurance athletes (Cox *et al.*, 2008), severity of chest infection, illness load and use of medications in male athletes (West *et al.*, 2009), induce an enhanced immune response to an influenza vaccine (French and Penny, 2009) and alleviate symptoms of atopic dermatitis (Weston and Halbert, 2005). Eventhough some strains have been associated with cholesterol metabolism, (Mikelsaar and Zilmer, 2009) some have been applied to treat urogenital infections in women (Reid, 2008).

There are tons of other benefits derived from consumption of fermented foods containing probiotics. They boost the immune system by increasing antibodies that fight infectious disease; the flora in probiotic foods form a barrier that covers the small intestine's inner lining and helps inhibit pathogenic organisms including *E.coli*, *Salmonella* and an unhealthy overgrowth of *Candida;* some fermentation create antioxidants that scavenge free radicals which are cancer precursors; fermentation transforms hard-to-digest lactose from milk to the more easily digested lactic acid; it neutralizes the anti-nutrients found in many foods including the phytic acid found in all grains, and generates new nutrients including omega-3 fatty acids as well as increase the folic acid, pyroxidine, B vitamins and riboflavin level in foods. To have the desired effect, scientists believe at least a million of each probiotic bacteria per gram of yoghurt or drink are needed (Shiby and Mishra, 2013).

In America, Europe and the Orient, there exist available technologies for commercial production of various types of probiotic foods. In Africa however, people of different regions, produce foods containing probiotic organisms, though at small scale level. These

include; dairy products such as *Nunu*, and non-dairy products such as *Togwa*, *Akamu* souring water, *Fufu* liquor, and fermented raffia palm sap (Adebolu *etal*., 2007; Prado *etal*., 2008).

Most probiotics have been documented to proliferate well in a dairy-based matrix due to the lactose-hydrolysing enzyme and proteolytic system involved in casein utilisation, which provides probiotic cells with a carbon source and essential amino acids for growth. Metabolism of these nutrients produced organic compounds that are essential for the development of flavour, preservation and appearance of the products (Yeo, 2011).

1.1 STATEMENT OF PROBLEM

Certain factors such as antibiotics, chlorinated water, alcoholic drinks and starvation deplete the intestinal bacteria resulting in imbalance between the pathogens and the health promoting bacteria. Hence, there is need to re-establish the balance through the consumption of probiotic foods.Although the probiotic properties and safety of strains of *Lactobacillus*species from different sources have been extensively studied and well documented in developed countries (and a few documented on *L. fermentum*), in Nigeria, detailed assessment of probiotic properties of *L. fermentum*are rarely dealt with in scientific publications. In Nigeria also, the market for fermented milk products is large and keeps growing because consumers are being more health conscious and have also increased interest in self-care. It is, therefore, worthwhile to leverage on this consumer trend to create a fermented milk with benefits that go beyond the basic nutrition. According to Rogelj (2000), dairy-fermented products such as yogurt, probiotic beverages and cheese-containing lactic acid bacteria and their constituents such as omega-3 fatty acid, phytosterols, isoflavones, conjugated linoleic acid (CLA), minerals and vitamins have a prominent position in the development of functional foods.*L. fermentum*are prevalentin many of the Nigerian indigenous fermented foodshowever, little is known about the specific health benefits they confer or the properties of their strains.Since *L. fermentum* has been observed to demonstrate a significant pH and bile tolerance (Srinu *et al.,* 2013; Mathara *et al.,* 2008) it becomes pertinent to investigate thesespecies for their probiotic properties.

1.2 AIM OF STUDY

The aim of this study was to assess the probiotic properties of *Lactobacillusfermentum* isolates from some fermented foods for a probiotic fermented milk production,while the objectiveswere:

1. To isolate *L. fermentum* from the fermented foods:*Gari* FCM (Fermented cassava mash for Gari production), *Nunu* and *Akamu*.
2. To determine their probiotic potential through *in-vitro* and *in-vivo*assays.
3. To produce fermented milk with probiotic properties using *L. fermentum.*

1.3 SIGNIFICANCE OF STUDY

The findings of this study will positively impact the society considering that our overall well-being hinges on the beneficial bacteria that live within the gastro-intestinal tract. These beneficial bacteria influence our health bycarrying signals to our organs, influencing our brain chemistry, and helping to break down the foods that we eat for our body to use as fuel and energy. These bacteria are unfortunately constantly under stress due to our lifestyle. To help support the growth of the healthy bacteria in the gut, we need to consume probiotic fermented foods. This study will thus provide probiotic fermented milk that could prevent malnutrition byoptimizing nutrient absorption in our body for proper growth and development especially in children and the elderly. It will also provideprobiotic fermented milk that could prevent the occurrence of cardiovascular disease by serving as a natural method of maintaining good cholesterol levels.

CHAPER 2

2.0 LITERATURE REVIEW

2.1 An overview of some indigenous fermented foods

Fermentation is a metabolic process of deriving energy from organic compounds without the involvement of an exogenous oxidizing agent (Bourdichon *et al*., 2012). Fermentation can be applied to designing and manufacturing of functional foods, which are foods that are a normal part of the diet but have components that confer particular health benefits on the consumer (Salovaara and Simonson, 2004).

In Nigeria, fermented cereal products such as *Kunu-zaki, Burukutu, Pito, Akamu* are popularly consumed by different ethnic groups. *Akamu,* is a porridge prepared from fermented maize, sorghum or millet in Nigeria as well as other West African countries. It is a staple food and also serves as a weaning food for infants. The traditional preparation of *Akamu* involves soaking of corn kernels in water for 1-3 days followed by wet milling and sieving to remove bran, hulls and germ. The pomace is retained on the sieve and later discarded as animal feed while the filtrate is fermented for 2-3 days to yield *Akamu*, which is a sour, white starchy sediment. *Akamu* is often marketed as a wet cake wrapped in leaves or transparent polythene bags. It is diluted to a solid content of 8 - 10% and boiled into a pap, or cooked and turned into a stiff gel called *agidi* or *eko* prior to consumption. The wet fermented porridge is prepared and consumed as Ogi, Akamu and Akassan among the Yorubas, Ibos and Hausas in the west, east and northern Nigeria, respectively (Parveen and Hafiz, 2003).

Microbiological and nutritional studies by Ijabadeniyi (2007), showed that the molds isolated from the fermenting maize varieties were *Aspergillus niger*,*Penicillium* sp., *Mucor mucedo* and*Rhizopus stolonifer*,and the yeast isolated was*Saccharomyces cerevisiae***.** The bacteria that were isolated were *Corynebacterium* sp., *Lactobacillus plantarum***,***Lactobacillus fermentum, Leuconostoc mesenteroides, Clostridium*

bifermentans and *Staphylococcus aureus*. During the secondary fermentation, the microorganisms werereduced to *Lactobacillus plantarum*, *Lactobacillus fermentum* and yeast, *Saccharomyces cerevisiae*.

Cassava (*Manihot esculenta*), also called yuca or manioc, is a woody shrub of the Euphorbiaceae (spurge family) native to South America but was introduced to West Africa in the late 16th century. It is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates. Cassava is a very unique and important root crop because not only do they grow quickly but are able to remain unharvested for as long as three years without deteriorating; thus making it a good reserve food against shortage (Igbinosa and Igiehon, 2015).

Traditionally, cassava is processed before consumption. Processing is necessary for several reasons. Firstly, it serves as a means of removing or reducing the potentially toxic cyanogenic glucosides present in fresh cassava. Secondly, it serves as a means of preservation. Thirdly, processing yields products that have different characteristics, which creates variety in cassava diets.Cassava roots are prepared into an amazing variety of foods. Traditional preparation techniques vary by region, and by ethnic group within a given region. Cassava is an important staple in Ghana, Ivory Coast, Sierra Leone, Liberia, Guinea, Senegal, Cameroon and Nigeria (Aworh, 2008). The majority of the cassava-based foods made in Africa rely on fermentation in one form or another. Two common products are *Gari*, a granular meal and *Fufu*, a sticky dough made by pounding cooked fermented roots into a paste, and *Lafun*, a flour made from soaked roots.

Gari is one of the most popular foods derived from fermented cassava. Itis a creamy-white, granular flour with a slightly fermented flavor and a slightly sour taste made from fermented, fresh cassava tubers. It is widely known in Nigeria and other West African countries. *Gari* is commonly consumed either by being soaked in cold water with sugar, coconut, roasted groundnuts, dry fish, or boiled cowpea as complements or as a paste made with hot water and eaten with vegetable sauce. When properly stored, it has a shelf-life of six months or more (IITA, 2005).

Gari is consumed by millions of people in West Africa. Its cheapness, longer shelflife, lower bulk and ease of preparation for consumption account for its popularity in the urban areas. The traditional production of *Gari* involves peeling, grating, fermentation at ambient

temperature, pressing, sieving and roasting. During fermentation, endogenous linamarase present in cassava roots and microorganisms hydrolyze linamarin and lotaustralin (cyanogenic glucosides) releasing hydrogen cyanide (HCN). Crushing of the tubers exposes the cyanogens which are located in the cell vacuole to the enzyme which is located on the outer cell membrane, facilitating their hydrolysis. Most of the cyanide in cassava tubers is eliminated during the peeling, pressing and frying operations. Processing cassava roots into *Gari* is the most effective traditional means of reducing cyanide content to a safe level by WHO standards (FAO/WHO, 1991) of 10 ppm, and is more effective than heap fermentation and sun drying, commonly used in eastern and southern Africa (Cardoso *et al.*, 2005).

The fermented cassava paste is roasted to destroy enzymes and microorganisms, to drive off cyanide gas, and to dry the product. However, preservation is also achieved by heat during the roasting. A low moisture content inhibits recontamination by bacteria and packaging is needed, especially in areas of high humidity, to retain the low moisture content (IITA, 2005). The fermentation process is now recognized as a lactic process involving the activities of other microorganisms, including the yeasts, all of which have different roles to play. Many of the LAB isolated from cassava are known to be involved in acidification and flavor development process and have been confirmed to be capable of producing detoxifying linamarase enzyme (Oyewole and Odunfa, 1990). *Lactobacillus plantarum, Lactobacillus fermentum, Leuconostoc fallax, Leuconostoc mesenteroides, Corynebacterium* spp*, Geotrichum candidum, Streptococcus faecium* have been isolated by Kostinek *et al.* (2005).

Although there is no perfect food known, milk is the most nearly perfect food. It is the most important foodstuff for a mammal and has always been the first food of the newborn. Milk from eight species of domesticated mammals (cow, buffalo, sheep, goat, horse, camel, yak, and zebu) has been used to make traditional fermented milk products throughout the world (Widyastuti *et al*., 2014). From a biological standpoint, fermented milks are characterized by the accumulation of microbial metabolic products. The fermentation process increases the shelf-life of the product, as well as adds to the taste and improves the digestibility of milk (Sharma *et al*., 2012; Steele *et al*., 2013).

In many modern societies, fermented dairy products make up a substantial proportion of the total daily food consumption. Furthermore, it has long been believed that consuming yogurt and other fermented milk products provides various health benefits (Adolfsson, *et al*., 2004). Fermented milk products can be divided into traditional and non-traditional types. Traditional fermented milk products have a long history and are known and made all over the world whenever milk of animals is kept. Their production is a crude art. In contrast, nontraditional fermented milk products are recently developed. They are based on known scientific principles, their microbial cultures are known, and their quality can be optimized. This is not the case with traditional products made with ill-defined cultures, where you have to take what you get out of the fermentation.

Fermented milk products are particularly important in African countries were malnutrition is prevalent and are common all over Africa. *Kindirmo*, *Nunu* and *Warankasi* are common fermented milk products in Nigeria. *Warankasi* is known among indigenous African consumers as cheese just as *Kindirmo* and *Nunu* are considered as the equivalent of yoghurt.

Nunu is the Hausa name for the natural yoghurt which is usually sold among the Fulanis. The Fulanis themselves call it kosap. It is used as a staple food amongst the Saharan tribes of West African Sub-region, and is also popular amongst the inhabitants of the Mediterranean region and the Middle East where it is known as *Dahi* or *Lassi*. Traditionally, *Nunu*is prepared by inoculating freshly drawn cow milk with a little of the leftover as a starter and then allowed to ferment for about 24h at room temperature. During fermentation, some of the lactose are converted to lactic acid. At the end of fermentation period, the milk butter is removed by churning for further use, giving rise to the fermented skimmed milk, *Nunu*,which is a sour but delicious and refreshing beverage (Akabanda *et al*., 2014).

Most of the organisms involved in the fermentation process are usually of three main groups; bacteria, yeast and mould. *Lactobacillus fermentum*is the dominant LAB throughout the fermentation with *Lactobacillus plantarum* and *Leuconostoc mesenteroides* playing prominent roles during the first 6-8 h of fermentation as well. Less frequently isolated LAB include*Lactobacillus helveticus, Enterococcus faecium, Enterococcus italicus, Weissella confusa* and a putatively novel *Lactococcus* spp. The yeasts involved

were identified as *Candida parapsilosis, Candida rugosa, Candida tropicalis, Galactomyces geotrichum, Pichia kudriavzevii* and *Saccharomyces cerevisiae* with *P. kudriavzevii* and *S. cerevisiae* being the dominant yeast species (Akabanda *et al*., 2014).

Nunu has a sharp acid taste and is, therefore, usually taken with sugar and fura which is made up of millet flour compressed into balls and cooked for about 20-40min. The cooked fura is crumbled in a bowl of Nunu giving rise to a product refered to as fura de nunu. Nunu is an excellent source of protein, rich in essential amino acids and a good source of calcium, phosphorous and vitamins A, C, E and B complex. However, like other milk products, it is poor in ascorbic acid and iron (Nebedum and Obiakor, 2007). *Nunu*, if well prepared and well preserved, could serve as an equally good alternative but cheaper source of dairy product. It is at present being prepared and hawked mostly by the nomadic Hausa/Fulani cattle rearers, who control over 80% of the country's cattle production and only available within walking distance of their settlements. Nunu is thus more available in the Northern part of Nigeria than in the South, and as such only a small percentage of non-Fulanis has acquired the taste for it. Traditional fermented milk product however, do not appeal to majority of the people because of the apparent unhygienic conditions in which they are prepared, and also their poor shelf life (Sudi, 2013).

2.2. The use of lactic acid bacteria as starter cultures

Pathogens have been isolated especially, from many African indigenous fermented foods as a result of poor sanitary conditions during preparation. These come from raw materials or from the handlers. For these pathogens to grow in fermented foods, which may result in foodborne diseases, the microorganisms must overcome such hurdles as low pH, low water activity (aw, in solid-state fermentation), and in some cases, heat treatments and natural antimicrobial compounds (Gadaga *et al.,* 2004). Foodborne pathogens are able to grow to high levels during the early stages of fermentation due to the low acid levels.

Some of the approaches that can be used to minimize the risk of food borne diseases through consumption of fermented foods include improved hygiene, use of protective cultures, and the use of starter cultures. Starter culturesare microbiological culture preparations to assist the beginning of the fermentation process in preparation of various foods and fermented drinks (Farnworth and Mainville, 2003).

The use of lactic acid bacteria starter cultures in the production of fermented foods ensures rapid growth of the bacteria with the resultant reduction in pH to below 4, which is critical for controlling pathogens (Gadaga *et al*., 2004). The lacticacid bacteria (LAB) comprise a clade of Gram-positive, low-GC, acid-tolerant, generally non-sporulating, non-respiring rods or cocci that are associated by their common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and lactic products, produce lactic acid as the major metabolic end-product of carbohydrate fermentation. This trait has, throughout history, linked LAB with food fermentations.

Historically, bacteria from the genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* are the main LAB species involved in food fermentation. Numerous reports indicate that *Lactobacillusbrevis, Lactobacillusfermentum, Lactobacillusplantarum, Lactobacillusreuteri, Pediococcuspentosaceus* and *Pediococcusacidilactici* exhibit superior performance in lactic fermented cereal and vegetable products. This is quite possibly the case for root crops, while the initiation of milk fermentations is typically associated with *Lactococcuslactis*, followed by *Lactobacilluscasei* (*paracasei*) and other *Lactobacillus* spp. during maturation (Holzapfel, 2002).

The benefits of lactic fermentation which may contribute to the safety of lactic fermented foods include; production of organic acids, bacteriocins, reduction of antinutritive factors, and degradation or inactivation of toxins. The LAB, therefore, are of major importance among bacteria associated with traditional fermented foods. Their association with the human environment and their beneficial interactions, both in food and in the human intestinal tract, combined with the long tradition of lactic fermented foods in many cultures, have led to the conclusion that these foods may be 'Generally Regarded As Safe' (GRAS).

The lactic acid bacteria (LAB) have been widely used as starter cultures for manufacturing various fermented foods such as dairies, beverages, meat, and vegetables. These starters, however, are not yet commercially available for the small-scale fermentation of traditional African foods. The LAB are not only of major economic significance, but are also of value

in maintaining and promoting human health. Those lactic acid bacteria with scientifically supported health claims are known as probiotics (Rashid*et al.*, 2007).

2.3 Probiotics: A historical overview

The term probiotics was introduced by Lilly and Stillwell (1965), to describe growth-promoting factors produced by microorganisms. It is derived from a Greek word which means pro-life. Probiotics are live microorganisms in foodstuffs which, when consumed at certain levels in nutrition, stabilize the gastrointestinal tract microflora thereby conferring health benefits on the consumer (FAO/WHO, 2001). These definitions imply that probiotic ingestion provides benefits for host health (Vandenplas, 2015).The works of Metchnikoff and Tissier were the first to make scientific suggestions about the probiotic use of bacteria. However the first clinical trials were done in the 1930s on the effect of probiotics on constipation (Koop-Hoolihan, 2001).

2.4 Microorganisms used as probiotics

Members of the genera *Lactobacillus* and *Bifidobacterium* are mainly used as human probiotics. Other organisms used are members of the genera *Lactococcus* and *Enterococcus* (Vandenplas*etal*., 2015). The Lactobacilliare considered as indigenous microorganisms colonizing the small intestine as they are found within the first week of life. Some of the important representatives are listed in Table 1a and b.

The use of lactic acid bacteria (LAB) as probiotics for human and animal consumption has been documented (Savadogo *etal*., 2006). *Bifidobacterium* species, in particular, strains of *Bifidobacterium animalis, Bifidobacterium lactis, Bifidobacterium bifidum, Bifidobacterium breve* and *Bifidobacterium longum* biotypes *infantis* and *longum* are often implemented in probiotic products in combination with other lactic acid bacteria (Masco *etal*., 2005). *Bifidobacterium* sppare commonly isolated from feaces of humans, animals, birds and are present in high numbers in breastfed babies. Bifidobacteria share many metabolic properties of LAB such as being fermentative and producing lactate, among other acids, and are commonly included in this group in many discussions on probiotics (Vankerckhoven *etal*., 2008).

It is essential to note that since probiotic activities are strain-specific, strain identification is recommended in order to establish their suitability and performance for industrial

application. This is achieved by a combination of phenotypic tests followed by genetic identification using molecular techniques eg. DNA/DNA hybridization and 16SRNA sequencing.

2.5 Desirable probiotic properties

In order for a potential probiotic strain to be able to exert its beneficial effects, it is expected to exhibit certain desirable properties. The ones currently determined by in vitro tests are:

1. Acid and bile tolerance which is crucial for oral administration.
2. Adhesion to mucosal and epithelial surfaces, an important property for successful immune modulation, competitive exclusion of pathogens, as well as prevention of pathogen adhesion and colonisation.
3. Antimicrobial activity against pathogenic bacteria.
4.Antibiotic susceptibility test.

Table 1a: Bacterial organisms considered as probiotics

Lactobacillus species	*Bifidobacterium* species
L. acidophilus *L. casei* *L. crispatus* *L. gallinarum* *L. gasseri* *L. johnsonii* *L. paracasei* *L. plantarum* *L. reuteri* *L. rhamnosus*	*B. adolescentis* *B. animalis* *B. bifidum* *B. breve* *B. infantis* *B. lactis* *B. longum*

Adapted from Holzapfel *et al*., 2001.

Table 1b: Microorganisms considered as probiotics

Lactic acid bacteria	Non lactic acid organisms
Enterococcus faecalis	
E. faecium	*Bacillus cereus var. to yoi*
Lactococcus lactis	*Escherichia coli strain nissle*
Leuconostoc mesenteroides	*Propionibacterium freudenreichii*
Pediococcus acidilactici	*Saccharomyces cerevisiae*
Sporolactobacillus inulinus	*S. boulardii*
Streptococcus thermophilus	

Adapted from Holzapfel *et al*., 2001

As far as the final product is concerned, the probiotic dose levels should be based on the ones found to be efficacious in human studies and the colony forming units per gram of product is an important parameter. Although the information about the minimum effective concentrations is still insufficient, it is generally accepted that probiotic products should have a minimum concentration of 10^6 CFU/mL or gram(Kechagia, 2013).

2.6 Mechanisms of action of probiotics

Probiotics have several mechanisms of action.The exact manner in which they exert their effects is still not fully elucidated. These mechanisms of action include: bacteriocin and short chain fatty acid production, lowering of gut pH, and nutrient competition, stimulation of mucosal barrier function and immunomodulation. The latter in particular has been the subject of numerous studies and there is considerable evidence that probiotics influence several aspects of the acquired and innate immune response by inducing phagocytosis and IgA secretion, modifying T-cell responses, enhancing Th1 responses, and attenuating Th2 responses (Kechagia*et al*., 2013)

2.5 Beneficial effects of probiotics

The beneficial effects of probiotics may be mediated by direct antagonistic effect against specific groups of organisms or by an effect on their metabolism or by stimulation of immunity. Probiotics antagonize pathogens through production of antimicrobial and antibacterial compounds such as cytokines and butyric acid (Kailasapathy and Chin, 2000); reduce gut pH by stimulating the lactic acid producing microflora, compete for binding and receptor sites that pathogens occupy (Kailasapathy and Chin, 2000); improve immune function and stimulate immunomodulatory cells (Rolfe, 2000); compete with pathogens for available nutrients and other growth factors (Rolfe, 2000); or produce lactase which aids in lactose digestion (Oyetayo and Oyetayo, 2005).

Probiotics have been shown to have a wide range of beneficial effects on human health. Some of these effects include:

1. Reduction in the risk of colon cancer.

2. Improvement in serum cholesterol levels and blood pressure

3. Improve immune function by increasing the number of IgA-producing plasma cells , increasing or improving phagocytosis as well as increasing the proportion of T lymphocytes and Natural Killer cells.

4. Decrease the risk of infections of the upper aero-digestive tract such as respiratory infections and *Helicobacter pylori* infections.

5. Decrease the incidence of and improve the treatment of both infantile diarrhoea and antibiotic associated diarrhoea.

6. Modulate inflammatory and hypersensitivity responses by regulating cytokine function, improving milk allergies, decreasing the risk of atopic eczema and preventing reoccurrences of Inflammatory Bowel Disease.

7. Improving lactose digestion among those who are lactose intolerant (Shiby and Mishra, 2013).

Colon Cancer: The etiology of colon cancer is complex comprising of a well-defined series of histological changes paralleled with mutational activation of oncogenes and inactivation of tumor suppressor genes regulated by an interplay between diet, environment, carcinogenic chemicals, and mutagens (Sankpal *et al*., 2012; Raman *et al*., 2013). Probiotic bacteria with antimutagenic and/or antigenotoxic activities, have been found to exert prophylactic effect against colon cancer (Papadimitriou *et al*., 2015).

Milk cultured with Lactobacilli strains have been shown to have anti-mutagenic effects in laboratory experiments including animal studies, reducing mutagenicity and chromosome damage by approximately 80%.Several laboratory studies have demonstrated that various species of lactic acid bacteria are capable of binding these mutagenic chemicals, even in human gastric juice, thus rendering them harmless. Interestingly, anti colon cancer

potential of probiotic strains has been attributable to metabiotics that have epigenetic, antimutagenic, immunomodulatory, apoptotic, and antimetastatic effects (Sharma and Shukla, 2016).Fermented milks and probiotics could modify the colonic environment beneficially through changes in colonic microflora and decreases in bacterial enzymes that activate carcinogens. Such changes have been observed in both human volunteers and animal studies.

The azoxymethane (AOM), dimethylhydrazine (DMH), and heterocyclic aromatic amines (HAAs) are carcinogens usedin most animal studies. When male or female BD6 rats were fed freeze-dried milk fermented with *L. bulgaricus* before and following DMH administration, this probiotic (2.5 g) reduced colon tumor incidence and multiplicity by over 40% in female but not in male rats. A different strain of *L. bulgaricus* reduced total intestinal tumors in both sexes. In another study, 10^{10}cfu/g of freeze-dried *L. acidophilus*, *L. casei* spp., *L.rhamnosus*, *Streptococcus thermophilus*, or a mixture of *L. acidophilus* and *B. animalis*were given to 5-week-old Sprague-Dawley rats for 4 weeks prior to DMH administration. Although tumor incidence was unchanged, tumor burden was reduced by 70% with *L. acidophilus* administration (Saikali *et al*., 2004).

Administration of *B. longum* also reducedDMH-induced colorectal tumor development in transgenicCB6F1-Tg-Hras2 mice (Ohno *et al*., 2001). *B. animalis* in milk or water ($6x10^9$cells per animal per day) and skim milk (6% of thediet) reduced ACF formation by over 50% when comparedwith administration of water as a control (Saikali *et al*., 2004).

Microbial metabolic end-products, which account for one third of the metabolites present in the human blood, play an important role in gut homeostasis and have an impact on host metabolism and health (Sharon *et al*., 2014 and Richards *et al.*, 2016). The short-chain fatty acids (SCFAs) acetate, butyrate, and propionate are quantitatively and metabolically the most important microbial end-products of the human colon fermentation process (Louis *et al*., 2014), as they display several physiological effects.

Antimutagenic potential of probiotics has primarily been attributed to binding of live bacteria with mutagens but now there is increasing evidence that even cell free supernatants might either scavenge the reactive carcinogen intermediates or influence the

ability of carcinogen activating/deactivating enzymes (Wollowski *et al.*, 2001). Supernatants of probiotic cultures supplemented with prebiotics were reported to substantially reduce the genotoxicity of human fecal slurry (Burns and Rowland, 2004). Similarly, metabolites produced in soymilk fermented by mixed culture of *Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacterium infantis, Bifidobacterium longum* have also been found to exhibit high antimutagenicity against mutagen 3, 2-dimethyl-4-amino-biphenyl (Hsieh and Chou, 2006). Further, it was observed that colon cells treated with supernatant of inulin fermentation by lactic acid bacteria elevated Glutathione *S*-transferase-pi [(GST)- pi] activity, a chemopreventive enzyme against mutagens (Scharlau *et al*., 2009).

Mutagen binding potential of probiotics (lactobacilli and bifidobacteria) has been found to be associated with cellular components such as peptidoglycans and polysaccharides but the antimutagenic activity very much depends upon the growth phase, cell number of bacterial strain and mutagen type (Raman *et al*., 2013). Researchers in Germany exposed rats to the carcinogens MNNG or DMH (chemicals used to induce colon cancer) which subsequently caused damage to the DNA in their intestinal cells. When they fed the rats lactic acid bacteria or yoghurt, however, this DNA damage was prevented. Because cancer initiation occurs due to mutations in DNA, this anti-mutagenic action of lactic acid bacteria lends support to the notion that it may contribute towards preventing cancer of the colon (Sharma and Shukla, 2016).

Yoghurt and other fermented dairy foods have shown to be protective against colon cancer in a handful of case-control studies. A study compared the diets of 746 colon cancer patients in California with 746 cancer-free people of the same age. A higher calcium intake was associated with a decreased risk, however the only single food which showed to be significantly protective was yoghurt. Another case control study in France found that yoghurt was the only food found to decrease the risk of colon adenomas (precancerous tumours) in a comparison between 208 cases and 462 controls. Moderate consumption decreased the risk by 40%, whilst higher consumption decreased the risk by 50% (Boutron *et al.*, 1996).

Cardiovascular disease (CVD) - Cholesterol:High level of serum cholesterol has been associated with risks of coronary heart disease (Pereira and Gibson, 2002; Pereira *et al*.,

2003). People affected with hypercholesterolemia may avert the use of cholesterol-lowering drugs by practising dietary control or supplementation of probiotics and/or prebiotics. In a study evaluating the effect of *L. plantarum* PH04, isolated from infant faeces, on cholesterol, Nguyen *et al.* (2007), administered *L. plantarum* (4×10^8 CFU/ml dose per mouse daily) to twelve male mice for 14 days. The authors found a significant ($P< 0.05$) reduction of total serum cholesterol (reduced by 7%) compared to the control. El-Shafie *etal*. (2009), showed the effect of *Lactobacillus plantarum*NRRL B-4524 used as single or mixed with *Lactobacillus paracasei* and/or other strains of bacteria in rat diets in lowering blood serum cholesterol. Fazeli *etal*. (2010), showed that the consumption of *L. plantarum* A7 (10^8 CFU ml^{-1}) for 14 day is effective in lowering serum lipid levels in rats. Taranto *etal*. (2000), reported that, administration of *Lactobacilllus reuteri* was effective in preventing hypercholesterolemia in mice and observed a decrease in total cholesterol (22%). Hung *etal*. (2008), showed that use of probiotic combination in fermented soybean meal resulted in reduction in total cholesterol in forty eight pigs.

Arun *etal*. (2006), showed that dietary supplementation of *Lactobacillus sporogenes* ($6x10^8$ spore per gram) at 100 mg kg^{-1} diet significantly lowered total cholesterol concentrations in the serum of broiler chickens. Supplementation of probiotics (*Lactobacillus acidphilus, Bifidobacterium bifidum* and *Aspergillus oryzae*) at 100 mg kg^{-1} in the diet of broiler chickens significantly reduced the serum cholesterol concentration. Kalavathy *etal*. (2003), reported that, dietary supplementation of a mix culture of 12 strains of *Lactobacillus* at 1% in the basal diet of broilers resulted in lowered serum cholesterol concentration.

L. fermentum SM-7 isolated from a fermented milk drink (koumiss) was found to significantly reduce serum total cholesterol (TC)in mice. Another study also consistently showed significant reduction by about 25% of serum TC in rats fed *L. fermentum* 9-41-A. The strain was isolated from faeces of healthy adults and selected for its probiotic characteristics (Pan *et al*., 2011). Vijayendra and Gupta (2012), observed a significant reduction of serum cholesterol level of 2.63, 4.1 and 4.68 mg/100 ml at the end of 30 days in rats fed with yoghurt, probiotic *dahi* and probiotic yoghurt, respectively, indicating the hypocholesterolaemic effect of the probiotic cultures.

The hypocholesterolemic potential of probiotics has also been evaluated using human subjects. Xiao *et al.* (2003), evaluated the effects of a low-fat yogurt containing 10^8 CFU/g of *B. longum* BL1 on lipid profiles of thirty-two subjects (body weight 55.4–81.8 kg, aged 28–60 years old). Results from this randomized, single-blind, placebo-controlled and parallel study showed a significant ($P< 0.05$) decline in serum total cholesterol after 4-weeks. A meta-analysis of 30 randomized controlled trials conducted by Young and Jeongseon (2015), also found participants receiving probiotic bacteria supplementation to have a significantly lower concentrations of total cholesterol compared to the control subjects.

Several mechanisms proposed for the cholesterol-lowering effects of probiotics includes; the enzymatic deconjugation of bile acids by bile-salt hydrolase of probiotics, the ability to bind cholesterol in the small intestines, the incorporation of cholesterol into the cellular membranes during growth and the conversion of cholesterol in the intestines to coprostanol, which is directly excreted in faeces. This decreases the amount of cholesterol being absorbed, leading to a reduced concentration in the physiological cholesterol pool. However, the mechanism underlying the hypocholesterolemic effect of probiotics might be strain-specific.

Cardiovascular disease (CVD) - Blood Pressure: Probiotics and their potential role in maintaining cardiovascular health has received much attention among the scientific communities. Numerous studies have shown either moderate or significant reduction in the ratios of systolic blood pressure/diastolic blood pressure (SBP/DBP). A mean reduction of SBP 5.2 (±8.1) mmHg and DBP 1.7 mmHg has been recorded in borderline hypertensive men (aged 23–59 years) given sour milk fermented with *L. helveticus* and *Saccharomyces cerevisiae* containing tripeptides (Mizushima *et al.,* 2004). *L. helveticus* (LBK-16H strain) fermented sour milk containing ACE-inhibitory tripeptides attenuated the development of hypertension in spontaneously hypertensive rats (Sipola *et al.,* 2001). In a study, milk fermented with *L. casei* strain Shirota and *Lactococcus lactis* YIT 2027 significantly reduced the mean SBP (17.4±4.3 mmHg) and DBP (7.5±5.7 mmHg) in mildly hypertensive patients (Inoue *et al.,* 2003). Furthermore, a meta-analysis based on 14 randomized placebo-controlled clinical trials has shown that probiotic fermented milk significantly reduced both SBP and DBP in pre-hypertensive and hypertensive subjects (Dong *et al.,* 2013). Tanida *et al.*(2005),showed that intraduodenal injection of

Lactobacillus johnsonii La1 (1×10^8CFU/day), or its metabolites, reduced hypertension. In a double-blind, randomized placebo-controlled trial, consumption of a *Lactobacillus plantarum* 299v (2×10^{10}/CFU/mL/day) fermented food product by 36 smokers for 6 weeks significantly reduced SBP (13±4 mmHg, $P<0.001$) (Naruszewicz *et al.,* 2002). Lactic acid bacteria are able to metabolize the complex milk protein and aid in the release of short bioactive peptides which have ACE-inhibitory activity, thereby contributing to the modulation of hypertension (Donkor *et al.,* 2007; Korhonen, 2009). In another study, fermented soy milk probiotic cocktail (*L. casei*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *S. thermophilus*, and *Bifidobacterium longum*) enriched with whey-separated bioactive peptides with high ACE-inhibitory activity positively reduced SBP in rats after 8 weeks of oral application (Tsai *et al.,* 2006).

In a placebo-controlled trial involving hypertensive patients, 8 weeks of consuming sour milk fermented by *Lactobacillus helveticus* and *Saccharomyces cerevisiae* resulted in significant reductions in both systolic and diastolic blood pressure (Seppo *et al.,* 2002). Several studies provide preliminary evidence to suggest that consumption of lactic acid bacteria fermented dairy foods along with a host of other functional foods known to have beneficial effects on blood pressure, could be incorporated into dietary strategies used to complement medical treatments for hypertensive patients (Seppo *et al.,* 2003).

Immune function:There is a significant body of evidence from clinical and laboratory investigations to suggest that consumption of lactic acid bacteria may have favourable effects on immune function. One beneficial mechanism involves antibodies such as Immunoglobulin A (IgA). These are produced by plasma cells of the immune system and are involved in protecting the body from potentially harmful microbes. *Lactobacillus casei*, *Lactobacillus acidophilus* and yogurt have been shown to enhance the number of IgA-producing plasma cells in a dose-dependent manner as well as increasing sIgA levels in mice and humans. Another beneficial mechanism that lactic acid bacteria may have on immune function is the ability to enhance a process known as phagocytosis, which is where certain types of white blood cells known as macrophages literally engulf and ingest "invaders" such as harmful bacteria and other disease causing microbes (pathogens). Probiotics therefore can enhance nonspecific cellular immune response characterized by activation of macrophages, natural killer (NK) cells, antigen-specific cytotoxic T-

lymphocytes, and the release of various cytokines in strain-specific and dose-dependent manner (Ashraf and Shah, 2014).

Investigation have shown that macrophage numbers increased in mice fed cultures of *L. acidophilus* or *L. casei* (Adolfsson *et al.,* 2004). Furthermore, *Lactobacillus acidophilus* and *Bifidobacterium longum* have been found to enhance phagocytic function of human macrophages *in vitro* whilst animal studies have demonstrated that *L. acidophilus, L. casei* and *Streptococcus thermophiles* either enhanced or increased phagocytosis in the macrophages of rodents. In a double blind, placebo-controlled trial designed to determine the effects of lactic acid bacteria on immune function, the results demonstrated that after 6 weeks, those who received the probiotic milk had significantly improved markers of natural immunity such as enhanced levels of interferon-alpha and increases in the phagocytic capacity of certain immune cells (Arunachalam *et al.,* 2000).

A similar trial in New Zealand demonstrated that elderly volunteers given the same strain of *B. lactis* showed significant improvements in the immune function including an increase in the proportion of immune cells known as T lymphocytes, especially helper and activated T-cells, as well as natural killer cells; a type of white cell known to attack tumours (Gill *et al.,* 2001a). The same researchers found both an increase in the number of natural killer cells as well as a 101 and 62% enhancement of their immune cells ability to attack tumours when elderly people were given either *B. lactis* HN019 or *Lactobacillus rhamnosus* HN001 respectively, for as little as 3 weeks (Gill *et al.,* 2001b). These probiotic strains have also demonstrated significant immune enhancing ability in animal studies (Gill *et al.,* 2001c).

In a randomised, double blind, placebo controlled study in Finland, 857 healthy children aged 1-6 years in 18 day-care centres throughout Helsinki were studied for 7 months to see whether consumption of a probiotic milk could reduce the incidence of respiratory infections. The children that were given milk with *L. rhamnosus* GG had 16% fewer days absent due to illness. Furthermore, there were 17% fewer cases of respiratory tract infections, and a 19% reduction in the need for antibiotic treatment for respiratory infections among the children that received the probiotic compared to those that did not (Hatakka *et al.,* 2001). Additionally, the children who received the probiotic had 44% fewer dental carries than those who received the placebo (normal milk), presumably

because *Lactobacillus* GG acts as an antagonist to the bacteria that cause dental problems (Nase *et al.,* 2001). In a trial involving 209 volunteers, daily consumption of a probiotic drink resulted in a 19% reduction in the occurrence of potentially disease causing bacteria found in the nasal tract (Gluck and Gebbers, 2003) – a part of the body that can harbour pathogenic microbes such as those that cause pneumonia, haemolytic anaemia as well as *Staphylococcus aureus* "golden staph".

Helicobacterpylori:Ingestion of lactic acid bacteria has also been found to be beneficial in people infected with the bacterium *Helicobacter pylori* which is responsible for gastritis and peptic ulcers. Various strains of lactic acid bacteria probiotics such as those isolated from yoghurt (Oh *et al.,* 2002) have been proven to reduce the growth of *H. pylori* in vitro (Midolo *et al.,* 1995), in animal studies (Sgouras *et al.,* 2004; Johnson-Henry *et al.,* 2004; Aiba *et al.,* 1998) and human clinical trials, presumable by producing selectively anti-bacterial substances known as bacteriocins (Pinchuk *et al.,* 2001) and by inhibiting binding ability (Mukai *et al.,* 2002). *Lactobacillus johnsonii* is probably the most successful species of probiotic shown to reduce *H. pylori* infection. For example, *L. johnsonii* has been shown to reduce *H. pylori* infection in children in Santiago, Chillie (Cruchet *et al.,* 2003).

In a double blind trial in Switzerland, patients with *H. pylori* infections, given *L. johnsonii* probiotic experienced a modest improvement such as decreases in the severity and activity of antral gastritis, decrease of *H.pylori* density and increased mucous thickness (Pantoflickova *et al.,* 2003). The authors concluded that regular ingestion of fermented milk containing *L. johnsonii* may reduce the risk of developing disorders associated with high degrees of gastric inflammation and mucus depletion. Further investigations in Switzerland have found that *L. johnsonii* probiotics are capable of producing a favourable effect on *H.pylori* gastritis in human subjects (Felley *et al.,* 2001) regardless of whether it was combined with a standard medication used to treat *H. pylori* called Omeprazole, or with a placebo.

Similar investigation demonstrated a modest suppressive effect on *H.pylori* growth in patients given *L. casei* Shirota strain for 3 weeks in the Netherlands (Cats *et al.,* 2003) whilst Japanese researchers found that consuming yoghurt containing *Lactobacillus gasseri* OLL2716 also resulted a suppression of *H. pylori* as well as a reduction in gastric

mucosal inflammation in 31 patients for 8 weeks (Sakamoto *et al.,* 2001). Similarly, the results of a clinical trial in China revealed that compared to those given a placebo, *H. pylori* growth was significantly inhibited after 6 weeks in 59 patients who consumed yoghurt containing *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 (Wang *et al.,* 2004).

Other investigations have found that the addition of probiotics to standard drug treatments can enhance their effectiveness (Tursi *et al.,* 2004) or reduce the severity of the drug-related side effects (Armuzzi *et al.,* 2001).A trial involving 120-infected patients in Italy found that patients given the conventional medical treatments (Rabeprazole, Clarithromycin and Amoxicillin) had a 72% successful eradication of the infection after 1 week, whereas those given the same treatment plus *L. acidophilus* probiotics had an 88% rate of success (Tursi *et al.,* 2004). Consumption of *Lactobacillus* and *Bifidobacterium*-containing yoghurt was shown to improve drug treatment in a Taiwanese study where the rate of successful eradication after 1 week of treatment in those given only drugs was 78% compared to 91% in those also given the yoghurt (Sheu *et al.,* 2002).

Diarrhoea: Diarrhoea is one of the most common causes of sickness in young children, often caused by rotavirus infections. It results in approximately 3 million doctors visits per year and contributes roughly 13% of hospitalizations among children under 5. The results of randomized, double blind placebo-controlled trials have found that administration of probiotics containing *Lactobacillus* GG (Guandalini *et al.,* 2000), *Lactobacillus reuteri, L. acidophilus* or *L. acidophilus* combined with *L. bulgaris* (Simakachorn *et al.,* 2000) have proven effective in the treatment of diarrhoea in children between 1 month and 2.5 years old, by reducing the duration and severity of symptoms.

Consumption of lactic acid bacteria has also been shown to reduce the risk of developing a type of diarrhoea which commonly occurs as a result of antibiotic treatments. The results of various randomized double-blind placebo-controlled trials have demonstrated that probiotics containing *Lactobacillus* GG may prevent antibiotic associated diarrhoea in both adults (Armuzzi *et al.,* 2001) and children. A meta-analysis of these trials reported a 60% average reduction in the incidence of antibiotic-associated diarrhoea in people given probiotics concurrently (Cremonini *et al.,* 2002).

Several studies have also found that consumption of yoghurt during antibiotic treatment can also halve the likelihood of getting diarrhoea, half the duration of diarrhoea symptoms (Beniwal *et al.,* 2003) as well as decrease some of the other side effects associated with antibiotics such as abdominal distress, stomach pain and flatulence. Similar randomized trials have demonstrated that consumption of lactic acid bacteria can protect against diarrhoea in healthy people as well. One such recent study involved 541 young soldiers, 275 of whom were given yoghurt containing *Lactobacillus casei,* the other 266 received ordinary non-probiotic yoghurt. Throughout the duration of the study, there were approximately 25% fewer cases of diarrhoea among those given the probiotic yoghurt (Pereg *et al.,* 2005).

Inflammation and allergies: Several studies have shown that probiotics can alter the production of behavioural and communication molecules called cytokines that are released from certain cells of the immune system and are involved in immune regulation as well as inflammatory responses. Some of the many beneficial effects that probiotics have on mediating immune regulation include balancing the control of pro-inflammatory and anti-inflammatory cytokines. Studies indicate that probiotics can be used as innovative tools to alleviate intestinal inflammation, normalize dysfunctional of the mucosa, and down-regulate hypersensitivity reactions such as allergies (Isolauri *et al.,* 2001). Human studies have found that administration of *Lactobacillus rhamnosus* GG can enhance the cellular immune response to intestinal micro-organisms whilst causing a decrease in the production of pro-inflammatory cytokines but an increase in anti-inflammatory cytokines (Schultz *et al.,* 2003).

It has been hypothesized that an increase in the occurrence of childhood allergies may be due to an increase in hygiene, and thus a reduction in the exposure to microbes early in life. Therefore it was suggested that exposure to “friendly” bacteria early in life might reduce the subsequent risk of allergies. In a randomised double-blind, placebo-controlled study in Finland, 132 pregnant women with a family history of atopic (allergic) eczema were given either *L. rhamnosus* GG or a placebo for several weeks prior to giving birth, as well as to their infants after birth. After both 6 months (Kalliomaki *et al.,* 2001) and a follow up 4 years later, (Kalliomaki *et al.,* 2003) there was a 50% reduction in frequency of atopic eczema in the group given the probiotic compared to the placebo group. This is

significant because early childhood atopic eczema is an indicator of other allergies later in life.

Other studies conducted by researchers in Finland also demonstrated a significantly greater reduction in the symptoms from atopic eczema and cow's milk allergy (Kirjavainen *et al.*, 2003) as well as a decrease in markers of inflammation (Majamaa *et al.*, 2003) in infants given *Lactobacillus* GG compared to those given a placebo.

Inflammatory bowel disease (IBD): Another potentially beneficial use for probiotics has been found to be in the treatment of Inflammatory Bowel Diseases (IBD), which include Chrohn's Disease (an inflammation of the small intestine), Ulcerative Colitis (an inflammation of the Colon resulting in ulceration) and pouchitis (an inflammation of the pouch created as treatment of a patient with ulcerative colitis)(Cheifetz and Itzkowitz, 2004).

There isevidence that commensal enteric bacteria and their products create a local environment that affects the course of IBD (Thompson-Chagoyán *et al.*, 2007). These high bacterial concentrations in IBD patients are characterized by decreased numbers of LAB and bifidobacteria and increased numbers of *E. coli*, coliforms, and bacteroides in the colon (Thompson-Chagoyán *et al.*, 2005).

A controlled clinical trial in Italy involved 40 patients which had undergone a procedure called ileal pouch-anal anastomosis for ulcerative colitis. They were randomised to receive either a highly concentrated probiotic supplement or a placebo and monitored for a year. By the end of the trial, only 10% of patients given the probiotic had reported an episode of acute pouchitis compared with 40% of those given the placebo (Gionchetti *et al.*, 2003).

Zeuthen *et al*. (2008),reported that the combination of *L. acidophilus* X37, *L. paracasei* Z11, *L. casei* CRL431, LGG, *B. longum* Q46, *B. bifidum* Z9, *B. breve* 20091, and *B. bifidum* 20082a decreased interleukin (IL)-12 and tumor necrosis alpha (TNF-α) concentrations in culture supernatants. Furthermore, a cell-free culture supernatant (CFS) from *Bifidobacterium breve* CNCM I-4035 also provides immunomodulatory effects on human intestinal dendritic cells(DCs)(Bermudez-Brito, 2012; Bermudez-Brito, 2013).

A specific probiotic bacterial strain could improve the state of the intestine by facilitating epithelial barrier functions, inhibiting regulatory T (T_{reg}) cell-mediated mucosal

inflammation and increasing production of interleukin (IL)-10 and transforming growth factor beta (TGF-β). This inflammation reduction may prevent IBD (Mercer *et al.,* 2012; Rauch and Lynch, 2012).

2.6 *Lactobacillus fermentum*as probiotic

Lactobacillus fermentum has been identified as potential probiotic (Mikelsaar, 2009). A few strains are considered probiotic or "friendly" bacteria in animals (Reque, 2000) and at least one strain has been applied to treat urogenital infections in women (Gardiner*et al.,* 2002a).It can also be a normal inhabitant of the human intestinal tract and some strains have been associated with cholesterol metabolism (Mikelsaar, 2009). In general, they are seen as beneficial to the host's body and the human health. Some commercialized strains of *L. fermentum* used as probiotics include *L. fermentum* PCC and*L. fermentum* ME-3

L. fermentum demonstrates a significant pH and bile tolerance in terms of its consideration as a probiotic. Testing of *Lactobacillus fermentum* against different pH concentration solutions revealed that it has a strong pH tolerance by its ability to grow and survive a few hours after being incubated in a pH 3 solution. Strains of *Lactobacillus fermentum* have also been tested in different bile concentrations and demonstrated to have good bile tolerance when incubated with 3 g/L of bile salt. The stomach has a pH between 2 and 4, and the upper intestine contains 3-5g/L of bile. *Lactobacillus fermentum* has been found to survive in these conditions further supporting the idea that it can act as a probiotic (Pan *et al.,* 2011).

One of the ways in which *Lactobacillus fermentum* has been seen as a probiotic is by its ability to reduce cholesterol levels. Tests conducted using several strains of *Lactobacillus* and cholesterol broths demonstrated that *Lactobacillus fermentum* had the largest removal of cholesterol. One of the mechanisms by which *L. fermentum* may remove cholesterol through in vivo is by the absorption of cholesterol, which as a result accelerates cholesterol metabolism. Another method is by the incorporation of cholesterol in the host body into its cell membrane or walls. This would also increase resistance of the bacterial cell membranes to environmental challenge. A third mechanism is by causing the body to consume more cholesterol. *L. fermentum* would interfere with the recycling of bile salt and facilitate its elimination, which as a result would increase the demand for bile salt made from cholesterol (Pan *et al.,* 2011).

The strain *Lactobacillus fermentum* ME-3 has been discovered and identified as an antimicrobial and antioxidative probiotic. Tests conducted on the ME-3 strain in different bile concentrations found that it was able to survive without large loss in numbers. It has also been found that *Lactobacillus fermentum* ME-3 has a tolerance to survive drops of pH levels. It could withstand a drop in values from 4.0 to 2.5 without decreasing in numbers. These characteristics of tolerance to bile concentrations and pH levels serve to classify ME-3 as a probiotic(Mikelsaar, 2009).

Lactobacillus fermentum ME-3 has also been found to have the capability to suppress mainly gram-negative bacteria. Research on the antioxidant properties of strain ME-3 in soft cheese products revealed that it prevented spoilage (Mikelsaar, 2009). Experimentation has also been conducted on the consumption of the ME-3 strain. The consumption had a positive influence on the microbiota of the gut. Volunteers were given goat milk fermented by strain ME-3 and capsulated ME-3. After three weeks analysis of fecal samples revealed that the ME-3 strain increased the number of beneficial *Lactobacilli* in comparison to those who were given non-fermented milk (Truusalu *et al.*, 2010). *L. fermentum* ME-3 also has the potential to lower the risk of cardiovascular disease that is tightly associated with maintenance of plasma lipid profile. In an eight-week study, consumption of kefir with the antioxidative probiotic *L. fermentum* ME-3, was reported to reduce serum LDL-C and TG values in clinically healthy volunteers with borderline-high lipid profile indices (Mikelsaar, 2015).

In general, strains of *Lactobacillus* have been considered safe because of their association with food and because they are normal inhabitants of the human microflora. They have also been identified to have a low pathogenic potential further reinforcing the idea that they are safe microbes (Truusalu *et al.*, 2010). Research with regard to the safety of *Lactobacillus fermentum* has been carried out on mice. Mice were fed (intragastrically) different concentrations of *Lactobacillus fermentum* for twenty-eight days and blood samples were taken from the mice and analyzed. There was no health difference observed between the control mice and those fed *Lactobacillus fermentum* in terms of blood biochemistry, protein, albumin, and glucose. Also no negative side effects during the experiment such as change in feed intake, or clinical signs such as diarrhea and ruffled fur, were observed. The ingestion of *Lactobacillus fermentum* in mice appeared safe which led

to further support that the use of *Lactobacillus fermentum* in food is also safe (Park *et al.*, 2005).

In order for *L. fermentum* to be considered as a potential probiotic, it must not contain any transferable resistant genes as this could lessen the effect of the use of antibiotics (Zeng *et al.*, 2010). According to Zhou *et al*. (2005), a strain of *L. fermentum, L. fermentum* A8 was found to be susceptible to chloramphenicol, erythromycin, gentamicin, streptomycin and tetracycline.Experiments conducted by introducing the strain ME-3 of *Lactobacillus fermentum* into dairy products as a probiotic ingredient revealed that it was able to suppress the reputed contaminants of food such as pathogenic *Salmonella*spp., *Shigella* spp., and urinary tract infections that are caused by *E. coli*and*Staphylococcus* spp. (Truusalu *et al.,* 2010). In 2008, Truusalu *et al.* eradicated *Salmonella typhimurium* infection in a murine model of typhoid fever with the combination of probiotic *Lactobacillus fermentum* ME-3 and ofloxacin.

Another strain of *L. fermentum*, *Lactobacillus fermentum* PCC, has been demonstrated to induce a protective immune response. In a randomized, double-blind, placebo-controlled study with 20 highly-trained distance runners, capsules of 12 billion CFU/day of *Lactobacillus fermentum* PCC was established to reduce total number of days with respiratory illness in the endurance athletes (Cox *et al.*, 2008) and may also reduce the severity of chest infection, illness load and use of medications in male athletes (West *et al.*, 2009). *Lactobacillus fermentum* PCC has also been found to induce an enhanced immune response to an influenza vaccine (French and Penny, 2009) and alleviate symptoms of atopic dermatitis (Weston *et al.*, 2005).

Administration of a follow-on formula containing human milk probiotic *Lactobacillus fermentum* has been proven to reduce the incidence of gastrointestinal and upper respiratory tract infections in infants between the ages of 6 and 12 months (Maldonado *et al.*, 2012). *Lactobacillus fermentum* ACA-DC 179 has also been reported to display probiotic potential in vitro and protect against trinitrobenzene sulfonic acid (TNBS)-induced colitis and *Salmonella* infection in murine models (Zoumpopoulou *et al.*, 2008).

Probiotic organisms are expected to possess the following characteristics: easy reproducibility; ability to survive the environmental conditions of the location where they

are active; genetically stable without plamid transfer; the absence of allergic, toxic, mutagenic or carcinogenic reactions, with neither its fermentation products nor its cell components being deleterious after consumption by the host; ability to remain viable during processing and ability to adhere to and colonize the location where they are active (Havenaar and Huis in't Veld, 1992; Wolfgang *etal*., 1999).

2.7 Safety of probiotics

Probiotics are viable organisms, and therefore, it is feasible that they could infect the host (Reid *et. al.,* 2003). Historical data indicates that probiotic Lactobacilli and Bifidobacteria administered in food are safe for human use (Reid, 2002). Their occurrence as normal commensals of the mammalian microbiota and their established safe use in diverse food and supplement products worldwide support this conclusion. Nevertheless, side effects have been reported, including rare systemic infections. Care must be taken when administering live bacteria to immunocompromised subjects and those with intestinal bleeding (Marteau, 2002). Care must also be taken to ensure that excessive immune stimulation is not induced in individuals who are susceptible to the development of arthritis or other complications (Reid *et al.,* 2003).

The issue of safety becomes more critical with organisms such as *Enterococcus* spp. as probiotics (Araujo and Ferreira, 2013). These bacteria are present in relatively high numbers in the intestine and are often included in the so-called probiotic cocktails, particularly in animal feed. However, Enterococci have emerged as an important cause of nosocomial infections, and isolates are increasingly vancomycin resistant (Gardiner *et al.,* 2002a). The safety of an organism to be used as a probiotic should be a major concern of the producer to ensure that the organism(s) contemplated for human use are not to be a significant risk. A form of safety may involve minimizing the transfer of drug resistance genes (Reid *et al.,* 2003).

Lactic acid bacteria traditionally used in cereal products (Saavedra, 2001) and fermented dairy products have a long history of safe use. Over the past few years, young children have exponentially increased their consumption of fermented milks (yoghurt) with no record of apparent adverse effects. A review in the USA identified 143 human clinical trials using multiple probiotic agents between 1961 and 1998, involving over 7,500 subjects with no adverse effects reported (Naidu *et al.,* 1999). Nevertheless, it is important

to establish the safety of long-term probiotic consumption by the general public and by high-risk groups if specific recommendations and indications are to be made. Few studies have closely followed large populations for long periods of time and monitored adverse events. From the studies, it was discovered that intakes of 10^6-10^9 colony-forming units daily of bifidobacteria and lactobacilli for ≤1 year resulted in no observed adverse effects. In addition, children receiving bifidobacteria not only tolerated the agent well from a gastrointestinal point of view, but generally experienced less frequent and less-hard bowel movements and a decreased frequency of diaper rash (Saavedra *et. al.*, 1998).

In order to establish safety guidelines for probiotic organisms, the FAO and WHO recommend that probiotic strains be characterized at a minimum with a series of tests, including antibiotic resistance patterns, metabolic activities, toxin production, haemolytic activity, infectivity in immunocompromised animal models, side effects in humans, and adverse incidents in consumers (FAO/WHO, 2002). One possible scheme for testing toxin production has been recommended by the European Union Scientific Committee on Animal Nutrition (2001). Given the rare incidence of side effects of *Lactobacillus* probiotics, large monitoring studies might prove useful. So far, there has been no reports of adverse overdose events caused by probiotics (Reid *et. al.*, 2003).

For improved safety and the production of fermented foods with consistent quality and beneficial health effects, a trend has emerged which involves the isolation of wild-type strains from traditional fermented products to be used as functional starter cultures in food fermentation (De Vuyst *et al.*, 2002; Okorie and Olasupo, 2013). These functional starter cultures are starters that possess inherent functional characteristics and can contribute to food quality and safety by offering one or more organoleptic, nutritional, technological or health advantage (probiotics) (Leroy and De Vuyst, 2004). Thus, the implementation of carefully selected strains as starter cultures or co-cultures in fermentation processes can help to achieve in situ expression of the desired property, maintaining a perfectly natural product and still function as probiotics where applicable.

2.8 Probiotics and their use in food formulations

Probiotics are now widely used in various food formulations with the aim of increasing the health promoting effects of such foods. It is anticipated that this kind of food called functional foods, will contribute to an overall better state of health for the consumers.

Yoghurt and milk to which probiotic bacteria have been added, such as acidophilus milk and fermented milk products such as kefirs, buttermilk, feta cheese (Xanthopoulos, *et al.*, 2000) are the primary food sources of probiotics in the United States. Europe and Asia lead the rest of the world in offering a variety of other food products containing probiotics (D.C.C., 2000). These include: Grain product such as traditional Sourdoughbreadsandfruit and vegetable products such as Sauerkraut(fermented cabbage).Many national versions of Sauerkrautinclude Korean Kimchi, Japanese Tsukemono, and French Choucroute. Also, bean products such as Miso (fermented soya bean paste used in Japan for making soups, adding flavor to sauces and as a spread for crackers), Tempeh (Indonesian fermented whole soya bean product with a cake-like form, used in soups, spreads, salads and sandwiches), and Natto (Fermented soybeans, traditionally from Japan, with a strong savory nutty taste and aged cheese-like smell) are popular probiotic foods in Asia.

In America, Europe and oriental countries particularly Japan where there exist the trained manpower and available technologies for commercial production of various types of probiotic (both dairy and non-dairy) foods, consumers normally access from a variety of such foods. In Africa, peoples of different regions inadvertently, produce foods containing probiotic organisms, though at small scale level. These include; dairy products such as Nunu, and non-dairy products such as Togwa, Akamu souring water, Fufu liquor, and fermented raffia palm sap. In many parts of Nigeria, nursing mothers do give their babies, Akamu liquor (water from fermented cereal pulp) and this causes the termination of their diarrhoea. Adebolu *etal*. (2007), evaluated the antibacterial activities of Akamu liquor from different grains against some common diarrhoeal bacteria in southwest Nigeria and discovered the inhibition of the pathogens by the Akamu liquor which contains a variety of organisms including *Lactobacillus* species. Another probiotic food, the Tanzanian Togwa is a starch-saccharified beverage made from maize flour and finger millet malt (Prado *etal*., 2008). Cereals and cereal components can be used as fermentation substrates for probiotic organisms imparting prebiotic effects (Lamsal and Faubion, 2009). This enhances the dietary value of the product as a whole.

Lactobacillus fermentum has been identified as the predominant lactic acid bacteria (LAB) species in several African cereal based fermented foods (Sawadogo-Lingani, 2007; Vieira-Dalodé *et al.,* 2007;Owusu-Kwarteng *et al.,* 2012). The predominance of *L. fermentum* during koko production, a millet-based fermented porridge in northern Ghana,

was reported by Lei and Jakobsen (2004), and the biodiversity of *L. fermentum* in their study was revealed by pulsed field gel electrophoresis (PFGE) and by multivariate data analysis. The technological roles of *L. fermentum* including acidification and aroma formation has also been described for Ghanaian fermented maize dough (Annan *et al.,* 2003). Despite the significant importance of *L. fermentum* in food fermentation, strains of this species isolated from spontaneously fermented food products in Africa are still rarely dealt with in scientific publications and detailed examinations of their technological properties, their ability to survive the passage of the gastrointestinal tract as well as their susceptibility to common antibiotics are still missing.

2.9 Food Matrix- a vehicle for delivering probiotic bacteria

In the production of probiotic food one of the important factors is the matrix of the food substrate. It acts as a medium to achieve the growth of microbes to at least 9 log cfu/g or ml (FAO/WHO, 2001), which is considered necessary to confer health benefits to the host (Santo *et al.,* 2011). Characterisation of specific probiotic strains, food matrix and dietary content interaction with the probiotics are the research areas for food technologists and industrialists (Isolauri, 2007). Composition of food substrate such as fat content, type of proteins, carbohydrates and pH can affect probiotic growth and survival. Charalampopoulos *et al.* (2003) suggested that the nature of food matrix could affect the stability of the probiotic microorganisms during gastrointestinal transit. Dairy and non-dairy substrates are considered as a vehicle for delivering probiotic bacteria to the human GIT and base for the development of probiotic foods.

Dairy matrices are an extremely promising source for the development of probiotic foods (Yeo *et al.,* 2011). Various food products have been developed as carriers for probiotics, mainly of dairy origin because consumers commonly associate them with fermented dairy products and perceive health benefits in the presence of probiotic cultures (Sanders, 2000). The base for the production of dairy fermented products is milk, which has a typical composition of 87.4% water, 4.7% lactose, 3.8% fat, 3.3% protein (80% casein and 20% whey protein), 0.2% citrate and 0.6% minerals, with pH in the range 6.5–6.7 (De Sukumar, 2007).

Most probiotics proliferate well in a dairy-based matrix due to the lactose-hydrolysing enzyme and proteolytic system involved in casein utilisation, which provides probiotic cells with a carbon source and essential amino acids for growth. Metabolism of these nutrients produced organic compounds that are essential for the development of flavour, preservation and appearance of the products (Yeo *et al.*, 2011). Some additives like prebiotics, pulses and cereal flours are used to speed up the acidification process and survivability of probiotics, as some lactobacilli are unable to consume lactose as a carbon source.

According to Rogelj (2000), dairy-fermented products such as yogurt, probiotic beverages and cheese-containing lactic acid bacteria and their constituents such as omega-3 fatty acid, phytosterols, isoflavones, conjugated linoleic acid, minerals and vitamins have a prominent position in the development of functional foods. In some cases, fermented milk products are fermented by monocultures of probiotic bacteria, but usually supporting cultures are applied to speed up the acidification process and provide the desired texture and flavour (Schmid *et al.,* 2006). Many lactobacilli and bifidobacteria survive in fermented milk products for 4–8 weeks in refrigerated storage. Probiotic dairy products, which contain health promoting lactic acid bacteria (LAB) in addition to traditionally used starter LAB, are good examples of successful fermented functional foods. Today, numerous commercial dairy-based beverages incorporate various strains of probiotic bacteria that are available for human consumption.

Increasing demand for new foods and tastes initiated development of non-dairy probiotic products that are part of the day-to-day normal diet to maintain the minimum therapeutic level (Lavermicocca, 2006). The application of probiotic microbial strains for fermentation of cereals and legumes is a rational approach for the development of functional foods. Cereals contain high levels of carbohydrates, which act as a source of carbon and energy for microbes during fermentation. Most of the carbohydrates in cereals are present as starch and only available for microbes after amylolytic hydrolysis. Endogenous cereal enzymes, malt or selected enzymes can be used to break down the starch to simple fermentable sugars (i.e., maltose and glucose), which can be utilised by probiotics as a carbon source (Salovaara and Simonson, 2004).

Pediococcus spp. VA403 (Pintado *et al.*, 1999), *Lactobacillus manihotivorans* (Ohkouchi and Inoue, 2006) and *Lactobacillus plantarum* (Thomsen and Guyot, 2007) are known as LAB, which have the ability to breakdown the starch and utilise it as a carbon source to produce lactic acid. Cereal-based products' ability to support the growth of probiotics is mainly due to their high concentration of fibres such as xylooligosaccharides, xylan and arabinoxylan, which may act as a growth substrate for probiotics. Besides carbohydrates, cereals also contain relatively high levels of minerals, vitamins, sterols, and other growth factors, which support the growth of microbes, including the LAB. Whole grains are also a source of many phytochemicals, including phytoestrogens, phenolic compounds, antioxidants and phytic acid (Katina *et al.*, 2007), which provide additional functionality to probiotic foods.

The nutritional quality of grains is sometimes inferior to that of milk because of its lower protein content, deficiency of certain essential amino acids, low starch availability, antinutrients (phytic acid and tannins) and the coarse nature of the grains (Blandino *et al.*, 2003). Fermentation has been postulated to decrease the level of starch as well as some non-digestible poly- and oligosaccharides, improve protein quality and increase the level of amino acids and group B vitamins. Fermentation also provides optimum pH conditions for enzymatic degradation of phytate and release minerals such as manganese (which is an important growth factor of probiotic), iron, zinc and calcium (Blandino *et al.*, 2003). Strains of *Lactobacillus* have been recognised as complex microorganisms that require fermentable carbohydrates, amino acids, vitamin B, nucleic acids and minerals to grow.

Charalapompoulos *et al.* (2003), conducted experiments with different cereals to determine the main parameters required for the growth of probiotic microorganisms, such as composition and processing of cereal grains, substrate formulation, growth capability and productivity of the starter culture, stability of the probiotic strain during storage, organoleptic properties and nutritional value of the final product. Different cereals were found to provide different conditions to support the growth of probiotics (Charalapompoulos *et al.*, 2003). It has been reported that *Lactobacillus reuteri*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* grow well in oat-based substrates (Martenson *et al.*, 2002). Yosa, a new oat-based fermented food similar to flavoured

yogurt or porridge, is considered as a health food due to its oat fibre, lactobacilli and bifidobacteria (Blandino *et al.*, 2003).

Helland *et al.* (2004b), studied the growth ability of probiotics in a corn-based fermented substrate and observed that maize fermentation induces fruity flavours in traditional Mexican foods, which could have good worldwide acceptance. Nyanzi *et al.* (2010), evaluated the sensory attributes of a maize beverage fermented by four species of probiotics and reported that the beverages fermented by *L. acidophilus* or *L. rhamnosus* were well accepted by trained and untrained panels.

Soy is an excellent raw material for the development of non-dairy probiotic foods to overcome the limitations associated with dairy products. The benefits of soy have drawn much attention recently and numerous soy products have been evaluated as possible probiotic vehicles. Experiments revealed that soy milk is a good food matrix for probiotics such as *Lactobacillus* spp., *L. casei, L. helveticus, L. fermenti*, *L. fermentum*, *L. reuteri* and *L. acidophilus* (Wang *et al.,* 2006).

Soy-based fermented foods may provide additional benefits for the consumer due to their various functional properties: they are hypolipidaemic, anticholesterolaemic and antiatherogenic and have reduced allergenicity (Lopez-Lazaro and Akiyama, 2002). According to Champagne *etal*. (2005), development of a fermented soy product containing probiotics requires strain selection for the ability to grow in the substrate, as well as the ability to compete or even establish a synergy between strains. Donkor *et al.* (2005), reported that the protein in fermented soy milk could encourage the growth of many probiotic strains such as *L. acidophilus*, *L. casei* and *S. thermophilus*.

Scientific research has shown that probiotic-containing soy-fermented beverages have good sensory acceptance for potential consumers (Shimakama *et al.,* 2003). Hauly *et al.* (2005), reported that soy yoghurt supplemented with fructooligosaccharide had an acceptance index above 70%. The texture and taste of soy yoghurt are essential attributes for product acceptability (Donkor *et al.,* 2007). Gel formation of soy milk proteins is a key process step in the manufacture of a non-dairy fermented product like yoghurt. The rheological properties of set gels determine the texture, organoleptic properties and shelf

life of the product (Lee and Lucey, 2006; Cayot *et al.,* 2008). Soy milk has a low acidification rate and slow growth of probiotic bacteria, which take longer to complete fermentation and produce undesirable changes in the product that are not acceptable to the consumer (Donkor *et al.,* 2007).

Addition of certain additives like prebiotics (inulin and fructooligosaccharide) and whey protein concentrate improves the textural and sensory characteristics of fermented soy yoghurt (Hauly *et al.,* 2005; Donkor *et al.,* 2007). Soy is the most studied matrix for the formulation of probiotic food, but other substrates like peanut have also been explored for the development of probiotic food (Mustafa *et al.,* 2009).

Fruits and vegetables are a rich source of minerals, vitamins, dietary fibres and antioxidants (Yoon *et al.,* 2004). Therefore, there has been increasing interest in the application of vegetable and fruit juices as alternative carriers of probiotics. A number of studies found that probiotic strains have the capability to grow in fruit and vegetable matrices (Rivera-Espinoza and Gallardo-Navarro, 2010). Researchers also observed significant differences in the acid resistance of lactobacilli and bifidobacteria in orange, pineapple, cranberry, bitter gourd, carrot and other juices. According to Sheehan *etal*. (2007), *Lactobacillus casei*, *Lactobacillus rhamnosus* and *Lactobacillus paracasei* survived longer in orange and pineapple juice than in cranberry juice. They survived at levels above 7.0 and 6.0 log cfu/ml in orange juice and pineapple juice for at least 12 weeks at refrigerated storage temperature.

Sheehan *etal*. (2007), reported that fruit juices appear as a more complex system for the development of probiotic foods, due to the more acidic pH of the products. Thus, the selection of probiotic strains that are more resistant to acidic environments is crucial in the development of a probiotic juice (Yeo *et al.,* 2011). Microencapsulation has been shown to provide protection to acid-sensitive probiotics. Ding and Shah (2008), studied the effect of microencapsulation on the viability of probiotic bacteria in orange and apple juices and reported that encapsulated probiotic bacteria was found to survive over 6 weeks of cold storage with counts of more than 10^5cfu/ml or g, while free probiotic cells lost their viability within 5 weeks. The addition of prebiotics can also improve the viability and stability of the probiotics (Vergara *et al.,* 2010). Kyung *etal*. (2005), developed a probiotic

red beet beverage using *Lactobacillus acidophilus* and *Lactobacillus plantarum* and reported that both strains reduced the pH of the juice from an initial value of 6.3 to less than 4.5 after 48 h of fermentation, due to their ability to produce a greater amount of lactic acid.

2.10 Processing, packaging and storage of probiotic functional food

Processing, packaging and storage of probiotic functional food is very important in the production of a probiotic food. The incorporation and viability of probiotic bacteria during storage is a constant challenge for the food industry and requires the understanding of all intrinsic and extrinsic factors associated with processing (Da Cruz *et al.,* 2007). From a technological perspective, it would be advantageous if microbial cultures were capable of growing in substrate media, survived during processing and maintained their efficiency throughout the storage (Stanton *et al.,* 2003). In addition, probiotic strains should be suitable for large-scale industrial production and must have good stable properties so that they can be cultured and incorporated into a range of food matrices without losing viability and functionality and creating unpleasant flavours or textures in the product (Kailasapathy *et al.,* 2010). Selection criteria for probiotic bacteria include an ability to survive the transition through the GIT, including acid and bile resistance, attachment abilities to intestinal epithelial cells, human intestinal colonisation, antimicrobial substance production and conveyance of beneficial effects on human health (Pineiro and Stanton *et al.,* 2007; Prado *et al.,* 2008). It should be certified as GRAS (generally regarded as safe) status (Liong *et al.,* 2008). Probiotics must grow well in simple media to sufficiently high cell concentrations and survive during various processes like centrifugation, freeze drying and freezing (Savini *et al.,* 2010).

Processing of microbial systems for functional foods is dependent on the composition and processing history of the raw material used as a substrate, the viability and productivity of the starter cultures applied, and processing and storage conditions of the final food products (Knorr, 1998). Pre-treatment for the development of a fermentable substrate for dairy probiotic food involves heat treatment (pasteurisation), homogenisation, ultrafiltration, stirring (incorporation of air) and addition of additives. Soaking, amylolytic treatment, grinding, sieving/ultrafiltration and pasteurisation/steaming are basic steps for the development of fermentable substrate for probiotic non-dairy foods.

Processing conditions (i.e., heating, homogenisation and packaging) affect the starter culture growth, fermentation time and subsequent handling. Most commonly, substrate fortification, pH adjustment, competitive microbial flora, thermal processing or aseptic packaging are employed during the manufacture of fermented functional foods. Bacteria must be added at a suitable concentration to remain greater than 10^9cfu/g or ml for the shelf life of the product, which is the requirement for the probiotic claim of any products (Yeo *et al.*, 2011). A number of factors, such as incubation temperature, incubation time, acidity, hydrogen peroxide produced by bacteria, concentration of lactic and acetic acid, and antagonistic and synergistic interaction of the probiotic species and starters can affect the survival of probiotic bacteria in dairy and non-dairy fermented foods (Shah *et al.*, 2001; Vinderola *et al.*, 2002). Probiotic fermentation of raw substrate allows the bacteria to multiply and impart distinctive flavours and organoleptic changes to the food (Kailasapathy, 2010). The quality of the final products depends upon selection of probiotic strains, type and amount of acids and other metabolites produced by it. Taste, flavour, appearance and composition will determine the acceptability of products among consumers. The various steps involved in the development of fermented functional foods can be summarised as:

- Formulation of fermentable substrate for probiotic bacteria
- Inoculation of probiotic bacteria
- Incubation at the optimum temperature
- Ceasing the fermentation by maintaining low temperature
- Sensory analysis
- Packaging and storage
- Evaluation of nutritional and health claims

Packaging is often described as a silent salesman and is defined as a device to contain what it sells or of a device which sells what it contains (Joshi and Mokashi, 1999). Careful considerations should always be given to the packaging of probiotic fermented products to provide suitable environmental conditions to maintain the viable numbers.

Mattila-Sandholm *etal*. (2002), reported that the packaging material and the storage conditions are important factors for the quality of fermented functional foods. The oxygen

content in the product and oxygen permeation through the package are considered the most significant factors affecting the viability of probiotics. The use of oxygen-impermeable packaging, microencapsulation of nutrients (Desmond *et al.*, 2002) and selection of stress-resistant strains (Shah, 2001) are applied to solve these problems. In this respect, the use of oxygen-scavenging plastics as chemical barriers to permeation should provide products equivalent to canned foods. Plastic materials having low oxygen permeability dominate food packaging materials in the dairy and non-dairy sector. Fermented foods may become contaminated with components or degraded products due to product package interactions. In fermented products where lactic acid is produced, it could penetrate into the structure of the plastic polymer packaging and form stubborn bacterial films on polymer surfaces (Steinka *et al.*, 2006).

Tetra Pak, and probiotic tubes and films are the latest invention in the field of probiotic beverage packaging. Unistraw's unique system stores the probiotics as dry, stable UniBeads in the straw, where they are kept in position by filters located at both ends of the straw. The UniBeads dissolve in the liquid as it passes through the straw when sipping it and shelf life of this straw is 12 months. There are some innovative packaging such as active packaging and antimicrobial packaging being used for the preservation of fermented functional foods. Active packaging is an area of food technology that can confer many preservative benefits of fermented food products. The objectives of this technology are to maintain sensory quality and the shelf life extension of foods, while maintaining nutritional quality and ensuring microbial safety (Lutter and Dewey, 2003). Antimicrobial packaging systems are particularly important in fermented foods to inhibit growth of spoilage and pathogenic microorganisms, contribute to the improvement of food safety and extend the shelf life of packaged food.

Many factors need to be considered in designing an antimicrobial packaging system, however, most factors are closely related to the characteristics of antimicrobial agents, packaged foods and target microorganisms (Cooksey, 2005). Packaging of food in a modified atmosphere can offer extended shelf life and improved product presentation in a convenient pack, making the product more attractive to the retail consumer (Lee *et al.*, 2008). Storage temperature is a critical parameter to maintain the viability of probiotic foods and reduce undesirable changes in fermented food products. It must be kept low to

prevent further fermentation once optimum acidity is achieved. Freeze-drying or refrigerated storage are generally applied storage/distribution routes.

Development of fermented food is a multistage process that is affected by many factors, such as sensory acceptance, physical and microbial stability, and price. Probiotic food is one of the largest functional food markets and has growth potential in the food industry among dairy and non-dairy probiotic products; those made with milk and plant sources have been reported to have numerous health benefits. The future of probiotic foods will undoubtedly involve a continuation of the labelling of health claims and safety debates. As consumers become more health conscious, the demand and market value for health-promoting foods and food components is expected to grow. Before the full market potential can be realised, however, consumers need to be assured of the safety and efficacy of probiotic foods. There is a need to test bioactive ingredients, explore more options of fermentable substrates that have not yet been industrially utilised, and optimise products and processes for the development of fermented foods. Culture viability during storage, inoculum size and inoculum strength of probiotics are other major issues related to product development, which have to be studied.

2.11 Limitations of probiotics

Probiotics have limitations. They are restricted to products that contain live microorganisms (e.g., as freeze-dried cells or in a fresh or fermented product), improve the health, growth and well-being of humans or animals, and can affect all host mucosal surfaces, including the mouth and gastrointestinal tract (e.g., applied in food, pill, or capsule form), the upper respiratory tract (e.g., applied as an aerosol), or the urogenital tract. Though probiotics are "generally regarded as safe (GRAS)", side effects such as septicemia and fungaemia have rarely been reported in high-risk situations (Vandenplas *et al.*, 2007).

Not all probiotic strains are effective, and considerable strain-to-strain variation in properties relevant to probiotic efficacy is observed within bacterial species (Crittenden *et al.*, 2005). Just one type or strain of organism cannot provide all potential benefits. The choice of strain of microorganism is important to avoid removal of micronutrients from the food, to avoid production of adverse components such as vasoactive amines and to avoid

opportunistic lactic acid bacterial pathogens. Because of the potential side effects and interactions with medications, dietary supplements should be taken only under the supervision of a healthcare provider. Mild gastrointestinal upset may occur in some individuals who take more than 1 to 2 billion *L. acidophilus* cells per day.

It is interesting to note that under similar genus of a microorganism there may be wide range of species and within each of these species are separate strains of which there can be hundreds, which may have different effects on health. To have any effect in the colon, the bacteria in probiotic foods should survive food processing and storage in large numbers, then survive the passage through the acids and digestive enzymes in the stomach and small intestine in appreciable numbers, and still survive once they reach the colon. Limited evidences regarding the survival of bacteria in the colon are available (Cooper, 2010).

To have the desired effect, scientists believe at least a million of each probiotic bacteria per gram of yoghurt or drink are needed e.g. if a yoghurt contains three different types of probiotic bacteria, it should contain at least a million of each of them per gram. The yoghurt, Vaalia contains three different types of bacteria at these desirable levels; Yoplus has two different bacteria and LC1 and Yakult have one bacterium at these levels. If a person is currently being treated with any of the Sulfasalazine, a medication used to treat ulcerative colitis, he/she should not use *Lactobacillus* or other probiotics without first talking to a healthcare provider. A laboratory study suggests that *L. acidophilus* speeds up metabolism of sulfasalazine (Bhadoria and Mahapatra, 2011).

Today probiotics are gaining importance because of the numerous benefits. The ability of probiotics to prevent diseases and improve health at all ages is increasing the market potential at a high rate. However, the development of successful probiotic products depends on proof of a probiotic effect as well as on the foods where high numbers of viable organisms survive at the time of consumption as well as at the time it reach to the colon. Identification and characterization of genus and species of probiotic organisms by using internationally accepted methodologies, such as DNA-DNA hybridization, sequencing of DNA encoding 16S rRNA, Pulsed Field Gel Electrophoresis or Randomly Amplified Polymorphic DNA and thereby labeling the product will help the consumers to know exactly what strains are present in the products. Keeping in mind the losses in cell viability during gastric transit, to deliver the relevant dose of live bacteria to the gut, the

probiotic food product should be regularly consumed in sufficient quantity (Ross *et al.*, 2005).

Finally, quality, safety and acceptability of traditional fermented foods, such as from milk origin, may be significantly improved through the use of *Lactobacillus fermentum* cultures selected on the basis of multifunctional considerations, taking into account the probiotic concept and possibilities offered for improved health benefits.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1COLLECTION OF SAMPLES OF NUNU, AKAMUAND FERMENTED CASSAVA MASH FOR GARRI PRODUCTION.

Sampling was done in Anambra State. The fresh Nunusamples (15) were obtained from Fulani settlers at 29 Squadron, Police Mobile Force, Agu-Awka and also from street vendors in Onitsha. Akamu samples (35) were obtained from the following markets; Ose market, Nkpor market, 3-3 market, Ochanja market, Abata-Nsugbe market, Eke Adazi and Eke Awka. The fermented cassava mash for Gari production (GariFCM) (20) was obtained from local producers in Onitsha, Adazi-Nnukwu, Nsugbe and Nkwelle.Fresh Nunu samples were also purchased for use as a control in the sensory evaluation. All samples were collected in sterile containers, labelled appropriately and taken immediatelyto the laboratory for analysis.

3.2 ISOLATION AND PRESERVTION OF *Lactobacillus* spp.

Fermented food samples, GariFCM, Nunuand Akamu,(10g or 10ml) were aseptically weighed and homogenized in 90ml sterile peptone water. From each homogenate, a 10-fold serial dilution was subsequently made. Then, 0.1 ml from each dilutions of $10^{-1}$to $10^{-6}$was subcultured in duplicate into the MRS agar supplemented with 0.02% sodium azide as described by McDonald *et al.* (1991) and incubated microaerophically at 37°C for 48 h.After incubation, discrete colonies were randomly subcultured and purified on fresh MRS agar plates. Cultures of the isolates were considered to be pure after three successive subcultures on MRS agar plates. Pure cultures of the bacterial isolates were subsequently sub-cultured in duplicates on MRS agar stabs in Bijou bottles. These were covered with sterile mineral oil and kept in the refrigerator as working and stock cultures.

CHARACTERIZATION OF THE *Lactobacillus* ISOLATES

Characterization of the *Lactobacillus* isolates weredone by observing their microscopic morphology and by tests of their biochemical and physiological characteristics. The characterized cultures were genetically identified.

3.3.1 Microscopic studies

Gram Staining: The isolates were Gram-stained according to the method described by Harrigan and McCance (1976).

3.3.2Biochemical and physiological studies

Each *Lactobacillus* isolate was tested for catalase activity by the method described by Harrigan and McCance (1976), nitrate reduction (Payne, 1973)and utilization of citrate using Simon Citrate Agar (Samelis *et al.*,1994).

Growth at temperatures 15°C and 45°C:A 24h oldculture of isolatewas inoculated into10%(v/v) MRS broth and incubated at 15°C and 45°C for 48 h. Growth was identified by turbidity compared with control (media without organism).

Growth at 6.5% and 9.6% sodium chloride (NaCl) broth: The isolates were inoculated in MRS broths having 6.5% and 9.6% NaCl concentrations and incubated at 37°C for48h. The culture tubes were observed for the presence or absence of growth.

Sugar fermentation pattern of isolated lactobacilli cultures: The sugar utilization pattern of the isolates suspected to be Lactobacilli was evaluated. In all, 13 sugars were used for the tentative identification of *Lactobacillusfermentum* and these include: arabinose, fructose, galactose, lactose, maltose, manitol, mannose, melibiose, raffinose, sorbitol, sucrose, glucose and xylose. The sugar utilization patterns were compared with those given for *Lactobacillus* species in the Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

3.3.3 Genetic identification of *Lactobacillus* isolates

Genetic identification was done using 16SrDNAregion sequencing analysis by Macrogen incorporated.

Primer Information
PCR Primer Name Primer Sequences
27F 5' (AGA GTT TGA TCM TGG CTC AG) 3'
1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Sequencing Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'

Procedure

The primers 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30$\mu\ell$ reaction mixture by using a EF-Taq(SolGent, Korea) as follows: activation of Taq polymerase at 95 °C for 2minutes, 35 cycles of 95 °C for 1min, 55°C, and 72 °C for 1min each were performed, finishing with a 10-min step at 72 °C. The amplification products were purified with a multiscreen filter plate(MilliporeCorp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA).

3.4 *IN-VITRO* EVALUATION OF PROBIOTIC PROPERTIES OF THE *Lactobacillus fermentum* ISOLATES.

3.4.1 Antibiotic susceptibility test: The antibiotic disk susceptibility test was done according to Kirby-Bauer method (Kirby*et al.*, 1966). Seventy-fivestrains of*Lactobacillusfermentum*isolated from this study were screened for possible resistance against the following commonly used antibiotics:Erythromycin(5μg), Gentamycin(10 μg), Augmentin(30 μg), Streptomycin(10 μg), Tetracycline(10 μg), Chloramphenicol(30 μg), Cotrimoxazole(25 μg), and Cloxacillin(5 μg). Using sterile forceps, antibiotic disks were placed on the surface of the inoculated plate andimmediately pressed down lightly with the instrument to ensure complete contact between the disk and the agar surface. The assay was carried out using multiple disks on the same plate to eliminate differential effects from growth time and temperature. Care was taken to ensure purchase of good quality and unexpired antibiotic disks from a reputable manufacturer. The antibiotics disks used were manufactured by Abtek, Biologicals Ltd. (Liverpool, UK)

3.4.2 Tolerance to acidic pH: Tolerance to acidic pH of the 30 antibiotic susceptible strains of*Lactobacillus fermentum,* was determined by growing bacteria in acidic MRS broth. Ten millilitreof MRS broth was dispensed into test tubes and adjusted to pH 4.0, 3.0 and 2.0 with 5M HCl. pH 6.5 was used as control. Subsequently, 0.1ml of 24 h old broth culture of each antibiotic susceptible strain of *Lactobacillus fermentum* was inoculated into

each broth tube. Thirty antibiotic susceptible strains of*Lactobacillus fermentum*, were screened for tolerance to acidic pH 4.0 and 3.0. The acid-tolerant strains were further exposed to pH 2.Test tubes were incubated at 37°C for 3h. After incubation, a 10-fold serial dilutionwas donein 0.1% peptone water. Then, 0.1 ml from each dilutions of $10^{-1}$to $10^{-5}$was cultured on MRS agar.Viable number of bacteria were enumerated after 48 h by pour plate of all samples (Desai, 2008). All experiments were repeated in triplicate. Data obtained from the study was expressed in terms of log10 CFU/ml.

3.4.3 Tolerance to bile: The test for bile tolerance was carried out by growing 0.1ml of the four acid-tolerant*Lactobacillusfermentum* strains in 10ml of MRS broth containing 3%, 5% and 10% (v/v) of fresh bovine bile for 6 h at 37°C. The series of bile concentrations were employed in this study considering the fluctuation of bile concentration at different times. MRS broth with no bile served as control. Viable counts of *Lactobacillus* strains were determined by pour plate counts of all the samples using 10-fold serial dilutions prepared in 0.1% peptone water. All the plates were incubated on MRS agar at 37°C for 48 h. All experiments were carried out in triplicates. Data obtained from the study was expressed in terms of log10 CFU/ml.

3.4.4. Cell surface hydrophobicity assay: This assay was carried out according to the method of Rosenberg*et al.*(1980), to measure the ability of the cells of *Lactobacillus fermentum* strains to adhere to intestinal mucosa. A 24 h old cultures of the four acid-tolerant*Lactobacillusfermentum* strains were centrifuged at 5000 x g for 15 min. The cells were washed three times with phosphate buffer saline (PBS) and optical densities of the bacteria were measured at 540nm and adjusted to an optical density of 1.0. One ml of bacterial cell suspension was added to 1 ml of xylene (Avondale, Oxon, England). The mixture was vortexed for 30sec.After phase separation (30 min),the optical density of the aqueous phase was again measured and compared with the initialvalue. Percentage hydrophobicity was calculated:

$$\% \text{ hydrophobicity} = \frac{A_0}{A_0} \left(\frac{A_0 - A}{1} \right) \times \frac{100}{}$$

A_0 = Initial absorbance value before addition of xylene.

A =Finalabsorbance value after addition and removal of xylene.

3.4.5 Antimicrobial activity of the isolates: The inhibitory effects of the four acid-tolerant*Lactobacillusfermentum* strains on selected pathogens and starter cultureswere determined by the Agar well-diffusion method. A 10 ml MRS broth was inoculated with each strain of the acid-tolerant*Lactobacillusfermentum*and incubated microaerophilically at 37^0C for 24 h. After incubation, the culture was subjected to centrifugation (5000 x g for 15 min), followed by decantation of the supernatant to obtain the cell-free supernatant (CFS). For preparation of plates containing pathogens, Nutrient Agar was used for *Salmonella, E. coli, Pseudomonas,* and *Staphylococcus* species sourced from NAFDAC Laboratory, Agulu. Nutrient Agar supplemented with lactose was used for *Streptococcus* sourced from commercial yoghurt, MRS agar for *Lactobacillus* sourced from commercial yoghurt, and Sabouraud Dextrose Agar for the *Candida* sp. sourced from Mercy Hospital Onitsha.Twenty millilitre of the appropriate agar media was autoclaved, allowed to cool and then vigorously mixed with 0.2 ml of a24h old culture of the pathogens or starter cultures. Wells of about 6 mm in diameter were cut into the agar layer, and the CFS (0.2 ml) from each test *Lactobacillusfermentum*strain was placed in each well. Plates were incubated aerobically at 37^0C for 24 h, except for the *Lactobacillus* starter which was incubated microaerophilically at 37^0C for 48 h, and the diameters of the zones of inhibition around the wells were observed and recorded (Vinderola *et al*., 2008*).*

3.5 *IN VIVO*EVALUATION OF PROBIOTIC PROPERTIES OF *Lactobacillusfermentum*STRAINS.

Four *Lactobacillusfermentum* strains(*L.fermentum* F-6, *L.fermentum* CECT 5716, *L.fermentum* cc IMAU:80780 and *L.fermentum* MGB 32-1) which were very acid and bile tolerant with other essential probiotic characteristics were selected for *in vivo* studies.

3.5.1 Two-week feeding trial: Using the method ofNguyen *et al*. (2007), ninety-six male albino rats aged 4-5 weeks procured from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were randomly assigned to treatment groups according to an approximately equal mean body weight. The rats were housed in plastic cages and kept in the animal house of the School of Basic Medical Sciences, NAUTH,Nnewi. They were acclimatized on basal diet (Vital Feed;Appendix ii) and water for one week *ad libitum*

before treatment. The fermented skimmed milk was prepared by inoculating sterile skimmed milk (10% w/v) with 0.1ml of each strain of *Lactobacillusfermentum* and incubated for 18 h at 37^0C. The concentration of the bacteria in the fermented milk was between 10^8-10^{10}cfu/ml. The sixteen groups of six rats per cageareas follows:

Group C was placed on basal diet alone (Control 1)

Group CM was placed on basal diet and 0.1ml, 0.5ml or 1ml sterile skimmed milk daily (Control 2)

Group LF1 was placed daily on basal diet and orally administered 0.1ml, 0.5ml or 1ml of skimmed milk fermented by *Lactobacillusfermentum* strain MGB 32-1

Group LF2 was placed daily on basal diet and orally administered0.1ml, 0.5ml or 1ml of skimmed milk fermented by *Lactobacillusfermentum* F-6

Group LF3 was placed daily on basal diet and orally administered0.1ml, 0.5ml or 1ml of skimmed milk fermented by *Lactobacillusfermentum* CECT 5716

Group LF4 was placed daily on basal diet and orally administered0.1ml, 0.5ml or 1ml of skimmed milk fermented by *Lactobacillusfermentum* cc IMAU:80780

The treatment was carried out for 14 days and a post-feeding period of 7 days was observed. Individual weight of rats were taken once a week and the mean weight of the rats determined. Faecal samples were aseptically taken from each group on weekly basis during acclimatization period, feeding period and post feeding period. At the end of the post feeding period of 7 days, the rats were decapitated by cervical dislocation and blood samples were taken from the heart. The blood samples were collected in plain sterile plastic bottles for the liver function tests and serum cholesterol level determination.

3.5.2Determination of viable bacterial count in faecalsamples of rats: The effect of the administered *Lactobacillusfermentum*on viable count of enterobacteria and lactobacilli was determined using freshly voided faeces. One gramme of freshly voided faecal samples of the albino rats were homogenized in sterile peptone water and a 10-fold serial dilution was done. 0.1ml of the diluted homogenates were plated on MacConkey Agar for the enumeration of enterobacteria and MRS Agar for the enumeration of *Lactobacillus*. This was done to confirm that the *Lactobacillusfermentum*strains were able to survive the stress within the gastrointestinal tract (GIT). The plates were incubated at 37^0C for 24 h and colony forming units on the plates were recorded (Cokasova *et al.,* 2012; Okafor and Umeh, 2013).

3.5.3 Thirteen-week subchronic oral toxicity study: For this study, forty-eight albino rats (male and female) aged 4-5 weeks were procured from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were acclimatized on basal diet (Appendix ii) and water for one week *ad libitum*. The rats were then, randomly assigned to treatment groups, according to an approximately equal mean body weight.The fermented skimmed milk was prepared by inoculating sterile skimmed milk (10% w/v) with 0.1ml of each strain of *Lactobacillusfermentum* and incubated for 18h at 37^0C. The concentration of the bacteria in the fermented milk was between 10^8-10^{10} cfu/ml. Each gender was assigned to six groups of four rats per cage as follows:

Group C was placed on basal diet alone (Control 1)

Group CM was placed on basal diet and 1ml sterile skimmed milk daily (Control 2)

Group LF1 was placed daily on basal diet and orally administered 1ml of skimmed milk fermented by *Lactobacillus fermentum* strain MGB 32-1

Group LF2 was placed daily on basal diet and orally administered1ml of skimmed milk fermented by *Lactobacillus fermentum* F-6

Group LF3 was placed daily on basal diet and orally administered1ml of skimmed milk fermented by *Lactobacillus fermentum* CECT 5716

Group LF4 was placed daily on basal diet and orally administered1ml of skimmed milk fermented by *Lactobacillus fermentum* cc IMAU:80780

The treatment was carried out for 13 weeks. The animals were housed in plastic cages. Individual weight of rats were taken once a week and the mean weight of the rats per group determined. The rats were decapitated by cervical dislocation and blood samples were taken from the heart. The blood samples were collected in plain sterile plastic bottles for the liver function tests and serum cholesterol level determination. Blood samples were also collected in EDTA bottles for the analysis of the haematological parameters(Endres *et al*., 2009).

3.5.4 Determination of serum aspartate aminotransferase activity

Aspartate Aminotransferase (AST) is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4dinitrophenylhydrazine. The Aspartate Aminotransferase was assayed according to the colorimetric method of Reitman and Frankel (1957) using Randox Diagnostic Kit, AST-Test, Randox laboratory Ltd.UK. The

measurement was done against reagent blank. The reagent blank consisted of 0.05ml of distilled water and 0.25ml of reagent B(1) (Appendix ii). The assay mixture consisted of 0.05ml blood serum of rats and 0.25ml of reagent B(1) followed by incubation at 37^0C for 30 min. Reagent B(2) (0.25ml) was added to both the reagent blank and the assay mixture and was mixed properly and allowed to stand at room temperature for another 20 min. Subsequently, 2.5 ml of 0.4 M NaOH solution was added to each test tube and the absorbance read at 546 nm against the blank after 5 min. The activity of the enzyme was extrapolated from an absorbance-enzyme activity table of values provided by the manufacturer in the kit. Enzyme activity was expressed in IU/L protein. (Reitman and Frankel, 1957).

3.5.5 Determination of serum alanine aminotransferase activity

Alanine aminotransferase (ALT) was assayed according to the colorimetric method of Reitman and Frankel (1957) using Randox Diagnostic Kit, ALT-Test, Randox laboratory Ltd.UK.The measurement was done against reagent blank. The reagent blank consisted of 0.05ml of distilled water and 0.25ml of reagent A(1) (Appendixii). The assay mixture consisted of 0.05ml blood serum of rats and 0.25ml of reagent A(1) followed by incubation at 37^0C for 30 min. Reagent B(2) (0.25ml) was added to both the reagent blank and the assay mixture and was mixed properly and allowed to stand at room temperature for another 20 min. Subsequently, 2.5 ml of 0.4 M NaOH solution was added to each test tube and the absorbance was read at 546 nm against the blank after 5 min. The activity of the enzyme was extrapolated from an absorbance-enzyme activity table of values provided by the manufacturer in the kit. Enzyme activity was expressed in IU/L protein. (Reitman and Frankel, 1957).

3.5.6 Determination of serum alkaline phosphatase activity

The serum alkaline phosphataseconcentration was determined using the standard method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie (1972). Serum sample (0.02ml)was mixed with 1 ml of the reagent at 30^0C. Using a spectrophotometer, the initial absorbance was taken. Absorbance readings were taken again at 1 min interval for 3 min at 405 nm using a timer. ALP activity was subsequently determined using the formula as provided in the manual.

U/l = 2760 x Change in Absorbance at 405 nm

3.5.7 Determination of total serum cholesterol level

The serum cholesterol level was analysed using Biosystems kit, Spain. The principle of the method comprises a reaction between the cholesterol in the sample and the components of the reagent (Appendix ii) giving rise to free and esterified cholesterol, a coloured complex which can be measured by spectrophotometry. The reagent was first keptat room temperature. For the blank, 1ml of the reagent was used. For the standard, 10ul of the cholesterol standard was mixed with 1ml of the reagent. For the sample, 10ul was mixed with 1ml of the reagent. All test tubes were incubated for 10 mins at room temperature. The absorbances of the standard and sample were measured at 500nm against the blank. The cholesterol concentration in the sample was calculated using the formula as provided in the manual (Meiattini *et al.,* 1978).

$$\frac{A\ Sample}{A\ Standard} \times 5.18 = C\ Sample$$

Where

A=Absorbance

C=Cholesterol

3.5.8 Determination of haematological parameters

Blood samples were obtained from the Albino rats at the end of the study and collected in EDTA bottles for the analysis of the following haematological parameters;

3.5.8.1Determination of haemoglobin concentration: Blood hemoglobin concentration (Hgb) was analyzed following the Cyanmethemoglobin method using Drabkin's fluid. 0.01ml of whole blood of the albino rats was incubated with 2.5 ml of Drabkin's fluid. After at least 15 min at room temperature the absorbance was measured using a spectrophotometer at wavelength of 540 nm (Drabkin and Austin, 1982).

3.5.8.2Determination of white blood cell count: The counting of total white blood cells was done using a diluting fluid (Turks fluid) in a ratio of 1:20 and then counted with an improved Neubauer counting chamber under a light microscope using a x10 objective lens in an area of 4sqmm. The cells appeared as small black dots(Akinnuga *et al.*, 2011). The number was thus calculated:

$$\text{White blood cell} = \frac{\text{Cells counted x blood dilution x } chamber \text{ depth}}{\text{Area of chamber counted}}$$

3.5.8.3 Determination of red blood cell count: The red blood cells (RBC) count was done using the conventional method of Dacie and Lewis (2001). Blood was diluted to 1:200 with Hayem's fluid which preserved the corpuscles and then counted with an improved Neubauer counting chamber under a light microscope using a x40 objective lens in an area of 5 sqmm. Their characteristic pink-red colour was used for their identification. The number was then calculated as follows:

$$\text{Red blood cells} = \frac{\text{Cells counted x } blood \text{ dilution x chamber depth}}{\text{Area of chamber counted}}$$

3.5.8.4 Determination of packed cell volume: The packed cell volume (PCV) or haematocrit was determined by the use of micro-haematocit method(Cheesbrough, 2000). A capillary tube was filled to ¾ of the tube with blood, sealed with plasticine and spun in a haematocrit centrifuge at 15000RPM for 5min to completely pack the cells. The tube was held against a ruler and the haematocrit is obtained by the following formula:

$$\text{PCV} = \frac{\text{Length of red cell column in mm}}{\text{Length of total column in mm}} \times 100$$

3.5.8.5Determination of platelets: The platelets were determined by diluting the blood 1:20 with one percent (1%) ammonium oxalate which haemolysed the red blood cells. The platelets were then counted in a definite area using the rulings of an improved Neubauer counting chamber. Their characteristic Mauve-pink colour was used in their identification (Dacie and Lewis, 2001).The platelet count is calculated as follows:

$$\text{Platelet count} = \frac{\text{Number of platelets counted X dilution}}{\text{Volume of square counted}}$$

3.5.8.6 Determination of mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and mean corpuscular volume: The mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated from the values obtained from red blood cells (RBC), packed cell volume (PCV) and Haemoglobin (Hb) content (Adedeji and Adegbile, 2011). They were calculated thus:

$$\text{Mean corpuscular haemoglobin (MCH)} = \frac{\text{Haemoglobin content}}{\text{Red blood cell count}} \times 100$$

$$\text{Mean corpuscular haemoglobin concentration(MCHC)} = \frac{\text{Haemoglobin content}}{\text{Packed cell volume}} \times 100$$

$$\text{Mean corpuscular volume (MCV)} = \frac{\text{Packed cell volume}}{\text{Red blood cell count}} \times 100$$

3.5.9 Determination of relative organ weight

Different organs namely the heart, liver, brain, spleen and kidneys were carefully dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as follows (Endres *et al*., 2009):

$$\text{Relative Organ Weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

3.5.10 Histopathplogical examination

This was carried out to evaluate the effect of the probiotic fermented milk samples on the liver and kidney tissues. A toxic effect will usually be apparent in the liver and kidney because they are the main sites of detoxification and excretion of toxic materials of administered substances.For the histopathological studies, tissue specimens were obtained from liver and kidney. The tissue specimens were fixed in 10% neutral formalin for 48h, embedded in paraffin, sectioned and stained with Hematoxyline and Eosin (H & E) according to the method described by Drury *et al*. (1976). Histological sections were examined using light microscope and were photographed.

3.6 Preparation of inocula

Inocula of thestrainsof the probiotic,*Lactobacillus fermentum,*obtained in this study was prepared for milk fermentation. The *Lactobacillus fermentum* strains to be used as inocula were prepared by transferring a loopful of a 24h old culture from MRS agar into 10 ml MRS broth and incubated at 37°C for 24 h. 0.1ml of the 24 h old culture was transferred into another 10 ml MRS broth and incubated at 37°C for 18 h. Subsequently, the cells were harvested by centrifugation at 5000 xg for 10 min (4°C) and washed three times with 20 ml sterile saline solution, [pH 7.2 ± 0.2; NaCl 0.85% (w/v)]before finally being suspended in 10 ml of the sterile diluent, to obtain a concentration approximately 10^8 CFU/ml.This served as the inoculum(Sawadogo-Lingani *et al*., 2008; Soma,2014).

3.7 Fermentation of milk

Skimmed milk (500 ml) was prepared by reconstituting skimmed milk powder (Marvel Original, Premier Foods, London) in sterile water at 10% (w/v), heating at 90°C for 5 min, and cooling to 37°C. Inoculum (0.1%) was added and the inoculated milk sets were then incubated at 37°C for 18h, following which the pH was determined using the pH meter (Shah, 2000). Analysis of the proximate composition of the probiotic fermented milk was also determined.

3.8 Chemical analysis

pH and Titratable Acidity

The pH of the fermented milk samples was determined in triplicates using a pH meter after standardization with pH 4 and pH 7 buffers.

The titratable acidity (expressed as % lactic acid) ofthe fermented milkduring productionwas determined in triplicates by titrating 10ml of thesampleswith a mixture of 3-4 drops of phenolphthaleinand 0.1M NaOH until a pink colour appeared. Each ml of 0.1 M NaOH is equivalent to 90.08 mg of lactic acid (AOAC, 1990).

The acidity of the samples was calculated by using the following equation:

$$\text{Titratable acidity (\%)} = \frac{0.0090 \text{ x volume of NaOH used}}{\text{Weight of the sample}} \times 100$$

3.9 Proximate analysis

The moisture content, crude protein, crude fat, total solids and ash contents oftheprobiotic fermented milksamples produced were determined in triplicates using standard procedures.This was done to obtain information about the major indicators of nutritional value of food namely, Protein, Fat, Ash and Moisture.

3.9.1 Determinationof moisture content

The moisture content of the fermented milk samples was determined using air oven method (AOAC, 2010). Aluminum dish was washed, dried in oven at 100°C until a constant weight was obtained, on the weighing balance. The sample (2.0g) was kept in the aluminum dish and kept in the oven at 105°C until a constant weight of the sample was obtained. The dried sample was cooled in a desiccator and the weight taken.The moisture content was then expressed as the percentage (%) of the dry weight of sample.

$$\%\ \text{moisture} = \frac{W1 - W2}{W1} \times 100$$

Where; W1= Initial weight of the sample
W2= Weight of the dried sample

3.9.2 Determination of ash content:

The ash content was determined according to the standard method of Association ofOfficial Analytical Chemists (AOAC, 2010). Crucible was sterilized, cooled and weighed. The fermented milk samples(2.0g) were weighed into a crucible of a known weight and placed on a bunsen flame in a fume cupboard to char the samples. Then, the charred sample was placed in a preheated muffle furnace at 550 ºC until the colour of the samples change to light gray ash. They were cooled in a desiccator and the weight recorded.The % ash contents were calculated as;

$$\%\ \text{Ash} = \frac{W1 - W2}{W1} \times 100$$

Where; W1= Initial weight of the sample
W2= Weight of the dried sample

3.9.3 Determination of protein content:

The crude protein of the fermented milk samples was determined using the Kjeldahl method (AOAC, 2010).The fermented milk sample (2.0g) was weighed in Kjeldahl flask and a tablet of Kjeldhal catalystwas added together with 25 ml of concentrated sulphuric acid (H_2SO_4). Contents of the Kjedahl flask were heated in a fume chamber until a clear solution was obtained. The clear solution was cooled and transferred into a 250 ml volumetric flask and the level made up by adding distilled water.The distillation apparatus was set up and 5 ml of 2 % boric acid containing 2-3 drops of methyl red indicator was added in the conical flask and placed under the condenser. The digest (5.0ml) was pipetted and placed into distillation apparatus using a funnel, then washed down with distilled water. Five millilitre of 60 % NaOH (Sodium hydroxide) solution was added. The digestion flask was heated until 100 ml of distillate (Ammonium sulphate) was collected into the flask. The solution in the flask was titrated with 0.04 M H_2SO_4 to get pink colour. The same was carried out on the blank.The % of crude protein in the sample was calculated as

$$\%\ \text{Nitrogen of sample}\ (\%\ \text{N}) = \frac{(\text{VS} - \text{VB})}{\text{W} \times \text{N acid}} \times 0.0140 \times 100$$

Where

VS = volume of acid required to titrate the sample

VB = volume of acid required to titrate the blank

N acid = normality of acid (0.1 N)

W = weight of sample

% Crude protein = % N × 6.38 (conversion factor).

3.9.4 Determination of fat content:

The fat content of the sampleswere determined using the Soxhlet extraction method (AOAC, 2010). The samples (2.0g) were placed in the thimble. ASoxhlet extractor with a reflux condenser and a 500 ml round bottom flask was fixed. Petroleum ether (300ml) was collected at the top of the Soxhlet extractor and drained into a container for re - use.The flask when free of ether was dried at 105ºC for one hour in an oven, then cooled in adesiccator and thereafter weighed.

$$\% \text{ Fat} = \frac{\text{Weight of extracted fat}}{\text{Weight of sample used}} \times 100$$

3.10SENSORY EVALUATIONOF THE MILK SAMPLES FERMENTED BY STRAINS OF *Lactobacillus fermentum*

Sensory evaluation was carried out on the probiotic fermented milk samples prepared from skimmed milk fermented with the four *L. fermentum* strains. Spontaneously fermented milk samples of Nunu served as control. The test was carried out to evaluate the overall acceptance of the samples. All prepared samples ofthe fermented milkand control were assessed for organoleptic qualities by a 12 man panel using 9-point hedonic scale (where 1 = like extremely, 2 = like very much, 3 = like moderately, 4=like slightly, 5 = neither like nor dislike, 6= dislike slightly, 7 = dislike moderately, 8= dislike very much and 9= dislike extremely). The samples were presented in a random order and water was used for mouth rinse between samples. The panellists were trained to have the same understanding of what was desirable. They were instructed on how to use the scale. The panellists were instructed to sniff and taste a sample. They were also allowed to re-taste and change their previous scores, if needed. The parameters used were, colour, aroma, taste and texture. All the panellists were persons accustomed to yoghurt. (Tamime & Robinson, 1999).

3.11STATISTICAL ANALYSIS

Analysis of variance (ANOVA) was carried out using the Statistical Package for Social Sciences (SPSS 20) for the weights of rats, relative organ weight, faecal bacteria count, serum biomarkers, total serum cholesterol level, haematological parameters of the albino rats. This was also done for the sensory scores ofthe fermented milksamples produced in this study.

CHAPTER 4

4.0 RESULTS

4.1 Characterization of the *Lactobacillus*isolates

A total of one hundred and ninety-nine (199) lactic acid bacteria isolates were isolated from the fermented food samples [Akamu**(35)**, Nunu**(15)** *and* Garri *FCM***(20)**] used. The isolates were gram-positive and catalase negative. Table2 shows the morphological, biochemical and physiological characteristics of the Lactic acid bacteria isolated from Nunu, Akamu and GarriFCM.Based on their physiological characteristics and sugar fermentation pattern,seventy-fivestrains of *Lactobacillus fermentum* were identified, twenty-two from Nunu, thirteen from Garri and forty from Akamu.Four *Lactobacillus fermentum*strains, which showed greater than 50% survival at pH 2, were further confirmed using 16SrDNA region sequencing analysis as LN12=*L.fermentum* F-6, LN43=*L.fermentum* CECT 5716, LA4=*L.fermentum* ccIMAU:80780 and LG11=*L.fermentum* strain MGB 32-1.

4.2 *In-vitro* evaluation of probiotic properties of the*Lactobacillusfermentum*isolates.

4.2.1Antibiotic susceptibility test:

All strains of *Lactobacillus fermentum* isolated were screened for their susceptibilty to commonly used antibiotics (Tables3a-c). Only 5 isolates from Nunu, 7 from Garri FCM and 18 from Akamu were found susceptible to the antibiotics used. Allisolates from Nunuwere sensitive to Chloramphenicol and Erythromycin. However, the isolates were resistant to Gentamycin(9.1%), Augmentin (36.4%), Streptomycin (45.5%), Tetracycline (27.3%), Cotrimoxazole(40.9%) and Cloxacillin (77.3%). The isolates from Gari (FCM) were sensitive to Chloramphenicol (100%) but resistant to Erythromycin (15.4%), Gentamycin (30.8%), Augmentin (30.8%), Streptomycin (46.2%), Tetracycline (46.2%), Cotrimoxazole (23.1%) and Cloxacillin (46.2%). Allisolates from Akamu were also sensitive to Chloramphenicol and Erythromycin. However, they were resistant to Gentamycin (20%), Augmentin (17.5%), Streptomycin (30%), Tetracycline (30%), Cotrimoxazole (35%) and Cloxacillin (55%).In general, all *Lactobacillus fermentum* isolates used in this study were observed to show good sensitivity to Chloramphenicol.

The highest susceptibility (100%) was observed inChloramphenicol andthe lowest susceptibility (40%) was found inCloxacillin antibiotics.

Table2: Morphological, biochemical and physiological characteristics of *Lactobacillus* spp. isolated from Nunu, Akamu and Garri FCM.

Tentative I.D	*L. plantarum*	*L. fermentum*	*L.helviticus*	*L. brevis*	*L.acidophilus*	*L. pentosus*
No. of Isolates	62	75	15	12	26	9
Morphology	R	R	R	R	R	R
Gram stain reaction	+	+	+	+	+	+
Catalase activity	-	-	-	-	-	-
Citrate utilization	+	+	-	-	+	+
Nitrate reduction	-	-	-	-	-	-
Growth at 15°C	+	-	-	+	-	-
Growth at 45°C	-	+	+	-	+	+
Growth in6.5%NaCl	+	-	+	-	+	+
Growth in9.6% NaCl	-	-	-	-	-	-
Fermentation of sugars						
Glucose	+	+	+	+	+	+
Arabinose	+	+	-	+	-	+
Fructose	+	+	-	+	+	+
Galactose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Maltose	+	+	-	+	+	+
Mannitol	+	+	-	-	-	+
Mannose	+	+	-	+	+	+
Melibiose	+	+	-	+	+	+
Raffinose	+	+	-	-	+	+
Xylose	+	-	-	+	-	+
Sorbitol	+	-	-	+	-	+
Sucrose	+	+	-	+	+	+

Key:+ =Positive; - = Negative;R=Rods

Table 3a: Antibiotic susceptibility profile of strains of *Lactobacillusfermentum*isolated from Nunu.

Isolate codes	Zones of Inhibition by Antibiotics (mm)							
	ERY	GEN	AUG	STR	TET	CHL	COT	CXC
LN2	11.3±1.2	12.7±0.6	14.3±1.5	R	R	21±1.7	R	R
LN5	14.7±0.6	12±1.0	R	R	11.3±1.2	12.3±1.5	R	R
LN51	18.3±2.5	15±1.7	14±3.6	12.3±1.5	12.7±3.1	19±2.0	22±2.0	9.3±1.2
LN11	17.3±2.3	8.6±0.6	R	9.7±0.6	8.7±1.2	16±1.0	R	R
LN8	15.7±1.5	R	R	R	R	16.7±1.5	8±0.0	R
LN15	23.7±1.5	13.7±1.2	8.7±0.6	R	9.3±1.2	21±1.0	R	R
LN19	21.3±0.6	9±1.0	8.3±0.6	8±0.0	R	17.7±2.5	R	R
LN22	10±1.0	11±1.7	R	10.7±1.5	9±1.0	20±2.6	8.7±0.6	R
LN23	19.7±1.5	R	8.7±0.6	R	8±0.0	13±1.0	R	R
LN28	12.3±1.2	16.3±1.5	R	9.3±1.5	8.6±0.6	22.7±1.5	8.3±0.6	R
LN12	17±3.0	16.3±1.5	15.7±3.1	11±1.7	12±3.0	19±2.6	22±2.6	9±1.0
LN34	14.6±0.6	11.7±1.5	11±1.0	R	R	22+2.0	9±1.0	R
LN37	21±1.0	12.3±1.5	R	8±0	10.3±2.1	22±0	R	R
LN41	22±3.0	13.7±0.6	10.7±1.2	R	9±1.7	14.7±1.5	12.3±0.6	R
LN32	24±1.0	17.3±1.2	15.7±1.5	9.7±1.5	19.3±3.1	28.3±0.6	25.3±1.5	9±1.0
LN46	13.7±1.5	15.3±3.0	R	11.3±1.5	10.3±1.5	16±2.0	9±1.7	R
LN10	13.7±0.6	9.3±1.5	8.7±1.2	8.3±0.6	10±1.0	21.3±0.6	9.7±1.5	8.3±0.6

Key: ERY=Erythromycin, GEN=Gentamycin, AUG=Augmentin, STR=Streptomycin, TET=Tetracycline, CHL=Chloramphenicol, COT=Cotrimoxazole, CXC=Cloxacillin. LN=*Lactobacillus fermentum* isolates from Nunu, R=Resistant**.**

Table: 3a (continued) Antibiotic susceptibility profile of strains of *Lactobacillusfermentum* isolated from Nunu.

Isolate codes	Zones of Inhibition by Antibiotics (mm)							
	ERY	GEN	AUG	STR	TET	CHL	COT	CXC
LN53	10.3±0.6	13.3±1.5	17.7±0.6	9.3±1.2	14.3±2.1	21.7±1.5	9.3±0.6	R
LN43	16±1.0	11.7±1.2	8.3±0.6	9±1.0	17.3±2.3	19.7±1.5	9.3±1.5	9.7±2.1
LN47	13.3±1.5	11.7±1.5	11±1.0	R	R	20±2.6	R	R
LN6	15±1.7	16.7±0.6	14.7+1.5	R	10±1.0	21.7±1.5	R	R
LN17	20±2.0	8.7±0.6	R	R	R	19.3±2.1	8.3±0.6	R

Key: ERY=Erythromycin

GEN=Gentamycin

AUG=Augmentin

STR=Streptomycin

TET=Tetracycline

CHL=Chloramphenicol

COT=Cotrimoxazole

CXC=Cloxacillin

LN=*Lactobacillus fermentum* isolate from Nunu

R=Resistant

Table 3b: Antibiotic susceptibility profile of strains of *Lactobacillusfermentum* isolated from Garri FCM.

Isolate codes	Zones of Inhibition by Antibiotics (mm)							
	ERY	GEN	AUG	STR	TET	CHL	COT	CXC
LG11	21±1.7	19±1.0	13±2.6	13.3±3.5	15±2.6	22.3±2.1	20.7±2.1	8.3±0.6
LG6	21.7±1.5	13.3±1.2	8.3±0.6	R	R	25.3±2.1	14.7±2.5	R
LG38	R	R	R	R	R	19.7±1.5	8.7±0.6	R
LG36	22.3±1.5	20±2.0	14±2.1	13±2.0	16±1.7	24±2.6	21±2.0	9.3±1.5
LG28	15.3±1.2	9±0.0	9.3±1.5	8.3±0.6	8.7±0.6	26.7±0.6	9±1.0	8.7±0.6
LG25	16.7±0.6	8.7±0.6	18.3±2.5	R	R	22	11.7±1.5	R
LG30	14.3±1.2	10±1.0	8.7±0.6	9±1.0	10.3±0.6	24.3±2.1	8.3±0.6	8.7±0.6
LG2	18±2.0	11.3±2.3	8.7±0.6	10±1.0	15.7±3.2	19.7±1.5	8.7±0.6	8.7±1.2
LG17	18.3±1.5	13.7±2.5	11±1.0	10.7±2.1	8.7±0.6	18.3±1.5	16.7±1.2	8.7±0.6
LG22	13.7±1.5	R	R	R	R	17.3±2.1	R	R
LG40	24.3±2.5	13±1.7	19±2.6	11.7±1.2	10.7±1.2	27.3±2.1	19.7±1.5	9.7±0.6
LG15	18.7±2.1	R	R	R	R	25.3±1.5	R	R
LG33	R	R	R	R	R	20.7±0.6	R	R

Key: ERY=Erythromycin

GEN=Gentamycin

AUG=Augmentin

STR=Streptomycin

TET=Tetracycline

CHL=Chloramphenicol

COT=Cotrimoxazole

CXC=Cloxacillin

LG=*Lactobacillus fermentum* isolates from Garri FCM.

R=Resistant

Table3c: Antibiotic susceptibility profile of strains of *Lactobacillusfermentum*isolatedfrom Akamu

Isolate codes	Zones of Inhibition by Antibiotics (mm)							
	ERY	GEN	AUG	STR	TET	CHL	COT	CXC
LA98	14.7±2.1	14±1.0	8±0.0	8.7±0.6	R	25.3±2.1	R	R
LA8	19.7±0.6	R	R	R	R	19.7±2.5	R	R
LA25	18.3±1.5	18±2.0	8.3±0.6	R	R	17.7±1.5	R	R
LA4	18.3±2.9	14±3.6	11±2.6	10.7±2.1	8.3±0.6	17.7±2.5	16.3±3.8	8.7±0.6
LA80	16.7±2.1	R	14.3±1.5	R	R	23.3±2.1	R	R
LA27	13.7±1.5	12±1.7	8.3±0.6	9.3±0.6	20±2.0	21.3±1.2	9.3±2.3	9.3±1.5
LA42	13±2.0	13.7±2.5	19.7±2.1	10.3±1.5	15.7±2.5	17.6±1.5	R	R
LA73	16.7±1.5	R	R	10.7±2.1	R	21.3±3.1	R	R
LA21	18.3±2.1	13.3±3.1	10.7±2.1	10±1.7	8±0.0	17.7±2.5	18±2.6	9.7±1.2
LA45	21.7±2.1	18.7±1.2	10±2.0	10.3±1.5	8.7±1.2	21.7±1.5	8.7±0.6	9±1.0
LA23	20.3±3.5	14±2.0	10±1.0	10.3±2.3	8.3±0.6	24.3±1.5	9.7±1.5	8.7±1.2
LA61	24.7±2.5	12±2.6	R	R	14.3±1.5	22.7±1.2	R	R
LA49	19.3±2.1	15.7±0.6	14.7±1.5	10.3±1.5	12±1.0	21.3±1.2	9.3±0.6	9.3±1.2
LA65	19±2.0	10.3±0.6	9.7±1.2	8.7±0.6	9.3±1.2	23.7±1.5	9.3±1.5	10±1.0
LA20	18.3±2.5	11.3±1.2	R	11.7±0.6	22±0	25.3±1.5	R	R
LA79	9.7±1.5	14.7±0.6	11.3±2.5	R	14±2.0	15.7±0.6	R	R
LA68	11.3±1.2	11±1.0	14.3±1.5	11±2.0	R	23.7±2.1	R	R

Key: ERY=Erythromycin, GEN=Gentamycin, AUG=Augmentin, STR=Streptomycin, TET=Tetracycline, CHL=Chloramphenicol, COT=Cotrimoxazole, CXC=Cloxacillin. LA=*Lactobacillus fermentum* isolates from AkamuR=Resistant.

Table 3c: (Continued) Antibiotic susceptibility profile of strains of *Lactobacillusfermentum*isolated from Akamu

Isolate codes	Zones of Inhibition by Antibiotics (mm)							
	ERY	GEN	AUG	STR	TET	CHL	COT	CXC
LA37	20.3±2.1	R	R	R	8.7±0.6	17.7±1.5	R	R
LA58	19.7±0.6	9.3±0.6	10.3±2.3	9±1.7	9.7±0.6	17.3±1.5	19.7±2.1	9.7±1.5
LA44	17.7±1.5	19.7±2.5	8.3±0.6	13±1.0	R	19±3.6	9.3±1.2	R
LA35	19.3±2.1	13±1.0	R	R	8.7±0.6	19.3±2.5	9.7±0.6	R
LA52	24±1.0	13.7±1.5	10.7±2.1	10±1.7	9±1.0	18.3±1.5	8.7±0.6	8.7±0.6
LA22	20.3±1.5	12.3±2.5	9.3±1.5	9.7±2.1	9.3±1.5	25±1.0	9.7±1.2	10±2.6
LA30	14.7±0.6	14.3±2.5	10.7±0.6	9.7±1.5	R	14.3±2.1	8.3±0.6	R
LA87	16±1.0	13.7±1.2	17.7±1.5	9.3±0.6	9.3±0.6	20.3±2.1	20.7±0.6	8.3±0.6
LA5	19.7±1.8	17.7±1.7	13±1.2	13.7±1.3	15.7±1.4	24±2.3	21.7±2.4	8.7±0.9
LA46	22±1.7	18±1.0	8.7±0.6	11±1.0	9.7±2.1	22.3±1.5	9.3±2.3	9±1.7
LA72	11.7±0.6	R	R	R	R	26.3±1.5	R	R
LA59	19.3±1.2	18±2.0	8.3±0.6	R	R	15±1.7	15±1.0	R
LA29	21±1.0	14.3±1.2	11.7±0.6	10.3±1.5	15.7±1.2	25±1.0	23±1.7	10±1.7
LA92	15.3±0.6	16.3±1.5	11.7±1.5	9.7±0.6	8.7±0.6	23±1.0	12.3±2.5	R
LA95	14.3±1.5	R	9.7±1.2	R	R	25.6±1.2	17.7±0.6	R
LA34	20.7±2.1	R	12.3±0.6	R	R	16.3±1.5	8.7±0.6	R

Key: ERY=Erythromycin, GEN=Gentamycin, AUG=Augmentin, STR=Streptomycin, TET=Tetracycline, CHL=Chloramphenicol, COT=Cotrimoxazole, CXC=Cloxacillin. LA=*Lactobacillus fermentum* isolates from AkamuR=Resistant.

Table 3c: (Continued) Antibiotic susceptibility profile of strains of *Lactobacillusfermentum* from Akamu

Isolate codes	Zones of Inhibition by Antibiotics (mm)							
	ERY	GEN	AUG	STR	TET	CHL	COT	CXC
LA70	21.7±0.6	14.7±1.5	12	R	9.3±0.6	18.7±1.5	R	R
LA14	24.7±1.2	18.7±1.5	13.3±3.1	10.7±1.5	22±2.6	28.3±1.5	23.3±0.6	8.3±0.6
LA18	15.3±2.9	R	9±1.0	11.7±1.5	8.7±0.6	25±1.7	R	R
LA55	18.7±1.5	15.3±2.5	9.3±1.5	9.3±0.6	10.3±1.5	25.3±0.6	18.3±2.5	9.3±2.3
LA13	18±2.0	13±1.0	15.3±2.5	12.3±2.5	10.6±1.2	24.7±0.6	8±0.0	R
LA63	13.3±3.1	9.7±1.5	10±2.6	10.7±1.2	13.3±2.5	24.3±3.5	23.6±2.1	9.7±1.2
LA9	15±3.0	11±1.7	16.7±2.5	9.3±1.2	11.3±2.1	21±3.6	24±1.7	9.3±1.5

Key: ERY=Erythromycin, GEN=Gentamycin, AUG=Augmentin, STR=Streptomycin, TET=Tetracycline, CHL=Chloramphenicol, COT=Cotrimoxazole, CXC=Cloxacillin. LA=*Lactobacillus fermentum* isolates from AkamuR=Resistant.

4.2.2 Tolerance to Acidic pH:

Results of the concentrations of strains grown in MRS broth at pH 4, 3 and 2 after 3 h exposure show that all the strains of *Lactobacillus fermentum* isolated from the fermented foods used in this study survived at pH 4 with a concentration of between 6.52 logcfu/ml and 9.09 logcfu/ml and a percentage survival range of between 74.6% and 98.8% (Table 4a-c). The viability of *Lactobacillus fermentum* decreased after incubation at pH 3, resulting in a concentration of between 2.75 logcfu/ml and 6.74 logcfu/ml and a percentage survival of between 31.5% and 74.5%. It was observed that the *L. fermentum* strains from Nunu had a percentage survival range of between 82% and 98% after 3h incubation at pH4.However, a percentage survival range of between 41.1% and 66.1% and between 39.7% and 56.5% was observed in the strains when incubated at pH 3 and pH 2 respectively. The highest survival was seen in strain LN 43, followed by LN 12 while the lowest was seen in strain LN 32 at pH 3. At pH 2 the highest percentage survival range was observed in strain LN12. The *L. fermentum*strains isolated from Akamu grew well at pH 4 and had a percentage survival range of between 74.6% and 99.7%. But, at pH 3 and pH 2, a percentage survivalrange of between 31.5% and 71.3% and between 24.9% and 53.9% respectively was observed. The highest survival was seen in strain LA 49 at pH3 and in strain LA 4 at pH 2. The strains from Garri (FCM) were observed to have a percentage survival range of between 76.1% and 98.3% at pH 4, between 33.7% and 74.5% at pH 3 and between 31.2% and 61.3% at pH 2. The highest survival was seen in strain LG 11 at pH3 and pH 2. It was observed that 3 strains from Nunu (LN12, LN43 and LN10), 10 strains from Akamu(LA49, LA21, LA45, LA4, LA58, LA87, LA5, LA29, LA14 and LA9) and 2 strains from Garri(FCM) (LG17 and LG11) showed greater than 50% survival after incubation at pH3. Out of 15 strains assayed for tolerance to pH 2, only 4 strains were found capable of surviving greater than 50% in pH 2. *L.fermentum strain MGB 32-1* recorded the highest survival rate of 61.9% at pH 2 while *L.fermentum cc IMAU:80780*recorded the lowest survival rate of 53.9%(Table 4a-c).

Table 4a: Viable count of *Lactobacillus fermentum* strains isolated from Nunu at different pH values

	Log10 cfu/ml			
Strains	pH6.5	pH4	pH3	pH2
LN51	8.85±0.1	7.31±0.19	4.22±0.17	N.D
LN12	9.2±0.14	9.09±0.25	5.82±0.12	5.2±0.15
LN43	9.28±0.16	8.73±0.02	6.13±0.19	5.1±0.13
LN32	9.02±0.13	7.76±0.12	3.71±0.05	N.D
LN10	8.96±0.06	8.26±0.1	4.66±0.08	3.56±0.08

Key: N.D= Not determined

LN= *Lactobacillus fermentum* isolate from Nunu

Table 4b: Viable count of *Lactobacillus fermentum* strains isolated from Akamu at different pH values

	Log cfu/ml			
Strains	pH6.5	pH4	pH3	pH2
LA49	8.98±0.16	8.62±0.21	6.41±0.25	2.54±0.07
LA21	8.91±0.11	8.69±0.22	5.25±0.23	3.85±0.09
LA27	8.56±0.05	7.7±0.13	3.32±0.15	N.D
LA45	8.92±0.18	8.38±0.04	6.11±0.21	3.25±0.2
LA23	9.06±0.07	7.77±0.07	3.26±0.07	N.D
LA4	8.9±0.07	8.87±0.08	6.31±0.06	4.8±0.05
LA65	9.18±0.23	7.02±0.13	4.4±0.14	N.D
LA58	8.35±0.13	8.01±0.09	5.53±0.28	4.03±0.24
LA52	9.09±0.08	8.15±0.16	3.19±0.17	N.D
LA22	9.15±0.11	8.02±0.22	4.02±0.12	N.D
LA87	9.2±0.07	8.88±0.05	6.21±0.14	3.87±0.16
LA5	8.88±0.33	8.41±0.15	6.14±0.12	2.81±0.13
LA46	8.74±0.08	6.52±0.08	2.75±0.15	N.D
LA29	8.65±0.16	8.28±0.1	6.02±0.2	3.22±0.13
LA14	8.81±0.11	8.21±0.05	5.15±0.08	3.11±0.26
LA55	8.95±0.07	7.04±0.07	4.06±0.05	N.D
LA63	8.62±0.13	6.82±0.17	4.04±0.17	N.D
LA9	9.11±0.15	8.44±0.04	6.05±0.25	2.27±0.07

key:

N.D= Not determined

LA=*Lactobacillus fermentum* isolate from Akamu

Table 4c: Viable count (log CFU/mL) of *Lactobacillus fermentum* strains isolated from Garri(FCM) at different pH values

	Logcfu/ml			
Strains	pH6.5	pH4	pH3	pH2
LG17	8.76±0.21	7.05±0.06	5.2±0.18	2.73±0.22
LG36	9.05±0.19	8.09±0.06	4.11±0.12	N.D
LG28	8.87±0.12	6.75±0.12	4.28±0.21	N.D
LG30	9.01±0.15	8.28±0.18	3.04±0.25	N.D
LG2	8.65±0.07	7.29±0.11	3.64±0.07	N.D
LG11	9.05±0.09	8.9±0.15	6.74±0.06	5.55±0.17
LG50	8.71±0.2	7.45±0.18	3.99±0.24	N.D

Key:

N.D= Not determined

LG= *Lactobacillus fermentum* isolate from Gari

4.2.3 Tolerance to Bile

Results of the determination of viable count of the *Lactobacillus fermentum*strains after 6 h exposure to MRS broth containing 3%, 5% and 10% bovine bile concentration showed thatthe viable countof all strains at 3% bile ranged from 6.2logcfu/ml-8.1logcfu/ml with a survival rate of between 70.5% and 88.5%. At 5% bovine bile concentration, all 4 strains suffered reduction in viability (5logcfu/ml-6.5logcfu/ml) with a survival rate of 56.9% to 71%. At 10% bovine bile concentration however, there was further reduction in viability as can be observed in the decrease in viable cell count (4.6logcfu/ml-5.5logcfu/ml) and survival rate of between 51.6% to 60.1% (Figures 1-3). It was observed that *Lactobacillus fermentum*-f6 showed the highest viable count at 3% bovine bile concentrationwhile *Lactobacillus fermentum* CECT 5716showed the least.A similar observation was made at 5% and 10%bovine bile concentrations.

4.2.4 Cell surface hydrophobicity assay

Results from the cell surface hydrophobicity assay showed a variable degree of hydrophobicity by the isolated strains as seen in Table 5. Hydrophobicity of the strains ranged from 47.7$\pm$1.5% to 71$\pm$2.6%. *Lactobacillus fermentum* CECT 5716 recorded the highest level of hydrophobicity while *Lactobacillus fermentum* cc IMAU:80780 had the lowest.

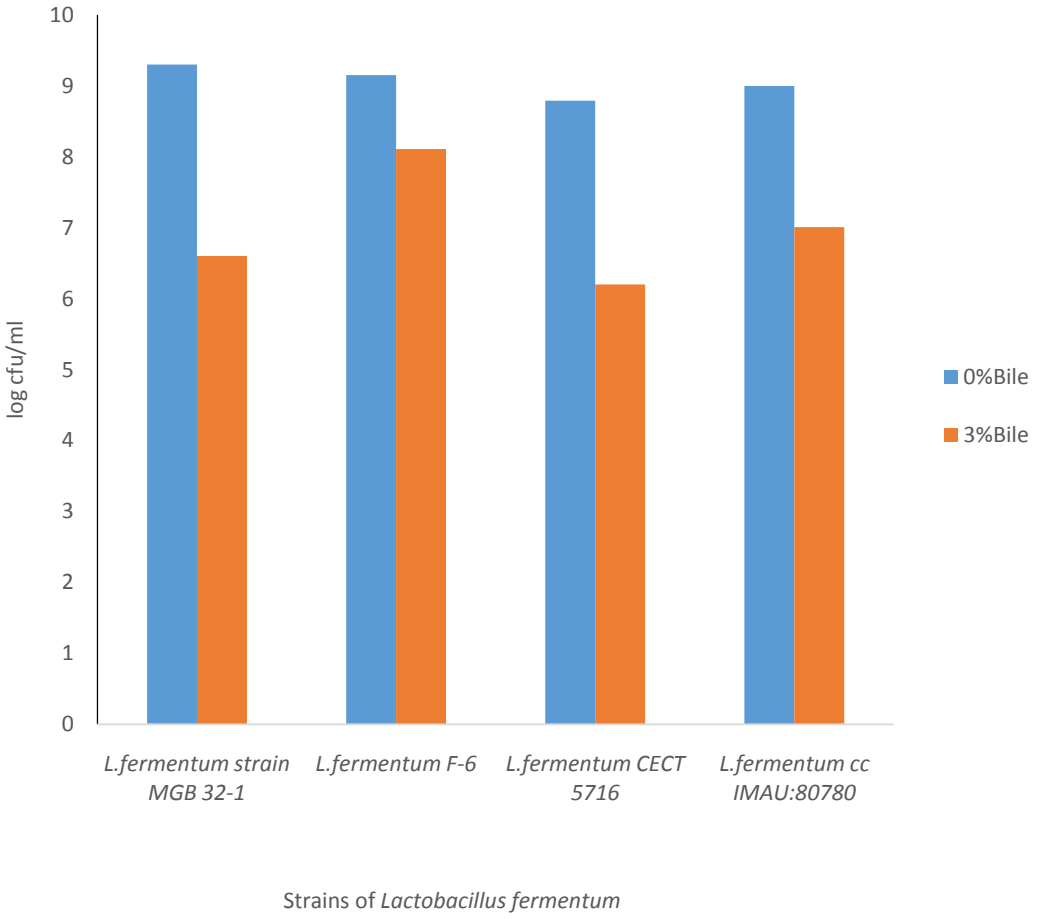


Figure 1: Viability of *Lactobacillus fermentum strains* at 3% Bovine bile concentration for 6 hours

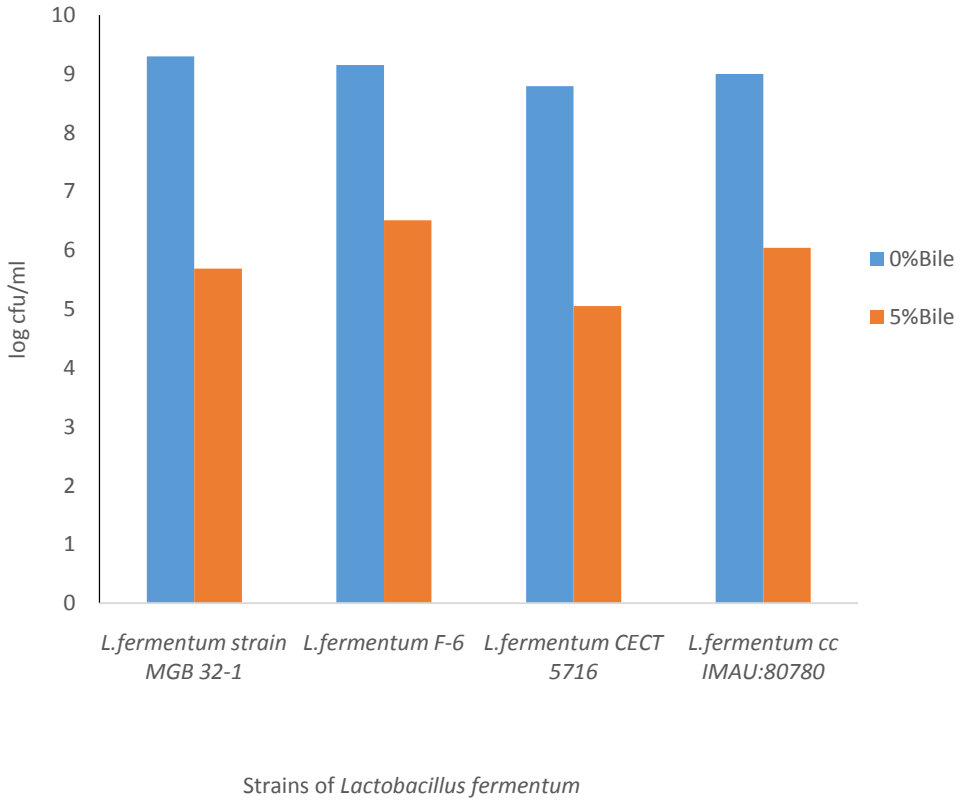


Figure 2: Viability of *Lactobacillus fermentum strains* at 5% Bovine bile concentration for 6 hours

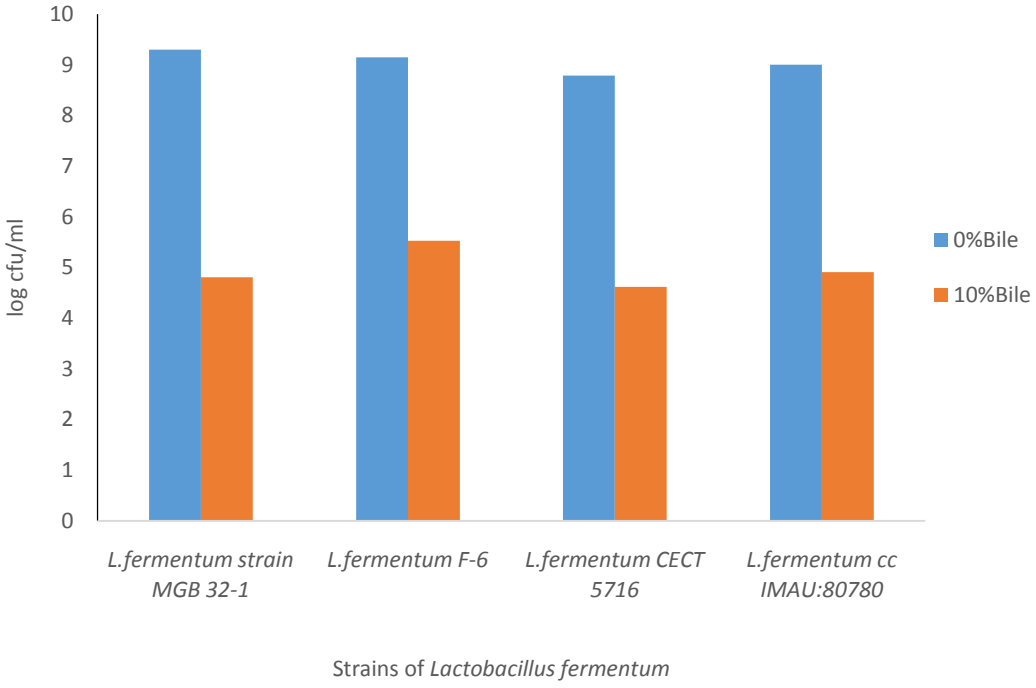


Figure 3: Viability of *Lactobacillus fermentum strains* at 10% Bovine bile concentration for 6 hours

Table5: Cell surface hydrophobicity of *Lactobacillus fermentum* strains

L. fermentum strains	Hydrophobicity(%)
L. fermentum MGB 32-1	52.3±2.5
L. fermentum F-6	51.7±1.5
L. fermentum CECT 5716	71±2.6
L. fermentum cc IMAU:80780	47.7±1.5

4.2.5 Antimicrobial activity of the cell free supernatant obtained from *Lactobacillus fermentum* strains

Results of the antimicrobial activity of the cell free supernatant (CFS) obtained from *Lactobacillus fermentum* strains against certain pathogens and commercial strains revealed that all isolates except *L.fermentum* strain MGB 32-1 showed good inhibition against *Salmonella* sp. *L.fermentum*cc IMAU:80780 had the most inhibitory effect against *Salmonella*sp. with a zone of inhibition of 17±1mm. *L.fermentum* CECT 5716 had inhibitory activity against the Gram negative bacteria (*Salmonella*sp. and *E.coli*) and yeast used in the study (Table 6). It, however, did not inhibit the growth of *Staphylococcus* sp. All isolates displayed inhibitory activity against *Candida* sp. *L.fermentum* CECT 5716had the most inhibitory effect of 18.3±1.5 against *Candida*sp. while *L.fermentum* MGB 32-1 had the least. Only *L. fermentum* F-6 inhibited *Staphylococcus* sp. No inhibitory activity was observed against *Lactobacillus* and *Streptococcus* species sourced from commercial yoghurt (Table 6).

4.3. *IN VIVO*EVALUATION OF PROBIOTIC PROPERTIES OF *Lactobacillus* ISOLATES

4.3.1 Two-week feeding trial

4.3.1.1 Mean weights per group of the rats fed 0.1ml, 0.5ml and 1ml of fermented milk for 2 weeks

Results of the mean weights per group of the rats fed 0.1ml of fermented milk for 2 weeks revealed that the initial mean weight of the rats ranged between 60.94g and 67.26g while the final mean weights were between 193.4g and 220.57g (Figure 4). The final mean weights of the treatment groups were all significantly higher ($p<0.05$) than the controls. The mean weights of the rats fed 0.5ml of fermented milk for 2 weeks showed that the group administered *L.fermentum*f6-fermented milk had the highest weight (223.6g). The final mean weights of the treatment groups were all significantly higher ($p<0.05$) than the controlgroups (Figure 5). Results of thefinal mean weights per group of rats fed 1ml of fermented milk for 2 weeks revealed a range of 193.4g to 227.88g (Figure 6). The final mean weights of the treatment groups were all significantly higher ($p<0.05$) than the controlgroups and the highest weight gain was observed in *Lactobacillus fermentum* F-6 at the end of the post-feeding period (Appendix iii).

Table6: Antimicrobial activity of the cell free supernatant (in mm) obtained from *Lactobacillus fermentum* strains.

Test organisms	Zones of inhibition (mm)			
	L.fermentum strain MGB 32-1	*L.fermentum* F-6	*L.fermentum* CECT 5716	*L.fermentum* cc IMAU:80780
Salmonella sp	0	11.7±0.6	15.7±2.5	17±1.0
Staphylococcus sp	0	16.3±3.2	0	0
Pseudomonas sp	18.3±2.5	0	0	0
E.coli	12.3±1.5	0	12.7±1.2	0
Streptococcus sp	0	0	0	0
Lactobacillus sp	0	0	0	0
Candida sp	10±1.7	15±1.0	18.3±1.5	11.7±1.5

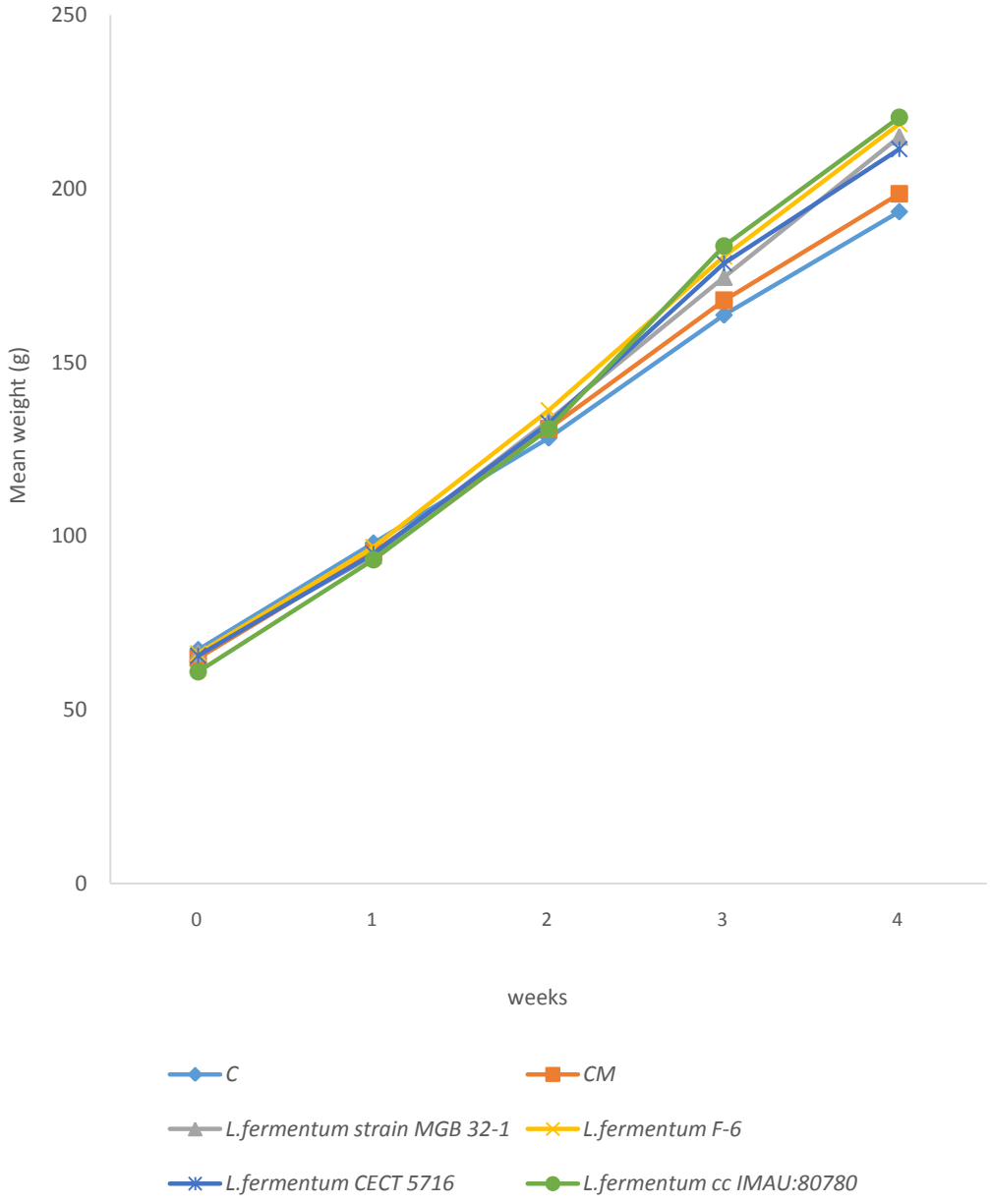


Figure 4: Mean weights of the rats fed 0.1ml of fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

Week 0-1: Acclimatization period.

Week 1-3: Feeding/treatment period.

Week 3-4: Post feeding period.

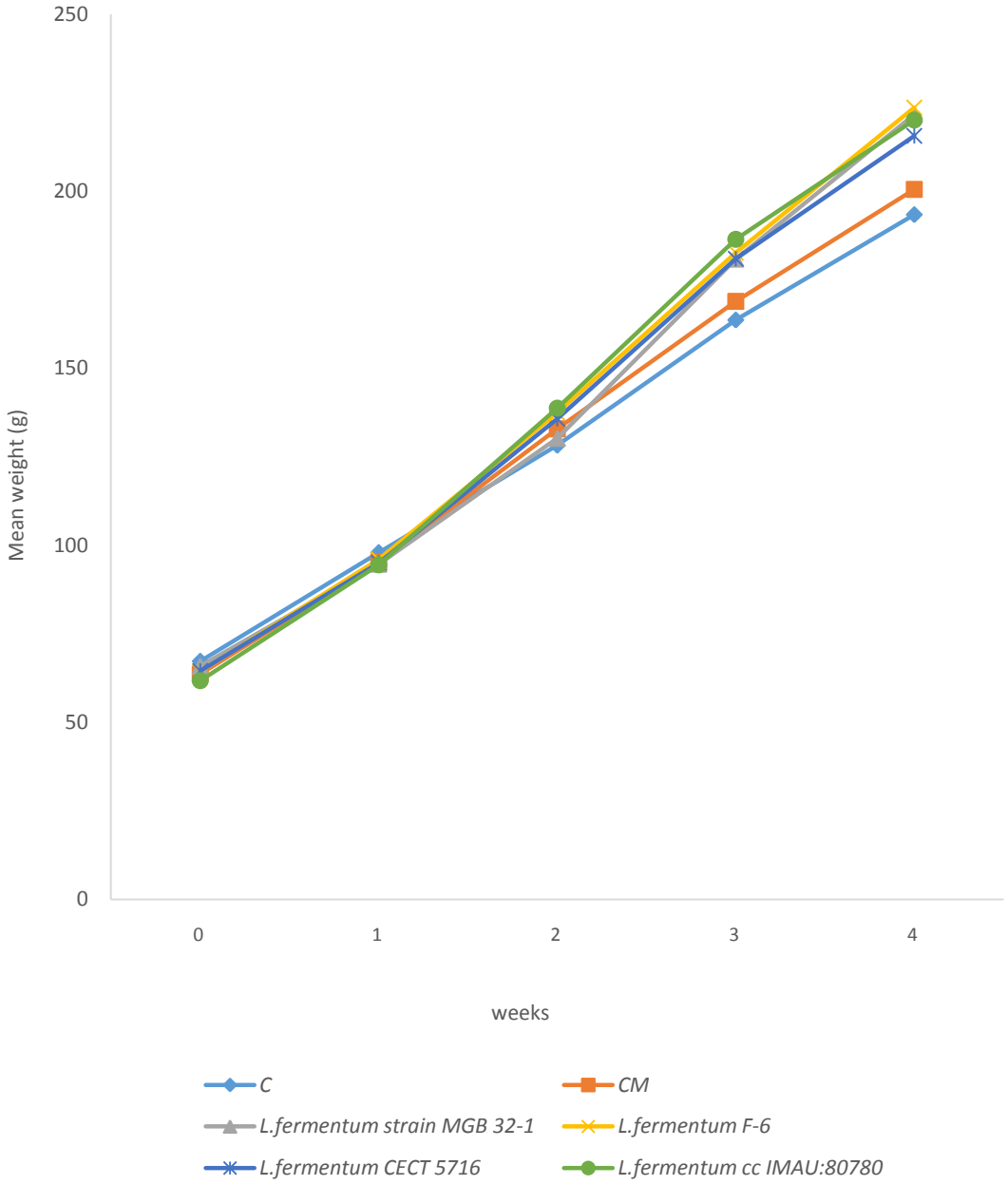


Figure 5: Mean weights of the rats fed 0.5ml of fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

Week 0-1: Acclimatization period.

Week 1-3: Feeding/treatment period.

Week 3-4: Post feeding period.

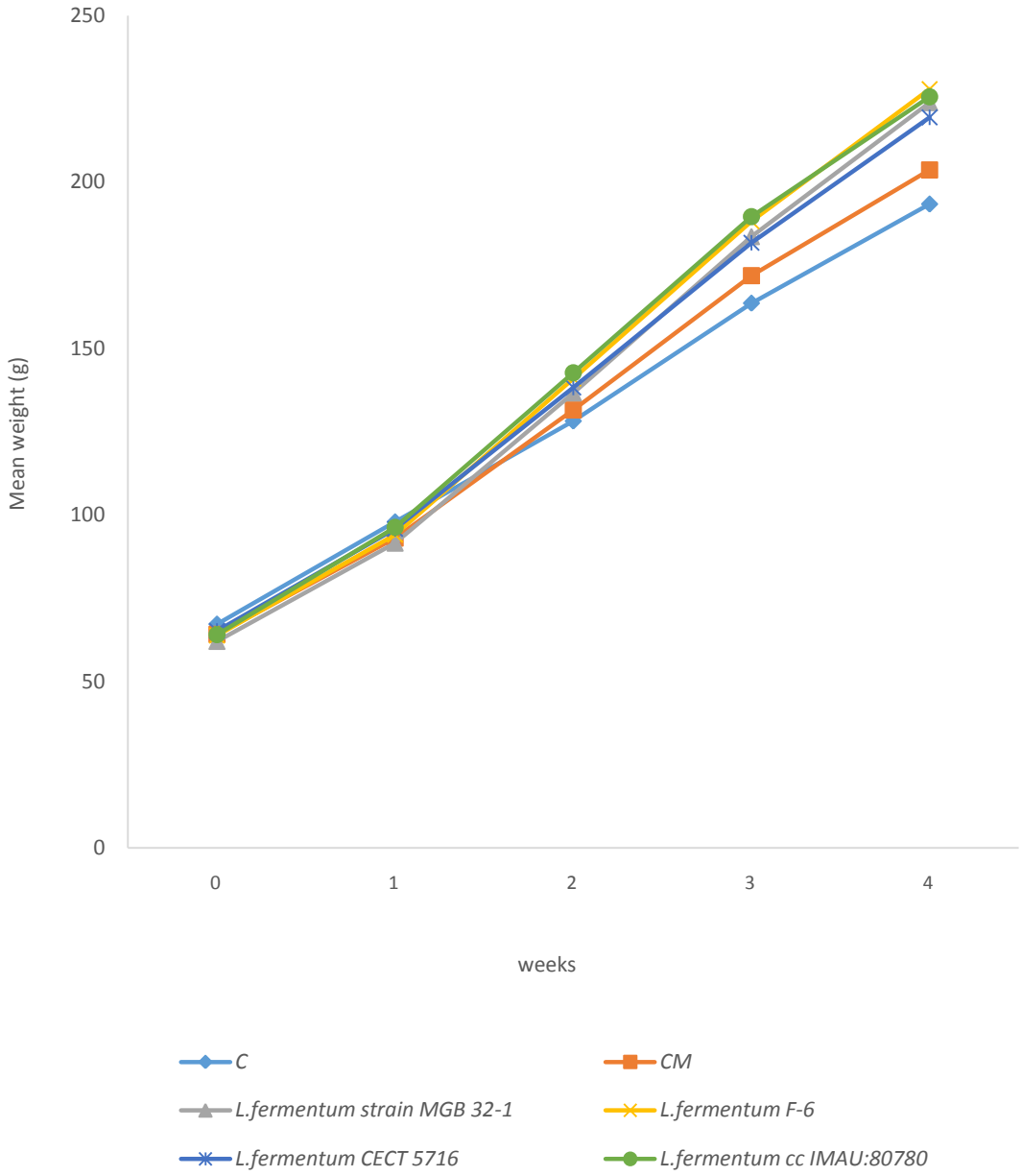


Figure 6: Mean weights of the rats fed 1ml of fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

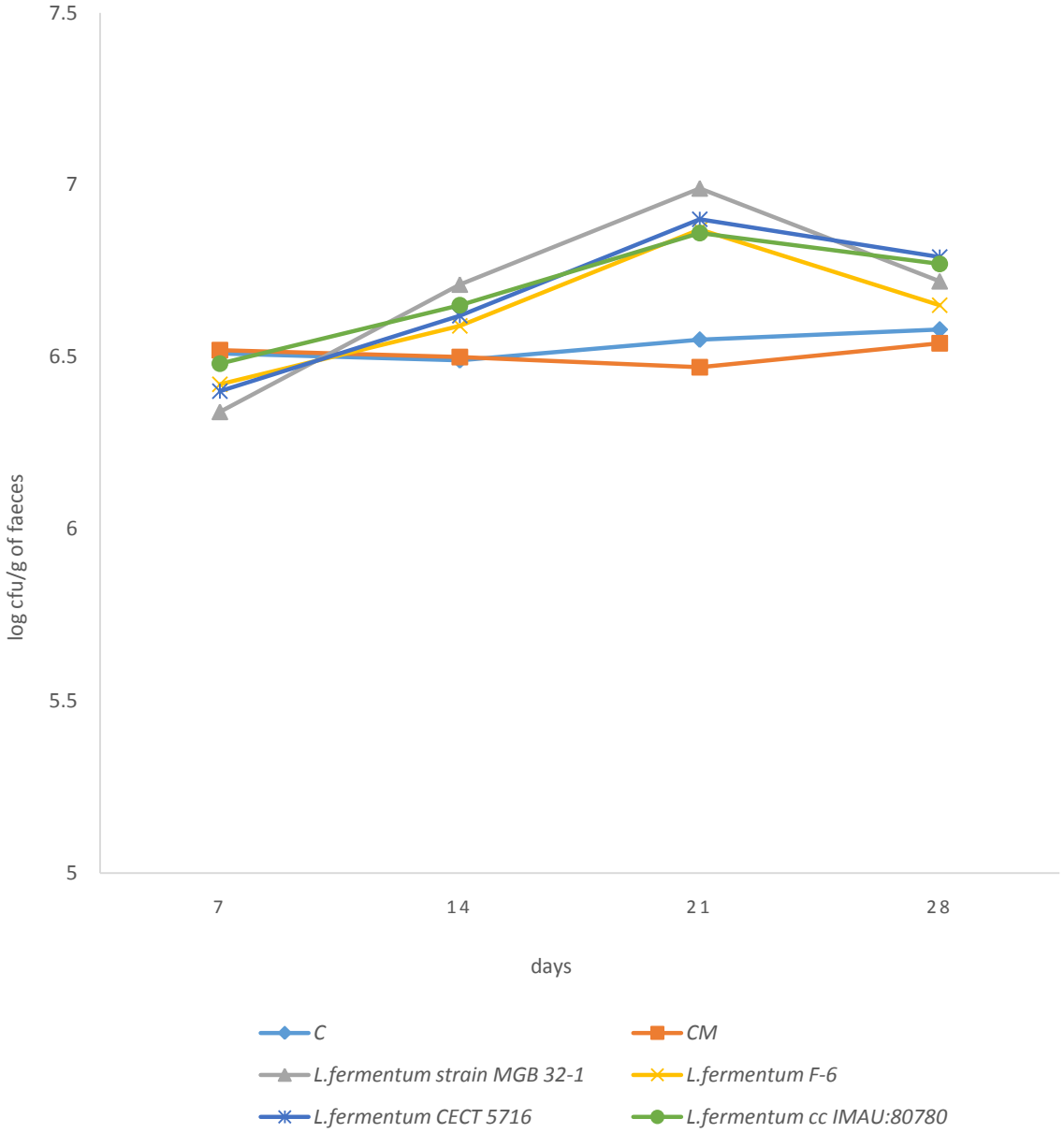
Week 0-1: Acclimatization period.

Week 1-3: Feeding/treatment period.

Week 3-4: Post feeding period.

4.3.1.2Determination of viable lactobacillicount in the faecal samples of rats

The viable count of the lactobacilli isolated from the faecal samples of rats fed 0.1ml milk/fermented milk ranged from 6.34 log cfu/g of faeces to 6.52 log cfu/g of faeces sampled on day 7. On the 21st day, the lactobacilli count increased in the treatment groups more than the control. At the end of the post-feeding period, the rats fed *Lactobacillus fermentum* CECT 5716-fermented milk recorded the highest count (6.79 log cfu/g) while *Lactobacillus fermentum* F-6 (6.65 log cfu/g) fed rats recorded the lowest (Figure 7). Only *Lactobacillus fermentum* F-6 group showed no significantly higher counts ($p>0.05$)than the two controls. For the rats fed 0.5ml milk/fermented milk, at the end of the post-feeding period, *Lactobacillus fermentum* cc IMAU:80780-fermented milk had the highest count of lactobacilli (7.06 log cfu/g) whereas *Lactobacillus fermentum* F-6 had the lowest (6.8 log cfu/g) (Figures 8). All treatment groups showed significantly higher counts ($p<0.05$) than the controls. Figure 9, shows the viable count of lactobacilli in the faecal samples of the rats fed 1ml of milk/fermented milk. Here, the rats fed milk fermented with *Lactobacillus fermentum* CECT 5716 maintained the highest count of lactobacilli up to the 28th day (7.65 log cfu/g) followed by *Lactobacillus fermentum* F-6 (7.33 log cfu/g). The peak period was observed to be on 21st day in all treatment groups administered 0.1ml, 0.5ml and 1ml of fermented milk. All treatment groups showed significantly higher counts ($p<0.05$) than the controls (Appendix iv). There was significantly higher counts of *Lactobacillus* in the group fed 1ml of fermented milk than in the groups fed 0.1ml and 0.5ml of fermented milk (Appendix v).



Values are mean for each group

Figure 7: Viable count of Lactobacilli in faecal samples of the rats fed 0.1ml of fermented milk at various periods.

Key:

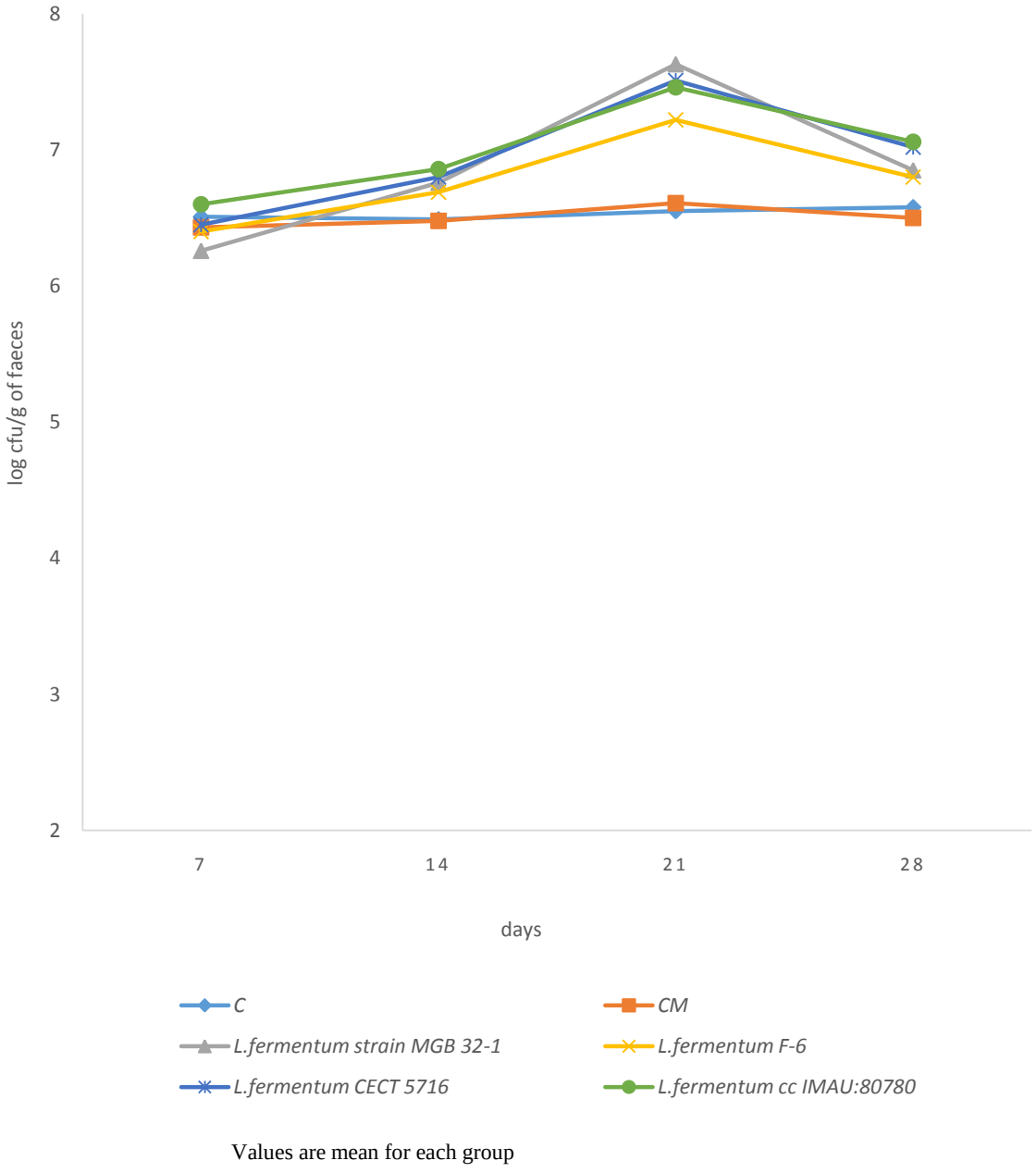
C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk.

Days 0-7: Acclimatization period.

Days 8-21: Feeding/treatment period.

Days 22-28: Post feeding period.



Values are mean for each group

Figure 8: Viable count of Lactobacilli in faecal samples of the rats fed 0.5ml of fermented milk at various periods.

Key:

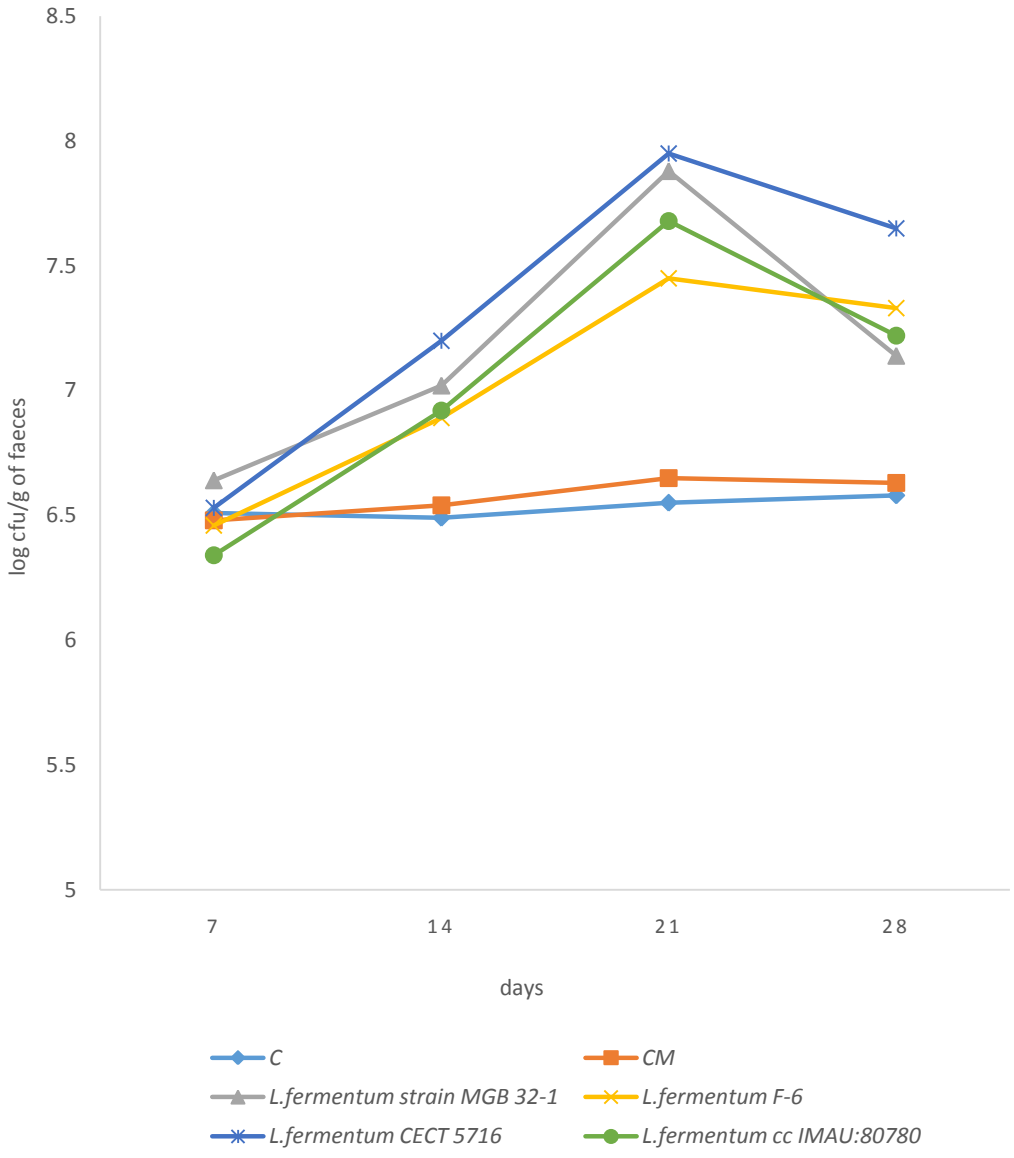
C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk.

Days 0-7: Acclimatization period.

Days 8-21: Feeding/treatment period.

Days 22-28: Post feeding period.



Values are mean for each group

Figure 9: Viable count of Lactobacilli in faecal samples of the rats fed 1ml of fermented milk at various periods.

Key:

C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk.

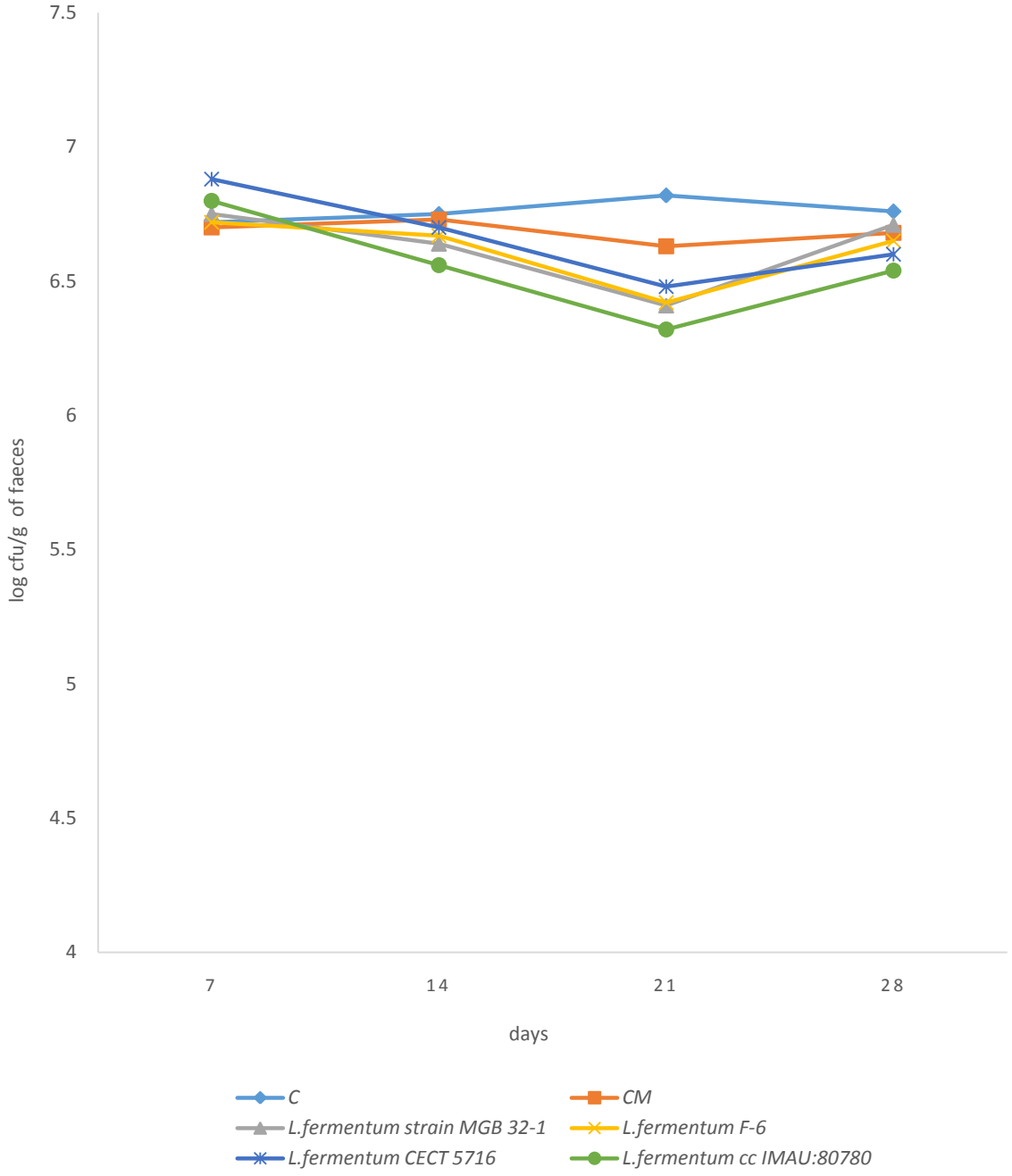
Days 0-7: Acclimatization period.

Days 8-21: Feeding/treatment period.

Days 22-28: Post feeding period.

4.3.1.3 Determination of viable enterobacteria count in the faecal samples of rats

Result of the determination of viable count of enterobacteria in the faecal samples of rats showed that during the acclimatization period, the viable count of enterobacteria in the group fed 0.1ml of milk/fermented milk ranged from 6.56 log cfu/g to 6.88 log cfu/g of faeces (Figure 10). Generally, there was a decrease in viable count of enterobacteria in all the treatment groups at the end of the post-feeding period compared to the initial count at day 7. A similar observation was made in the group fed 0.5ml (Figure 11) and 1ml (Figure 12) of milk/fermented milk. This trend can easily be distinguished from the control in the different volumes of fermented milk used. The group fed 0.5ml and 1ml had significantly lower counts of enterobacteria ($p>0.05$) at the end of the post-feeding period compared to the control (appendix iv). There was significantly lower counts of enterobacteria in the group fed 1ml of fermented milk than in the groups fed 0.1ml and 0.5ml of fermented milk (Appendix v).



Values are mean for each group

Figure 10: Viable count of enterobacteria in faecal samples of the rats fed 0.1ml of fermented milk at various periods.

Key:

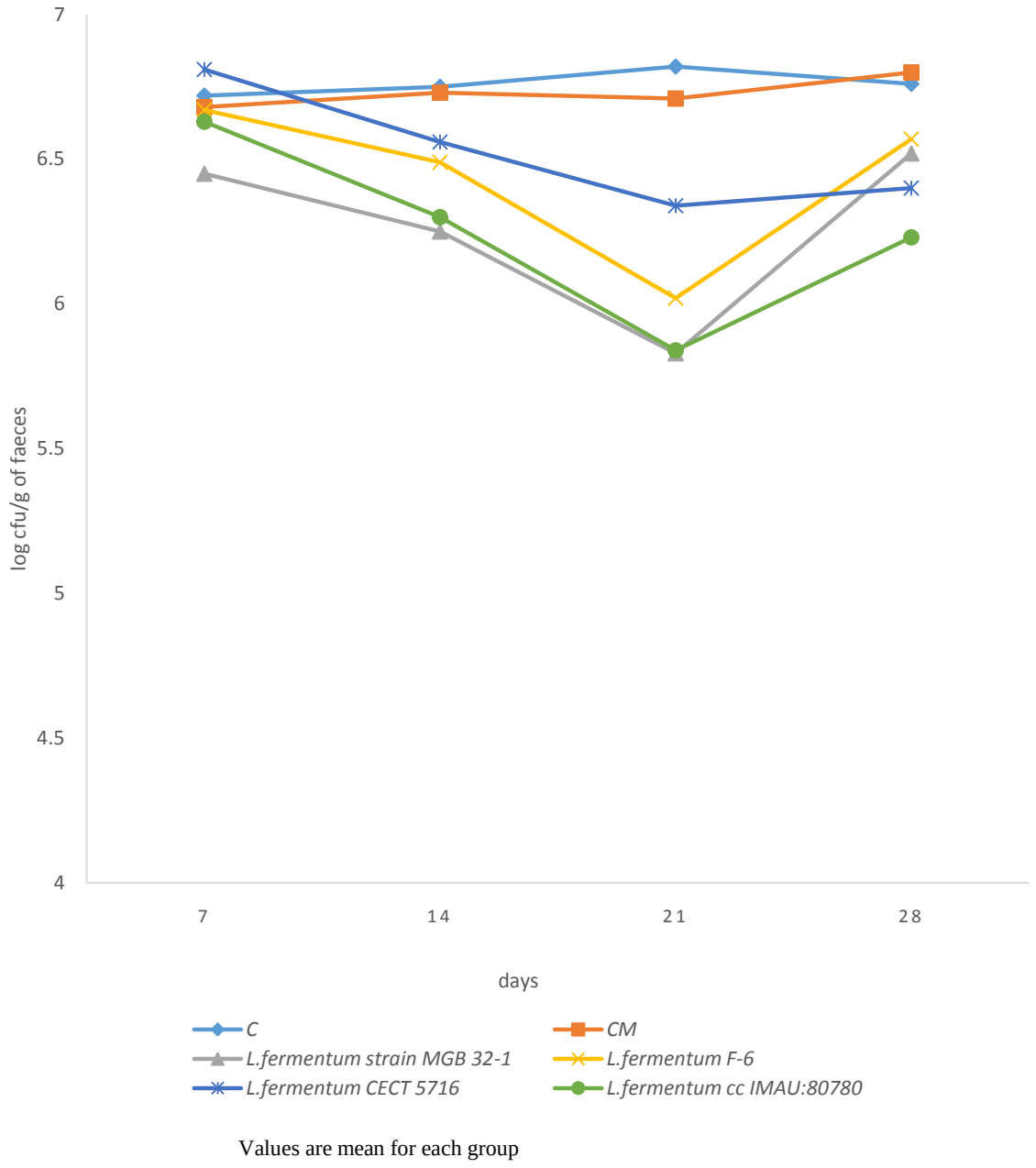
C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk.

Days 0-7: Acclimatization period.

Days 8-21: Feeding/treatment period.

Days 22-28: Post feeding period.



Values are mean for each group

Figure 11: Viable count of enterobacteria in faecal samples of the rats fed 0.5ml of fermented milk at various periods.

Key:

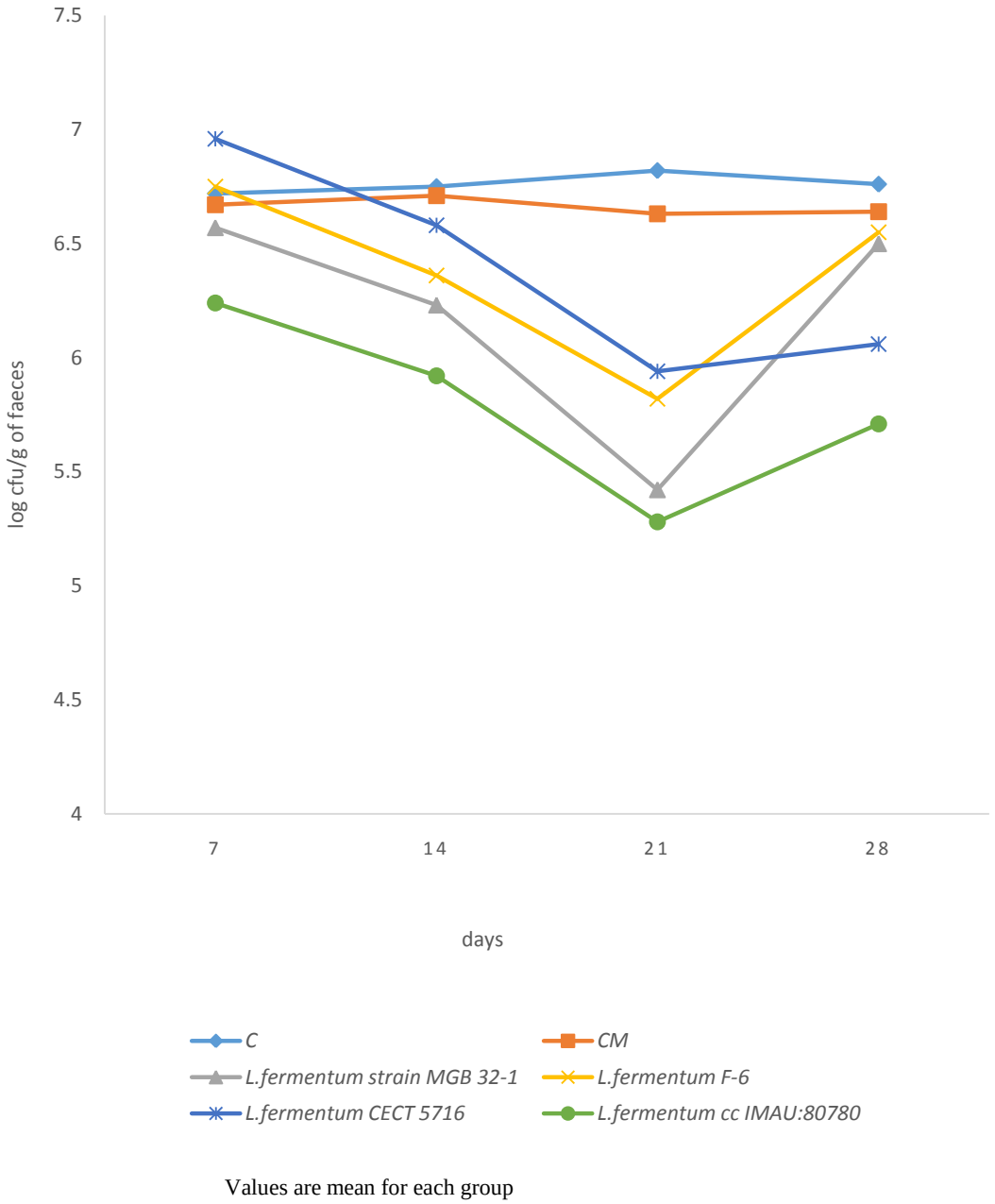
C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk.

Days 0-7: Acclimatization period.

Days 8-21: Feeding/treatment period.

Days 22-28: Post feeding period.



Values are mean for each group

Figure 12: Viable count of Enterobacteria in faecal samples of the rats fed 1ml of fermented milk at various periods.

Key:

C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk.

Days 0-7: Acclimatization period.

Days 8-21: Feeding/treatment period.

Days 22-28: Post feeding period.

4.3.1.4 Level of Alanine aminotransferase in the serum of male rats fed 0.1ml,0.5ml and1ml fermented milk for 2 weeks

Results ofthe level of Alanine aminotransferase (ALT) in the serum of rats fed 0.1ml fermented milk for 2 weeks revealed that*Lactobacillus fermentum* F-6 had the lowest level followed by *Lactobacillus fermentum* MGB 32-1 (Figure 13).From the result of the rats fed 0.5ml milk/fermented milk (Figure 14), *Lactobacillus fermentum* F-6 had the lowest level of ALT. The level of Alanine aminotransferase in the serum of rats fed 1ml milk/fermented milk for 2 weeks is shown in Figure 15. The lowest level of ALT was also observed in *Lactobacillus fermentum* F-6. The ALT levels of the rats fed 0.1ml, 0.5ml and 1ml of fermented milk were not significantly different from the control (Appendix vii).

4.3.1.5 Level of Aspartateaminotransferase in the serum of male rats fed 0.1ml,0.5ml and1ml fermented milk for 2 weeks

The results from the investigation of the level of Aspartate aminotransferase (AST) in the serum of rats fed 0.1ml fermented milk for 2 weeks (Figure 16),revealed that *Lactobacillus fermentum* CECT 5716 had the lowest level. All the treatment groups had lower levels of AST in the rat serum when compared to the controls. Figure 17, shows thelevel of Aspartate aminotransferase in the serum of rats fed 0.5ml milk/fermented milk for 2 weeks. A similar observation was also made, with *Lactobacillus fermentum* CECT 5716 having the lowest level of AST in the serum. Lower levels of AST (Figure 18), were observed in all treatment groups. The AST levels of the rats fed 0.1ml, 0.5ml and 1ml of fermented milk were not significantly different from the control (Appendix vi).

4.3.1.6 Level of Alkaline phosphatase in the serum of male rats fed 0.1ml, 0.5ml and1ml fermented milk for 2 weeks

Results of thelevel of Alkaline phosphatase (ALP) in the serum of rats fed 0.1ml fermented milkrevealed thatthe least level of ALP was seen in *Lactobacillusfermentum cc* IMAU: 80780 followed by *Lactobacillus fermentum* F-6. For the group fed 0.5ml of milk/fermented milk (Figure 20), the lowest level was seen in *Lactobacillus fermentum* MGB 32-1 and this was followed by *Lactobacillus fermentum cc* IMAU: 80780. Figure 21, shows thelevel of Alkaline phosphatase (ALP) in the serum of rats fed 1ml milk/fermented milk. In this case, the group fed fermented milk, by *Lactobacillus fermentum* CECT 5716 had the lowest level followed by the group fed fermented milk, by

Lactobacillus fermentum cc IMAU: 80780. The observed differences in the ALP levels at the different concentrations were not significant when compared to the controls (Appendix viii).

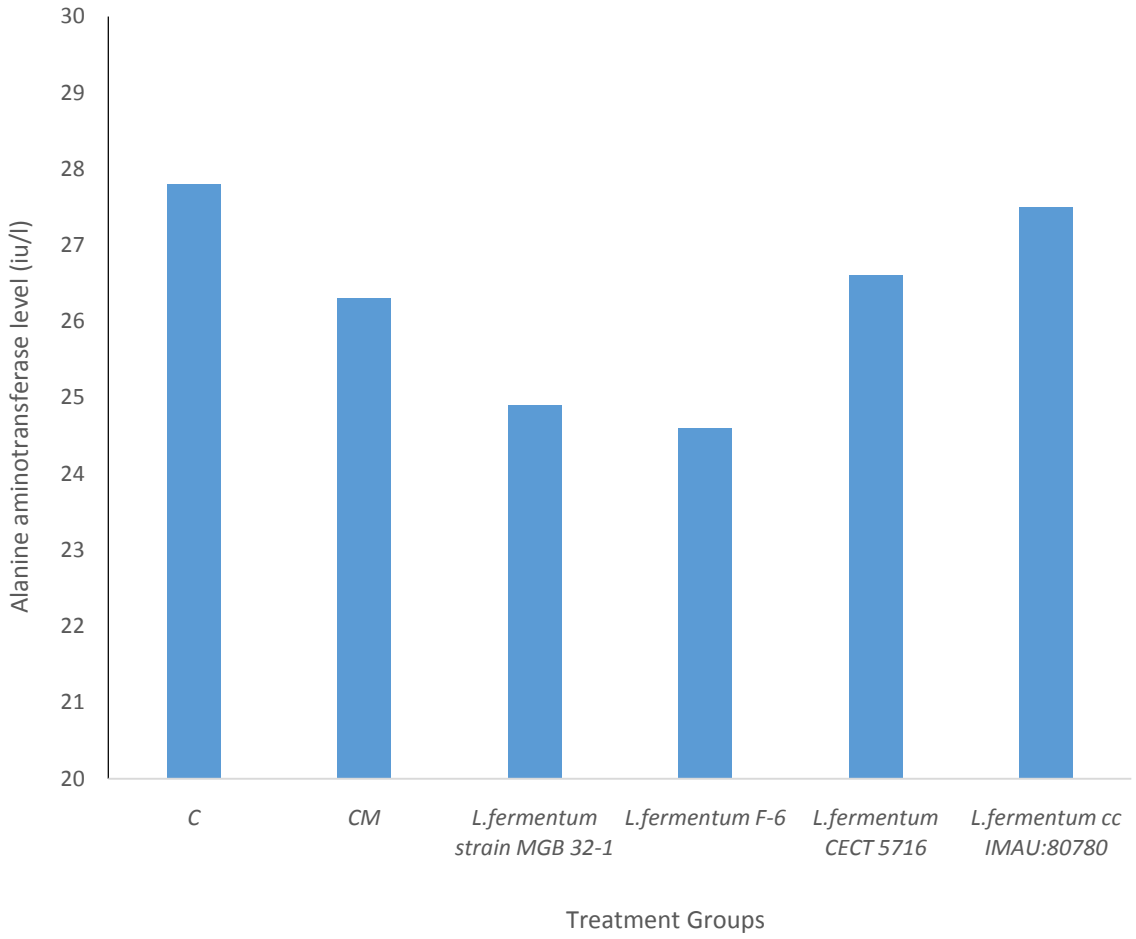


Figure 13: Level of Alanine aminotransferase in serum of rats fed 0.1ml of fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

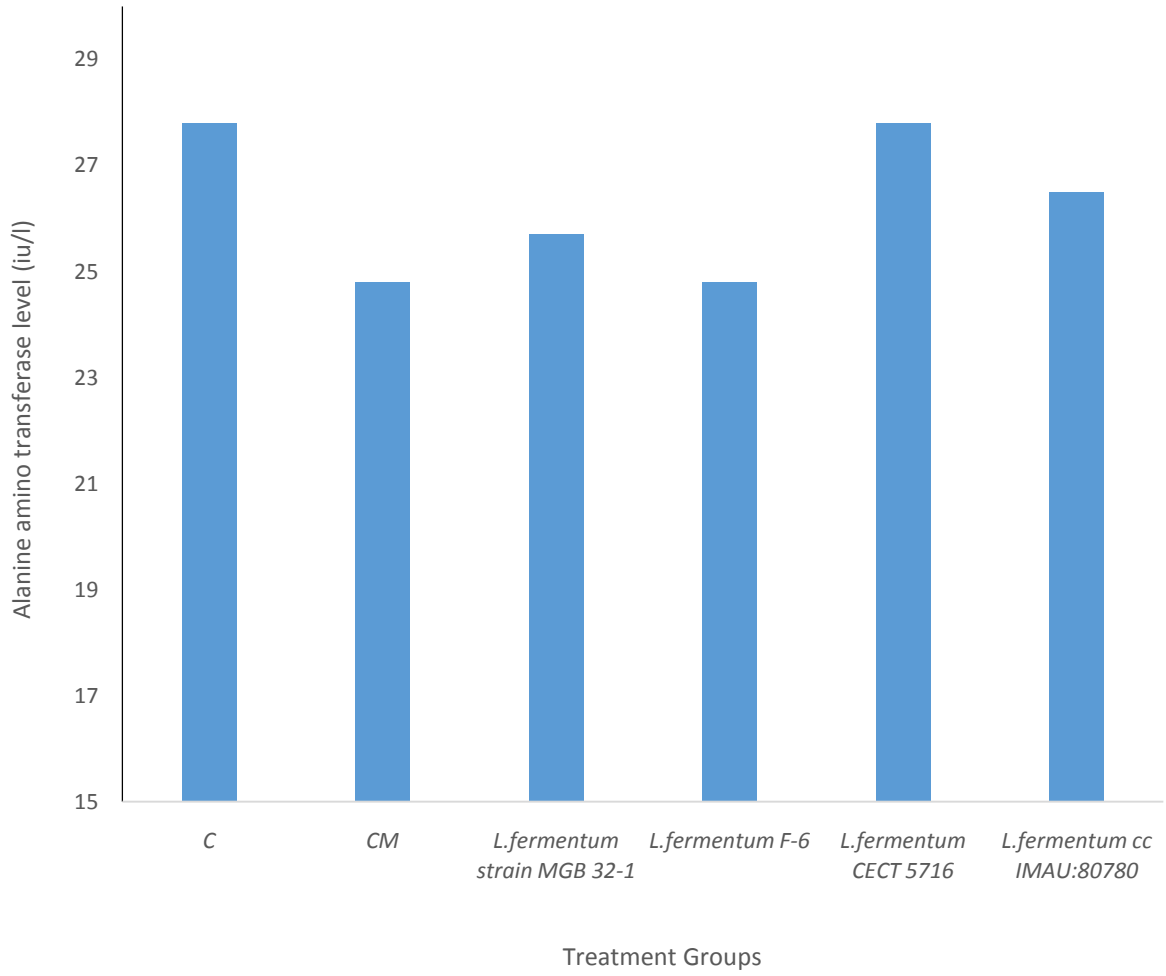


Figure 14: Level of Alanine aminotransferase in serum of rats fed 0.5ml fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

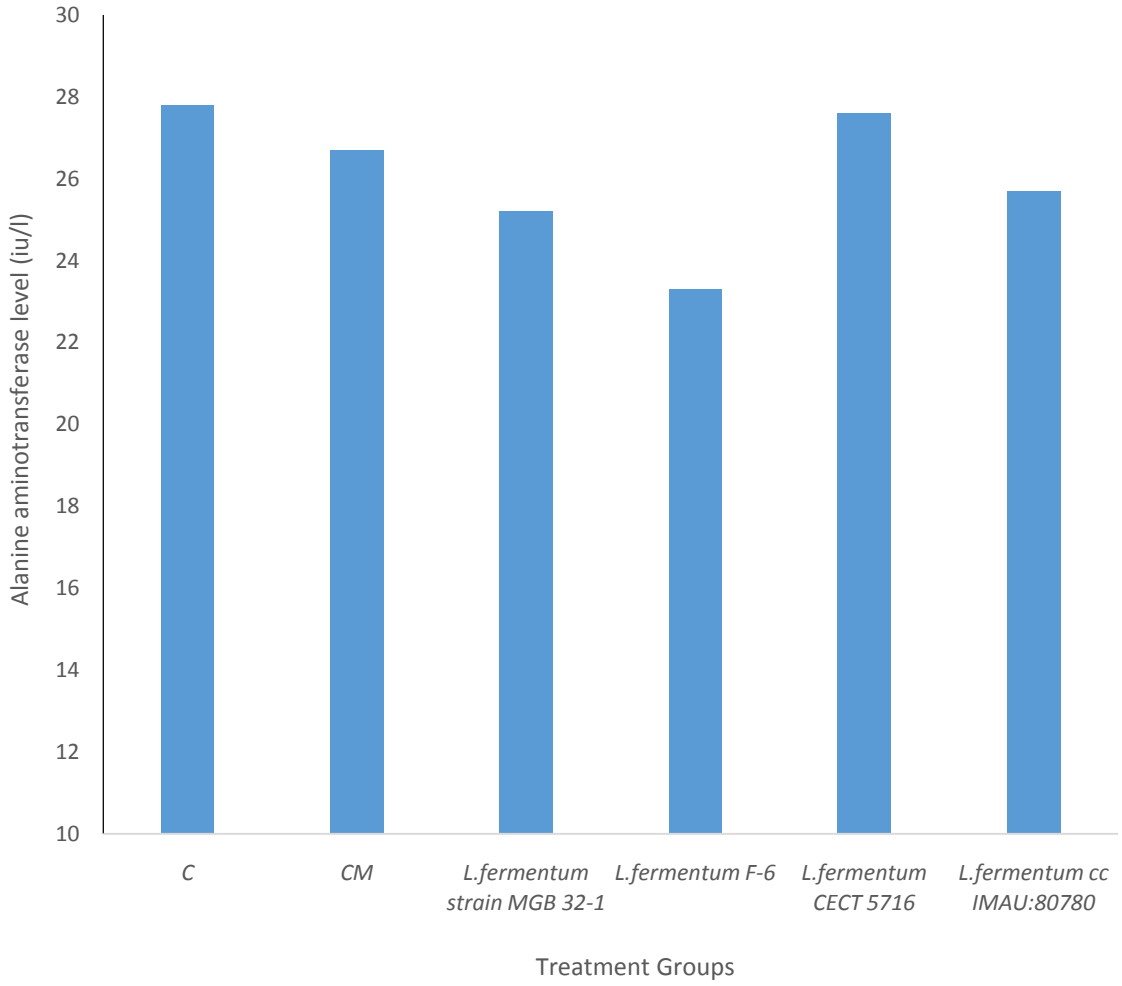


Figure 15: Level of Alanine aminotransferase in serum of the rats fed 1ml of fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

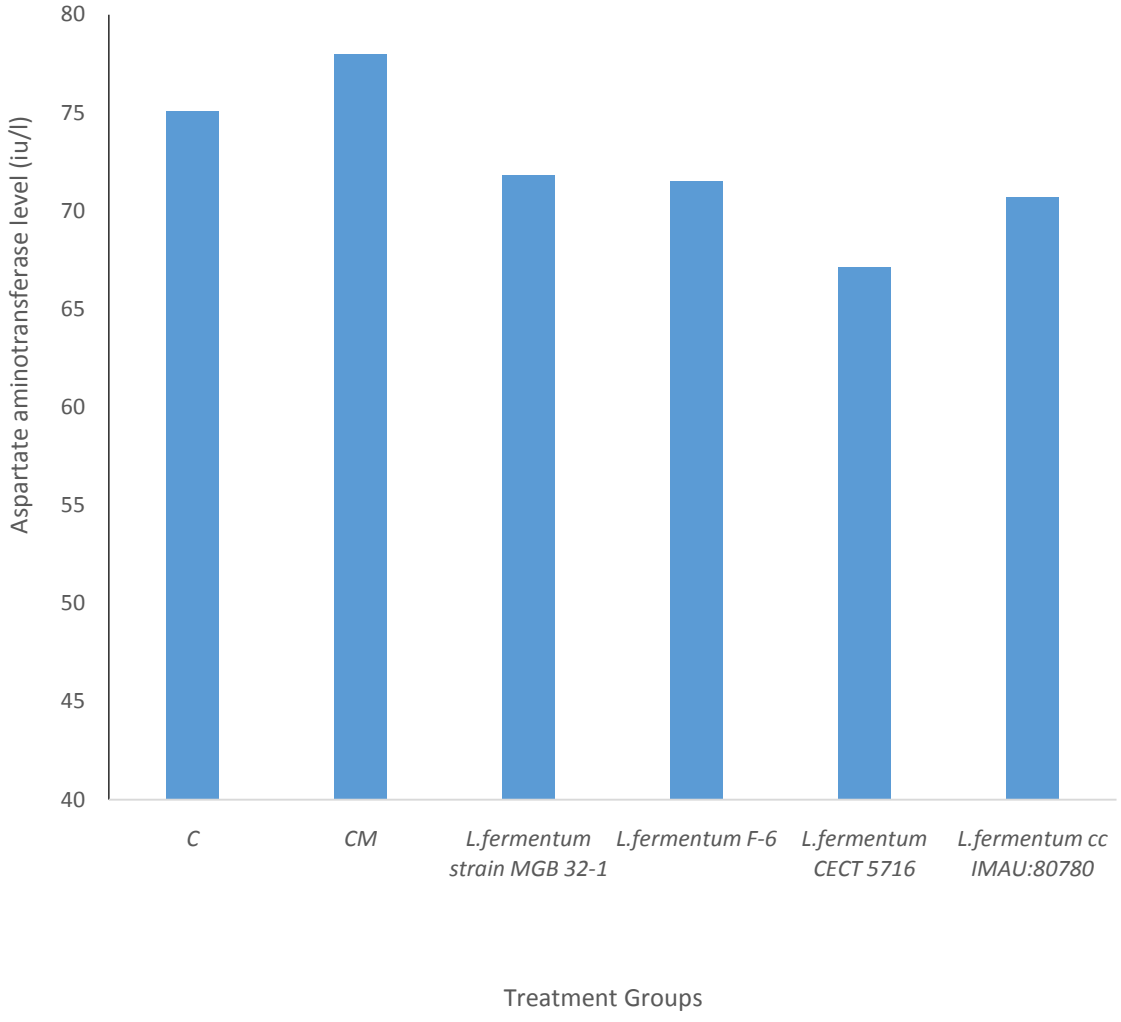


Figure 16: Level of Aspartate aminotransferase in serum of rats fed 0.1ml fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

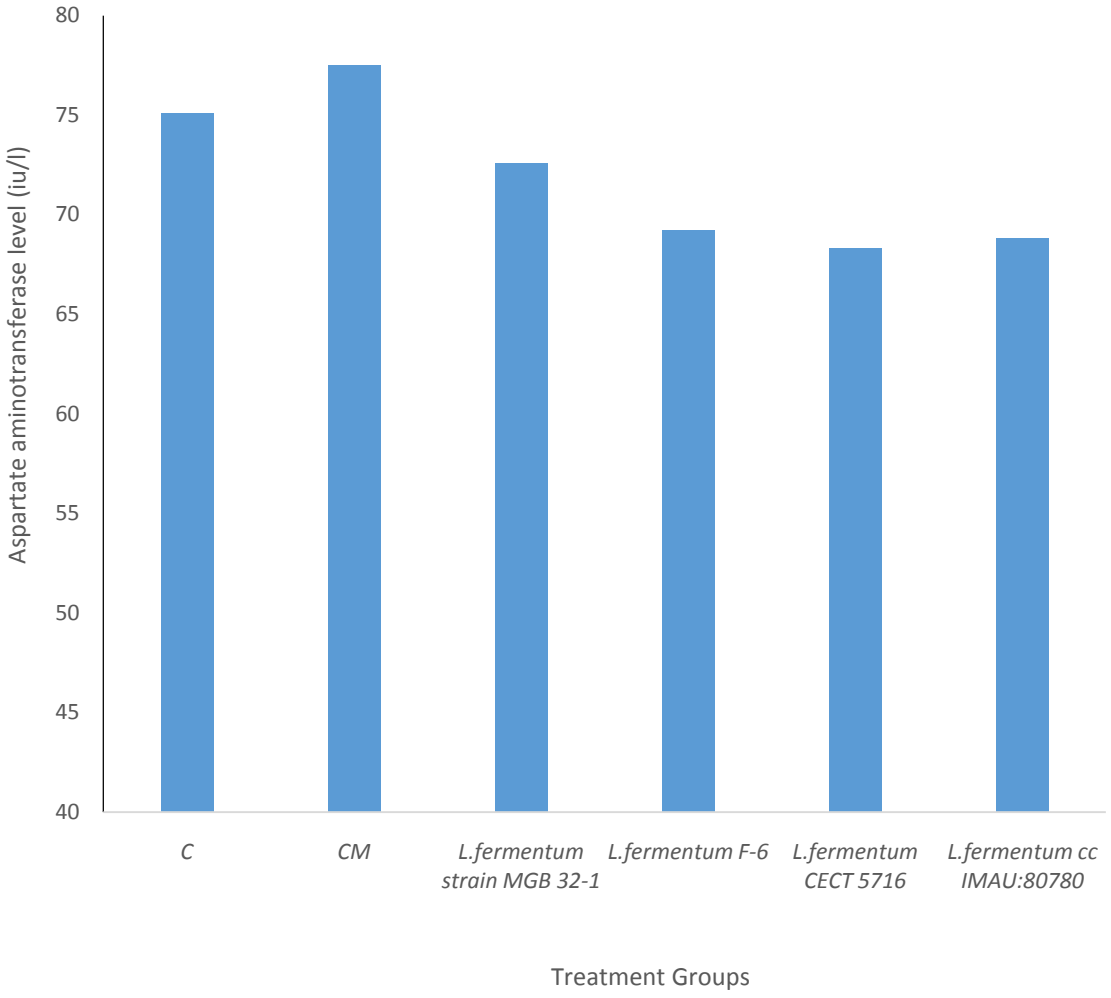


Figure 17: Level of Aspartate aminotransferase in serum of rats fed 0.5ml fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

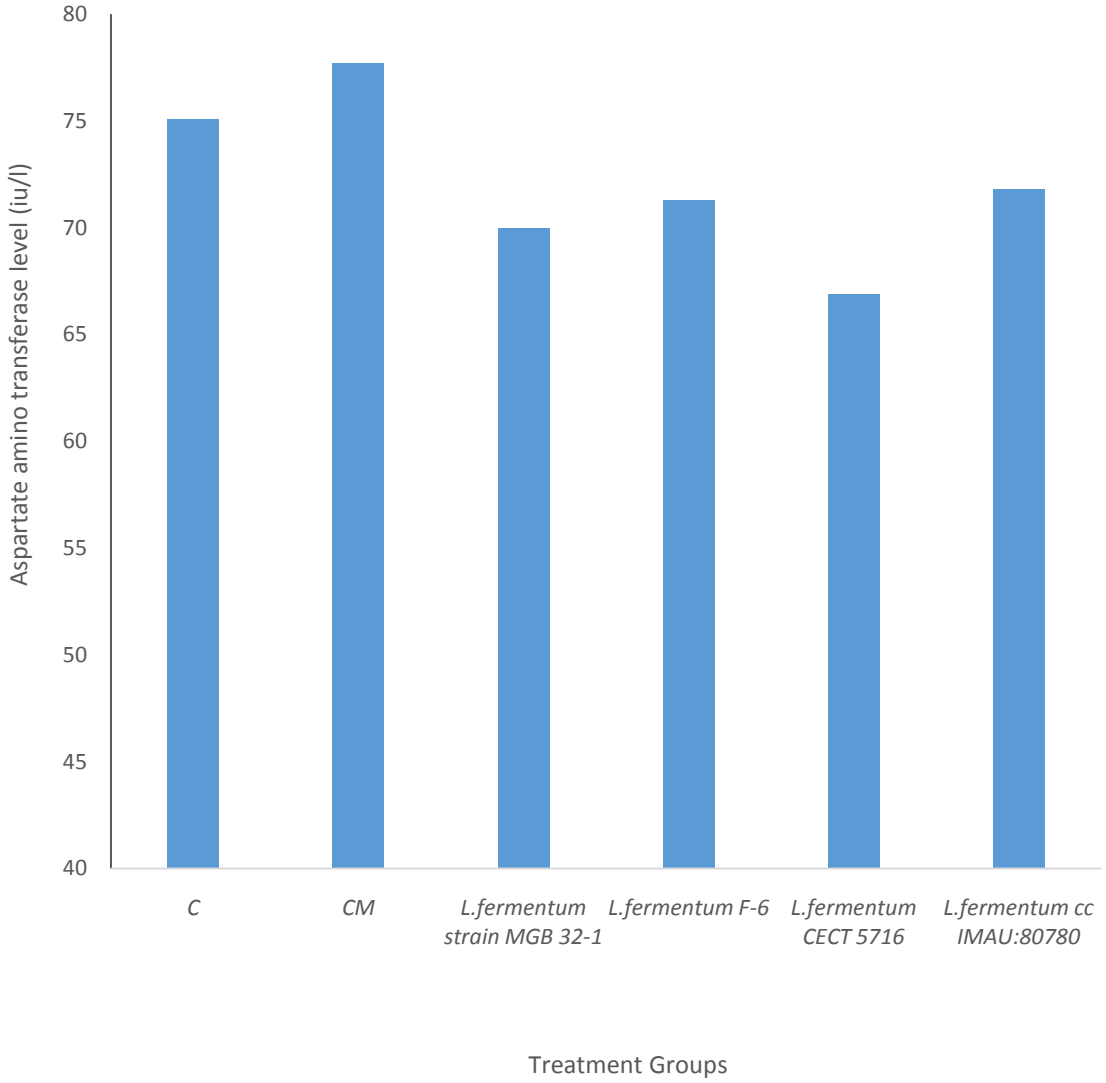


Figure 18: Level of Aspartate aminotransferase in serum of rats fed 1ml of fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

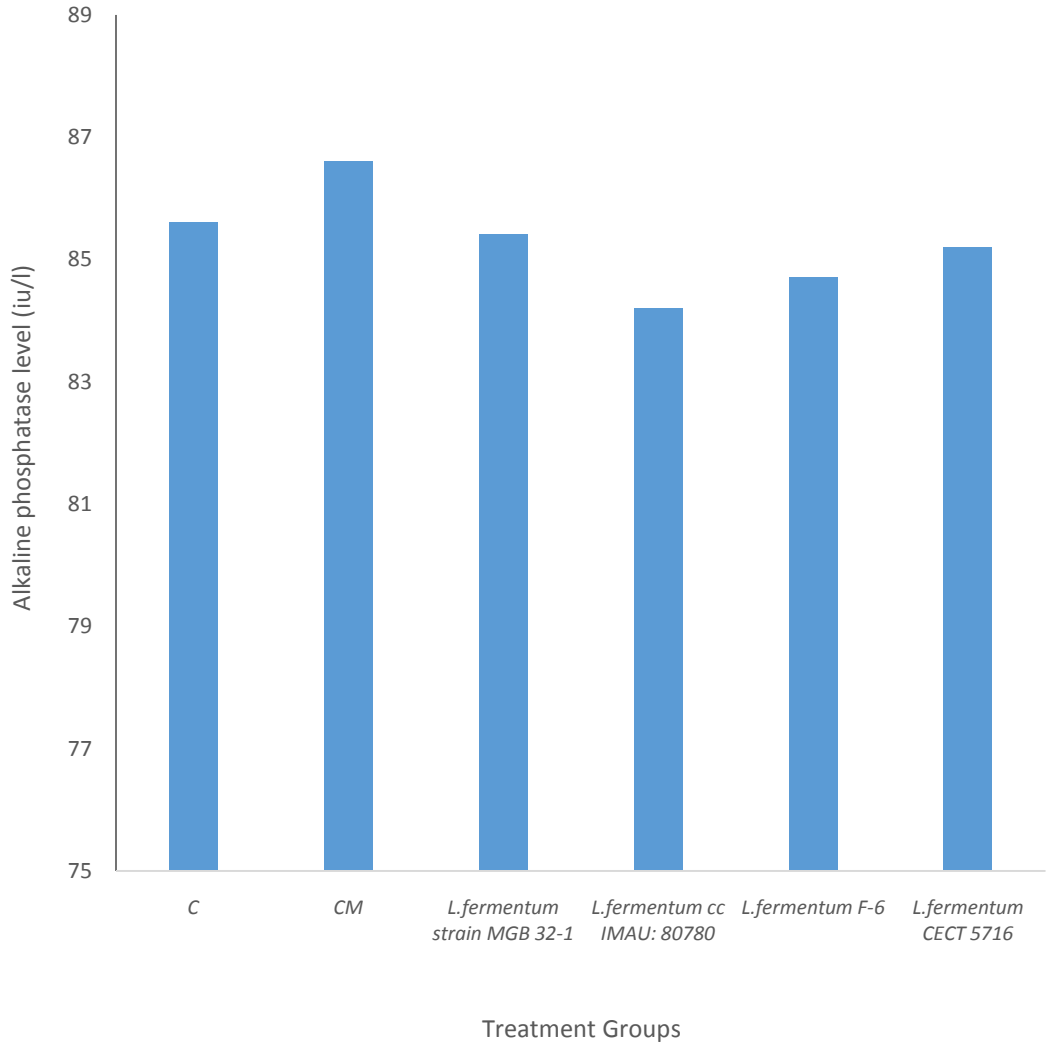


Figure 19: Level of Alkaline phosphatase in serum of rats fed 0.1ml fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

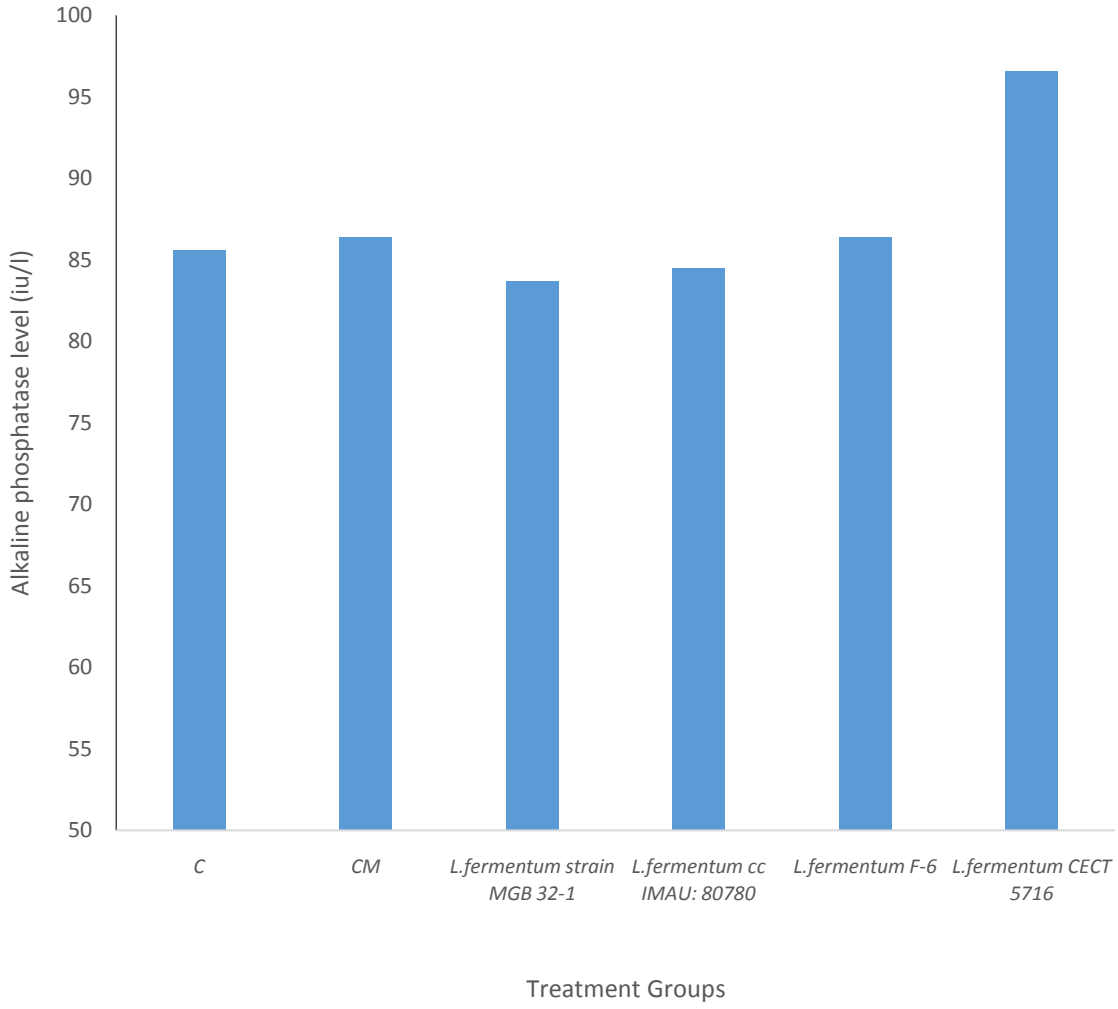


Figure 20: Level of Alkaline phosphatase in serum of rats fed 0.5ml of fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

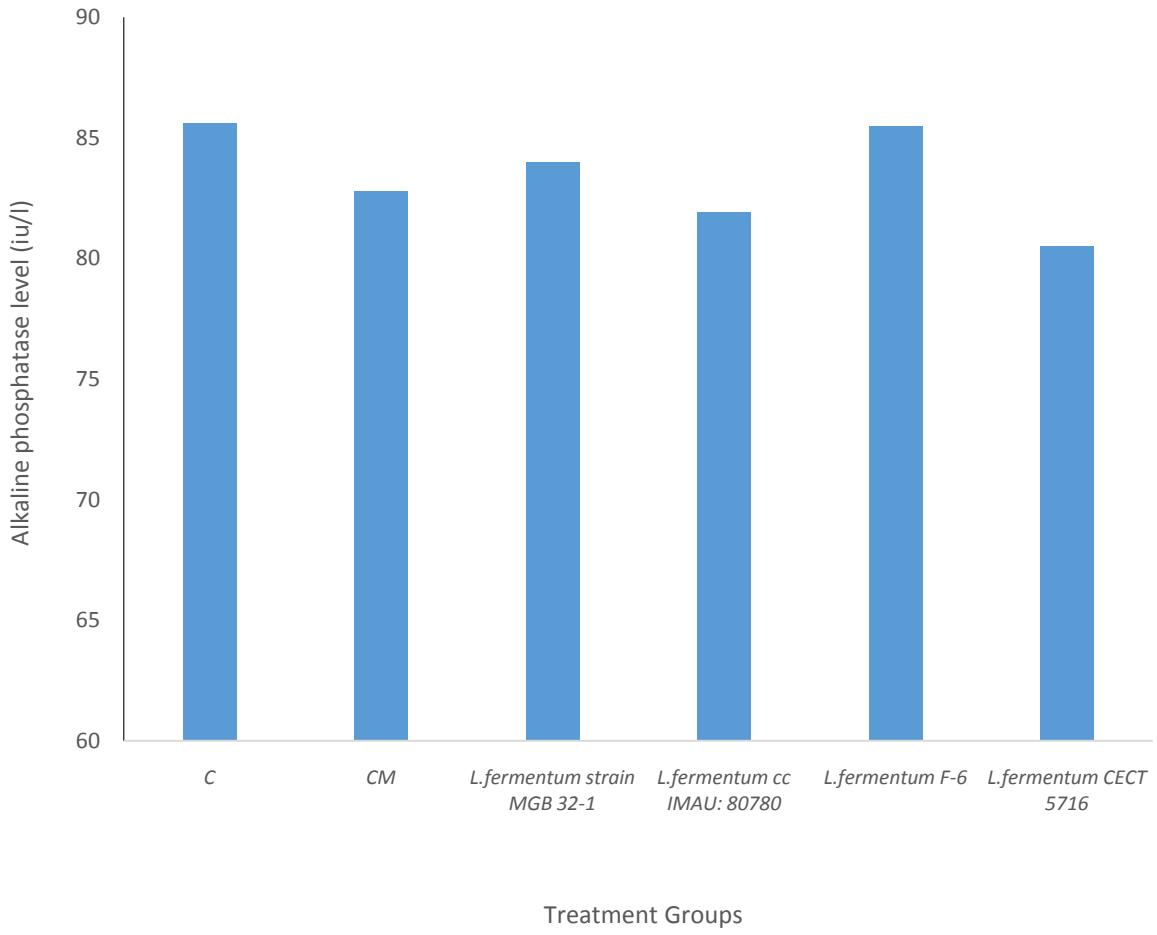


Figure 21: Level of Alkaline phosphatase in serum of rats fed 1ml fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

4.3.1.7 Level of total serum cholesterol of male rats fed 0.1ml, 0.5ml and 1ml of fermented milk for 2 weeks

The levels of serum cholesterol of rats fed 0.1ml of fermented milk for 2 weeks are shown in Figure 22. There were no significant difference in the level of serum cholesterol of the treatment groups when compared to the controls at this concentration (Appendix ix). A significant difference was noted only in the group fed 0.5ml of *Lactobacillus fermentum* F6 -fermented milk when compared to the control (Figure 23). Results from this study also show that only *Lactobacillus fermentum* CECT 5716 was not able to reduce serum cholesterol level significantly ($p \leq 0.05$) after 2 weeks of consumption of 1ml of fermented milk (Figure 24). The determination of the level of serum cholesterol of rats fed 0.5ml and 1ml of fermented milk respectively for 2 weeks revealed that *Lactobacillus fermentum* F-6 and *Lactobacillus fermentum* cc IMAU: 80780 group had the lowest levels and were significantly lower than the controls when fed 1ml of the fermented milk.

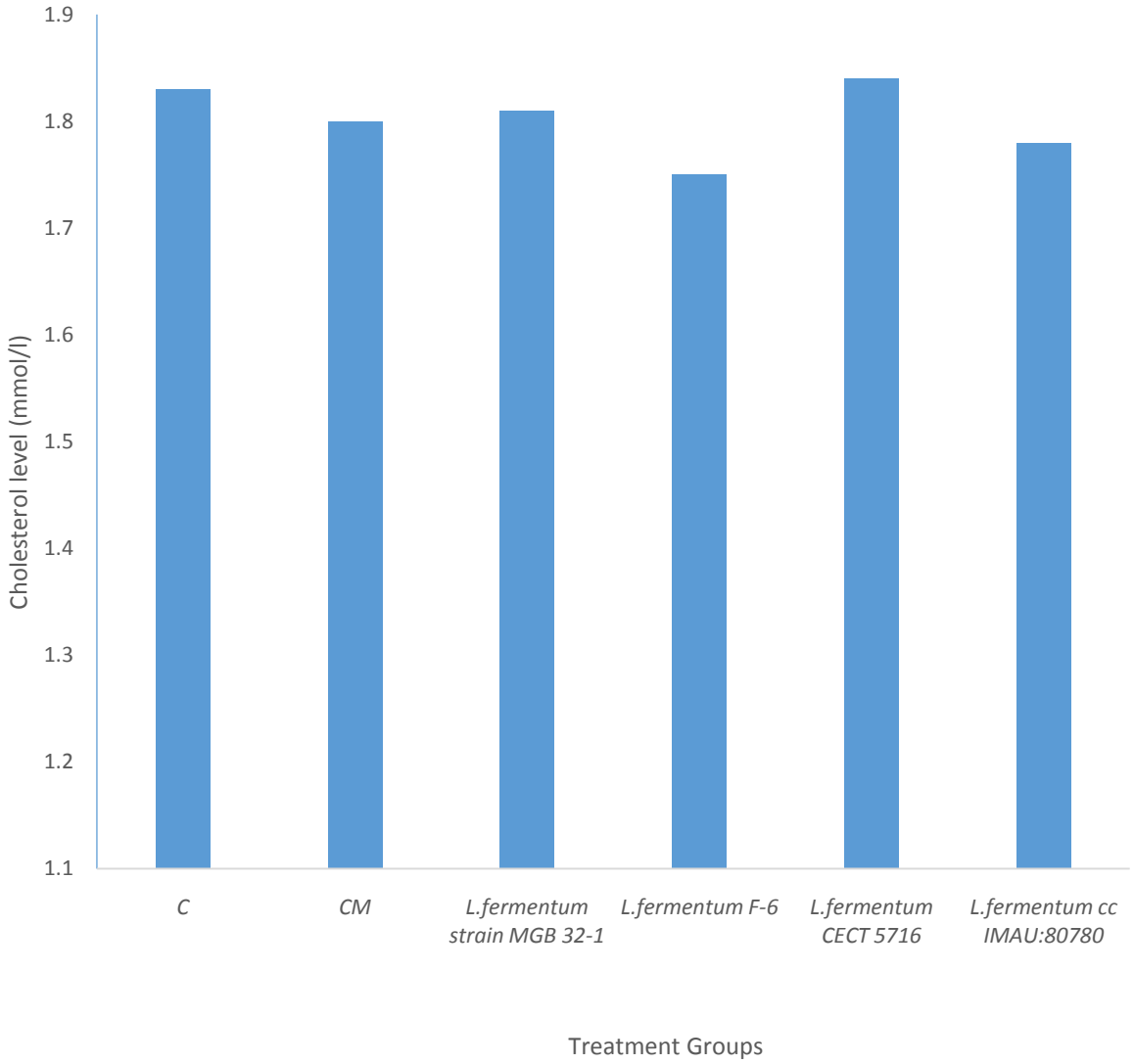


Figure 22: Level of serum cholesterol of rats fed 0.1ml of fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

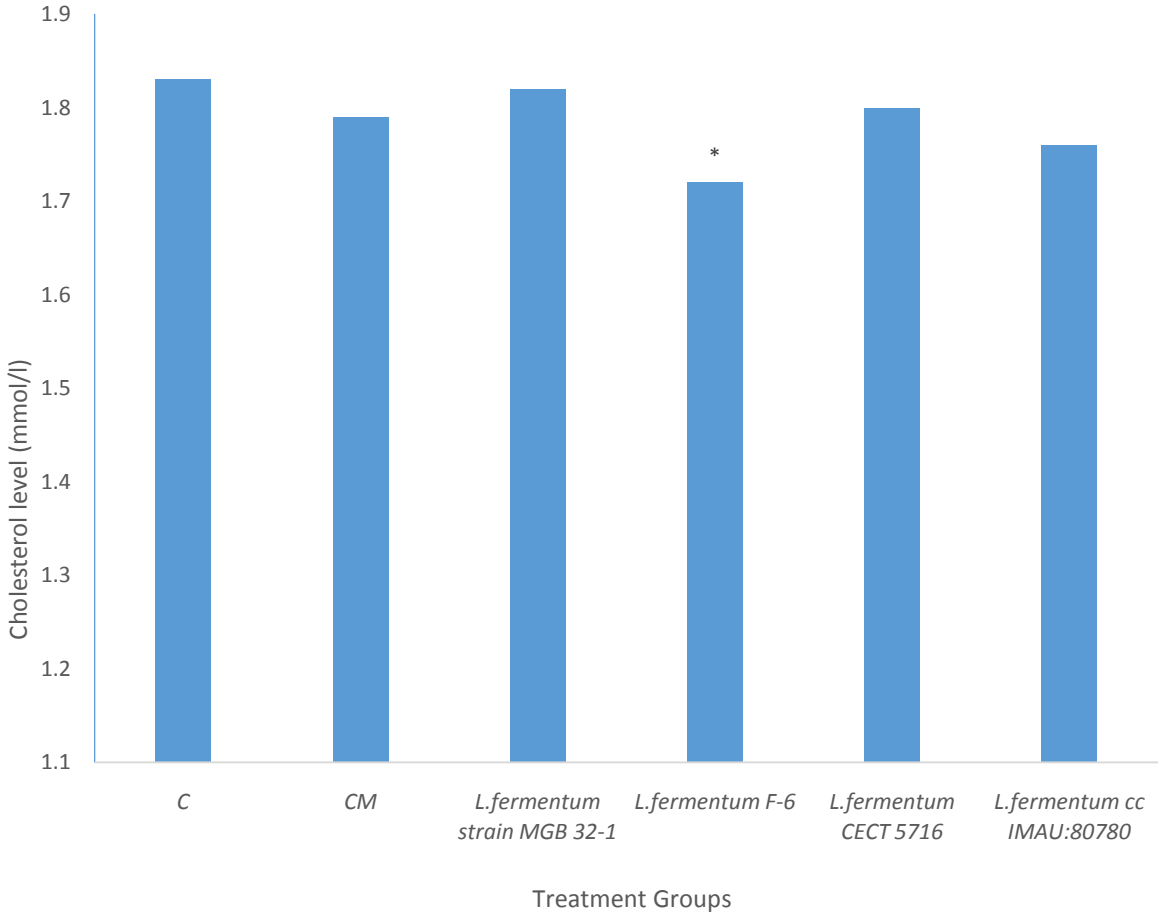


Figure 23: Level of serum cholesterol of rats fed 0.5ml of fermented milk for 2 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

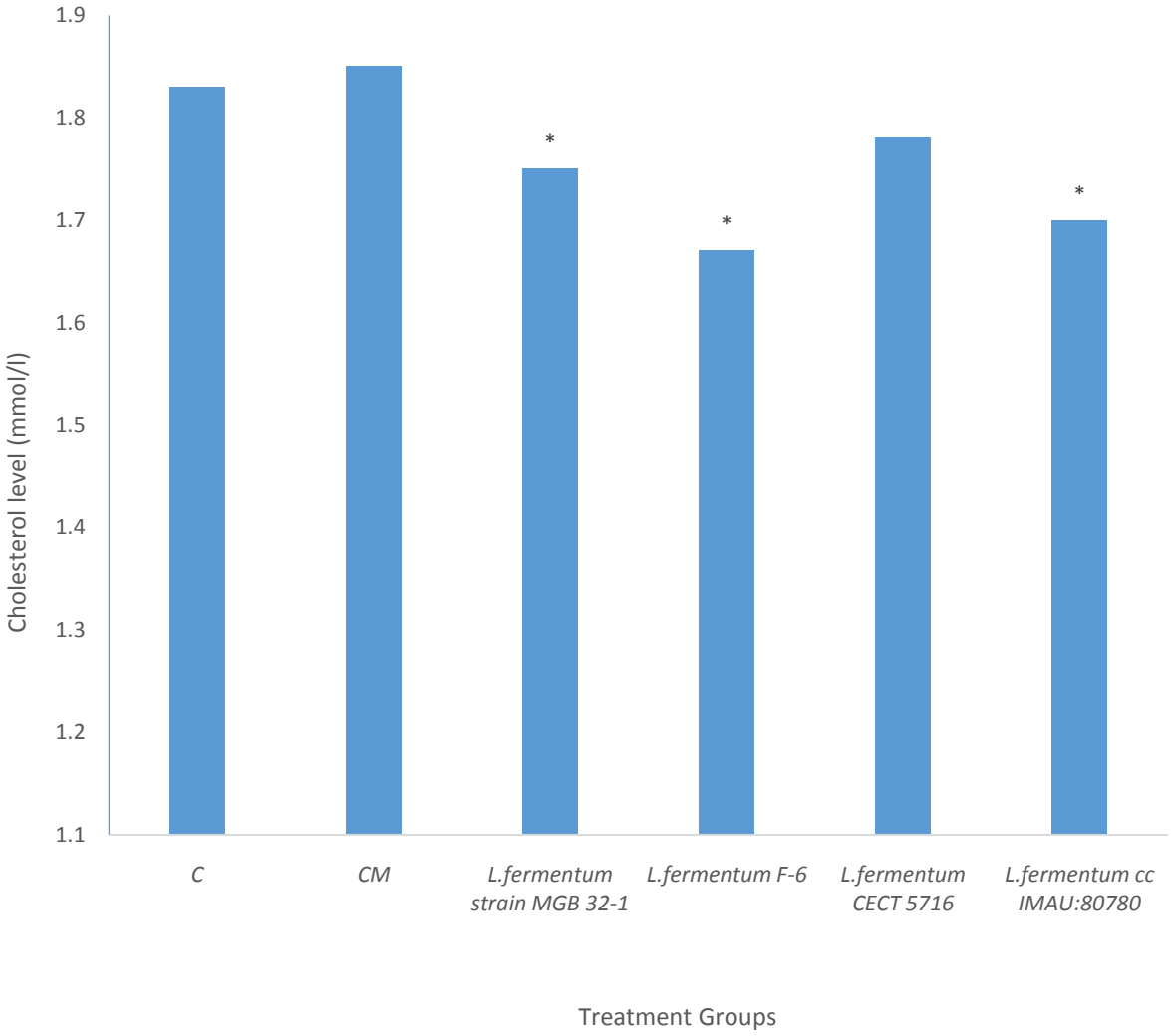


Figure 24: Level of serum cholesterol of rats fed 1ml of fermented milk for 2weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

4.3.2 Thirteen-Week Subchronic Oral Toxicity Study

4.3.2.1 Mean weights of male and female rats after 13 weeks consumption of 1ml of fermented milk

The mean weights of the male rats fed 1ml of fermented milk for13 weeks can be seen in Figure 25. At the end of the treatment period, the weight of all treatment groups,*L.fermentum* strain MGB 32-1 (390.3g), *L.fermentum* F-6 (399.26g), *L.fermentum* CECT 5716 (377.4g) and*L.fermentum* ccIMAU:80780 (393.41g), were all observed to be significantly higher than control C (300.23g) and control CM (351.31g). The highest weight gain was observed in *L. fermentum* F-6 (324.55g). The mean weights of the female rats fed 1ml of fermented milk for 13 weeks can be seen in Figure 26. At the end of the treatment period, the weight of all treatment groups,*L.fermentum* strain MGB 32-1 (281.51g), *L.fermentum* F-6 (292.55g), *L.fermentum* CECT 5716 (272.38g) and*L.fermentum* ccIMAU:80780 (285.66g), were all observed to be significantly higher than control C (203.38g) and controlCM (248.38g). The highest weight gain was also recorded in *L. fermentum* F-6 (222.05g) (Appendix x).

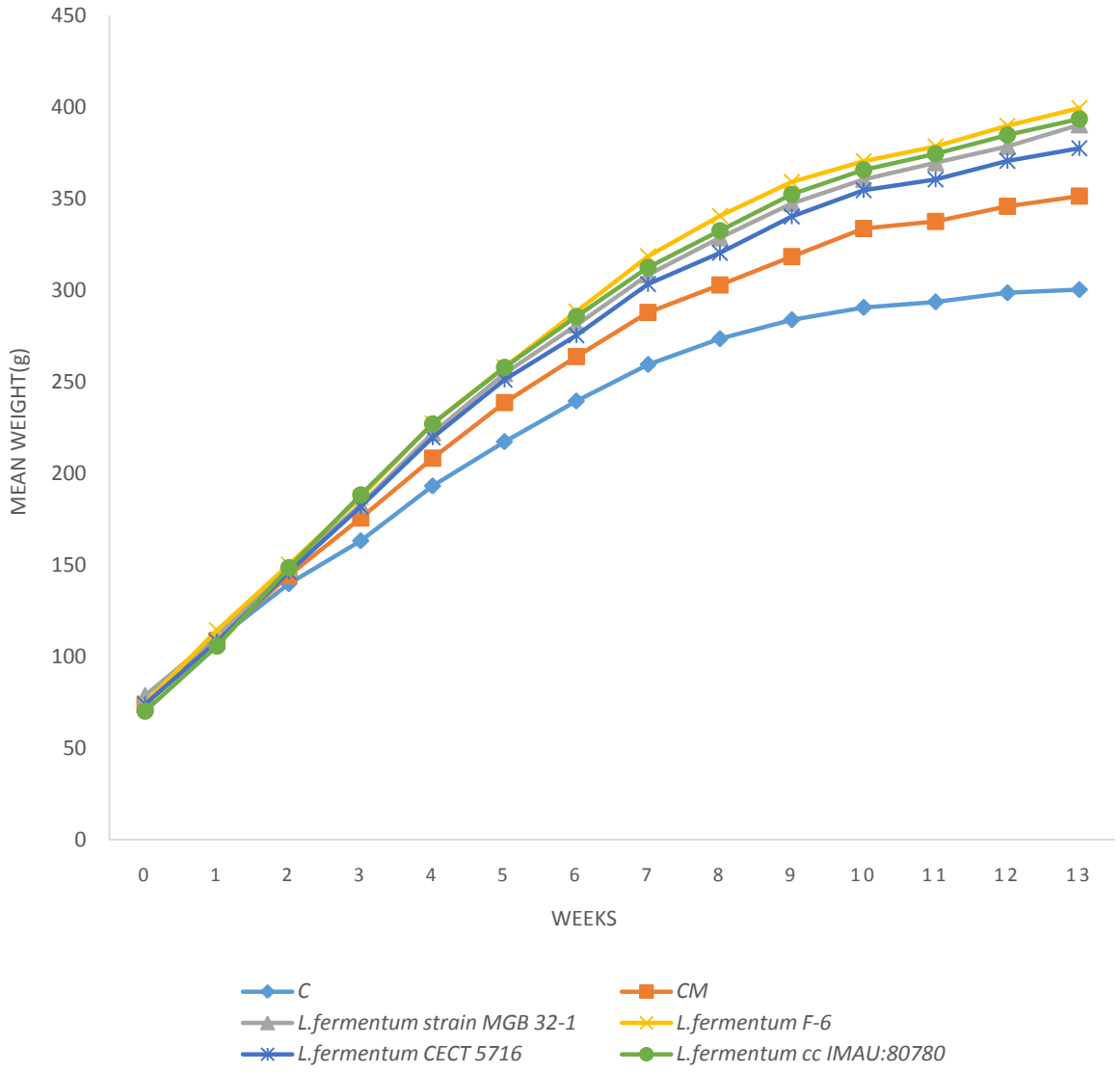


Figure 25: Mean weights of the male rats fed 1ml of fermented milk in the 13-week subchronic oral toxicity study.

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

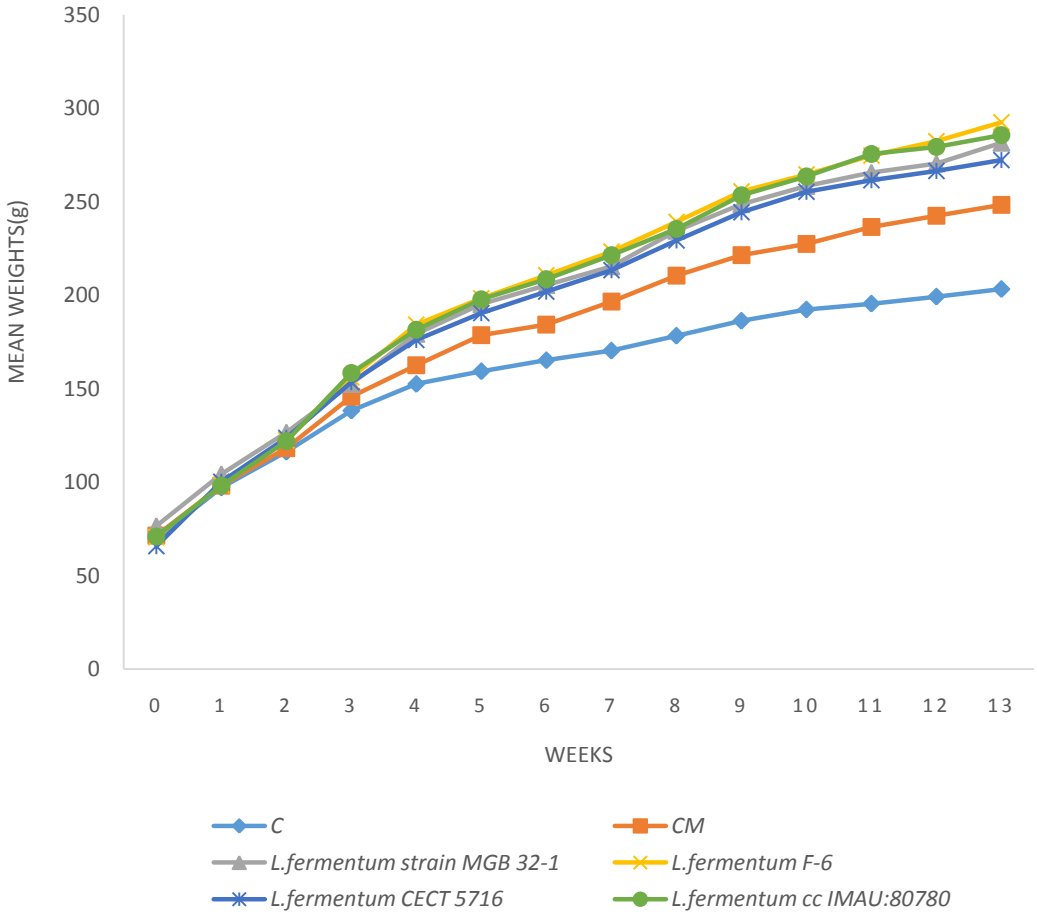


Figure 26: Mean weights of the female rats fed 1ml of fermented milk in the 13-week sub-chronic oral toxicity study

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

4.3.2.2 Level of Alanineaminotransferase activity in serum of male and female rats fed 1ml fermented milk for 13 weeks.

Results of the determination of the Alanine aminotransferase (ALT) levels in male rats fed fermented milk for 13 weeks (Figure 27), shows that the lowest levels were observed in *Lactobacillus fermentum* MGB 32-1 group and *Lactobacillus fermentum* F-6. The group of female rats fed *Lactobacillus fermentum* MGB 32-1-fermented milk for 13 weeks also had the lowest level of ALT (Figure 28). However, no significant differences were observed in both male and female groups (Appendix xii).

4.3.2.3 Level of Aspartate aminotransferase activity in serum of male and female rats fed 1ml fermented milk for 13 weeks.

Results of the determination of the Aspartate aminotransferase(AST) levels in male rats fed fermented milk for 13 weeks (Figure 29), shows that the lowest level was observed in *Lactobacillus fermentum* IMAU: 80780 group.The group of female rats fed *Lactobacillus fermentum* F-6-fermented milk for 13 weeks had the lowest level of AST (Figure 30). Again no significant differences were observed when compared to the controls (Appendix xi).

4.3.2.4 Level of Alkaline phosphatase activity in serum of male and female rats fed 1ml fermented milk for 13 weeks.

Results of the determination of the level ofAlkaline phosphatase(ALP) in male rats fed fermented milk for 13 weeks showed thatthe lowest level was observed in *Lactobacillus fermentum* MGB 32-1 group (Figure 31). No significant differences were observed between all treatment groups and the control groups.The group of female rats fed *Lactobacillus fermentum* CECT 5716-fermented milk for 13 weeks had the lowest level of ALP (Figure 32) and no significant differences were noted between the treatment groups and the control (Appendix xiii).

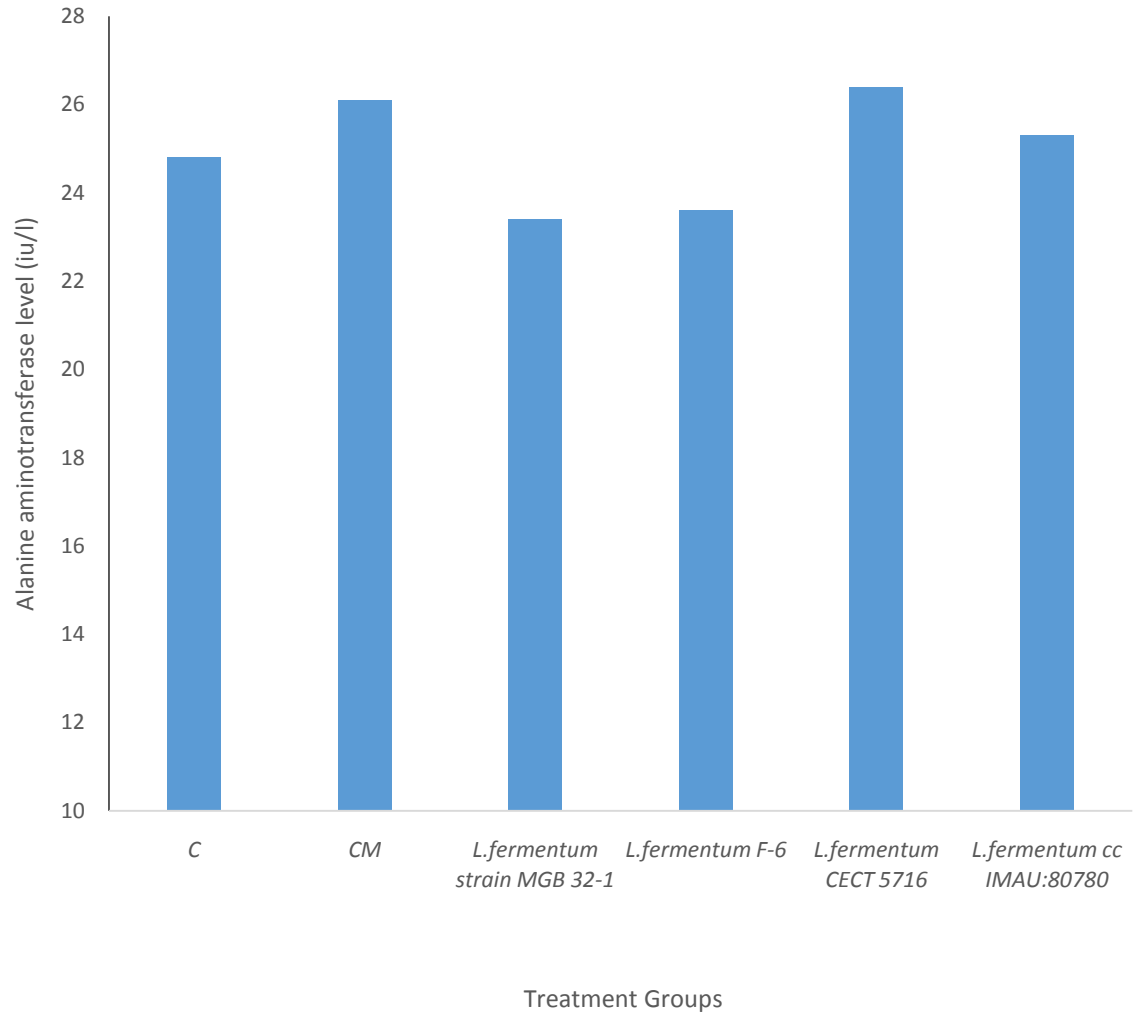


Figure 27: Level of Alanine aminotransferase in serum of male rats fed 1ml fermented milk for 13 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

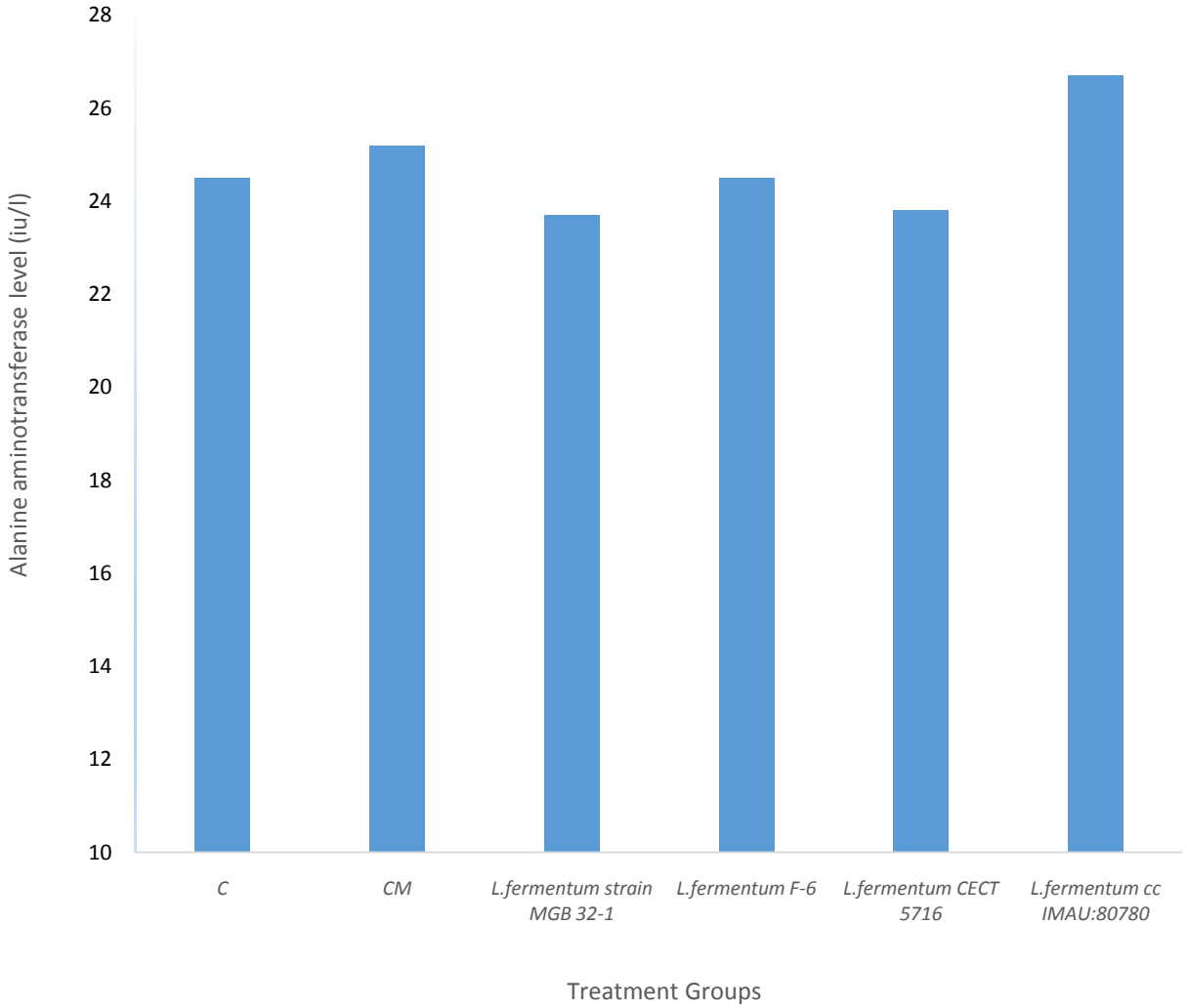


Figure 28: Level of Alanine aminotransferase in serum of female rats fed 1ml of fermented milk for 13 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

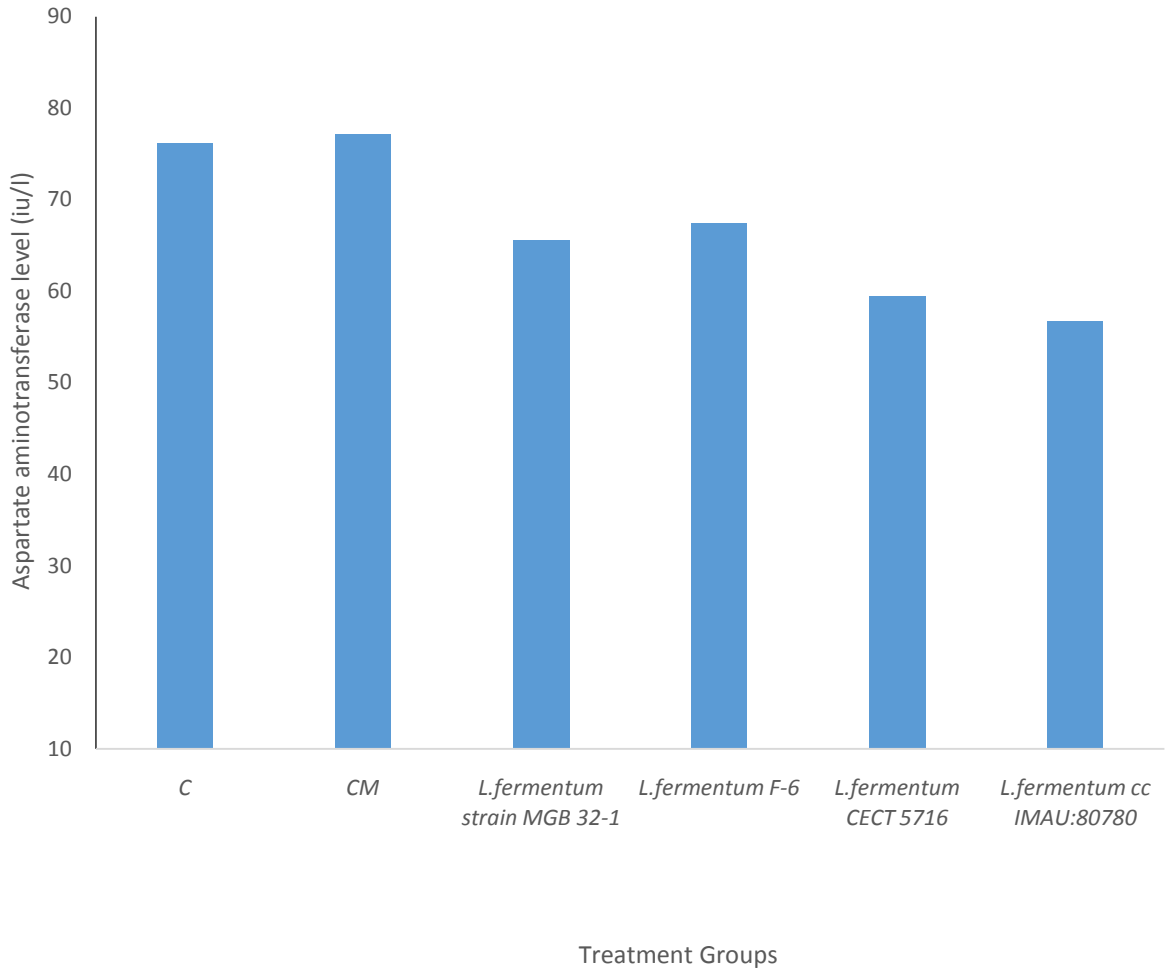


Figure 29: Level of Aspartate aminotransferase in serum of male rats fed 1ml fermented milk for 13 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

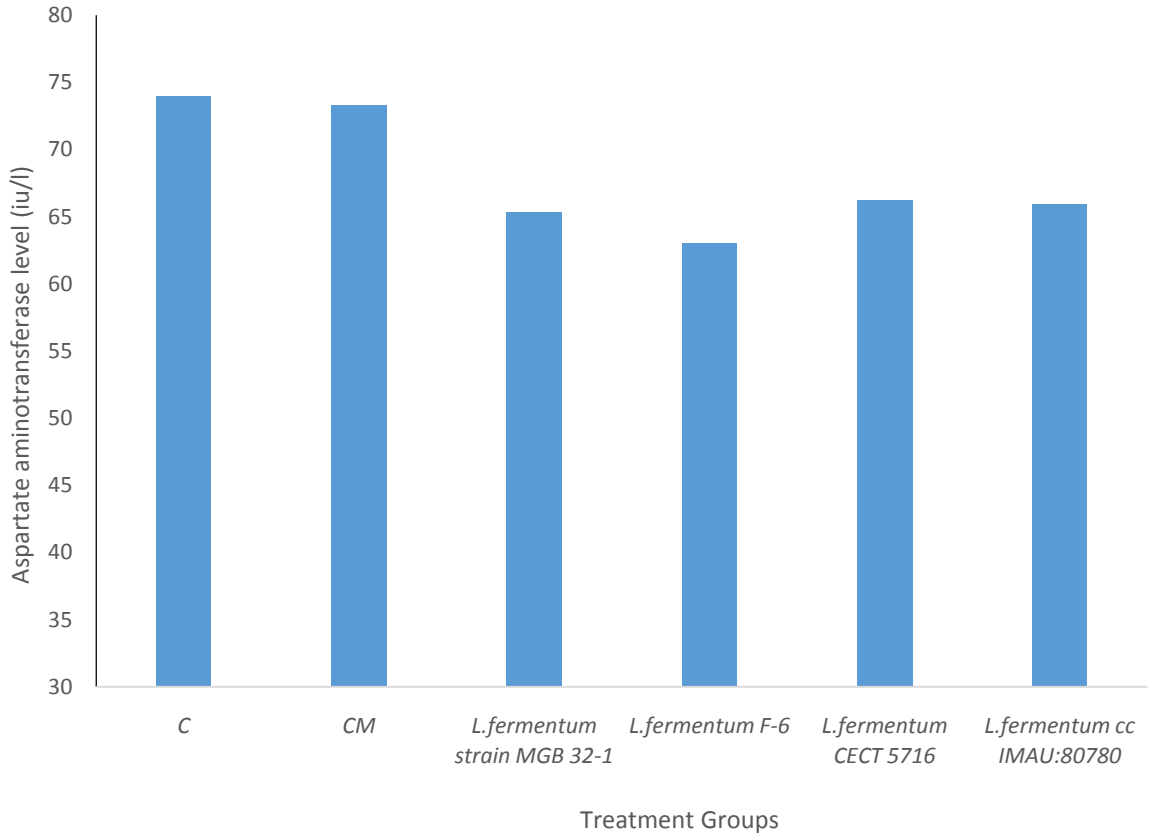


Figure 30: Level of Aspartate aminotransferase in serum of female rats fed 1ml of fermented milk for 13 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

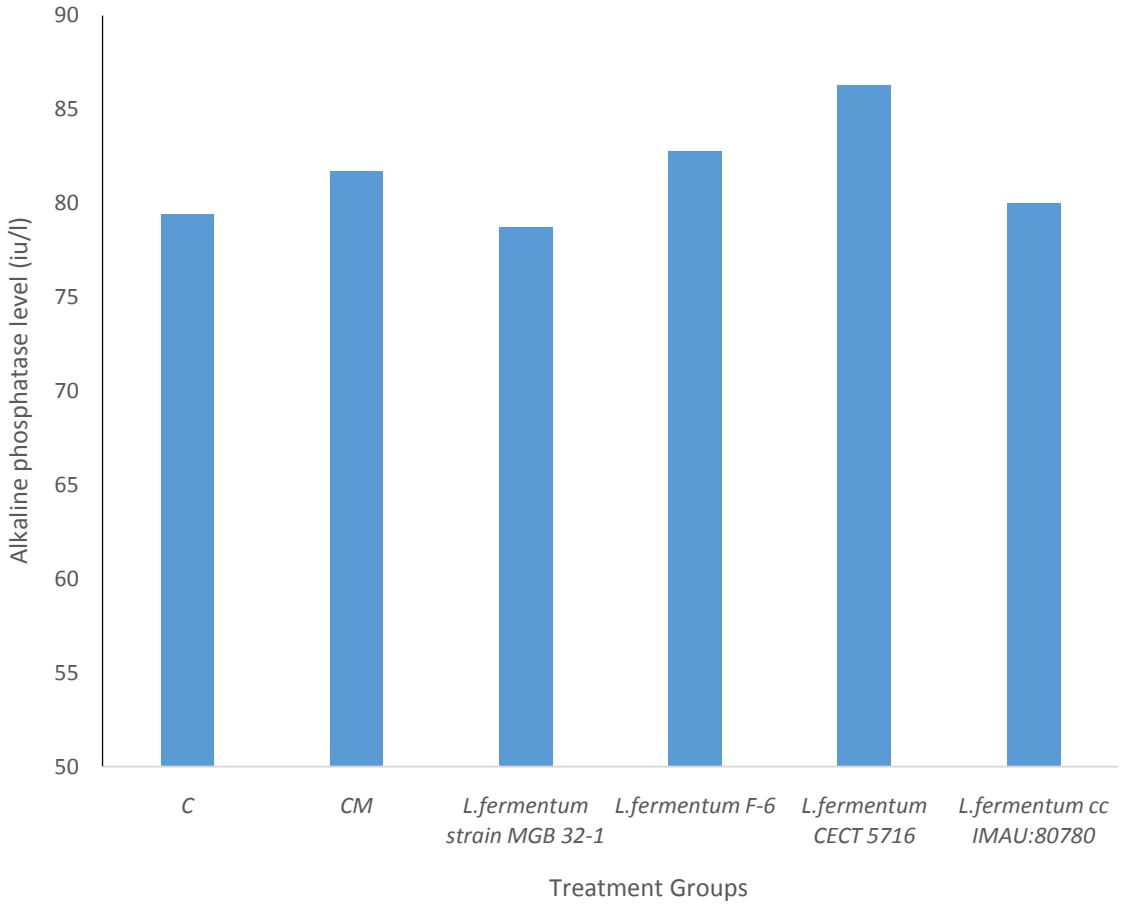


Figure 31: Level of Alkaline phosphatase in serum of male rats fed 1ml of fermented milk for 13 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

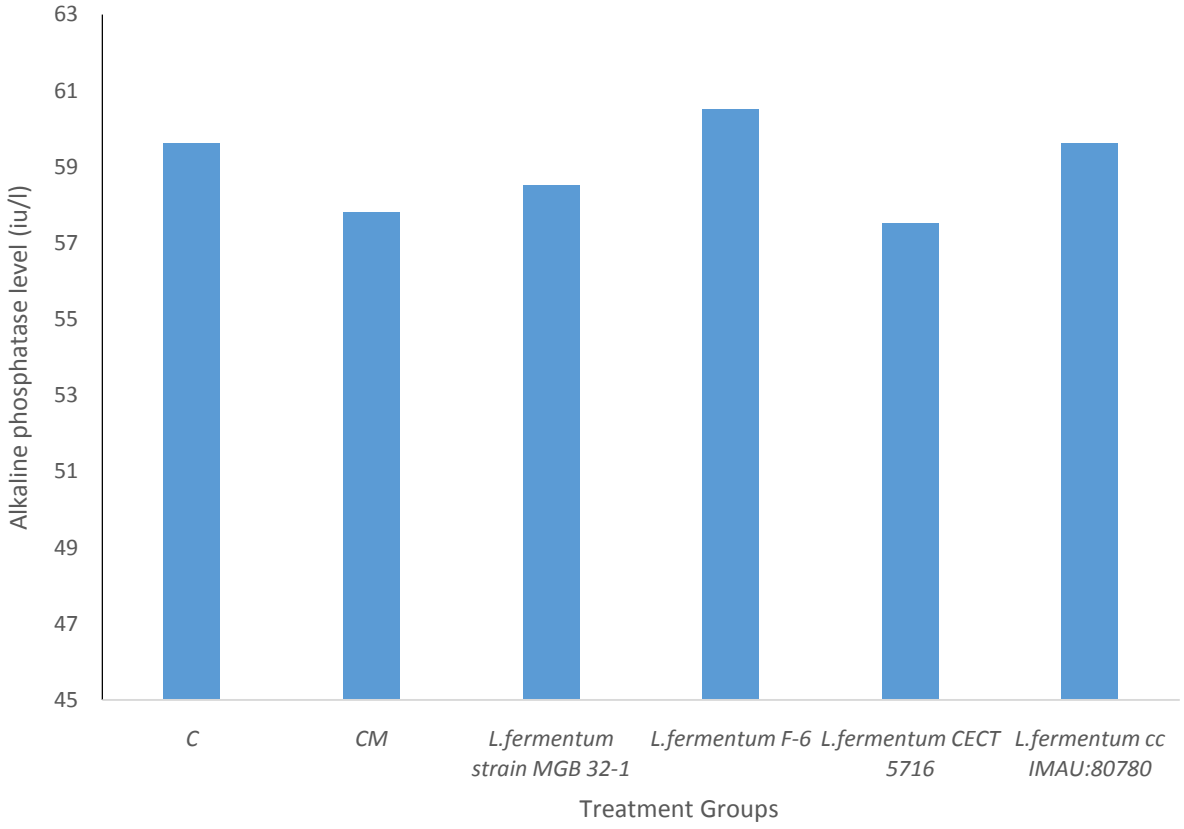


Figure 32: Level of Alkaline phosphatase in serum of female rats fed 1ml fermented milk for 13 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

4.3.2.5 Haematology of male and female rats fed 1ml fermented milk for 13 weeks

The haematology of male rats fed 1ml fermented milk for 13 weeks showed that the HGB, RBC, PCV, MCV, MCH and MCHC of all treatment groups were all significantly higher ($p<0.05$)than the controls while the WBC count was significantly lower ($p<0.05$)(Appendix xiv). There were, however, no significant differences ($p>0.05$)in the platelet count when compared to the controls (Table 7). The haematology of the female rats fed 1ml fermented milk for 13 weeks is presented in Table 8. The HGB, RBC, PCV, MCV and MCH of all treatment groups were all significantly higher ($p<0.05$)than the controls while the MCHC, platelet and WBC count were not significant ($p>0.05$) (Appendix xv).

4.3.2.6 Relative organ weights of male and female rats after 13 weeks consumption of 1ml of fermented milk

Results of the relative organ weights of male rats studied after 13 weeks consumption of the fermented milk showed no significant changes ($p>0.05$) in the relative weights of the heart, liver, spleen, kidney and brain of all the treated rats when compared to the control groups (Appendix xvi). No significant changes ($p>0.05$) in the relative weights of the heart, liver, spleen, kidney and brain of all thefemale rats in the treatment groups were observed in relation to the control groups (Appendix xvii).

Table 7: Haematology of male rats fed 1ml of fermented milk for 13 weeks

GROUP	HGB(g/dl)	RBC(10^{12}/l)	PCV(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)	
CONTROL	10.04±0.544	5.33±0.192	33.1±1.73	62.5±1.2	18.8±0.37	30.1±0.5	424.
CONTROL+MILK	10.44±0.425	5.43±0.147	34.1±1.3	62.8±0.75	19.2±0.3	30.6±0.26	428
L.fermentum strain MGB 32-1	13.33±0.565*	6.42±0.223*	43±1.67*	66.9±0.39*	20.8±0.15*	31±0.16*	458.
L.fermentum F-6	14.1±0.345*	6.62±0.162*	45.1±0.93*	68.2±1.67*	21.3±0.61*	31.3±0.17*	455
L.fermentum CECT 5716	13.04±0.488*	6.34±0.166*	41.7±1.53*	65.8±0.93*	20.6±0.22*	31.3±0.31*	466
L.fermentum cc IMAU: 80780	12.79±0.45*	6.27±0.154*	41.4±1.88*	65.9±1.55*	20.4±0.22*	31±0.59*	444.

Key: HGB=Haemoglobin, RBC=Red blood cell, PCV= Packed cell volume, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration, PLTS=Platelets, WBC=White blood cell

Table 8: Haematology of female rats fed 1ml of fermented milk for 13 weeks

GROUP	HGB(g/dl)	RBC(10^{12}/l)	PCV(%)	MCV(fl)	MCH(pg)	MCHC(g/dl)	PLTS
CONTROL	9.78±0.79	5.2±0.27	32.4±2.39	62.2±1.38	18.8±0.54	30.3±0.24	415.5±38.3
CONTROL+ MILK	10.15±0.31	5.37±0.11	32.4±1.74	60.2±2.31	18.9±0.22	31.4±1.2	432±58.8
L.fermentum strain MGB 32-1	12.97±0.5*	6.31±0.18*	40.6±2.89*	64.3±2.81*	20.5±0.21*	32±1.16	453.5±53.5
L.fermentum F-6	13.7±0.3*	6.57±0.11*	43.9±1.06*	66.9±0.48*	20.9±0.13*	31.2±1.1	448.25±45.7
L.fermentum CECT 5716	12.35±0.44*	6.12±0.15*	39.1±2.15*	63.4±2.78*	20.2±0.22*	31.9±1.15	464.75±32.4
L.fermentum cc IMAU: 80780	11.99±0.56*	6±0.19*	38.3±2.27*	63.4±2.71*	20±0.29*	31.6±1.03	434.5±42.7

Key: HGB=Haemoglobin, RBC=Red blood cell, PCV= Packed cell volume, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration, PLTS=Platelets, WBC=White blood cell

Table 9: Relative organ weight of male rats fed 1ml of fermented milk for 13 weeks

	Relative organ weight of male rat (g)				
Group	Liver	Brain	Heart	Kidney	Spleen
CONTOL	2.459±0.011	0.546±0.014	0.353±0.0213	0.675±0.0221	0.189±0.0043
CONTROL+MILK	2.453±0.031	0.55±0.013	0.348±0.0076	0.6785±0.0021	0.1888±0.0045
L.fermentum strain MGB 32-1	2.453±0.068	0.547±0.01	0.346±0.0031	0.6683±0.0036	0.1843±0.0010
L.fermentum F-6	2.438±0.018	0.537±0.011	0.341±0.0039	0.6645±0.0042	0.184±0.0012
L.fermentum CECT 5716	2.451±0.014	0.55±0.013	0.346±0.0038	0.6725±0.0037	0.186±0.0078
L.fermentum cc IMAU: 80780	2.438±0.017	0.541±0.009	0.342±0.0031	0.6663±0.0036	0.1843±0.0005

Table 10: Relative organ weight of female rats fed 1ml of fermented milk for 13 weeks

	Relative organ weight of female rat (g)				
Group	Liver	Brain	Heart	Kidney	Spleen
Control	2.56±0.0888	0.963±0.0266	0.374±0.0088	0.851±0.0034	0.2575±0.0117
Control+Milk	2.46±0.0596	0.955±0.0066	0.37±0.0072	0.848±0.013	0.2588±0.0021
L.fermentum strain MGB 32-1	2.548±0.0702	0.949±0.0123	0.381±0.0081	0.84±0.0061	0.2513±0.0038
L.fermentum F-6	2.519±0.0877	0.948±0.0015	0.365±0.0032	0.839±0.0161	0.2548±0.0035
L.fermentum CECT 5716	2.52±0.0861	0.951±0.0087	0.372±0.0060	0.836±0.0085	0.2608±0.0057
L.fermentum cc IMAU: 80780	2.493±0.0618	0.948±0.005	0.369±0.0062	0.838±0.0079	0.2548±0.0025

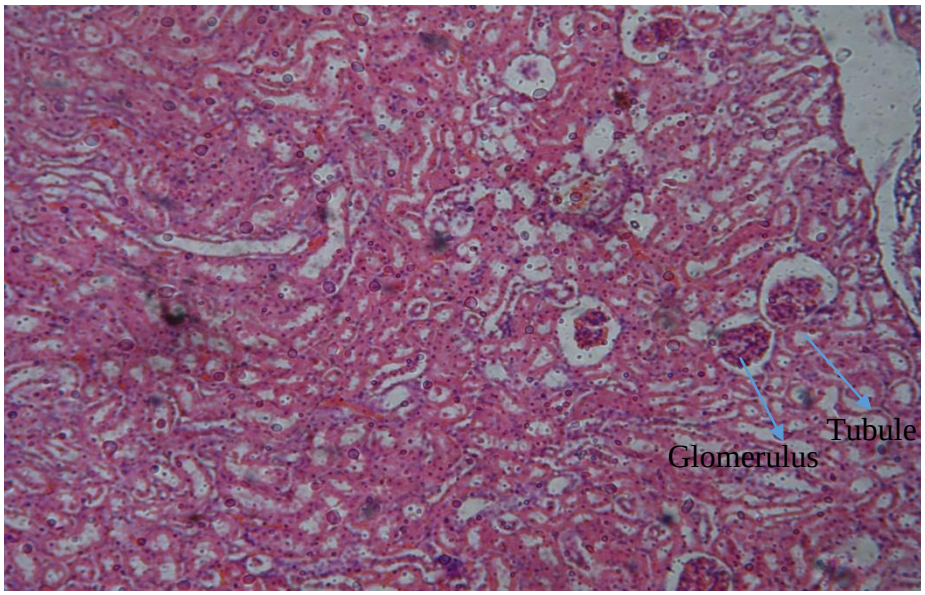
4.3.2.7 Histopathological examination of the kidney and liver

Sections of the kidneys showed normal glomeruli and tubules. The blood vessels are normal and the interstitium is free of inflammatory cells. The photomicrograph shows that the glomerulus in cortex and the tubules in the medulla of the control and all the kidney samples of the rats treated with different fermented milk samples all show no pathology (Plates 1-6). Photomicrograph shows normal histopathological architecture. Features are those of a normal renal biopsy.

Liver sections show normal portal tracts, central veins and the sinusoids. The photomicrograph (Plates 7-12) shows that the liver sections of the control groups and all the liver sections of the rats treated with different fermented milk samples had no pathology. Photomicrograph shows normal histopathological architecture. Features are those of normal liver biopsy. Histopathological examination of the liver and kidney showed no evidence of pathologic changes as the tissues of the rats in treatment group were comparable to the control. This result is consistent with the results of the relative organ weights which revealed no abnormalities.

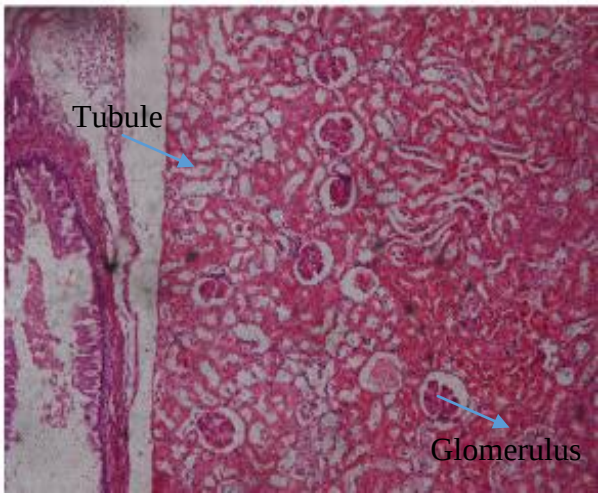
4.3.2.8 Level of total serum cholesterol of male and female rats administered 1ml fermented milk for 13 weeks

The determination of the level of serum cholesterol of male rats fed 1ml of fermented milk for 13 weeks revealed that *Lactobacillus fermentum* F-6 had the lowest level followed by *Lactobacillus fermentum* IMAU: 80780 (Figure 33). A similar observation was made in the female rats after the 13 week-feeding period (Figure 34). All the test strains displayed significant reduction ($p<0.05$) in the serum cholesterol level of both male and female rats after 13 weeks consumption of 1ml of fermented milk (Appendix xviii). According to the T-test conducted no significant difference was observed in the effect of the test strains on the male when compared with the female rats.



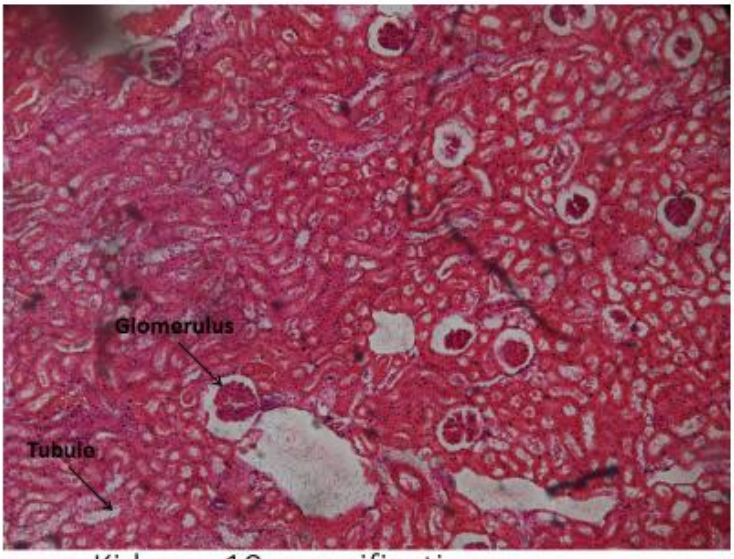
X20 magnification

Plate 1: Photomicrograph of the kidney of rats fed basal diet only (C)



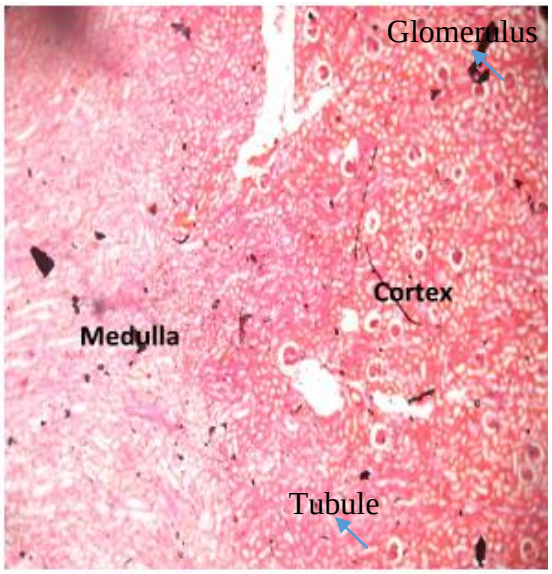
X10 magnification

Plate 2: Photomicrograph of the kidney of rats fed basal diet + milk (CM)



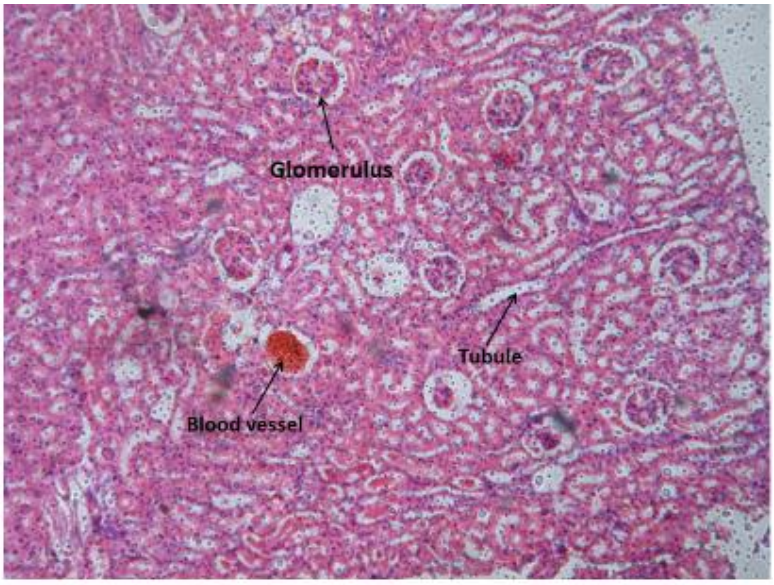
Kidney x10 magnification

Plate 3: Photomicrograph of the kidney of rats fed *L.fermentum* strain MGB 32-1-fermented milk



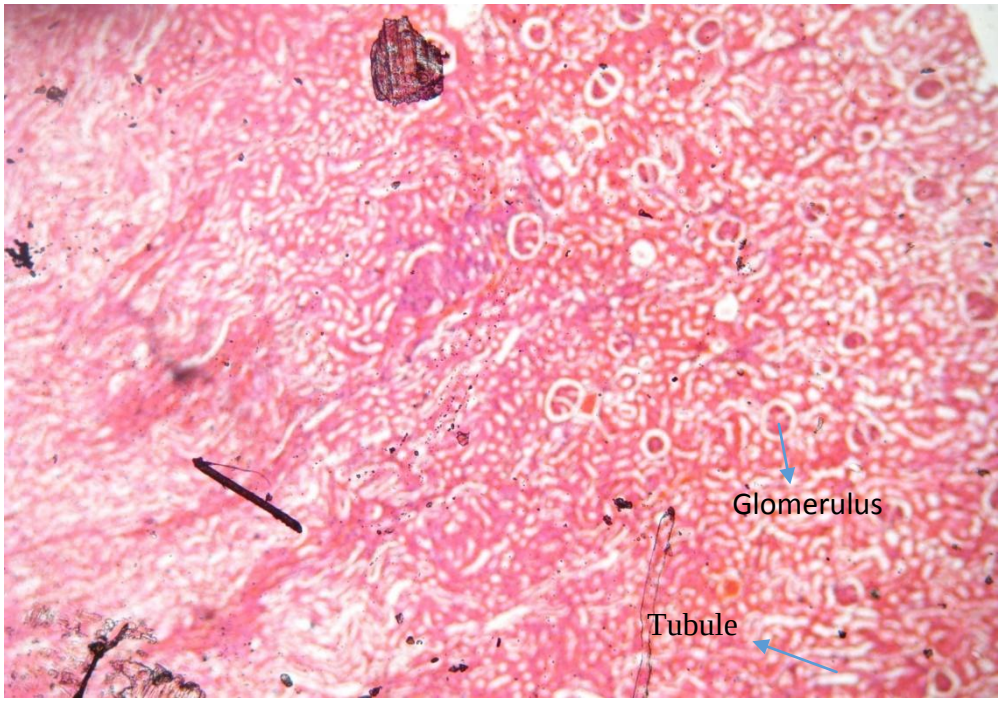
X5 magnification

Plate 4: Photomicrograph of the kidney of rats fed *L.fermentum* strain F6-fermented milk



X5 magnification

Plate 5: Photomicrograph of the kidney of rats fed *L.fermentum* CECT 5716-fermented milk



X5 magnification

Plate 6: Photomicrograph of the kidney of rats fed *L.fermentum* cc IMAU: 80780-fermented milk

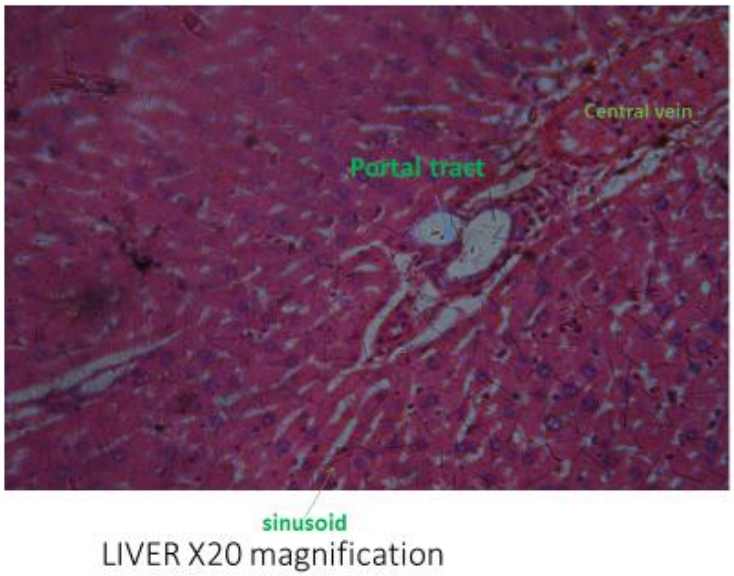
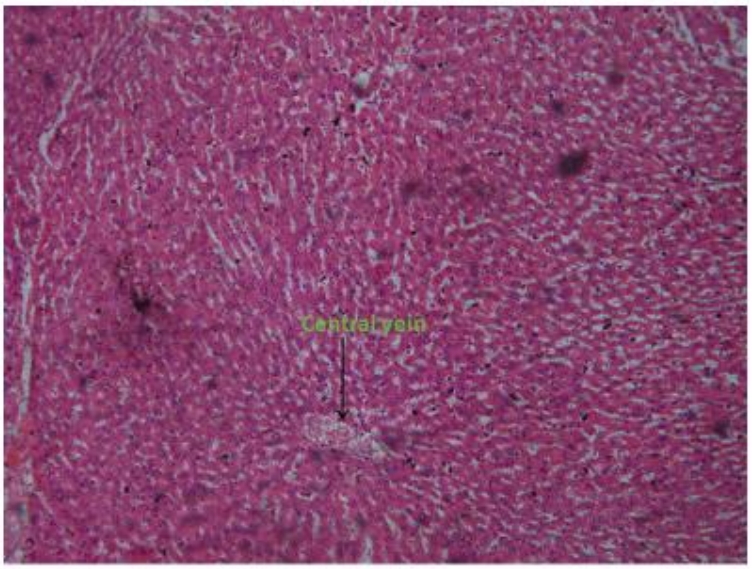
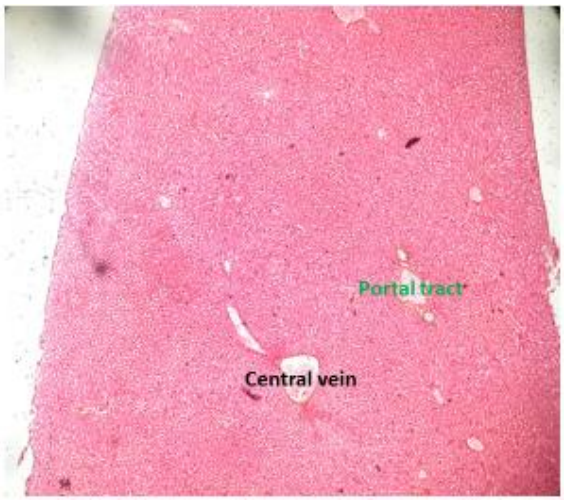


Plate 7: Photomicrograph of the liver of rats fed basal diet only (C)



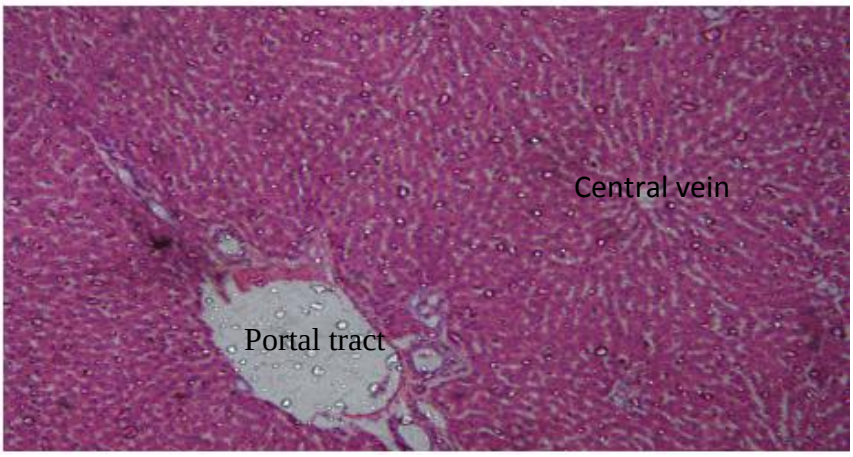
LIVER X10 magnification

Plate 8: Photomicrograph of the liver of rats fed basal diet + milk (CM)



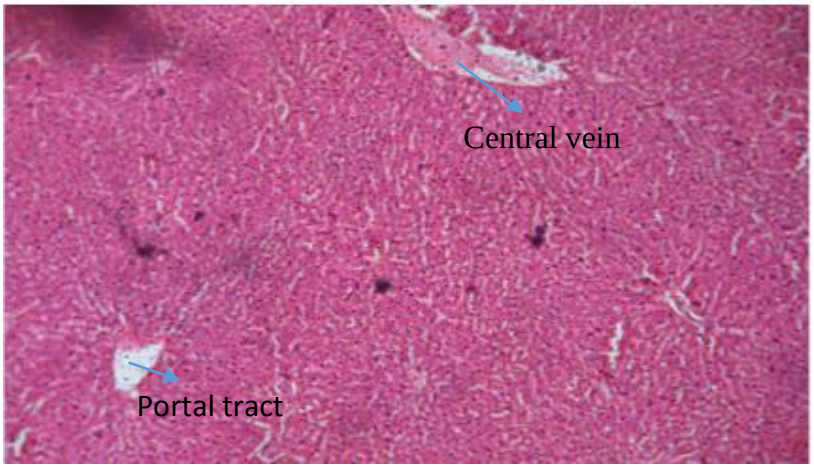
X5 magnification

Plate 9: Photomicrograph of the liver of rats fed *L.fermentum* strain MGB 32-1 -fermented milk



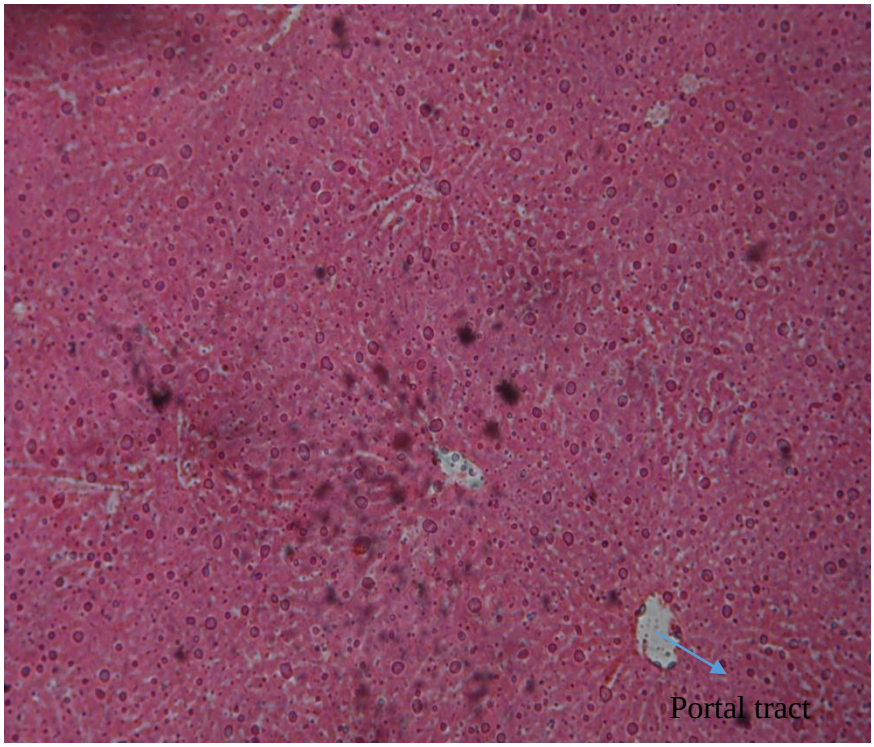
X10 magnification

Plate 10: Photomicrograph of the liver of rats fed *L.fermentum* F-6-fermented milk



X10 magnification

Plate 11: Photomicrograph of the liver of rats fed *L.fermentum* CECT 5716-fermented milk



X10 magnification

Plate 12: Photomicrograph of the liver of rats fed *L.fermentum* cc IMAU: 80780-fermented milk

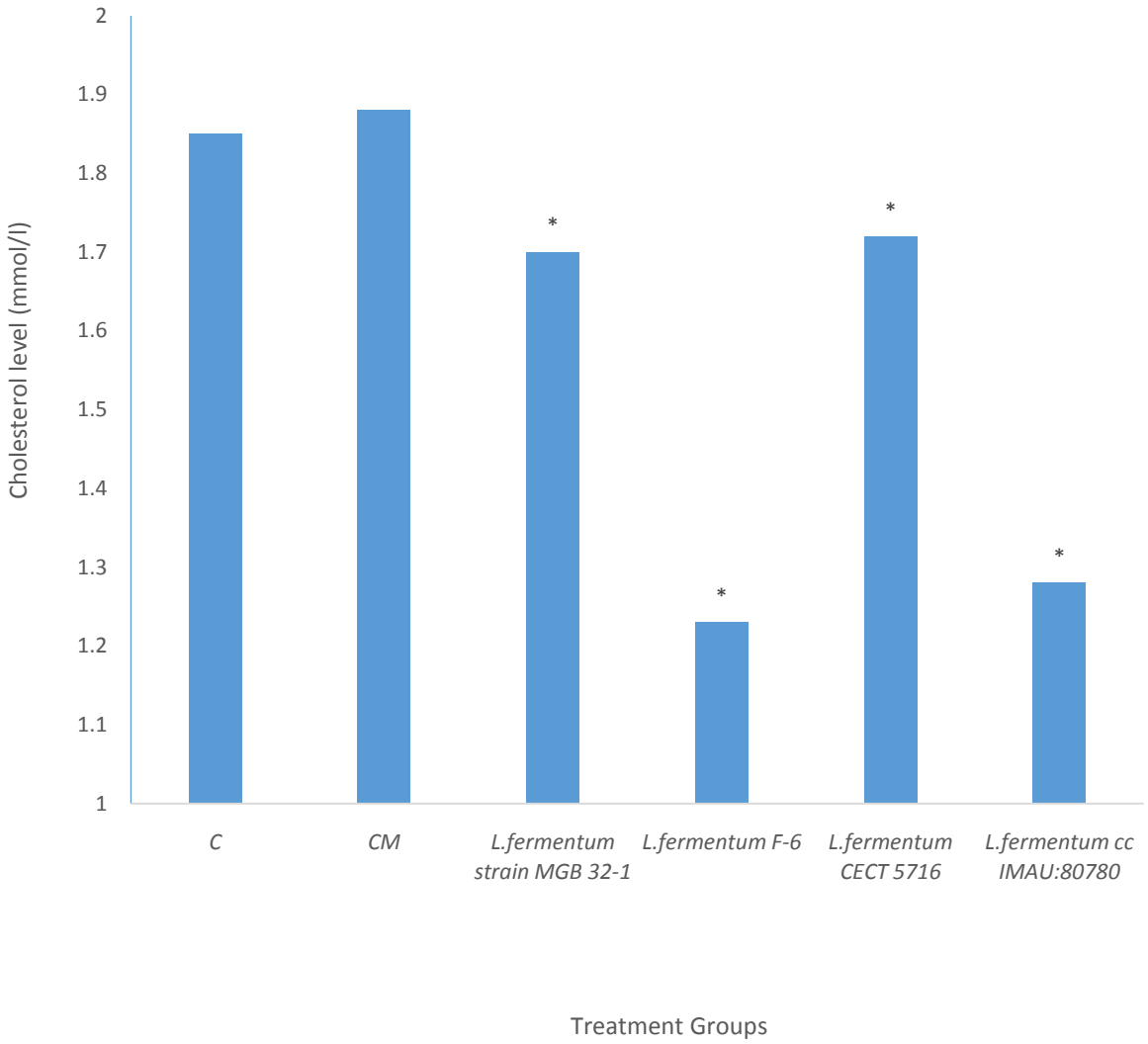


Figure 33: Level of serum cholesterol of male rats fed 1ml of fermented milk for 13 weeks

Key: C: Rats placed on basal diet alone.CM: Rats placed on basal diet and skimmed milk

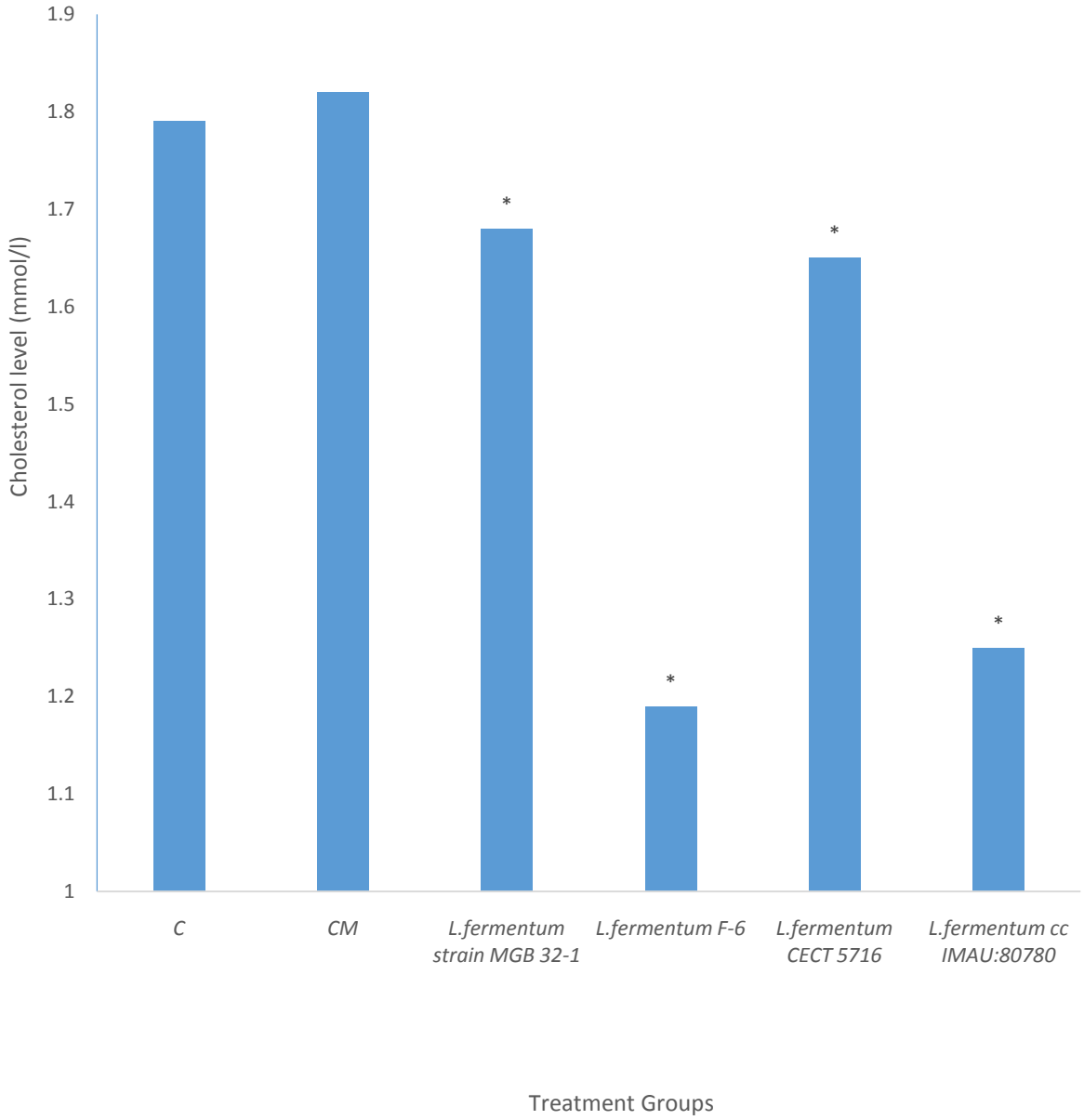


Figure 34: Level of serum cholesterol of female rats fed 1ml of fermented milk for 13 weeks

Key: C: Rats placed on basal diet alone.

CM: Rats placed on basal diet and skimmed milk

4.4 Chemical analysis of the *L. fermentum*-fermented milk samples

Chemical analysis of the fermented milk samples fermented by the strains of *Lactobacillus fermentum* showed thatthe pH of the fermented milk samples was between 4.49-4.69 (Table 11). The lowest pH was observed in *Lactobacillus fermentum* F-6. The titratable acidity of the samples ranged from 0.82-0.87, of which *Lactobacillus fermentum* F-6 gave the highest (0.82).

4.5 Proximate composition of the *L. fermentum*-fermented milk samples

The proximate composition of the fermented milk samples fermented by strains of *Lactobacillus fermentum*showed that the moisture content ranged from 87.08 to 87.71 whilethe protein content ranged between 3.47 and 3.5. The fat content in the samples ranged between0.59 and 0.61. The ash content of the samples in this study was between 0.69 and 0.72 (Table 12).

Table 11: Chemical analysis of the fermented milk samples fermented by strains of *Lactobacillus fermentum*

Sample	pH	TTA(g/100ml)
L.fermentum strain MGB 32-1	4.69	0.82
L.fermentum F-6	4.49	0.87
L.fermentum CECT 5716	4.58	0.84
L.fermentum cc IMAU: 80780	4.56	0.85

Key:

TTA=Titratable acidity

Table 12: Proximate composition of the fermented milk samples fermented by strains of *Lactobacillus fermentum*

Samples	Parameters(%)			
	Moisture	Protein	Ash	Crude Fat
L.fermentum strain MGB 32-1	87.3	3.5	0.7	0.6
L.fermentum F-6	87.08	3.48	0.72	0.59
L.fermentum CECT 5716	87.71	3.48	0.69	0.61
L.fermentum cc IMAU: 80780	87.48	3.47	0.71	0.6

4.6 Sensory evaluation of the *L. fermentum*-fermented milk samples

Data from the sensory evaluation (Figure 35), shows that the spontaneously fermented Nunu (control) were scored higher for taste than the test samples. This variation in taste was, however, non-significant ($p<0.05$) between the control and the test samples.For the other parameters, including overall acceptability, there was significant differences between the control and the test samples ($p<0.05$).The aroma and the texture of all test samples were rated significantly higher than the spontaneously fermented control. The colour was also rated significantly higher than the spontaneously fermented control. Based on the overall acceptance, the test samples were preferred to the spontaneously fermented control and there was no statistically significant preference among the test samples (Appendix xix).

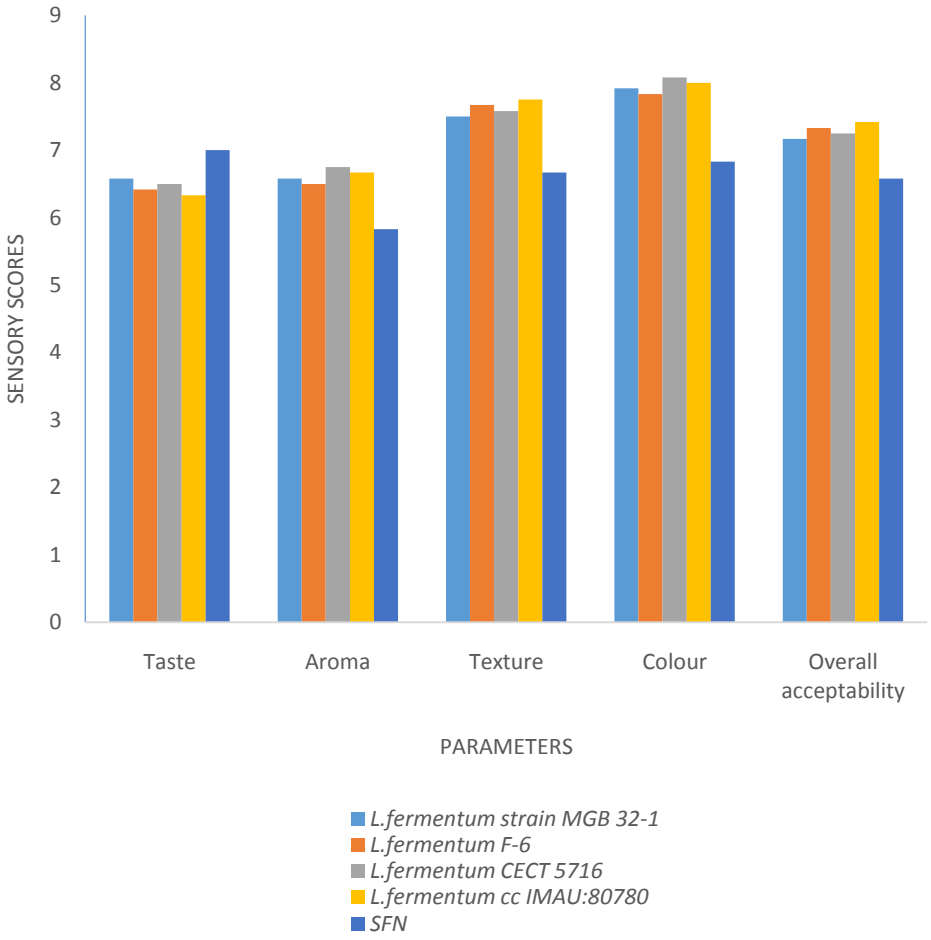


Fig. 35: Sensory evaluation of the fermented milk samples fermented by strains of *Lactobacillus fermentum*

CHAPTER 5

5.0 DISCUSSION

A total of seventy-five (75) strains of *Lactobacillus fermentum* were tentatively identified from the fermented foods (Akamu, Nunuand GariFCM) used in this study. Strains of *Lactobacillus fermentum* are prevalent in Nigerian indigenous fermented foods. It is one of the predominant lactic acid bacteria (LAB) in Nunu fermentation (Akabanda *et al.,* 2014; Owusu-Kwarteng*et al.,* 2013). It has also been isolated by other researchers from Akamu (Akinleye *et al.,* 2014; Ekwem, 2014) and Cassava (Kostinek *et al.,* 2005; Edward*et al.,* 2012). Some *Lactobacillus fermentum* strains have been widely used as probiotics for human and animals (Zarlok, 2016; Strompfova *et al.,* 2005).

Antibiotic sensitivity is considered an important part of safety assessment for the evaluation of probiotics. Safety concerns arise due to the possibility of transference of antibiotic resistant genes to intestinal pathogens (Curragh and Collins, 1992; Ammor *et al.,* 2007) and lessening of the effect of the use of antibiotics, which could lead to complications during treatment. For this reason, sensitivity to antibiotics was chosen in this study as a selection criterion for isolates to be included in further experiments. The *L. fermentum* strains isolated were first screened for their sensitivity to eight commonly used antibiotics and thirty (30) isolates were found susceptible (Tables 3a-c). The susceptibility of the *L. fermentum* isolates to antibiotics is beneficial as it minimizes the chances of disseminating resistance genes to pathogens both in the food matrix and/or in the gastrointestinal tract.

Results from the antibiotic sensitivity study are supported by the work of Zeng *et al.* (2010), who observed*L. fermentum* strains to be susceptible to erythromycin and chloramphenicol. In another study by Zhou *et al*. (2005), a strain of *L. fermentum, L. fermentum* A8, was also found to be susceptible to chloramphenicol, erythromycin, gentamicin, streptomycin and tetracycline assayed by the antibiotic disk diffusion tests. Similar findings were also reported by Kaktcham*et al*., (2012)and Udhayashree *et al.,* (2012).

The results from this study were also corroborated byHalder and Mandal, (2016). They found curd isolates of *L. fermentum*to be either sensitive or intermediately susceptible to

the antibiotics, chloramphenicol, gentamicin and tetracycline. Strains with intermediate susceptibility are safe to be used as probiotics because the chances of transferring low level of resistance (intermediate susceptibility) are limited since such resistance is intrinsic and not plasmid mediated (Halder and Mandal, 2016).

In order for probiotic bacteria to fulfil their physiological role in the gut, the bacteria must overcome a number of stresses before they reach the target site (Nagpal *et al.,* 2012). The acidic environments encountered both in food and in the gastrointestinal tract provide a significant survival challenge for probiotic organisms. The antibiotic susceptible strains of *L. fermentum* grew well at pH 4(Tables 4a-c). Their viability was however affected more at pH 3because of increased acidity. After 3h of incubation at pH3, it was observed that 60% of antibiotic susceptible strains of *L.fermentum*from Nunu, 55.6% from Akamuand 28.6% from GarriFCM were tolerant to pH3. Four strains showed greater than 50% survival rate at pH 2 and were selected for further studies. *L.fermentum strain MGB 32-1*recorded the highest survival rate of 61.9% at this pH while *L.fermentum cc IMAU:*80780recorded the lowest survival rate of 53.9%. According to Prasad *et al.*(1998) and Chan *et al*. (2011),the threshold point to state acid resistance in this study was set at pH = 2 for 3 hours incubation, as it simulates bacterial residency in the stomach.

Chan *et al*. (2011), reported that acids such as the hydrochloric acid (HCl) found also in human stomach, disrupt the biomolecules of cells such as fatty acids, proteins and DNA. Low pH environments can inhibit the metabolism and reduce the growth and viability of lactobacilli. Other studies also confirmed that exposure to acidic pH $\leq$ 2 after 3h incubation caused a reduction in the viable count of the bacteria intensively (Mandal*et al.,* 2006). Results of this study showed good survival ability in the strains of *L.fermentum*isolated from the fermented foods. This finding is supported by that of Srinu *et al.,* (2013) who noted that strains of *L. fermentum; L fermentum* 141 and *L fermentum* 156 obtained from National Dairy Research Institute (NDRI, Karnal) showed good survival abilities in tested acidic pH ranges 2 to 3.5. They also observed that the strain *L fermentum* 141 was able to grow even at pH of 1.5. According to Delgado *et al.* (2014), *Lactobacillus* species (*L. gasseri, L. fermentum, L. vaginalis* and *L. reuteri*) isolated from human stomach showed good tolerance and survival at low pH, indicating their capacity to survive in the human stomach.A number of other studies equally corroborate the findings from this study. Archer and Halami, (2015) observed high tolerance to acid and bile of

twelve*L. fermentum* isolates selected from indigenous fermented dairy products and infant faecal samples. Pereira and Gibson (2002),also demonstratedthe ability of *L. fermentum* KCto maintain viability for 2h at pH 2 and to grow in a medium with 4 mg of bile acids per litre.

The detergent property of bile confers potent toxicity, primarily through the dissolution of bacterial membranes (Begley *et al.,* 2005). Therefore, for a probiotic strain to be able to perform effectively in the gastrointestinal tract, it must overcome the antimicrobial challenge posed by bile. Thus in vitro resistance to bile has become necessary in screening potential probiotic strains as one of the physiologically relevant stresses in the gastrointestinal tract. According to Fuller (1992), bile, even at low concentrations, can inhibit the *in vitro* growth of microorganisms.

In the present study, the four strains selected were able to grow in the different concentrations of fresh bovine bile used (Figures 1-3). At the highest concentration of bile (10%), a survival rate of between 51.6% to 60.1% was observed. This resultagrees with previous reports of Srinu*et al.* (2013),who observed that all the lactic acid bacterial strains used in their study survived and tolerated bile salts concentrations of 0.3 to 2.0 % quite effectively. Similar observations were also reported by Barakat *et al.* (2011). In another related study by Mikelsaar and Zilmer,(2009), *L. fermentum* ME-3 was also reported to tolerate the tested bile concentrations (0.3–2.0%) similarly well during 24 h without any remarkable loss in viable counts. Also, *L. fermentum* FTL2311 and *L. fermentum* FTL10BR were reported by Klayraung and Okonogi(2009), to be able to tolerate relatively low concentrations of about 0.3-1% bile.

The mechanism of bile salt resistance could be the ability to deconjugate bile salts. This feature relates also to its capacity to remove cholesterol from the intestinal environment (Begley *et al*., 2005), reduce serum cholesterol and also to produce a detergent-shock protein that enables lactobacilli to survive exposure to bile (De Smet*et al*., 1995). Therefore, as with other *L. fermentum* strains, these fourstrains can be considered intrinsically resistant to human upper gastrointestinal transit.

Determination of microbial adhesion to hydrocarbons as a way to estimate the ability of strain to adhere to epithelial cells is a valid qualitative approach and considered an important characteristics of probiotic lactic acid bacteria. Adhesion verifies the potential of

the strain to inhabit the intestinal tract and to grow in intestinal conditions. Ly *et al*. (2008), confirmed that bacteria possess physicochemical surface properties such as hydrophobicity which are involved in physicochemical interactions between cells and interfaces.

In this study, variable degrees of hydrophobicity by the isolated strains were seen (Table 5). *Lactobacillus fermentum* CECT 5716 had the highest level of hydrophobicity. A high value of hydrophobicity could be a sign of a greater capability of bacteria to adhere to the epithelial cells of the host as indicated by Rosenberg *et al.* (1980).

*Lactobacillus fermentum*is a normal resident of the human gut microflora and has been reported to adhere to the epithelial cells, with a preference for the small intestine (Rojas *et al.,* 2002). It has also been shown by other researchers to colonize the intestine after oral administration (Reid *et al.,* 2001) and to promote the survival of healthy intestinal microflora (Wickstrom*et al.,* 2013).

As reported by Qing (2015), *L. fermentum* L9-1 exhibited cell surface hydrophobicity of 63.15 ± 0.57 while *L. fermentum* 9shgave 59.70 $\pm$ 1.78 when measured by xylene extraction. Tejpal (2009), on investigating the adhesive properties of potential probiotic lactobacilli isolated from food and faeces discovered that *L.fermentum* showed a remarkable 73% hydrophobicity in xylene. Conversely, in another study by Okafor and Umeh (2013), five strains of *Lactobacillus* evaluated for hydrophobicity towards xylene displayed variable degrees of hydrophobicity with an average of 24.6%.

It is usually expected that potential probiotic lactobacilli should be capable of inhibiting the growth of pathogens (Mahasneh and Abbas, 2010; Khay *et al*., 2011; Kazemipoor *et al*., 2012; Rushdy and Gomaa, 2013). The prevention of gastrointestinal tract colonization by a variety of pathogens is a primary mechanism of beneficial effects mediated by probiotics (Lu and Walker, 2001; Ljungh and Wadstrom, 2006). It has been shown that the large spectrum of different metabolites is responsible for the suppression of the growth of pathogens *in vitro* and for their competitive exclusion in animal models. Many of the metabolites produced by lactic acid bacteria have a broad antimicrobial activity against some other species, especially Gram-negative ones (Ouwehand *et al.,* 2005). In this study, all isolates except *L. fermentum* strain MGB 32-1 showed good inhibition against *Salmonella* sp. (Table 6). *L. fermentum* CECT 5716 had inhibitory activity against the

Gram negative bacteria used in the study but did not inhibit the growth of *Staphylococcus* sp. Only *L. fermentum* F-6 inhibited *Staphylococcus* sp.

No inhibitory activity was observed against *Lactobacillus* and *Streptococcus* species sourced from commercial yoghurt.This shows that there exists no antagonism between the *L. fermentum* strains and the starter cultures(Table 6). Therefore, both can exist together in fermented products if they are to be used as adjunct cultures. This agrees with the work of Annuk (2002), who demonstrated that *L. fermentum* ME-3 has the ability to suppress mainly gram-negative bacteria and to some extent *Staphylococcus aureus.* Other studies by Abbas and Mahasneh (2014), have also demonstrated the inhibitory potential of *L. fermentum* isolates against Gram-positive bacteria *Bacillus cereus,* methicillin resistant *S. aureus* (MRSA) and Gram-negative bacteria (*E. coli* ATCC 25922 and *S. typhimurium* ATCC 14028).

Lactobacilli are the dominant bacteria of a healthy human vagina. They have antagonistic effect on potentially pathogenic microorganisms and vaginal pathogens (Purkhayasthai *et al*., 2015). All isolates in this study displayed inhibitory activity against *Candida* sp. The work of Rönnqvist *et al.*(2007), confirms this report. They stated that*L. fermentum* Ess-1 has great potential to be used as a probiotic to treat symptomatic vulvo-vaginal candidiasis (VVC) or to prevent recurrent VVC infection, and have shown exceptional fungistatic properties against the two most common yeast species associated with VVC, *C. albicans* and *C. glabrata*.

Other *invivo* studies have also shown the effectiveness of probiotics in *Candida* infection. Wagner *et al*. (1997), and Matsubara *et al.*(2012), demonstrated that the inoculation of probiotics (*L. acidophilus, L. reuteri, L. casei* GG, and *B. animalis*) in immunodeficient mice reduced the incidence of systemic candidiasis, and prolonged the survival of adult and neonatal mice. Abedin *et al.*(2013),have also demonstrated that a naturally occurring probiotic *L. fermentum* strain in human dairy food has a potential and promising anti-fungal activity.

Probiotics have been used as growth promoters due to their ability to suppress the growth and activities of growth depressing microflora and their ability in enhancing the absorption of nutrients through the production of digestive enzymes (Fuller and Gibson, 1997). In this study, it can be stated that the strains of *L. fermentum* promoted the growth of the rats

since after 2 weeks administration of fermented milk, the final mean weight of the treatment groups were all observed to be significantly higher than the controls (Figures 4-6). The mean weights of the male and female rats fed 1ml of fermented milk for 13 weeks (Figures 25 and 26) were also noted to be significantly higher than controls.

In support of this study, Xie *et al*. (2011), found that the administration of lactobacilli significantly improved weight again and food efficiency compared to the control group. Similar findings were made in rats by Aboderin and Oyetayo (2006), and Akanbi and Agarry (2014), using *Lactobacillus plantarum* and Guo (2012),using *L. salivarius* G1-1,*L.reuteri* G22-2 and *L. reuteri* G8-5. Studies by Vijayendra (2012), indicate that supplementation of probiotic cultures helped to improve the body weight of albino rats (Wister) after 30 days of feeding, with a weight gain of 123.33 and 129.33 g with probiotic yogurt and probiotic Dahi, respectively. Also, Anukam (2005) found a 30% improvement in the birth weight of newborn Sprague-Dawley albino pups, whose mothers were fed probiotic *Lactobacillus* strains GR-1 and RC-14, when compared to controls.

These resultsare in line with the findings of other studies that used broiler chickens (Islam *et al*., 2004; Singh *et al*., 1999). Consumption of probiotics have also been found to result in an increase in the daily weight gain, the egg production, shell weight, shell thickness and yolk of leghorn chickens (Panda *et al*., 2003). Kalavathy *et al*. (2003), also reported that, dietary supplementation of a mix culture of twelve strains of *Lactobacillus* at 1% in the basal diet of broilers resulted in higher body weight gain. It has also been reported that the consumption of *Lactobacillus sp.* by newborn ducks and chicks resulted in weight gain. A significant increase in body weight and liver mass was noted after a second dose of *Lactobacillus* administration in newborn chicks (Angelakis and Raoult, 2010). According to Strompfova (2005), the addition of *L. fermentum* AD1 strain increased the weight gain of quail by 14% after 7 days from the beginning of its application.

The findings of this work was further supported by that of Wang *et al.* (2009a),who revealed that *L. fermentum* I5007 improved weight gain. Okafor and Umeh (2013), also observed that the weight of the rats in the test groups which were fed milk fermented by *Lactobacillus* spp. were significantly higher ($p<0.05$) than control group (fed milk only). A similar observation was made by Oyetayo (2004), using rats dosed with *L. acidophilus* and simultaneously challenged with *E. coli* treatments and found that they had better performances when compared with the control for total weight gain.

Results from this study appear not to be supported by some other studies such as Chiu *et al*. (2006), where the groups of hamsters used showed no significant differences in body weight gain irrespective of administration of lactobacilli or not. Similarly, Wang *et al*. (2009b), found no significant differences in body weight gain in animals supplemented with a strain of *L. plantarum* and the control group.

The ability of isolates to protect the GIT against pathogens can be confirmed by monitoring the count of enterobacteria and beneficial bacteria especially lactobacilli. In this study, intestinal colonization ability and faecal microbiota changes were evaluated *invivo* using the rat model. *Lactobacillus* count was increased while enterobacteria count was reduced significantly in the treatment groups (Figures 7-12). The recovery rate in faeces suggests good colonization ability of the strains and bacterial adhesion to the intestinal mucosa. Similar reports by Wang *et al*. (2010) demonstrate significant increase in *Lactobacillus* and decrease in faecal coliform. Yang*et al*. (2005) also observed reduced faecal coliform counts due to appropriate beneficial role of *Lactobacillus* and *Bifidobacterium* proliferation and the inhibited invasion of pathogens in rat gut.

A similar observation was made in a study by Zavisic*et al.* (2012), who indicated that after 7 days of lactobacilli administration to Wistar rats, the strains G1 and G3 were re-isolated from the faecal samples, as well as from the ileum surface. It was also noted that after the 7-day treatment of the Wistar rats with the strain G1, a significant decrease in *E. coli* populations was observed. In another study by Gomathi (2014), three strains of LAB(*L. fermentum* AB1, *L. fermentum* TY5, and *L. salivarius* AB11)hada significant increase. According to Gomathi, (2014), the *Lactobacillus* count was increased while coliform count was reduced significantly in the groups of rats studied. Works of Okafor and Umeh (2013), also indicate that the *Lactobacillus* AC, AD and AE administered to the albino rats survived the gastrointestinal tracts of the rats during the feeding period.

Survival of the intestinal transit and at least transient colonization are the main preconditions for microorganisms to be of any beneficial effect after consumption (Iyer *et al*., 2010). A number of reports indicate that several probiotic agents are able to inhibit the adherence of pathogenic bacteria to intestinal epithelial cells through their ability to increase the production of intestinal mucins (Mack *et al*., 1999; Servin and Coconnier, 2003). Oyetayo (2004), noted a reduction in the count of enterobacteria in rats dosed with

L. casei after 3 days of feeding trials. This supports the findings made in this study where a reduction in enterobacteria count was also observed after 2 weeks of feeding trial.

Similar observations were also made by Casas and Dobrogosz (2000), who monitored the count of enterobacteria especially *E.coli* and beneficial bacteria especially lactobacilli in goats faeces and observed an increase in fecal lactobacilli count in goats treated with lactobacilli and a slight decrease in enteric bacteria count. Earlier reports have also shown that selected probiotic strain *L.reuteri* and *L.acidophilus* have an increasing effect in the numbers of enterobacteria in piglets (Ratcliff, 1958). The ability of lactobacilli to produce toxic metabolites such as lactic acid, hydrogen peroxide (H_2O_2) and bacteriocins has been suggested as being responsible for their ability to inhibit other bacteria (Juven, 1992). Other factors such as host immunomodulation also play a prominent role (Fujiwara, 2001).

Hepatocytes play a major role in absorbing and metabolizing many toxic chemicals (Eka *et al.*, 1994). They are therefore liable to injury by various chemicals including food. Alanine aminotransferase and aspartate aminotransferase are liver function parameters. Their increase in the general circulation of the body system implies liver dysfunction. In the present study, no dose-related effects and no significant differences were observed after 2 weeks of consumption of fermented milk (Figures 13-18). No significant differences were also observed in the levels of the enzymes in the serum of the albino rats after 13 weeks administration of the fermented milk (Figures 27-30).

Contrary reports to this study were made by Okafor and Umeh (2013), who observed a significant reduction in the level of ALT and AST in the serum of albino rats. A similar observation was made byKirpich *et al*. (2008) in a pilot study using alcoholic subjects and Islam *et al*. (2004), in broiler chickens. Other studies have also shown a significant decrease in serum levels of the liver enzymes ALT and AST, with the consumption of *Saccharomyces boulardii* in broilers (Agawane and Lonkar, 2004) and *L. acidophilus*, *Lactobacillus rhamnosus* ATCC 53103, *L. rhamnosus* DSM 6594 and*L. plantarum* DSM 9843 in an acute liver injury model (Adawi *et al.*, 2001).

However, in contrast to the findings made in this study,Harikrishnan *et al.*(2011),reported a rise in serum levels of these enzymes by *S. parauberis* (2.1×10^7 CFU/ml) in animal models. It seems that the effect of probiotics on serum levels of ALT and AST is dependent on the species and strains of probiotic. The addition of *Lactobacillus plantarum*

and *Bifidobacterium infantis* to the rat feed resulted in lowered levels of serum ALT, (Osman *et al.,* 2007) but consumption of *Saccharomyces cerevisiae* caused a significant increase in serum ALT levels in rats (Mannaa *et al.,* 2005).

In this study, the administration of fermented milk products to albino rats for 2 weeks and 13 weeks did not affect the liver enzymes. Other reports also corroborated this finding. In the sub-acute studies by Ohhira (2000), there was no significant differences in ALT and AST levels between rats in the control group and the treatment groups (rats fed with OMX probiotics capsules). Findings by Huang *et al.* (2014),also revealed no significant difference in ALT and AST between the dosage groups and the control group. Similarly, Asemi and Esmaillzadeh (2013), did not find any significant effect of probiotic yogurt consumption on serum AST and ALT levels compared with conventional yoghurt. Thomas and Lee (1999), recorded no significant difference in ALT levels between rats fed with lactic acid bacteria capsules and the control group. Also, Sadiek and Boehm (2001), demonstrated that the activities of AST and ALT were normally and nearly the same in control and probiotic-treated animals. Oo *et al.* (2016), recorded no significant difference for AST after 3months.

Results from this study were also in accord with the findings of Afify *et al.* (2012), who observed that the aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) showed non-significant changes between two treatments (biscuit with probiotic bacteria and without probiotic bacteria diets) and the normal control group used in their study. Similarly, the ALT, AST and ALP analyses results of Huang *et al.* (2014), for male rats revealed no significant difference between any of the dosage groups and the control group. For the female rats also, there was no significant difference in ALT and AST between any of the dosage groups and the control group.

The level of ALP in male and female rats fed fermented milk for 2weeks and 13 weeks showed no significant differences between the treatment groups and the control groups (Figures 19-21) and (Figures 31-32). From the results of the analyses of these liver enzymes,it could be deduced that the *Lactobacillus fermentum*-fermented milk had no deleterious effects on the animal health and are therefore presumed to be safe for consumption.

To further assess the safety of the bacteria strains, the haematological parameters of the rats were also investigated. For the male and female rats fed 1ml of fermented milk for 13 weeks (Tables 7 and 8), the HGB, RBC, PCV, MCV, MCH of all treatment groups were all significantly higher than the controls. A high level of Hb, PCV and RBC is an indication that the rats are not anaemic while a lower level is a sign of anaemia (Cheesborough,1991). The results of the haematological parameters show that rats dosed with *Lactobacillus fermentum*-fermented milk showed signs of better health. This could be due to increased nutrient absorption. These findings are consistent with those of Islam *et al*. (2004), Hossain *et al*. (2005) and Awad *et al*. (2009).The work of Salahuddin *et al.*(2013),showed a dose dependent increase in total erythrocyte count and haemoglobin concentration.Iyer *et al*. (2010),also found an increase in HCT, HGB, RBC in rats fed 10^7 cfu/ml of *Streptococcus thermophillus*-fermented milk compared to control. Similar results were obtained in another study with *Lactobacillus*strains by Okafor and Umeh, (2013).

Organ weights are widely accepted in the evaluation of test article-associated toxicity (Wooley, 2003). Changes in organ weights can be another sign for toxic effects of a test substrate in short-term toxicity tests (Abotsi *et al.,* 2011). In this study,there were no significant changes ($p>0.05$) in the relative weights of the heart, liver, spleen, kidney and brain of all the treated rats in relation to control groups (Tables 9 and 10). This indicates that the *Lactobacillusfermentum* strains did not cause any adverse effects on any of the organs. A similar finding was made by Shokryazdan *et al.*(2016). This result was also supported by histopathological examinations of the organs which did not reveal any abnormalities.

Exposure to bacterial products of intestinal origin leads to liver inflammation, hepatocyte injury and hepatic fibrosis (Shanab *et al.,* 2011). *Lactobacilli* can translocate and survive in the spleen, liver, and kidney (Bloskma, 1981). In the course of their translocation, they can cause cellular injury that may increase AST and ALT level in the serum. In this study no significant higher ALT and AST values were observed. Moreso, photomicrograph slides (Plates 1-12) showed no tissue inflammation in the liver and kidney. Histopathological evaluation of the liver and kidney shows that the fermented milk products were well tolerated by the rats used in the study and suggests no bacterial

translocation. No apparent differences were found between rats from the four study groups and the control groups and there were no histological indications of inflammation.

Probiotics have infact been suggested as a treatment for different types of chronic liver damage because of their abilities to augment intestinal barrier function and to prevent bacterial translocation (Cesaro *et al.,* 2011). The administration of probiotics have been reported to reducebacterial translocation in a rat model (Zhou *et al.,* 2010). The effect was suggested to be the result of an immune modulatory effect and the maintenance of gut barrier integrity (Generoso *et al.,* 2010). Diya *et al.* (2001) actually found *Lactobacillus plantarum* BJ0021 to decrease bacterial translocation.

It has been predicted that by 2030, cardiovascular diseases will remain the leading cause of death, affecting approximately 23.6 million people around the World (WHO, 2009). High level of serum cholesterol has been associated with risks of coronary heart disease. (Anderson and Gilliland, 1999; Agerholm *et al*., 2000; Pereira and Gibson, 2002; Pereira *et al*., 2003). People affected with hypercholesterolemia may avert the use of cholesterol-lowering drugs by practising dietary control or supplementation of probiotics and/or prebiotics.

Human studies have shown promising evidence that well-established probiotics possess hypocholesterolemic effects, while new strains of probiotics have been evaluated in animal models for their potential hypocholesterolemic effects. Many studies have used rats (Gallaher *et al.,* 2000), mice (Lichtman *et al.,* 1999), hamsters (Lin *et al.,* 2004), guinea pigs (Madsen *et al.,* 2007) and pigs (Patterson *et al.,* 2008)

This study showed that the albino rats administered 1ml each of fermented milk fermented by the test strains(*Lactobacillus fermentum* F-6, *Lactobacillus fermentum* MGB 32-1 and *Lactobacillus fermentum cc* IMAU: 80780)for 2 weeks had significant lower serum cholesterol levelsthan the control (Figure 24). All the test strains demonstrated significant reduction in the serum cholesterol level of both male and female rats after 13 weeks consumption of 1ml of fermented milk containing approximately 10^8cfu/ml daily (Figures 33-34). T-test conducted revealed that there was no significant difference in the effect of the treatment on the male rat when compared to the female rats.

Comparable findings have been reported by other researchers. In a study evaluating the effect of *L. plantarum* PH04 isolated from infant faeces, on cholesterol, Nguyen *et al.* (2007), administered *L. plantarum* (4×10^8 CFU/ml dose per mouse daily) to twelve male mice for 14 days. The authors found a significant ($P< 0.05$) reduction of total serum cholesterol (reduced by 7%) compared to the control. In another study, El-Gawad *et al.* (2005), fed forty-eight male albino rats (average weight 80–100g) with 50 g of yogurt [contained 0.07% (w/v) *Bifidobacterium longum* Bb-46] daily for 35 days. The administration of *B. longum* Bb-46-fermented buffalo milk-yogurt significantly reduced concentration of total cholesterol by 50.3%, compared to the control ($P< 0.05$). In another study, Fukushima *et al.* (1999), found that male Fischer rats (8 week old) fed with 30 g/kg of *L. acidophilus*-fermented rice bran significantly showed an improved lipid profile compared to the control.

Other studies equally corroborate data obtained from this study which shows a significant reduction in the serum cholesterol level in the rats administered 1ml of fermented milk, fermented by *L. fermentum*f6, *L. fermentum* MGB 32-1 and *L. fermentum* cc IMAU:80780 for 2 weeks. Fazeli *et al*. (2010) showed that the consumption of *L. plantarum* A7 (10^8 CFU ml^{-1}) for 14 days is effective in lowering serum lipid levels in rats. Taranto *et al.* (2000), reported that, administration of *Lactobacilllus reuteri* was effective in preventing hypercholesterolemia in mice and observed a decrease in total cholesterol (22%). Park *et al.* (2007), also found that the supplementation of *L. acidophilus* ATCC 43121 (2×10^6 CFU/day) for 21 days reduced total serum cholesterol by 25% compared to the control in 36 male Sprague-Dawley rats.

Numerous other reports confirm the hypocholesterolaemic effect of *L. fermentum* strains. *L. fermentum* SM-7 isolated from a fermented milk drink (koumiss) was found to significantly reduce serum total cholesterol in mice. Another study also consistently showed significant reduction by about 25% of serum total cholesterol in rats fed *L. fermentum* 9-41-A (Pan *et al*., 2011). Vijayendra and Gupta (2012), observed a significant reduction of serum cholesterol level of 2.63, 4.1 and 4.68 mg/100 ml at the end of 30 days in rats fed with yoghurt, probiotic *dahi* and probiotic yoghurt, respectively, indicating the hypocholesterolaemic effect of the probiotic cultures.Sindhu and Khetarpaul (2003), reported that the feeding of *L. casei* NCDC-19 (10^9 CFU) and *Saccharomyces boulardii* (10^9 CFU) caused a 19% reduction in the total serum cholesterol, after the 42 day feeding

trial. In another study, De Rodas *et al.* (1996), reported that pigs fed with *L. acidophilus* ATCC 43121 (2.5×10^{11} cells per feeding) for 15 days showed a reduced total blood cholesterol by 11.8% compared to the control.

Results from this study are further supported by that involving human subjects. According to Anderson and Gilliland (1999),daily consumption of 200 g of yogurt containing *L. acidophilus* L1 after each dinner for 10 weeks contributed to a significant ($P< 0.05$) reduction in serum cholesterol concentration compared to the placebo group. In another study, Xiao *et al.* (2003), evaluated the effects of a low-fat yogurt containing 10^8 CFU/g of *B. longum* BL1 on lipid profiles of thirty-two subjects and showed a significant ($P< 0.05$) decline in serum total cholesterol after 4-weeks.

Although numerous studies have demonstrated convincing cholesterol-lowering effects of probiotics in both animals and humans, contrary results exists. A study by Hatakka *et al.* (2008), refuted the purported hypocholesterolemic effect of probiotics, and reported that the administration of *L. rhamnosus* LC705 (10^{10} CFU/g per capsule; two capsules daily) did not influence blood lipid profiles in thirty-eight men with mean cholesterol levels of 6.2 mmol/L after a 4-week treatment period. Lewis and Burmeister (2005), conducted a randomized, placebo-controlled double blind and crossover designed study oneighty volunteers who consumed two capsules containing freeze-dried *L. acidophilus* (3×10^{10} CFU/2 capsules) three times daily for six weeks and found that *L. acidophilus* capsules did not significantly change plasma total cholesterol of the subjects.

Several mechanisms proposed for the cholesterol-lowering effects of probiotics include; the enzymatic deconjugation of bile acids by bile-salt hydrolase of probiotics, the ability to bind cholesterol in the small intestines, the incorporation of cholesterol into the cellular membranes during growth and the conversion of cholesterol in the intestines to coprostanol, which is directly excreted in faeces. This decreases the amount of cholesterol being absorbed, leading to a reduced concentration in the physiological cholesterol pool. However, the mechanism underlying the hypocholesterolemic effect of probiotics might be strain-specific.

pH is a measure of acidity or alkalinity of a product sample. The results of the pH analysis (Table 11) show that all the samples of fermented milk produced in this study using the test strains were in the acidic range of pH value with *L.fermentum* F-6-fermented milk

samples being most acidic with a pH value of 4.49. The titratable acidity of the samples ranged from 0.84 to 0.87. The significance of acidic pH value in foods cannot be overemphasized especially for fermented foods as they help in the preservation of food samples (Uriahand Izuagbe 1990). The presence of organic acid is responsible for low pH in the samples. These results are in line with the findings of Salji *et al.* (1985) and Sutherland and Varnam (1994) who reported yogurt pH as 4.50. Also, Sokolinska *et al* (2004), stated that the pH values of milk decreased during the manufacturing process which is as a result of the ability of lactic strains to ferment lactose into lactic acid with an increase in acidity and a decrease in pH of fermented milk. A similar report was made by Zourari *et al*. (1992), who stated that the lactic acid that is produced from the fermentation of lactose contributes to the sour taste of yoghurts by decreasing the pH and allows for the characteristic texture by acting on the milk protein.

Proximate analysis is used to determine if a food is within its normal compositional parameters. The proximate composition of the fermented milk samples fermented by strains of *Lactobacillus fermentum* (Table 12) showed that the moisture content ranged from 87.08 to 87.89. The high moisture content could be as a result of reconstitution of the milk prior to fermentation. Comparable results were made by Igwegbe *et al.* (2015), and Olugbuyiro and Oseh (2011), in commercial yoghurt. The protein content ranged between 3.47 and 3.5. These results are in line with the findings of Janhoj and Michael (2006), who reported that the protein contents of low-fat stirred yogurt ranged from 3.4 to 6.0%. The fat content in the test samples ranged between 0.59 and 0.61. The low fat content of the fermented milk samples is attributed to the low fat content of the skimmed milk used. According to USDA (2001), yoghurt with fat content within the range of 0.5 to 2.0% should be labeled “low fat yoghurt”. The ash content is a measure of the total amount of minerals present within a food. The quality of many foods depends on the concentration of minerals they contain. The ash content of the samples in this study was between 0.69 and 0.72. Comparable findings of between 0.26-0.71,were also made by Olugbuyiro and Oseh, (2011) with different commercial yoghurts.

The fermented milk samples, fermented by all the test strains had a firmer texture compared to the spontaneously fermented Nunu(Figure 35).This finding is supported by Domaga (2009), who reported that powdered milk yoghurt has a firm texture. The higher value in taste for Nunu may be due to its higher fat content and fat improves taste of

yoghurt. This is supported by the findings of Onweluzo and Nwakalor (2009), who reported that fat is known to promote good mouth feel. It was also noted from this study that the spontaneously fermented Nunu had the lowest aroma which may be due to off flavours introduced by contaminants. Based on the overall acceptance, the test samples were preferred to the spontaneously fermented control and there was no statistically significant preference among the test samples. Akabanda *et al.* (2014), stated that*Lactobacillus fermentum, L. helveticus,* and *L. plantarum* starter cultures, whether used alone or in combination, were able to produce yoghurt with desirable consumer sensory characteristics.

6.0 CONCLUSION

This preliminary study showed that out of seventy-five strains of *L. fermentum*isolated from the three fermented foods,four strains were found to have desirable probiotic characteristics. Results from the *in vivo* study indicate that these strains colonized the albinorats used in the study and influenced their well being.The present finding also showed that these 4 strains of *L. fermentum*displayedgood probiotic characteristics in terms of weight gain, safety of use and hypocholesterolemic effect. However, *L. fermentum* F6 was selected as the best of the four strains because itshowed signs of better health based on haematological status and performance in terms of weight gain. It recorded the highest weight gain in both male and female rats which suggeststhat it is the best growth promoter. It also had the most hypocholesterolemic effect which was observed to be dose dependent. This implies that *L. fermentum* F6-probiotic fermented milkcould also be included as part of a natural and safe method of preventing hypercholesterolaemia and maintaininggoodlevels of cholesterol in man and animals, with no adverse effects occurring.

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8.0 APPENDICES

Appendix i: Composition of basal diet

Ingredients	level in diet
Crude protein	19%
Fat	8.6%
Crude fibre	5.4%
Calcium	1.2%
Phosphorus	0.4%
Lysine	0.8%
Methionine	0.3%
Metabolisable energy,	kcal/kg 2,900

Manufactured by Vital Feed, Plateau State, Nigeria

Appendix ii: Composition of some reagents

A. Reagents for Alanine aminotransferase assay:

Reagent 1: Phosphate buffer (pH 7.4), L-alanine, alpha-oxoglutarate

Reagent 2: 2,4-dinitrophenylhydrazine

Reagent 3: Sodium hydroxide (0.4mol/l)

B. Reagents for Aspartate aminotransferase assay:

Reagent 1: Phosphate buffer (pH 7.4), L-aspartate, alpha-oxoglutarate

Reagent 2: 2,4-dinitrophenylhydrazine

Reagent 3: Sodium hydroxide (0.4mol/l)

C. Reagents for Alkaline phosphatase assay:

Reagent 1: Diethanolamine buffer 1 mol/l, (pH 9.8)

$MgCl_2$ (0.5mmol/l)

Reagent 2: p-nitrophenylphosphate (10 mmol/l)

D. Reagent for Serum cholesterol determination:

Reagent- Pipes, Sodium cholate, phenol, cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine, pH 7.0.

Cholesterol standard- Cholesterol (5.18mmol/L)

Appendix iii: Effect of 2 weeks consumption of *Lactobacillus fermentum*-fermented milk on the weight of rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
weight0.1	*c*	6	193.4000	12.17144	4.96897	180.6269	206.1731	177.55	209.14
	CM	6	198.5517	9.35423	3.81885	188.7350	208.3683	186.72	209.88
	L.fermentum strain MGB 32-1	6	214.9383	8.31127	3.39306	206.2162	223.6605	204.78	225.34
	L.fermentum F-6	6	218.6650	10.90991	4.45395	207.2158	230.1142	204.63	232.45
	L.fermentum CECT 5716	6	211.4267	9.47134	3.86666	201.4871	221.3662	200.38	222.22
	L.fermentum cc IMAU:80780	6	220.5717	7.24947	2.95958	212.9638	228.1795	211.39	229.65
	Total	36	209.5922	13.67312	2.27885	204.9659	214.2185	177.55	232.45
weight0.5	*c*	6	193.4000	12.17144	4.96897	180.6269	206.1731	177.55	209.14
	CM	6	200.5200	9.35243	3.81811	190.7052	210.3348	188.46	211.75
	L.fermentum strain MGB 32-1	6	221.6917	6.63507	2.70876	214.7286	228.6547	213.51	229.87
	L.fermentum F-6	6	223.6233	6.49857	2.65303	216.8035	230.4432	215.84	231.22
	L.fermentum CECT 5716	6	215.6283	5.96105	2.43359	209.3726	221.8841	208.32	222.90
	L.fermentum cc IMAU:80780	6	220.1667	7.64198	3.11983	212.1469	228.1864	210.31	229.68
	Total	36	212.5050	13.92805	2.32134	207.7924	217.2176	177.55	231.22
weight1	*c*	6	193.4000	12.17144	4.96897	180.6269	206.1731	177.55	209.14
	CM	6	203.6083	8.42594	3.43988	194.7659	212.4508	193.85	215.33
	L.fermentum strain MGB 32-1	6	223.8633	8.48446	3.46377	214.9594	232.7672	213.26	234.45
	L.fermentum F-6	6	227.8800	7.55792	3.08551	219.9484	235.8116	218.83	236.66
	L.fermentum CECT 5716	6	219.4650	6.54758	2.67304	212.5937	226.3363	211.41	227.40
	L.fermentum cc IMAU:80780	6	225.6067	7.27857	2.97146	217.9683	233.2451	216.82	234.70
	Total	36	215.6372	15.16122	2.52687	210.5074	220.7670	177.55	236.66

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
weight0.1	Between Groups	3713.349	5	742.670	7.873	.000
	Within Groups	2830.050	30	94.335		
	Total	6543.399	35			
weight0.5	Between Groups	4710.659	5	942.132	13.595	.000
	Within Groups	2079.007	30	69.300		
	Total	6789.666	35			
weight1	Between Groups	5824.707	5	1164.941	15.739	.000
	Within Groups	2220.485	30	74.016		
	Total	8045.192	35			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) Organism weight	(J) Organismweight	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
weight0.1	*c*	*CM*	-5.15167	5.60758	.366	-16.6039	6.3005
		L.fermentum strain MGB 32-1	-21.53833*	5.60758	.001	-32.9905	-10.0861
		L.fermentum F-6	-25.26500*	5.60758	.000	-36.7172	-13.8128
		L.fermentum CECT 5716	-18.02667*	5.60758	.003	-29.4789	-6.5745
		L.fermentum cc IMAU:80780	-27.17167*	5.60758	.000	-38.6239	-15.7195
	CM	*c*	5.15167	5.60758	.366	-6.3005	16.6039
		L.fermentum strain MGB 32-1	-16.38667*	5.60758	.007	-27.8389	-4.9345
		L.fermentum F-6	-20.11333*	5.60758	.001	-31.5655	-8.6611
		L.fermentum CECT 5716	-12.87500*	5.60758	.029	-24.3272	-1.4228
		L.fermentum cc IMAU:80780	-22.02000*	5.60758	.000	-33.4722	-10.5678
	L.fermentum strain	*c*	21.53833*	5.60758	.001	10.0861	32.9905
		CM	16.38667*	5.60758	.007	4.9345	27.8389

	MGB 32-1	*L.fermentum F-6*	-3.72667	5.60758	.511	-15.1789	7.7255
		L.fermentum CECT 5716	3.51167	5.60758	.536	-7.9405	14.9639
		L.fermentum cc IMAU:80780	-5.63333	5.60758	.323	-17.0855	5.8189
		c	25.26500*	5.60758	.000	13.8128	36.7172
		CM	20.11333*	5.60758	.001	8.6611	31.5655
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	3.72667	5.60758	.511	-7.7255	15.1789
		L.fermentum CECT 5716	7.23833	5.60758	.207	-4.2139	18.6905
		L.fermentum cc IMAU:80780	-1.90667	5.60758	.736	-13.3589	9.5455
		c	18.02667*	5.60758	.003	6.5745	29.4789
		CM	12.87500*	5.60758	.029	1.4228	24.3272
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-3.51167	5.60758	.536	-14.9639	7.9405
		L.fermentum F-6	-7.23833	5.60758	.207	-18.6905	4.2139
		L.fermentum cc IMAU:80780	-9.14500	5.60758	.113	-20.5972	2.3072
		c	27.17167*	5.60758	.000	15.7195	38.6239
		CM	22.02000*	5.60758	.000	10.5678	33.4722
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	5.63333	5.60758	.323	-5.8189	17.0855
		L.fermentum F-6	1.90667	5.60758	.736	-9.5455	13.3589
		L.fermentum CECT 5716	9.14500	5.60758	.113	-2.3072	20.5972
		CM	-7.12000	4.80625	.149	-16.9357	2.6957
	c	*L.fermentum strain MGB 32-1*	-28.29167*	4.80625	.000	-38.1073	-18.4760
		L.fermentum F-6	-30.22333*	4.80625	.000	-40.0390	-20.4077
		L.fermentum CECT 5716	-22.22833*	4.80625	.000	-32.0440	-12.4127
weight0.5		*L.fermentum cc IMAU:80780*	-26.76667*	4.80625	.000	-36.5823	-16.9510
		c	7.12000	4.80625	.149	-2.6957	16.9357
	CM	*L.fermentum strain MGB 32-1*	-21.17167*	4.80625	.000	-30.9873	-11.3560
		L.fermentum F-6	-23.10333*	4.80625	.000	-32.9190	-13.2877
		L.fermentum CECT 5716	-15.10833*	4.80625	.004	-24.9240	-5.2927

		L.fermentum cc IMAU:80780	-19.64667*	4.80625	.000	-29.4623	-9.8310
		c	28.29167*	4.80625	.000	18.4760	38.1073
		CM	21.17167*	4.80625	.000	11.3560	30.9873
	L.fermentum strain MGB 32-1	*L.fermentum F-6*	-1.93167	4.80625	.691	-11.7473	7.8840
		L.fermentum CECT 5716	6.06333	4.80625	.217	-3.7523	15.8790
		L.fermentum cc IMAU:80780	1.52500	4.80625	.753	-8.2907	11.3407
		c	30.22333*	4.80625	.000	20.4077	40.0390
		CM	23.10333*	4.80625	.000	13.2877	32.9190
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	1.93167	4.80625	.691	-7.8840	11.7473
		L.fermentum CECT 5716	7.99500	4.80625	.107	-1.8207	17.8107
		L.fermentum cc IMAU:80780	3.45667	4.80625	.478	-6.3590	13.2723
		c	22.22833*	4.80625	.000	12.4127	32.0440
		CM	15.10833*	4.80625	.004	5.2927	24.9240
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-6.06333	4.80625	.217	-15.8790	3.7523
		L.fermentum F-6	-7.99500	4.80625	.107	-17.8107	1.8207
		L.fermentum cc IMAU:80780	-4.53833	4.80625	.353	-14.3540	5.2773
		c	26.76667*	4.80625	.000	16.9510	36.5823
		CM	19.64667*	4.80625	.000	9.8310	29.4623
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-1.52500	4.80625	.753	-11.3407	8.2907
		L.fermentum F-6	-3.45667	4.80625	.478	-13.2723	6.3590
		L.fermentum CECT 5716	4.53833	4.80625	.353	-5.2773	14.3540
		CM	-10.20833*	4.96710	.049	-20.3525	-.0642
	c	*L.fermentum strain MGB 32-1*	-30.46333*	4.96710	.000	-40.6075	-20.3192
		L.fermentum F-6	-34.48000*	4.96710	.000	-44.6242	-24.3358
		L.fermentum CECT 5716	-26.06500*	4.96710	.000	-36.2092	-15.9208
weight1		*L.fermentum cc IMAU:80780*	-32.20667*	4.96710	.000	-42.3508	-22.0625
		c	10.20833*	4.96710	.049	.0642	20.3525
	CM	*L.fermentum strain MGB 32-1*	-20.25500*	4.96710	.000	-30.3992	-10.1108

	L.fermentum F-6	-24.27167*	4.96710	.000	-34.4158	-14.1275
	L.fermentum CECT 5716	-15.85667*	4.96710	.003	-26.0008	-5.7125
	L.fermentum cc IMAU:80780	-21.99833*	4.96710	.000	-32.1425	-11.8542
	c	30.46333*	4.96710	.000	20.3192	40.6075
	CM	20.25500*	4.96710	.000	10.1108	30.3992
L.fermentum strain MGB 32-1	*L.fermentum F-6*	-4.01667	4.96710	.425	-14.1608	6.1275
	L.fermentum CECT 5716	4.39833	4.96710	.383	-5.7458	14.5425
	L.fermentum cc IMAU:80780	-1.74333	4.96710	.728	-11.8875	8.4008
	c	34.48000*	4.96710	.000	24.3358	44.6242
	CM	24.27167*	4.96710	.000	14.1275	34.4158
L.fermentum F-6	*L.fermentum strain MGB 32-1*	4.01667	4.96710	.425	-6.1275	14.1608
	L.fermentum CECT 5716	8.41500	4.96710	.101	-1.7292	18.5592
	L.fermentum cc IMAU:80780	2.27333	4.96710	.650	-7.8708	12.4175
	c	26.06500*	4.96710	.000	15.9208	36.2092
	CM	15.85667*	4.96710	.003	5.7125	26.0008
L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-4.39833	4.96710	.383	-14.5425	5.7458
	L.fermentum F-6	-8.41500	4.96710	.101	-18.5592	1.7292
	L.fermentum cc IMAU:80780	-6.14167	4.96710	.226	-16.2858	4.0025
	c	32.20667*	4.96710	.000	22.0625	42.3508
	CM	21.99833*	4.96710	.000	11.8542	32.1425
L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	1.74333	4.96710	.728	-8.4008	11.8875
	L.fermentum F-6	-2.27333	4.96710	.650	-12.4175	7.8708
	L.fermentum CECT 5716	6.14167	4.96710	.226	-4.0025	16.2858

*. The mean difference is significant at the 0.05 level.

Appendix iv: Effect of 2 weeks consumption of *Lactobacillus fermentum*-fermented milk on the viable counts of *Lactobacillus* and enterobacteria.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lactoba 1m	*c*	6	6.5767	.08756	.03575	6.4848	6.6686	6.47	6.69
	CM	6	6.6250	.13035	.05321	6.4882	6.7618	6.46	6.81
	L.fermentum strain MGB 32-1	6	7.1417	.23853	.09738	6.8913	7.3920	6.88	7.52
	L.fermentum F-6	6	7.3333	.13441	.05487	7.1923	7.4744	7.19	7.54
	L.fermentum CECT 5716	6	7.6500	.11628	.04747	7.5280	7.7720	7.47	7.80
	L.fermentum cc IMAU:80780	6	7.2200	.09716	.03967	7.1180	7.3220	7.10	7.35
	Total	36	7.0911	.40868	.06811	6.9528	7.2294	6.46	7.80
Entero1 m	*c*	6	6.7567	.18446	.07531	6.5631	6.9502	6.54	6.97
	CM	6	6.6417	.08658	.03535	6.5508	6.7325	6.52	6.76
	L.fermentum strain MGB 32-1	6	6.5033	.13110	.05352	6.3658	6.6409	6.41	6.76
	L.fermentum F-6	6	6.5500	.14339	.05854	6.3995	6.7005	6.39	6.74
	L.fermentum CECT 5716	6	6.0583	.11035	.04505	5.9425	6.1741	5.93	6.24
	L.fermentum cc IMAU:80780	6	5.7083	.22221	.09072	5.4751	5.9415	5.39	5.96
	Total	36	6.3697	.39838	.06640	6.2349	6.5045	5.39	6.97
Lacto0.5 m	*c*	6	6.5767	.08756	.03575	6.4848	6.6686	6.47	6.69
	CM	6	6.5033	.09709	.03964	6.4014	6.6052	6.38	6.65
	L.fermentum strain MGB 32-1	6	6.8483	.07885	.03219	6.7656	6.9311	6.73	6.94
	L.fermentum F-6	6	6.8017	.07600	.03103	6.7219	6.8814	6.73	6.93
	L.fermentum CECT 5716	6	7.0233	.11518	.04702	6.9025	7.1442	6.87	7.15
	L.fermentum cc IMAU:80780	6	7.0633	.11725	.04787	6.9403	7.1864	6.97	7.28
	Total	36	6.8028	.22909	.03818	6.7253	6.8803	6.38	7.28
Entero0. 5m	*c*	6	6.7567	.18446	.07531	6.5631	6.9502	6.54	6.97
	CM	6	6.7967	.12028	.04910	6.6704	6.9229	6.62	6.95
	L.fermentum strain MGB 32-1	6	6.5233	.14459	.05903	6.3716	6.6751	6.38	6.76
	L.fermentum F-6	6	6.5700	.15582	.06361	6.4065	6.7335	6.43	6.85

	L.fermentum CECT 5716	6	6.4033	.13171	.05377	6.2651	6.5416	6.22	6.62
	L.fermentum cc IMAU:80780	6	6.2317	.16774	.06848	6.0556	6.4077	6.10	6.56
	Total	36	6.5469	.24264	.04044	6.4648	6.6290	6.10	6.97
	c	6	6.5767	.08756	.03575	6.4848	6.6686	6.47	6.69
	CM	6	6.5400	.20465	.08355	6.3252	6.7548	6.24	6.87
	L.fermentum strain MGB 32-1	6	6.7233	.14334	.05852	6.5729	6.8738	6.50	6.90
Lacto0.1m	*L.fermentum F-6*	6	6.6500	.06356	.02595	6.5833	6.7167	6.57	6.74
	L.fermentum CECT 5716	6	6.7850	.06156	.02513	6.7204	6.8496	6.72	6.87
	L.fermentum cc IMAU:80780	6	6.7733	.13880	.05667	6.6277	6.9190	6.56	6.96
	Total	36	6.6747	.15135	.02522	6.6235	6.7259	6.24	6.96
	c	6	6.7567	.18446	.07531	6.5631	6.9502	6.54	6.97
	CM	6	6.6750	.07120	.02907	6.6003	6.7497	6.60	6.80
	L.fermentum strain MGB 32-1	6	6.7133	.04179	.01706	6.6695	6.7572	6.66	6.76
Entero0.1m	*L.fermentum F-6*	6	6.6500	.10139	.04139	6.5436	6.7564	6.53	6.78
	L.fermentum CECT 5716	6	6.5983	.11017	.04498	6.4827	6.7139	6.41	6.71
	L.fermentum cc IMAU:80780	6	6.5350	.07232	.02952	6.4591	6.6109	6.44	6.63
	Total	36	6.6547	.12342	.02057	6.6130	6.6965	6.41	6.97

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lactoba1m	Between Groups	5.233	5	1.047	51.225	.000
	Within Groups	.613	30	.020		
	Total	5.846	35			
Entero1m	Between Groups	4.851	5	.970	41.333	.000
	Within Groups	.704	30	.023		
	Total	5.555	35			
Lacto0.5m	Between Groups	1.556	5	.311	33.292	.000
	Within Groups	.280	30	.009		
	Total	1.837	35			
Entero0.5m	Between Groups	1.365	5	.273	11.768	.000
	Within Groups	.696	30	.023		
	Total	2.061	35			
Lacto0.1m	Between Groups	.316	5	.063	3.899	.008
	Within Groups	.486	30	.016		
	Total	.802	35			

	Between Groups	.191	5	.038	3.340	.016
Entero0.1m	Within Groups	.342	30	.011		
	Total	.533	35			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) OrgLatoento	(J) OrgLatoento	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lactoba1m	*c*	*CM*	-.04833	.08252	.562	-.2169	.1202
		L.fermentum strain MGB 32-1	-.56500*	.08252	.000	-.7335	-.3965
		L.fermentum F-6	-.75667*	.08252	.000	-.9252	-.5881
		L.fermentum CECT 5716	-1.07333*	.08252	.000	-1.2419	-.9048
		L.fermentum cc IMAU:80780	-.64333*	.08252	.000	-.8119	-.4748
	CM	*c*	.04833	.08252	.562	-.1202	.2169
		L.fermentum strain MGB 32-1	-.51667*	.08252	.000	-.6852	-.3481
		L.fermentum F-6	-.70833*	.08252	.000	-.8769	-.5398
		L.fermentum CECT 5716	-1.02500*	.08252	.000	-1.1935	-.8565
		L.fermentum cc IMAU:80780	-.59500*	.08252	.000	-.7635	-.4265
	L.fermentum strain MGB 32-1	*c*	.56500*	.08252	.000	.3965	.7335
		CM	.51667*	.08252	.000	.3481	.6852
		L.fermentum F-6	-.19167*	.08252	.027	-.3602	-.0231
		L.fermentum CECT 5716	-.50833*	.08252	.000	-.6769	-.3398
		L.fermentum cc IMAU:80780	-.07833	.08252	.350	-.2469	.0902
	L.fermentum F-6	*c*	.75667*	.08252	.000	.5881	.9252
		CM	.70833*	.08252	.000	.5398	.8769
		L.fermentum strain MGB 32-1	.19167*	.08252	.027	.0231	.3602
		L.fermentum CECT 5716	-.31667*	.08252	.001	-.4852	-.1481

Entero1m	*L.fermentum CECT 5716*	*L.fermentum cc IMAU:80780*	.11333	.08252	.180	-.0552	.2819
		c	1.07333*	.08252	.000	.9048	1.2419
		CM	1.02500*	.08252	.000	.8565	1.1935
		L.fermentum strain MGB 32-1	.50833*	.08252	.000	.3398	.6769
		L.fermentum F-6	.31667*	.08252	.001	.1481	.4852
	L.fermentum cc IMAU:80780	*L.fermentum cc IMAU:80780*	.43000*	.08252	.000	.2615	.5985
		c	.64333*	.08252	.000	.4748	.8119
		CM	.59500*	.08252	.000	.4265	.7635
		L.fermentum strain MGB 32-1	.07833	.08252	.350	-.0902	.2469
		L.fermentum F-6	-.11333	.08252	.180	-.2819	.0552
		L.fermentum CECT 5716	-.43000*	.08252	.000	-.5985	-.2615
	c	*CM*	.11500	.08845	.203	-.0656	.2956
		L.fermentum strain MGB 32-1	.25333*	.08845	.008	.0727	.4340
		L.fermentum F-6	.20667*	.08845	.026	.0260	.3873
		L.fermentum CECT 5716	.69833*	.08845	.000	.5177	.8790
		L.fermentum cc IMAU:80780	1.04833*	.08845	.000	.8677	1.2290
	CM	*c*	-.11500	.08845	.203	-.2956	.0656
		L.fermentum strain MGB 32-1	.13833	.08845	.128	-.0423	.3190
		L.fermentum F-6	.09167	.08845	.308	-.0890	.2723
		L.fermentum CECT 5716	.58333*	.08845	.000	.4027	.7640
		L.fermentum cc IMAU:80780	.93333*	.08845	.000	.7527	1.1140
	L.fermentum strain MGB 32-1	*c*	-.25333*	.08845	.008	-.4340	-.0727
		CM	-.13833	.08845	.128	-.3190	.0423
		L.fermentum F-6	-.04667	.08845	.602	-.2273	.1340
		L.fermentum CECT 5716	.44500*	.08845	.000	.2644	.6256
		L.fermentum cc IMAU:80780	.79500*	.08845	.000	.6144	.9756
	L.fermentum F-6	*c*	-.20667*	.08845	.026	-.3873	-.0260
		CM	-.09167	.08845	.308	-.2723	.0890

		L.fermentum strain MGB 32-1	.04667	.08845	.602	-.1340	.2273
		L.fermentum CECT 5716	.49167*	.08845	.000	.3110	.6723
		L.fermentum cc IMAU:80780	.84167*	.08845	.000	.6610	1.0223
		c	-.69833*	.08845	.000	-.8790	-.5177
		CM	-.58333*	.08845	.000	-.7640	-.4027
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.44500*	.08845	.000	-.6256	-.2644
		L.fermentum F-6	-.49167*	.08845	.000	-.6723	-.3110
		L.fermentum cc IMAU:80780	.35000*	.08845	.000	.1694	.5306
		c	-1.04833*	.08845	.000	-1.2290	-.8677
		CM	-.93333*	.08845	.000	-1.1140	-.7527
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.79500*	.08845	.000	-.9756	-.6144
		L.fermentum F-6	-.84167*	.08845	.000	-1.0223	-.6610
		L.fermentum CECT 5716	-.35000*	.08845	.000	-.5306	-.1694
		CM	.07333	.05583	.199	-.0407	.1873
		L.fermentum strain MGB 32-1	-.27167*	.05583	.000	-.3857	-.1577
	c	*L.fermentum F-6*	-.22500*	.05583	.000	-.3390	-.1110
		L.fermentum CECT 5716	-.44667*	.05583	.000	-.5607	-.3327
		L.fermentum cc IMAU:80780	-.48667*	.05583	.000	-.6007	-.3727
		c	-.07333	.05583	.199	-.1873	.0407
		L.fermentum strain MGB 32-1	-.34500*	.05583	.000	-.4590	-.2310
Lacto0.5m	CM	*L.fermentum F-6*	-.29833*	.05583	.000	-.4123	-.1843
		L.fermentum CECT 5716	-.52000*	.05583	.000	-.6340	-.4060
		L.fermentum cc IMAU:80780	-.56000*	.05583	.000	-.6740	-.4460
		c	.27167*	.05583	.000	.1577	.3857
		CM	.34500*	.05583	.000	.2310	.4590
	L.fermentum strain MGB 32-1	*L.fermentum F-6*	.04667	.05583	.410	-.0673	.1607
		L.fermentum CECT 5716	-.17500*	.05583	.004	-.2890	-.0610
		L.fermentum cc IMAU:80780	-.21500*	.05583	.001	-.3290	-.1010

		c	.22500*	.05583	.000	.1110	.3390
		CM	.29833*	.05583	.000	.1843	.4123
		L.fermentum strain MGB 32-1	-.04667	.05583	.410	-.1607	.0673
	L.fermentum F-6	*L.fermentum CECT 5716*	-.22167*	.05583	.000	-.3357	-.1077
		L.fermentum cc IMAU:80780	-.26167*	.05583	.000	-.3757	-.1477
		c	.44667*	.05583	.000	.3327	.5607
		CM	.52000*	.05583	.000	.4060	.6340
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	.17500*	.05583	.004	.0610	.2890
		L.fermentum F-6	.22167*	.05583	.000	.1077	.3357
		L.fermentum cc IMAU:80780	-.04000	.05583	.479	-.1540	.0740
		c	.48667*	.05583	.000	.3727	.6007
		CM	.56000*	.05583	.000	.4460	.6740
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	.21500*	.05583	.001	.1010	.3290
		L.fermentum F-6	.26167*	.05583	.000	.1477	.3757
		L.fermentum CECT 5716	.04000	.05583	.479	-.0740	.1540
		CM	-.04000	.08793	.652	-.2196	.1396
		L.fermentum strain MGB 32-1	.23333*	.08793	.013	.0538	.4129
	c	*L.fermentum F-6*	.18667*	.08793	.042	.0071	.3662
		L.fermentum CECT 5716	.35333*	.08793	.000	.1738	.5329
		L.fermentum cc IMAU:80780	.52500*	.08793	.000	.3454	.7046
		c	.04000	.08793	.652	-.1396	.2196
		L.fermentum strain MGB 32-1	.27333*	.08793	.004	.0938	.4529
Entero0.5m		*L.fermentum F-6*	.22667*	.08793	.015	.0471	.4062
	CM	*L.fermentum CECT 5716*	.39333*	.08793	.000	.2138	.5729
		L.fermentum cc IMAU:80780	.56500*	.08793	.000	.3854	.7446
		c	-.23333*	.08793	.013	-.4129	-.0538
		CM	-.27333*	.08793	.004	-.4529	-.0938
	L.fermentum strain MGB 32-1	*L.fermentum F-6*	-.04667	.08793	.600	-.2262	.1329
		L.fermentum CECT 5716	.12000	.08793	.182	-.0596	.2996

		L.fermentum cc IMAU:80780	.29167*	.08793	.002	.1121	.4712
		c	-.18667*	.08793	.042	-.3662	-.0071
		CM	-.22667*	.08793	.015	-.4062	-.0471
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	.04667	.08793	.600	-.1329	.2262
		L.fermentum CECT 5716	.16667	.08793	.068	-.0129	.3462
		L.fermentum cc IMAU:80780	.33833*	.08793	.001	.1588	.5179
		c	-.35333*	.08793	.000	-.5329	-.1738
		CM	-.39333*	.08793	.000	-.5729	-.2138
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.12000	.08793	.182	-.2996	.0596
		L.fermentum F-6	-.16667	.08793	.068	-.3462	.0129
		L.fermentum cc IMAU:80780	.17167	.08793	.060	-.0079	.3512
		c	-.52500*	.08793	.000	-.7046	-.3454
		CM	-.56500*	.08793	.000	-.7446	-.3854
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.29167*	.08793	.002	-.4712	-.1121
		L.fermentum F-6	-.33833*	.08793	.001	-.5179	-.1588
		L.fermentum CECT 5716	-.17167	.08793	.060	-.3512	.0079
		CM	.03667	.07348	.621	-.1134	.1867
		L.fermentum strain MGB 32-1	-.14667	.07348	.055	-.2967	.0034
	c	*L.fermentum F-6*	-.07333	.07348	.326	-.2234	.0767
		L.fermentum CECT 5716	-.20833*	.07348	.008	-.3584	-.0583
		L.fermentum cc IMAU:80780	-.19667*	.07348	.012	-.3467	-.0466
		c	-.03667	.07348	.621	-.1867	.1134
Lacto0.1m		*L.fermentum strain MGB 32-1*	-.18333*	.07348	.018	-.3334	-.0333
		L.fermentum F-6	-.11000	.07348	.145	-.2601	.0401
	CM	*L.fermentum CECT 5716*	-.24500*	.07348	.002	-.3951	-.0949
		L.fermentum cc IMAU:80780	-.23333*	.07348	.003	-.3834	-.0833
		c	.14667	.07348	.055	-.0034	.2967
	L.fermentum strain MGB 32-1	*CM*	.18333*	.07348	.018	.0333	.3334
		L.fermentum F-6	.07333	.07348	.326	-.0767	.2234

		L.fermentum CECT 5716	-.06167	.07348	.408	-.2117	.0884
		L.fermentum cc IMAU:80780	-.05000	.07348	.501	-.2001	.1001
		c	.07333	.07348	.326	-.0767	.2234
		CM	.11000	.07348	.145	-.0401	.2601
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	-.07333	.07348	.326	-.2234	.0767
		L.fermentum CECT 5716	-.13500	.07348	.076	-.2851	.0151
		L.fermentum cc IMAU:80780	-.12333	.07348	.104	-.2734	.0267
		c	.20833*	.07348	.008	.0583	.3584
		CM	.24500*	.07348	.002	.0949	.3951
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	.06167	.07348	.408	-.0884	.2117
		L.fermentum F-6	.13500	.07348	.076	-.0151	.2851
		L.fermentum cc IMAU:80780	.01167	.07348	.875	-.1384	.1617
		c	.19667*	.07348	.012	.0466	.3467
		CM	.23333*	.07348	.003	.0833	.3834
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	.05000	.07348	.501	-.1001	.2001
		L.fermentum F-6	.12333	.07348	.104	-.0267	.2734
		L.fermentum CECT 5716	-.01167	.07348	.875	-.1617	.1384
		CM	.08167	.06168	.196	-.0443	.2076
	c	*L.fermentum strain MGB 32-1*	.04333	.06168	.488	-.0826	.1693
		L.fermentum F-6	.10667	.06168	.094	-.0193	.2326
		L.fermentum CECT 5716	.15833*	.06168	.015	.0324	.2843
		L.fermentum cc IMAU:80780	.22167*	.06168	.001	.0957	.3476
Entero0.1m		c	-.08167	.06168	.196	-.2076	.0443
		L.fermentum strain MGB 32-1	-.03833	.06168	.539	-.1643	.0876
	CM	L.fermentum F-6	.02500	.06168	.688	-.1010	.1510
		L.fermentum CECT 5716	.07667	.06168	.224	-.0493	.2026
		L.fermentum cc IMAU:80780	.14000*	.06168	.031	.0140	.2660

	c	-.04333	.06168	.488	-.1693	.0826
	CM	.03833	.06168	.539	-.0876	.1643
L.fermentum strain MGB 32-1	*L.fermentum F-6*	.06333	.06168	.313	-.0626	.1893
	L.fermentum CECT 5716	.11500	.06168	.072	-.0110	.2410
	L.fermentum cc IMAU:80780	.17833*	.06168	.007	.0524	.3043
	c	-.10667	.06168	.094	-.2326	.0193
	CM	-.02500	.06168	.688	-.1510	.1010
L.fermentum F-6	*L.fermentum strain MGB 32-1*	-.06333	.06168	.313	-.1893	.0626
	L.fermentum CECT 5716	.05167	.06168	.409	-.0743	.1776
	L.fermentum cc IMAU:80780	.11500	.06168	.072	-.0110	.2410
	c	-.15833*	.06168	.015	-.2843	-.0324
	CM	-.07667	.06168	.224	-.2026	.0493
L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.11500	.06168	.072	-.2410	.0110
	L.fermentum F-6	-.05167	.06168	.409	-.1776	.0743
	L.fermentum cc IMAU:80780	.06333	.06168	.313	-.0626	.1893
	c	-.22167*	.06168	.001	-.3476	-.0957
	CM	-.14000*	.06168	.031	-.2660	-.0140
L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.17833*	.06168	.007	-.3043	-.0524
	L.fermentum F-6	-.11500	.06168	.072	-.2410	.0110
	L.fermentum CECT 5716	-.06333	.06168	.313	-.1893	.0626

*. The mean difference is significant at the 0.05 level.

Appendix v: Effect of different volumes of fermented milk on the viable count of lactobacilli and enterobacteria

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Lactoba2	1m	36	7.0911	.40868	.06811	6.9528	7.2294	6.46	7.80
	0.5m	36	6.8028	.22909	.03818	6.7253	6.8803	6.38	7.28
	0.1m	36	6.6747	.15135	.02522	6.6235	6.7259	6.24	6.96
	Total	108	6.8562	.33151	.03190	6.7930	6.9194	6.24	7.80
Entero2	1m	36	6.3697	.39838	.06640	6.2349	6.5045	5.39	6.97
	0.5m	36	6.5469	.24264	.04044	6.4648	6.6290	6.10	6.97
	0.1m	36	6.6547	.12342	.02057	6.6130	6.6965	6.41	6.97
	Total	108	6.5238	.30015	.02888	6.4665	6.5811	5.39	6.97

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Lactoba2	Between Groups	3.275	2	1.637	20.265	.000
	Within Groups	8.484	105	.081		
	Total	11.759	107			
Entero2	Between Groups	1.491	2	.745	9.606	.000
	Within Groups	8.148	105	.078		
	Total	9.639	107			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) Conc lactoentero2	(J) Conc lactoentero2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lactoba2	1m	0.5m	.28833*	.06700	.000	.1555	.4212
		0.1m	.41639*	.06700	.000	.2835	.5492
	0.5m	1m	-.28833*	.06700	.000	-.4212	-.1555
		0.1m	.12806	.06700	.059	-.0048	.2609
	0.1m	1m	-.41639*	.06700	.000	-.5492	-.2835
		0.5m	-.12806	.06700	.059	-.2609	.0048
Entero2	1m	0.5m	-.17722*	.06566	.008	-.3074	-.0470
		0.1m	-.28500*	.06566	.000	-.4152	-.1548
	0.5m	1m	.17722*	.06566	.008	.0470	.3074
		0.1m	-.10778	.06566	.104	-.2380	.0224
	0.1m	1m	.28500*	.06566	.000	.1548	.4152
		0.5m	.10778	.06566	.104	-.0224	.2380

*. The mean difference is significant at the 0.05 level.

Appendix vi: Effect of 2 weeks consumption of *Lactobacillusfermentum*-fermented milk on AST levels

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
AST1ml	C	6	75.067	6.7580	2.7589	67.975	82.159	65.2	86.4
	CM	6	77.700	10.4292	4.2577	66.755	88.645	59.0	88.0
	L.fermentum strain MGB 32-1	6	69.967	8.5608	3.4949	60.983	78.951	54.5	80.8
	L.fermentum F-6	6	71.267	8.8827	3.6264	61.945	80.589	55.3	82.8
	L.fermentum CECT 5716	6	66.850	7.0293	2.8697	59.473	74.227	59.3	78.9
	L.fermentum cc IMAU:80780	6	71.750	6.4890	2.6491	64.940	78.560	60.7	79.4
	Total	36	72.100	8.3311	1.3885	69.281	74.919	54.5	88.0
AST0.5 ml	*C*	6	75.0667	6.75801	2.75895	67.9746	82.1588	65.20	86.40
	CM	6	77.4667	9.47685	3.86891	67.5213	87.4120	60.70	86.90
	L.fermentum strain MGB 32-1	6	72.5833	12.09585	4.93811	59.8895	85.2772	52.90	88.60
	L.fermentum F-6	6	69.1833	8.65689	3.53416	60.0985	78.2682	53.70	80.40
	L.fermentum CECT 5716	6	68.2833	7.67109	3.13171	60.2330	76.3337	58.80	81.40
	L.fermentum cc IMAU:80780	6	68.7833	7.77288	3.17327	60.6262	76.9405	56.80	81.00
	Total	36	71.8944	8.96010	1.49335	68.8628	74.9261	52.90	88.60
AST0.1 ml	*C*	6	75.0667	6.75801	2.75895	67.9746	82.1588	65.20	86.40
	CM	6	77.9667	8.79583	3.59088	68.7360	87.1973	62.70	87.90
	L.fermentum strain MGB 32-1	6	71.7500	8.94444	3.65155	62.3634	81.1366	55.80	83.20
	L.fermentum F-6	6	71.4667	8.83146	3.60543	62.1986	80.7347	54.70	80.80
	L.fermentum CECT 5716	6	67.0833	8.58101	3.50318	58.0781	76.0885	58.20	82.20
	L.fermentum cc IMAU:80780	6	70.7167	7.23310	2.95290	63.1260	78.3073	59.20	81.40
	Total	36	72.3417	8.37985	1.39664	69.5063	75.1770	54.70	87.90

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
AST1m	Between Groups	438.550	5	87.710	1.322	.282
	Within Groups	1990.730	30	66.358		
	Total	2429.280	35			
AST0.5m	Between Groups	429.939	5	85.988	1.084	.389
	Within Groups	2379.980	30	79.333		
	Total	2809.919	35			
AST0.1m	Between Groups	422.836	5	84.567	1.247	.312
	Within Groups	2034.932	30	67.831		
	Total	2457.768	35			

Appendix vii: Effect of 2 weeks consumption of *Lactobacillusfermentum*-fermented milk on ALT levels

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
ALT1ml	*C*	6	27.7667	3.52117	1.43751	24.0714	31.4619	21.00	31.10
	CM	6	26.7167	1.31060	.53505	25.3413	28.0921	24.50	28.20
	L.fermentum strain MGB 32-1	6	25.2333	1.69902	.69362	23.4503	27.0163	23.20	27.70
	L.fermentum F-6	6	23.3333	3.98280	1.62597	19.1536	27.5130	20.30	31.10
	L.fermentum CECT 5716	6	27.5833	2.12077	.86580	25.3577	29.8089	24.50	30.50
	L.fermentum cc IMAU:80780	6	25.6500	3.02043	1.23309	22.4803	28.8197	23.30	31.30
	Total	36	26.0472	3.00537	.50090	25.0303	27.0641	20.30	31.30
ALT0.5ml	*C*	6	27.7667	3.52117	1.43751	24.0714	31.4619	21.00	31.10
	CM	6	24.7500	1.34870	.55061	23.3346	26.1654	22.70	26.50
	L.fermentum strain MGB 32-1	6	25.6833	2.63015	1.07375	22.9232	28.4435	23.50	30.60
	L.fermentum F-6	6	24.7833	1.86378	.76088	22.8274	26.7392	23.10	28.20
	L.fermentum CECT 5716	6	27.7500	2.25100	.91897	25.3877	30.1123	25.40	31.20
	L.fermentum cc IMAU:80780	6	26.5333	2.81330	1.14853	23.5810	29.4857	24.40	31.80
	Total	36	26.2111	2.64043	.44007	25.3177	27.1045	21.00	31.80
ALT0.1ml	*C*	6	27.7667	3.52117	1.43751	24.0714	31.4619	21.00	31.10
	CM	6	26.8667	1.49354	.60974	25.2993	28.4340	25.40	29.50
	L.fermentum strain MGB 32-1	6	24.8667	1.16905	.47726	23.6398	26.0935	22.80	26.20
	L.fermentum F-6	6	24.6167	2.69178	1.09891	21.7918	27.4415	22.30	29.60
	L.fermentum CECT 5716	6	26.5500	1.64894	.67318	24.8195	28.2805	24.80	29.20
	L.fermentum cc IMAU:80780	6	27.4667	1.97754	.80733	25.3914	29.5420	25.50	30.70
	Total	36	26.3556	2.40208	.40035	25.5428	27.1683	21.00	31.10

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ALT1mm	Between Groups	83.698	5	16.740	2.161	.085
	Within Groups	232.432	30	7.748		
	Total	316.130	35			
ALT0.5mm	Between Groups	56.062	5	11.212	1.790	.145
	Within Groups	187.953	30	6.265		
	Total	244.016	35			
ALT0.1mm	Between Groups	52.592	5	10.518	2.113	.091
	Within Groups	149.357	30	4.979		
	Total	201.949	35			

Appendix viii: Effect of 2 weeks consumption of *Lactobacillusfermentum*-fermented milk on ALP levels

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
ALP1ml	*C*	6	85.5667	6.30608	2.57445	78.9488	92.1845	74.50	91.10
	CM	6	82.7833	5.23886	2.13876	77.2855	88.2812	74.50	88.30
	L.fermentum strain MGB 32-1	6	84.0167	4.66451	1.90428	79.1216	88.9118	79.30	91.10
	L.fermentum F-6	6	81.8833	6.66916	2.72267	74.8845	88.8822	71.80	88.30
	L.fermentum CECT 5716	6	85.4833	3.99270	1.63001	81.2932	89.6734	80.00	91.10
	L.fermentum cc IMAU:80780	6	80.5000	4.44432	1.81439	75.8360	85.1640	74.50	85.60
	Total	36	83.3722	5.26094	.87682	81.5922	85.1523	71.80	91.10
ALP0.5ml	*C*	6	85.5667	6.30608	2.57445	78.9488	92.1845	74.50	91.10
	CM	6	86.4000	3.64253	1.48706	82.5774	90.2226	81.70	91.10
	L.fermentum strain MGB 32-1	6	83.6500	7.12987	2.91076	76.1677	91.1323	74.90	92.50
	L.fermentum F-6	6	84.5333	6.16495	2.51683	78.0636	91.0031	76.40	92.80
	L.fermentum CECT 5716	6	86.3667	7.25249	2.96082	78.7556	93.9777	76.50	96.10
	L.fermentum cc IMAU:80780	6	86.6000	3.96232	1.61761	82.4418	90.7582	81.60	91.50
	Total	36	85.5194	5.58993	.93166	83.6281	87.4108	74.50	96.10
ALP0.1ml	*C*	6	85.5667	6.30608	2.57445	78.9488	92.1845	74.50	91.10
	CM	6	86.6333	6.17533	2.52107	80.1527	93.1139	77.30	94.70
	L.fermentum strain MGB 32-1	6	85.3667	7.71535	3.14978	77.2699	93.4634	74.50	95.50
	L.fermentum F-6	6	84.1900	5.16246	2.10757	78.7723	89.6077	77.30	91.10
	L.fermentum CECT 5716	6	84.6533	5.70560	2.32930	78.6657	90.6410	74.52	91.10
	L.fermentum cc IMAU:80780	6	85.1600	3.10805	1.26886	81.8983	88.4217	80.40	88.30
	Total	36	85.2617	5.48387	.91398	83.4062	87.1171	74.50	95.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ALP1m	Between Groups	123.006	5	24.601	.873	.511
	Within Groups	845.707	30	28.190		
	Total	968.712	35			
ALP0.5m	Between Groups	42.781	5	8.556	.244	.939
	Within Groups	1050.875	30	35.029		
	Total	1093.656	35			
ALP0.1m	Between Groups	21.086	5	4.217	.123	.986
	Within Groups	1031.464	30	34.382		
	Total	1052.551	35			

Appendix ix: Effect of 2 weeks consumption of *Lactobacillusfermentum*-fermented milk on serum cholesterol levels.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Chole1ml	*C*	6	1.8317	.12497	.05102	1.7005	1.9628	1.63	1.97
	CM	6	1.8467	.07285	.02974	1.7702	1.9231	1.76	1.95
	L.fermentum strain MGB 32-1	6	1.7500	.06325	.02582	1.6836	1.8164	1.65	1.83
	L.fermentum F-6	6	1.6667	.08618	.03518	1.5762	1.7571	1.55	1.79
	L.fermentum CECT 5716	6	1.7800	.08695	.03550	1.6888	1.8712	1.62	1.86
	L.fermentum cc IMAU:80780	6	1.7033	.08802	.03593	1.6110	1.7957	1.63	1.85
	Total	36	1.7631	.10534	.01756	1.7274	1.7987	1.55	1.97
Chole0.5ml	*C*	6	1.8317	.12497	.05102	1.7005	1.9628	1.63	1.97
	CM	6	1.7900	.08741	.03568	1.6983	1.8817	1.68	1.91
	L.fermentum strain MGB 32-1	6	1.8233	.05538	.02261	1.7652	1.8814	1.74	1.89
	L.fermentum F-6	6	1.7217	.07083	.02892	1.6473	1.7960	1.62	1.79
	L.fermentum CECT 5716	6	1.8017	.08256	.03371	1.7150	1.8883	1.69	1.93
	L.fermentum cc IMAU:80780	6	1.7600	.06387	.02608	1.6930	1.8270	1.67	1.85
	Total	36	1.7881	.08658	.01443	1.7588	1.8174	1.62	1.97
Chole0.1ml	*C*	6	1.8317	.12497	.05102	1.7005	1.9628	1.63	1.97
	CM	6	1.8017	.08256	.03371	1.7150	1.8883	1.68	1.90
	L.fermentum strain MGB 32-1	6	1.8133	.05715	.02333	1.7534	1.8733	1.73	1.89
	L.fermentum F-6	6	1.7500	.08438	.03445	1.6614	1.8386	1.67	1.91
	L.fermentum CECT 5716	6	1.8417	.07468	.03049	1.7633	1.9200	1.72	1.95
	L.fermentum cc IMAU:80780	6	1.7800	.09381	.03830	1.6816	1.8784	1.65	1.87
	Total	36	1.8031	.08792	.01465	1.7733	1.8328	1.63	1.97

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Chole1mm	Between Groups	.150	5	.030	3.779	.009
	Within Groups	.238	30	.008		
	Total	.388	35			
Chole0.5mm	Between Groups	.051	5	.010	1.454	.234
	Within Groups	.211	30	.007		
	Total	.262	35			
Chole0.1m	Between Groups	.035	5	.007	.879	.507
	Within Groups	.236	30	.008		
	Total	.271	35			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) CHOLESTEROL ORGANISM	(J) CHOLESTEROL ORGANISM	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Chole1ml	*C*	*CM*	-.01500	.05145	.773	-.1201	.0901
		L.fermentum strain MGB 32-1	.08167	.05145	.123	-.0234	.1868
		L.fermentum F-6	.16500*	.05145	.003	.0599	.2701
		L.fermentum CECT 5716	.05167	.05145	.323	-.0534	.1568
		L.fermentum cc IMAU:80780	.12833*	.05145	.018	.0232	.2334
	CM	*C*	.01500	.05145	.773	-.0901	.1201
		L.fermentum strain MGB 32-1	.09667	.05145	.070	-.0084	.2018
		L.fermentum F-6	.18000*	.05145	.001	.0749	.2851
		L.fermentum CECT 5716	.06667	.05145	.205	-.0384	.1718
		L.fermentum cc IMAU:80780	.14333*	.05145	.009	.0382	.2484
	L.fermentum strain MGB 32-1	*C*	-.08167	.05145	.123	-.1868	.0234
		CM	-.09667	.05145	.070	-.2018	.0084

		L.fermentum F-6	.08333	.05145	.116	-.0218	.1884
		L.fermentum CECT 5716	-.03000	.05145	.564	-.1351	.0751
		L.fermentum cc IMAU:80780	.04667	.05145	.372	-.0584	.1518
		C	-.16500*	.05145	.003	-.2701	-.0599
		CM	-.18000*	.05145	.001	-.2851	-.0749
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	-.08333	.05145	.116	-.1884	.0218
		L.fermentum CECT 5716	-.11333*	.05145	.035	-.2184	-.0082
		L.fermentum cc IMAU:80780	-.03667	.05145	.482	-.1418	.0684
		C	-.05167	.05145	.323	-.1568	.0534
		CM	-.06667	.05145	.205	-.1718	.0384
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	.03000	.05145	.564	-.0751	.1351
		L.fermentum F-6	.11333*	.05145	.035	.0082	.2184
		L.fermentum cc IMAU:80780	.07667	.05145	.147	-.0284	.1818
		C	-.12833*	.05145	.018	-.2334	-.0232
		CM	-.14333*	.05145	.009	-.2484	-.0382
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.04667	.05145	.372	-.1518	.0584
		L.fermentum F-6	.03667	.05145	.482	-.0684	.1418
		L.fermentum CECT 5716	-.07667	.05145	.147	-.1818	.0284

*. The mean difference is significant at the 0.05 level.

Appendix x: Effect of 13 weeks consumption of 1 ml of *Lactobacillusfermentum*-fermented milk on the weight of rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Maleweight 13weeks	*c*	4	300.2325	9.18397	4.59198	285.6188	314.8462	288.24	309.26
	CM	4	351.3050	9.66723	4.83361	335.9223	366.6877	339.37	362.51
	L.fermentum strain MGB 32-1	4	390.3000	9.63322	4.81661	374.9714	405.6286	378.61	401.13
	L.fermentum F-6	4	399.2600	9.21955	4.60977	384.5896	413.9304	388.34	409.15
	L.fermentum CECT 5716	4	377.4000	7.69457	3.84729	365.1562	389.6438	367.27	384.41
	L.fermentum cc IMAU:80780	4	393.4125	8.23151	4.11576	380.3143	406.5107	383.58	402.46
	Total	24	368.6517	35.96898	7.34214	353.4633	383.8400	288.24	409.15
Femaleweight13weeks	*c*	4	203.3750	13.93711	6.96856	181.1979	225.5521	188.48	218.30
	CM	4	248.3750	8.32675	4.16337	235.1253	261.6247	239.25	257.13
	L.fermentum strain MGB 32-1	4	281.5075	11.31364	5.65682	263.5050	299.5100	269.72	293.58
	L.fermentum F-6	4	292.5525	11.16782	5.58391	274.7820	310.3230	280.96	304.65
	L.fermentum CECT 5716	4	272.3825	7.35443	3.67721	260.6800	284.0850	264.38	280.30
	L.fermentum cc IMAU:80780	4	285.6575	10.93107	5.46553	268.2637	303.0513	273.77	297.69
	Total	24	263.9750	32.56566	6.64744	250.2237	277.7263	188.48	304.65

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Maleweight13weeks	Between Groups	28308.967	5	5661.793	70.396	.000
	Within Groups	1447.691	18	80.427		
	Total	29756.659	23			
Femaleweight13weeks	Between Groups	22322.396	5	4464.479	38.829	.000
	Within Groups	2069.617	18	114.979		
	Total	24392.013	23			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) Weightsex	(J) Weightsex	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Malew eight13 weeks	*c*	*CM*	-51.07250*	6.34142	.000	-64.3953	-37.7497
		L.fermentum strain MGB 32-1	-90.06750*	6.34142	.000	-103.3903	-76.7447
		L.fermentum F-6	-99.02750*	6.34142	.000	-112.3503	-85.7047
		L.fermentum CECT 5716	-77.16750*	6.34142	.000	-90.4903	-63.8447
		L.fermentum cc IMAU:80780	-93.18000*	6.34142	.000	-106.5028	-79.8572
	CM	*c*	51.07250*	6.34142	.000	37.7497	64.3953
		L.fermentum strain MGB 32-1	-38.99500*	6.34142	.000	-52.3178	-25.6722
		L.fermentum F-6	-47.95500*	6.34142	.000	-61.2778	-34.6322
		L.fermentum CECT 5716	-26.09500*	6.34142	.001	-39.4178	-12.7722
		L.fermentum cc IMAU:80780	-42.10750*	6.34142	.000	-55.4303	-28.7847
	L.fermentum strain MGB 32-1	*c*	90.06750*	6.34142	.000	76.7447	103.3903
		CM	38.99500*	6.34142	.000	25.6722	52.3178
		L.fermentum F-6	-8.96000	6.34142	.175	-22.2828	4.3628
		L.fermentum CECT 5716	12.90000	6.34142	.057	-.4228	26.2228
		L.fermentum cc IMAU:80780	-3.11250	6.34142	.629	-16.4353	10.2103
	L.fermentum F-6	*c*	99.02750*	6.34142	.000	85.7047	112.3503
		CM	47.95500*	6.34142	.000	34.6322	61.2778
		L.fermentum strain MGB 32-1	8.96000	6.34142	.175	-4.3628	22.2828

		L.fermentum CECT 5716	21.86000*	6.34142	.003	8.5372	35.1828
		L.fermentum cc IMAU:80780	5.84750	6.34142	.369	-7.4753	19.1703
		c	77.16750*	6.34142	.000	63.8447	90.4903
		CM	26.09500*	6.34142	.001	12.7722	39.4178
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-12.90000	6.34142	.057	-26.2228	.4228
		L.fermentum F-6	-21.86000*	6.34142	.003	-35.1828	-8.5372
		L.fermentum cc IMAU:80780	-16.01250*	6.34142	.021	-29.3353	-2.6897
		c	93.18000*	6.34142	.000	79.8572	106.5028
		CM	42.10750*	6.34142	.000	28.7847	55.4303
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	3.11250	6.34142	.629	-10.2103	16.4353
		L.fermentum F-6	-5.84750	6.34142	.369	-19.1703	7.4753
		L.fermentum CECT 5716	16.01250*	6.34142	.021	2.6897	29.3353
		CM	-45.00000*	7.58217	.000	-60.9296	-29.0704
	c	*L.fermentum strain MGB 32-1*	-78.13250*	7.58217	.000	-94.0621	-62.2029
		L.fermentum F-6	-89.17750*	7.58217	.000	-105.1071	-73.2479
		L.fermentum CECT 5716	-69.00750*	7.58217	.000	-84.9371	-53.0779
Female weight 13weeks		*L.fermentum cc IMAU:80780*	-82.28250*	7.58217	.000	-98.2121	-66.3529
		c	45.00000*	7.58217	.000	29.0704	60.9296
	CM	*L.fermentum strain MGB 32-1*	-33.13250*	7.58217	.000	-49.0621	-17.2029
		L.fermentum F-6	-44.17750*	7.58217	.000	-60.1071	-28.2479
		L.fermentum CECT 5716	-24.00750*	7.58217	.005	-39.9371	-8.0779

	L.fermentum cc IMAU:80780	-37.28250*	7.58217	.000	-53.2121	-21.3529
	c	78.13250*	7.58217	.000	62.2029	94.0621
	CM	33.13250*	7.58217	.000	17.2029	49.0621
L.fermentum strain MGB 32-1	*L.fermentum F-6*	-11.04500	7.58217	.162	-26.9746	4.8846
	L.fermentum CECT 5716	9.12500	7.58217	.244	-6.8046	25.0546
	L.fermentum cc IMAU:80780	-4.15000	7.58217	.591	-20.0796	11.7796
	c	89.17750*	7.58217	.000	73.2479	105.1071
	CM	44.17750*	7.58217	.000	28.2479	60.1071
L.fermentum F-6	*L.fermentum strain MGB 32-1*	11.04500	7.58217	.162	-4.8846	26.9746
	L.fermentum CECT 5716	20.17000*	7.58217	.016	4.2404	36.0996
	L.fermentum cc IMAU:80780	6.89500	7.58217	.375	-9.0346	22.8246
	c	69.00750*	7.58217	.000	53.0779	84.9371
	CM	24.00750*	7.58217	.005	8.0779	39.9371
L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-9.12500	7.58217	.244	-25.0546	6.8046
	L.fermentum F-6	-20.17000*	7.58217	.016	-36.0996	-4.2404
	L.fermentum cc IMAU:80780	-13.27500	7.58217	.097	-29.2046	2.6546
	c	82.28250*	7.58217	.000	66.3529	98.2121
	CM	37.28250*	7.58217	.000	21.3529	53.2121
L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	4.15000	7.58217	.591	-11.7796	20.0796
	L.fermentum F-6	-6.89500	7.58217	.375	-22.8246	9.0346
	L.fermentum CECT 5716	13.27500	7.58217	.097	-2.6546	29.2046

*. The mean difference is significant at the 0.05 level.

Appendix xi: Effect of 13 weeks consumption of 1ml of *Lactobacillusfermentum*-fermented milk on the serum AST levels

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
AST MALE	*C*	4	76.0500	11.47679	5.73839	57.7879	94.3121	60.70	88.50
	CM	4	77.1500	7.27026	3.63513	65.5814	88.7186	68.80	86.40
	L.fermentum strain MGB 32-1	4	65.5000	12.74912	6.37456	45.2133	85.7867	48.60	79.50
	L.fermentum F-6	4	67.3500	13.77595	6.88797	45.4294	89.2706	47.70	77.40
	L.fermentum CECT 5716	4	59.3500	11.59008	5.79504	40.9076	77.7924	50.60	76.00
	L.fermentum cc IMAU:80780	4	56.7250	1.00125	.50062	55.1318	58.3182	55.50	57.90
	Total	24	67.0208	12.19807	2.48992	61.8700	72.1716	47.70	88.50
AST FEMALE	*C*	4	73.9750	9.46022	4.73011	58.9217	89.0283	61.50	84.20
	CM	4	73.3000	6.63576	3.31788	62.7410	83.8590	66.50	82.40
	L.fermentum strain MGB 32-1	4	65.3000	7.77560	3.88780	52.9273	77.6727	56.80	75.50
	L.fermentum F-6	4	63.0250	5.63464	2.81732	54.0590	71.9910	55.30	68.40
	L.fermentum CECT 5716	4	66.2250	10.73448	5.36724	49.1440	83.3060	51.20	74.40
	L.fermentum cc IMAU:80780	4	65.9250	4.36759	2.18379	58.9752	72.8748	60.40	70.40
	Total	24	67.9583	8.05751	1.64473	64.5559	71.3607	51.20	84.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ASTMALE	Between Groups	1405.572	5	281.114	2.509	.068
	Within Groups	2016.668	18	112.037		
	Total	3422.240	23			
AST FEMALE	Between Groups	413.108	5	82.622	1.377	.279
	Within Groups	1080.130	18	60.007		
	Total	1493.238	23			

Appendix xii: Effect of 13 weeks consumption of 1ml of *Lactobacillusfermentum*-fermented milk on the serum ALT levels

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
ALT MALE	C	4	24.7750	1.77834	.88917	21.9453	27.6047	22.50	26.80
	CM	4	26.1250	1.20658	.60329	24.2051	28.0449	24.80	27.50
	L.fermentum strain MGB 32-1	4	23.3750	1.30224	.65112	21.3028	25.4472	22.30	25.20
	L.fermentum F-6	4	23.6250	1.99060	.99530	20.4575	26.7925	21.80	26.40
	L.fermentum CECT 5716	4	26.4000	2.33381	1.16690	22.6864	30.1136	24.50	29.40
	L.fermentum cc IMAU:80780	4	25.2750	1.70171	.85086	22.5672	27.9828	23.80	27.70
	Total	24	24.9292	1.94790	.39761	24.1066	25.7517	21.80	29.40
ALT FEMALE	C	4	24.4750	2.03204	1.01602	21.2416	27.7084	22.80	27.40
	CM	4	25.2250	2.57472	1.28736	21.1281	29.3219	23.20	28.80
	L.fermentum strain MGB 32-1	4	23.7000	.98995	.49497	22.1248	25.2752	22.30	24.60
	L.fermentum F-6	4	24.4500	1.99081	.99541	21.2822	27.6178	22.60	27.20
	L.fermentum CECT 5716	4	23.8000	2.33095	1.16548	20.0909	27.5091	21.50	26.70
	L.fermentum cc IMAU:80780	4	26.7250	1.61116	.80558	24.1613	29.2887	25.20	28.40
	Total	24	24.7292	2.04694	.41783	23.8648	25.5935	21.50	28.80

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ALTMALE	Between Groups	31.412	5	6.282	2.024	.124
	Within Groups	55.857	18	3.103		
	Total	87.270	23			
ALTFEMALE	Between Groups	25.177	5	5.035	1.273	.318
	Within Groups	71.193	18	3.955		
	Total	96.370	23			

Appendix xiii: Effect of 13 weeks consumption of 1ml of *Lactobacillusfermentum*-fermented milk on the serum ALP levels

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
ALP male	C	4	79.3500	4.73322	2.36661	71.8184	86.8816	74.50	85.60
	CM	4	81.6500	6.99452	3.49726	70.5202	92.7798	71.80	88.30
	L.fermentum strain MGB 32-1	4	78.6500	3.56324	1.78162	72.9801	84.3199	74.50	82.80
	L.fermentum F-6	4	82.8000	7.15216	3.57608	71.4193	94.1807	74.50	91.10
	L.fermentum CECT 5716	4	86.2500	4.73322	2.36661	78.7184	93.7816	80.00	91.10
	L.fermentum cc IMAU:80780	4	80.0500	5.95623	2.97811	70.5723	89.5277	71.80	85.60
	Total	24	81.4583	5.65539	1.15440	79.0703	83.8464	71.80	91.10
ALP Female	C	4	59.5750	5.15970	2.57985	51.3648	67.7852	53.50	65.70
	CM	4	57.8000	5.50212	2.75106	49.0449	66.5551	52.80	64.30
	L.fermentum strain MGB 32-1	4	58.4500	8.36361	4.18181	45.1416	71.7584	48.90	67.80
	L.fermentum F-6	4	60.4500	8.43900	4.21950	47.0217	73.8783	51.30	69.50
	L.fermentum CECT 5716	4	57.4500	3.97199	1.98599	51.1297	63.7703	52.70	62.20
	L.fermentum cc IMAU:80780	4	59.5750	6.73517	3.36758	48.8578	70.2922	51.60	67.50
	Total	24	58.8833	5.91576	1.20755	56.3853	61.3813	48.90	69.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ALPmale	Between Groups	156.448	5	31.290	.972	.461
	Within Groups	579.170	18	32.176		
	Total	735.618	23			
ALPFemale	Between Groups	27.308	5	5.462	.126	.985
	Within Groups	777.605	18	43.200		
	Total	804.913	23			

Appendix xiv: Effect of 13 weeks consumption of 1ml of *Lactobacillusfermentum*-fermented milk on the haematology of male albino rats

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	C	4	10.0350	.54440	.27220	9.1687	10.9013	9.42	10.72
	CM	4	10.4350	.42462	.21231	9.7593	11.1107	9.86	10.87
	L.fermentum strain MGB 32-1	4	13.3325	.56524	.28262	12.4331	14.2319	12.61	13.91
Male	*L.fermentum F-6*	4	14.0950	.34492	.17246	13.5462	14.6438	13.62	14.35
HGB	*L.fermentum CECT 5716*	4	13.0425	.48815	.24408	12.2657	13.8193	12.46	13.62
	L.fermentum cc IMAU:80780	4	12.7875	.45036	.22518	12.0709	13.5041	12.17	13.19
	Total	24	12.2879	1.59901	.32640	11.6127	12.9631	9.42	14.35
	C	4	5.3300	.19201	.09600	5.0245	5.6355	5.12	5.57
	CM	4	5.4275	.14728	.07364	5.1931	5.6619	5.23	5.58
	L.fermentum strain MGB 32-1	4	6.4200	.22256	.11128	6.0659	6.7741	6.13	6.65
Male	*L.fermentum F-6*	4	6.6150	.16176	.08088	6.3576	6.8724	6.45	6.81
RBC	*L.fermentum CECT 5716*	4	6.3425	.16601	.08300	6.0783	6.6067	6.15	6.55
	L.fermentum cc IMAU:80780	4	6.2750	.15438	.07719	6.0293	6.5207	6.06	6.42
	Total	24	6.0683	.53331	.10886	5.8431	6.2935	5.12	6.81
	C	4	33.3500	1.72530	.86265	30.6047	36.0953	31.30	35.10
	CM	4	34.1000	1.29872	.64936	32.0335	36.1665	32.50	35.60
	L.fermentum strain MGB 32-1	4	42.9750	1.67207	.83604	40.3144	45.6356	40.70	44.50
MalePC	*L.fermentum F-6*	4	45.1000	.92736	.46368	43.6244	46.5756	43.80	45.90
V	*L.fermentum CECT 5716*	4	41.7000	1.52534	.76267	39.2728	44.1272	40.20	43.50
	L.fermentum cc IMAU:80780	4	41.3750	1.87683	.93842	38.3885	44.3615	39.30	43.80
	Total	24	39.7667	4.73605	.96674	37.7668	41.7665	31.30	45.90
MaleMC	*C*	4	62.5250	1.20104	.60052	60.6139	64.4361	61.10	63.90
v	*CM*	4	62.8250	.75000	.37500	61.6316	64.0184	62.10	63.80

	L.fermentum strain MGB 32-1	4	66.9250	.38622	.19311	66.3104	67.5396	66.40	67.30
	L.fermentum F-6	4	68.2000	1.67133	.83566	65.5405	70.8595	67.20	70.70
	L.fermentum CECT 5716	4	65.7500	.92916	.46458	64.2715	67.2285	64.60	66.60
	L.fermentum cc IMAU:80780	4	65.9250	1.54785	.77392	63.4620	68.3880	64.90	68.20
	Total	24	65.3583	2.34445	.47856	64.3684	66.3483	61.10	70.70
	C	4	18.8250	.36856	.18428	18.2385	19.4115	18.40	19.30
	CM	4	19.2250	.29861	.14930	18.7498	19.7002	18.80	19.50
	L.fermentum strain MGB 32-1	4	20.7750	.15000	.07500	20.5363	21.0137	20.60	20.90
Male	*L.fermentum F-6*	4	21.3000	.60553	.30277	20.3365	22.2635	20.90	22.20
MCH	*L.fermentum CECT 5716*	4	20.5750	.22174	.11087	20.2222	20.9278	20.30	20.80
	L.fermentum cc IMAU:80780	4	20.3750	.22174	.11087	20.0222	20.7278	20.10	20.60
	Total	24	20.1792	.94039	.19196	19.7821	20.5763	18.40	22.20
	C	4	30.0750	.49917	.24958	29.2807	30.8693	29.40	30.60
	CM	4	30.6000	.25820	.12910	30.1891	31.0109	30.30	30.90
	L.fermentum strain MGB 32-1	4	31.0000	.16330	.08165	30.7402	31.2598	30.80	31.20
Male	*L.fermentum F-6*	4	31.2750	.17078	.08539	31.0032	31.5468	31.10	31.50
MCHC	*L.fermentum CECT 5716*	4	31.2750	.30957	.15478	30.7824	31.7676	31.00	31.70
	L.fermentum cc IMAU:80780	4	30.9500	.59161	.29580	30.0086	31.8914	30.10	31.40
	Total	24	30.8625	.53877	.10998	30.6350	31.0900	29.40	31.70
	C	4	424.2500	53.31901	26.65950	339.4076	509.0924	372.00	476.00
	CM	4	428.5000	52.23983	26.11992	345.3748	511.6252	370.00	486.00
	L.fermentum strain MGB 32-1	4	458.7500	39.44933	19.72467	395.9773	521.5227	419.00	497.00
Male PLTS	*L.fermentum F-6*	4	455.0000	46.74042	23.37021	380.6256	529.3744	404.00	506.00
	L.fermentum CECT 5716	4	466.5000	32.02603	16.01302	415.5394	517.4606	429.00	503.00
	L.fermentum cc IMAU:80780	4	444.2500	43.43865	21.71933	375.1294	513.3706	402.00	487.00
	Total	24	446.2083	42.96609	8.77042	428.0653	464.3513	370.00	506.00
Male	*C*	4	7.3425	.46636	.23318	6.6004	8.0846	6.88	7.85

WBC	*CM*	4	6.8300	.31801	.15901	6.3240	7.3360	6.45	7.20
	L.fermentum strain MGB 32-1	4	7.0125	.28814	.14407	6.5540	7.4710	6.65	7.34
	L.fermentum F-6	4	6.5500	.33297	.16648	6.0202	7.0798	6.18	6.97
	L.fermentum CECT 5716	4	6.5375	.39280	.19640	5.9125	7.1625	5.96	6.83
	L.fermentum cc IMAU:80780	4	6.7000	.35336	.17668	6.1377	7.2623	6.32	7.03
	Total	24	6.8287	.43149	.08808	6.6465	7.0110	5.96	7.85

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
MAleHGB	Between Groups	54.738	5	10.948	48.432	.000
	Within Groups	4.069	18	.226		
	Total	58.807	23			
MaleRBC	Between Groups	5.985	5	1.197	38.684	.000
	Within Groups	.557	18	.031		
	Total	6.542	23			
MalePCV	Between Groups	473.388	5	94.678	40.094	.000
	Within Groups	42.505	18	2.361		
	Total	515.893	23			
MaleMCv	Between Groups	101.798	5	20.360	14.885	.000
	Within Groups	24.620	18	1.368		
	Total	126.418	23			
MaleMCH	Between Groups	18.202	5	3.640	30.656	.000
	Within Groups	2.137	18	.119		
	Total	20.340	23			
MaleMCHC	Between Groups	4.224	5	.845	6.200	.002
	Within Groups	2.452	18	.136		
	Total	6.676	23			
MalePLTS	Between Groups	5783.708	5	1156.742	.568	.724
	Within Groups	36676.250	18	2037.569		
	Total	42459.958	23			
MaleWBC	Between Groups	1.907	5	.381	2.891	.044
	Within Groups	2.375	18	.132		
	Total	4.282	23			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) GenderOrgan	(J) GenderOrgan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
MAleGDL	*C*	*CM*	-.40000	.33618	.250	-1.1063	.3063
		L.fermentum strain MGB 32-1	-3.29750*	.33618	.000	-4.0038	-2.5912
		L.fermentum F-6	-4.06000*	.33618	.000	-4.7663	-3.3537
		L.fermentum CECT 5716	-3.00750*	.33618	.000	-3.7138	-2.3012
		L.fermentum cc IMAU:80780	-2.75250*	.33618	.000	-3.4588	-2.0462
	CM	*C*	.40000	.33618	.250	-.3063	1.1063
		L.fermentum strain MGB 32-1	-2.89750*	.33618	.000	-3.6038	-2.1912
		L.fermentum F-6	-3.66000*	.33618	.000	-4.3663	-2.9537
		L.fermentum CECT 5716	-2.60750*	.33618	.000	-3.3138	-1.9012
		L.fermentum cc IMAU:80780	-2.35250*	.33618	.000	-3.0588	-1.6462
	L.fermentum strain MGB 32-1	*C*	3.29750*	.33618	.000	2.5912	4.0038
		CM	2.89750*	.33618	.000	2.1912	3.6038
		L.fermentum F-6	-.76250*	.33618	.036	-1.4688	-.0562
		L.fermentum CECT 5716	.29000	.33618	.400	-.4163	.9963
		L.fermentum cc IMAU:80780	.54500	.33618	.122	-.1613	1.2513
	L.fermentum F-6	*C*	4.06000*	.33618	.000	3.3537	4.7663
		CM	3.66000*	.33618	.000	2.9537	4.3663
		L.fermentum strain MGB 32-1	.76250*	.33618	.036	.0562	1.4688
		L.fermentum CECT 5716	1.05250*	.33618	.006	.3462	1.7588

MaleRBC	*L.fermentum CECT 5716*	*L.fermentum cc IMAU:80780*	1.30750*	.33618	.001	.6012	2.0138
		C	3.00750*	.33618	.000	2.3012	3.7138
		CM	2.60750*	.33618	.000	1.9012	3.3138
		L.fermentum strain MGB 32-1	-.29000	.33618	.400	-.9963	.4163
		L.fermentum F-6	-1.05250*	.33618	.006	-1.7588	-.3462
	L.fermentum cc IMAU:80780	*L.fermentum cc IMAU:80780*	.25500	.33618	.458	-.4513	.9613
		C	2.75250*	.33618	.000	2.0462	3.4588
		CM	2.35250*	.33618	.000	1.6462	3.0588
		L.fermentum strain MGB 32-1	-.54500	.33618	.122	-1.2513	.1613
		L.fermentum F-6	-1.30750*	.33618	.001	-2.0138	-.6012
	C	*L.fermentum CECT 5716*	-.25500	.33618	.458	-.9613	.4513
		CM	-.09750	.12438	.443	-.3588	.1638
		L.fermentum strain MGB 32-1	-1.09000*	.12438	.000	-1.3513	-.8287
		L.fermentum F-6	-1.28500*	.12438	.000	-1.5463	-1.0237
		L.fermentum CECT 5716	-1.01250*	.12438	.000	-1.2738	-.7512
		L.fermentum cc IMAU:80780	-.94500*	.12438	.000	-1.2063	-.6837
	CM	*C*	.09750	.12438	.443	-.1638	.3588
		L.fermentum strain MGB 32-1	-.99250*	.12438	.000	-1.2538	-.7312
		L.fermentum F-6	-1.18750*	.12438	.000	-1.4488	-.9262
		L.fermentum CECT 5716	-.91500*	.12438	.000	-1.1763	-.6537
		L.fermentum cc IMAU:80780	-.84750*	.12438	.000	-1.1088	-.5862
	L.fermentum strain MGB 32-1	*C*	1.09000*	.12438	.000	.8287	1.3513
		CM	.99250*	.12438	.000	.7312	1.2538
		L.fermentum F-6	-.19500	.12438	.134	-.4563	.0663
		L.fermentum CECT 5716	.07750	.12438	.541	-.1838	.3388
		L.fermentum cc IMAU:80780	.14500	.12438	.259	-.1163	.4063
	L.fermentum F-6	*C*	1.28500*	.12438	.000	1.0237	1.5463
		CM	1.18750*	.12438	.000	.9262	1.4488

		L.fermentum strain MGB 32-1	.19500	.12438	.134	-.0663	.4563
		L.fermentum CECT 5716	.27250*	.12438	.042	.0112	.5338
		L.fermentum cc IMAU:80780	.34000*	.12438	.014	.0787	.6013
		C	1.01250*	.12438	.000	.7512	1.2738
		CM	.91500*	.12438	.000	.6537	1.1763
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.07750	.12438	.541	-.3388	.1838
		L.fermentum F-6	-.27250*	.12438	.042	-.5338	-.0112
		L.fermentum cc IMAU:80780	.06750	.12438	.594	-.1938	.3288
		C	.94500*	.12438	.000	.6837	1.2063
		CM	.84750*	.12438	.000	.5862	1.1088
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.14500	.12438	.259	-.4063	.1163
		L.fermentum F-6	-.34000*	.12438	.014	-.6013	-.0787
		L.fermentum CECT 5716	-.06750	.12438	.594	-.3288	.1938
		CM	-.75000	1.08660	.499	-3.0329	1.5329
	C	*L.fermentum strain MGB 32-1*	-9.62500*	1.08660	.000	-11.9079	-7.3421
		L.fermentum F-6	-11.75000*	1.08660	.000	-14.0329	-9.4671
		L.fermentum CECT 5716	-8.35000*	1.08660	.000	-10.6329	-6.0671
		L.fermentum cc IMAU:80780	-8.02500*	1.08660	.000	-10.3079	-5.7421
		C	.75000	1.08660	.499	-1.5329	3.0329
	CM	*L.fermentum strain MGB 32-1*	-8.87500*	1.08660	.000	-11.1579	-6.5921
MalePCV		*L.fermentum F-6*	-11.00000*	1.08660	.000	-13.2829	-8.7171
		L.fermentum CECT 5716	-7.60000*	1.08660	.000	-9.8829	-5.3171
		L.fermentum cc IMAU:80780	-7.27500*	1.08660	.000	-9.5579	-4.9921
		C	9.62500*	1.08660	.000	7.3421	11.9079
		CM	8.87500*	1.08660	.000	6.5921	11.1579
	L.fermentum strain MGB 32-1	*L.fermentum F-6*	-2.12500	1.08660	.066	-4.4079	.1579
		L.fermentum CECT 5716	1.27500	1.08660	.256	-1.0079	3.5579
		L.fermentum cc IMAU:80780	1.60000	1.08660	.158	-.6829	3.8829

	L.fermentum F-6	*C*	11.75000*	1.08660	.000	9.4671	14.0329
		CM	11.00000*	1.08660	.000	8.7171	13.2829
		L.fermentum strain MGB 32-1	2.12500	1.08660	.066	-.1579	4.4079
		L.fermentum CECT 5716	3.40000*	1.08660	.006	1.1171	5.6829
		L.fermentum cc IMAU:80780	3.72500*	1.08660	.003	1.4421	6.0079
	L.fermentum CECT 5716	*C*	8.35000*	1.08660	.000	6.0671	10.6329
		CM	7.60000*	1.08660	.000	5.3171	9.8829
		L.fermentum strain MGB 32-1	-1.27500	1.08660	.256	-3.5579	1.0079
		L.fermentum F-6	-3.40000*	1.08660	.006	-5.6829	-1.1171
		L.fermentum cc IMAU:80780	.32500	1.08660	.768	-1.9579	2.6079
	L.fermentum cc IMAU:80780	*C*	8.02500*	1.08660	.000	5.7421	10.3079
		CM	7.27500*	1.08660	.000	4.9921	9.5579
		L.fermentum strain MGB 32-1	-1.60000	1.08660	.158	-3.8829	.6829
		L.fermentum F-6	-3.72500*	1.08660	.003	-6.0079	-1.4421
		L.fermentum CECT 5716	-.32500	1.08660	.768	-2.6079	1.9579
	C	*CM*	-.30000	.82698	.721	-2.0374	1.4374
		L.fermentum strain MGB 32-1	-4.40000*	.82698	.000	-6.1374	-2.6626
		L.fermentum F-6	-5.67500*	.82698	.000	-7.4124	-3.9376
		L.fermentum CECT 5716	-3.22500*	.82698	.001	-4.9624	-1.4876
		L.fermentum cc IMAU:80780	-3.40000*	.82698	.001	-5.1374	-1.6626
	CM	*C*	.30000	.82698	.721	-1.4374	2.0374
		L.fermentum strain MGB 32-1	-4.10000*	.82698	.000	-5.8374	-2.3626
		L.fermentum F-6	-5.37500*	.82698	.000	-7.1124	-3.6376
		L.fermentum CECT 5716	-2.92500*	.82698	.002	-4.6624	-1.1876
		L.fermentum cc IMAU:80780	-3.10000*	.82698	.001	-4.8374	-1.3626
	L.fermentum strain MGB 32-1	*C*	4.40000*	.82698	.000	2.6626	6.1374
		CM	4.10000*	.82698	.000	2.3626	5.8374
		L.fermentum F-6	-1.27500	.82698	.141	-3.0124	.4624
		L.fermentum CECT 5716	1.17500	.82698	.172	-.5624	2.9124

MaleMCv

		L.fermentum cc IMAU:80780	1.00000	.82698	.242	-.7374	2.7374
		C	5.67500*	.82698	.000	3.9376	7.4124
		CM	5.37500*	.82698	.000	3.6376	7.1124
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	1.27500	.82698	.141	-.4624	3.0124
		L.fermentum CECT 5716	2.45000*	.82698	.008	.7126	4.1874
		L.fermentum cc IMAU:80780	2.27500*	.82698	.013	.5376	4.0124
		C	3.22500*	.82698	.001	1.4876	4.9624
		CM	2.92500*	.82698	.002	1.1876	4.6624
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-1.17500	.82698	.172	-2.9124	.5624
		L.fermentum F-6	-2.45000*	.82698	.008	-4.1874	-.7126
		L.fermentum cc IMAU:80780	-.17500	.82698	.835	-1.9124	1.5624
		C	3.40000*	.82698	.001	1.6626	5.1374
		CM	3.10000*	.82698	.001	1.3626	4.8374
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-1.00000	.82698	.242	-2.7374	.7374
		L.fermentum F-6	-2.27500*	.82698	.013	-4.0124	-.5376
		L.fermentum CECT 5716	.17500	.82698	.835	-1.5624	1.9124
		CM	-.40000	.24367	.118	-.9119	.1119
		L.fermentum strain MGB 32-1	-1.95000*	.24367	.000	-2.4619	-1.4381
	C	*L.fermentum F-6*	-2.47500*	.24367	.000	-2.9869	-1.9631
		L.fermentum CECT 5716	-1.75000*	.24367	.000	-2.2619	-1.2381
		L.fermentum cc IMAU:80780	-1.55000*	.24367	.000	-2.0619	-1.0381
		C	.40000	.24367	.118	-.1119	.9119
MaleMCH		*L.fermentum strain MGB 32-1*	-1.55000*	.24367	.000	-2.0619	-1.0381
	CM	*L.fermentum F-6*	-2.07500*	.24367	.000	-2.5869	-1.5631
		L.fermentum CECT 5716	-1.35000*	.24367	.000	-1.8619	-.8381
		L.fermentum cc IMAU:80780	-1.15000*	.24367	.000	-1.6619	-.6381
		C	1.95000*	.24367	.000	1.4381	2.4619
	L.fermentum strain MGB 32-1	*CM*	1.55000*	.24367	.000	1.0381	2.0619
		L.fermentum F-6	-.52500*	.24367	.045	-1.0369	-.0131

		L.fermentum CECT 5716	.20000	.24367	.423	-.3119	.7119
		L.fermentum cc IMAU:80780	.40000	.24367	.118	-.1119	.9119
		C	2.47500*	.24367	.000	1.9631	2.9869
		CM	2.07500*	.24367	.000	1.5631	2.5869
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	.52500*	.24367	.045	.0131	1.0369
		L.fermentum CECT 5716	.72500*	.24367	.008	.2131	1.2369
		L.fermentum cc IMAU:80780	.92500*	.24367	.001	.4131	1.4369
		C	1.75000*	.24367	.000	1.2381	2.2619
		CM	1.35000*	.24367	.000	.8381	1.8619
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.20000	.24367	.423	-.7119	.3119
		L.fermentum F-6	-.72500*	.24367	.008	-1.2369	-.2131
		L.fermentum cc IMAU:80780	.20000	.24367	.423	-.3119	.7119
		C	1.55000*	.24367	.000	1.0381	2.0619
		CM	1.15000*	.24367	.000	.6381	1.6619
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.40000	.24367	.118	-.9119	.1119
		L.fermentum F-6	-.92500*	.24367	.001	-1.4369	-.4131
		L.fermentum CECT 5716	-.20000	.24367	.423	-.7119	.3119
		CM	-.52500	.26101	.059	-1.0734	.0234
		L.fermentum strain MGB 32-1	-.92500*	.26101	.002	-1.4734	-.3766
	C	*L.fermentum F-6*	-1.20000*	.26101	.000	-1.7484	-.6516
		L.fermentum CECT 5716	-1.20000*	.26101	.000	-1.7484	-.6516
		L.fermentum cc IMAU:80780	-.87500*	.26101	.004	-1.4234	-.3266
MaleMCHC		*C*	.52500	.26101	.059	-.0234	1.0734
		L.fermentum strain MGB 32-1	-.40000	.26101	.143	-.9484	.1484
	CM	*L.fermentum F-6*	-.67500*	.26101	.019	-1.2234	-.1266
		L.fermentum CECT 5716	-.67500*	.26101	.019	-1.2234	-.1266
		L.fermentum cc IMAU:80780	-.35000	.26101	.197	-.8984	.1984
	L.fermentum strain	*C*	.92500*	.26101	.002	.3766	1.4734

	MGB 32-1	*CM*	.40000	.26101	.143	-.1484	.9484
		L.fermentum F-6	-.27500	.26101	.306	-.8234	.2734
		L.fermentum CECT 5716	-.27500	.26101	.306	-.8234	.2734
		L.fermentum cc IMAU:80780	.05000	.26101	.850	-.4984	.5984
		C	1.20000*	.26101	.000	.6516	1.7484
	L.fermentum F-6	*CM*	.67500*	.26101	.019	.1266	1.2234
		L.fermentum strain MGB 32-1	.27500	.26101	.306	-.2734	.8234
		L.fermentum CECT 5716	.00000	.26101	1.000	-.5484	.5484
		L.fermentum cc IMAU:80780	.32500	.26101	.229	-.2234	.8734
		C	1.20000*	.26101	.000	.6516	1.7484
	L.fermentum CECT 5716	*CM*	.67500*	.26101	.019	.1266	1.2234
		L.fermentum strain MGB 32-1	.27500	.26101	.306	-.2734	.8234
		L.fermentum F-6	.00000	.26101	1.000	-.5484	.5484
		L.fermentum cc IMAU:80780	.32500	.26101	.229	-.2234	.8734
		C	.87500*	.26101	.004	.3266	1.4234
	L.fermentum cc IMAU:80780	*CM*	.35000	.26101	.197	-.1984	.8984
		L.fermentum strain MGB 32-1	-.05000	.26101	.850	-.5984	.4984
		L.fermentum F-6	-.32500	.26101	.229	-.8734	.2234
		L.fermentum CECT 5716	-.32500	.26101	.229	-.8734	.2234
		CM	-4.25000	31.91841	.896	-71.3081	62.8081
		L.fermentum strain MGB 32-1	-34.50000	31.91841	.294	-101.5581	32.5581
	C	*L.fermentum F-6*	-30.75000	31.91841	.348	-97.8081	36.3081
		L.fermentum CECT 5716	-42.25000	31.91841	.202	-109.3081	24.8081
MalePLTS		*L.fermentum cc IMAU:80780*	-20.00000	31.91841	.539	-87.0581	47.0581
		C	4.25000	31.91841	.896	-62.8081	71.3081
		L.fermentum strain MGB 32-1	-30.25000	31.91841	.356	-97.3081	36.8081
	CM	*L.fermentum F-6*	-26.50000	31.91841	.417	-93.5581	40.5581
		L.fermentum CECT 5716	-38.00000	31.91841	.249	-105.0581	29.0581

		L.fermentum cc IMAU:80780	-15.75000	31.91841	.628	-82.8081	51.3081
		C	34.50000	31.91841	.294	-32.5581	101.5581
		CM	30.25000	31.91841	.356	-36.8081	97.3081
	L.fermentum strain MGB 32-1	*L.fermentum F-6*	3.75000	31.91841	.908	-63.3081	70.8081
		L.fermentum CECT 5716	-7.75000	31.91841	.811	-74.8081	59.3081
		L.fermentum cc IMAU:80780	14.50000	31.91841	.655	-52.5581	81.5581
		C	30.75000	31.91841	.348	-36.3081	97.8081
		CM	26.50000	31.91841	.417	-40.5581	93.5581
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	-3.75000	31.91841	.908	-70.8081	63.3081
		L.fermentum CECT 5716	-11.50000	31.91841	.723	-78.5581	55.5581
		L.fermentum cc IMAU:80780	10.75000	31.91841	.740	-56.3081	77.8081
		C	42.25000	31.91841	.202	-24.8081	109.3081
		CM	38.00000	31.91841	.249	-29.0581	105.0581
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	7.75000	31.91841	.811	-59.3081	74.8081
		L.fermentum F-6	11.50000	31.91841	.723	-55.5581	78.5581
		L.fermentum cc IMAU:80780	22.25000	31.91841	.495	-44.8081	89.3081
		C	20.00000	31.91841	.539	-47.0581	87.0581
		CM	15.75000	31.91841	.628	-51.3081	82.8081
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-14.50000	31.91841	.655	-81.5581	52.5581
		L.fermentum F-6	-10.75000	31.91841	.740	-77.8081	56.3081
		L.fermentum CECT 5716	-22.25000	31.91841	.495	-89.3081	44.8081
		CM	.51250	.25685	.061	-.0271	1.0521
		L.fermentum strain MGB 32-1	.33000	.25685	.215	-.2096	.8696
	C	*L.fermentum F-6*	.79250*	.25685	.006	.2529	1.3321
MaleWBC		*L.fermentum CECT 5716*	.80500*	.25685	.006	.2654	1.3446
		L.fermentum cc IMAU:80780	.64250*	.25685	.022	.1029	1.1821
		C	-.51250	.25685	.061	-1.0521	.0271
	CM	*L.fermentum strain MGB 32-1*	-.18250	.25685	.486	-.7221	.3571

		L.fermentum F-6	.28000	.25685	.290	-.2596	.8196
		L.fermentum CECT 5716	.29250	.25685	.270	-.2471	.8321
		L.fermentum cc IMAU:80780	.13000	.25685	.619	-.4096	.6696
		C	-.33000	.25685	.215	-.8696	.2096
		CM	.18250	.25685	.486	-.3571	.7221
	L.fermentum strain MGB 32-1	*L.fermentum F-6*	.46250	.25685	.089	-.0771	1.0021
		L.fermentum CECT 5716	.47500	.25685	.081	-.0646	1.0146
		L.fermentum cc IMAU:80780	.31250	.25685	.239	-.2271	.8521
		C	-.79250*	.25685	.006	-1.3321	-.2529
		CM	-.28000	.25685	.290	-.8196	.2596
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	-.46250	.25685	.089	-1.0021	.0771
		L.fermentum CECT 5716	.01250	.25685	.962	-.5271	.5521
		L.fermentum cc IMAU:80780	-.15000	.25685	.566	-.6896	.3896
		C	-.80500*	.25685	.006	-1.3446	-.2654
		CM	-.29250	.25685	.270	-.8321	.2471
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.47500	.25685	.081	-1.0146	.0646
		L.fermentum F-6	-.01250	.25685	.962	-.5521	.5271
		L.fermentum cc IMAU:80780	-.16250	.25685	.535	-.7021	.3771
		C	-.64250*	.25685	.022	-1.1821	-.1029
		CM	-.13000	.25685	.619	-.6696	.4096
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.31250	.25685	.239	-.8521	.2271
		L.fermentum F-6	.15000	.25685	.566	-.3896	.6896
		L.fermentum CECT 5716	.16250	.25685	.535	-.3771	.7021

*. The mean difference is significant at the 0.05 level.

Appendix xv: Effect of 13 weeks consumption of 1ml of *Lactobacillusfermentum*-fermented milk on the haematology of female albino rats.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	C	4	9.7825	.79315	.39658	8.5204	11.0446	8.99	10.72
	CM	4	10.1450	.31172	.15586	9.6490	10.6410	9.86	10.58
	L.fermentum strain MGB 32-1	4	12.9725	.49527	.24763	12.1844	13.7606	12.32	13.48
FemaleGDL	*L.fermentum F-6*	4	13.6950	.30425	.15212	13.2109	14.1791	13.33	14.06
	L.fermentum CECT 5716	4	12.3525	.43508	.21754	11.6602	13.0448	11.88	12.90
	L.fermentum cc IMAU:80780	4	11.9925	.55776	.27888	11.1050	12.8800	11.45	12.75
	Total	24	11.8233	1.52016	.31030	11.1814	12.4652	8.99	14.06
	C	4	5.1975	.26986	.13493	4.7681	5.6269	4.92	5.52
	CM	4	5.3725	.11236	.05618	5.1937	5.5513	5.25	5.52
	L.fermentum strain MGB 32-1	4	6.3100	.18367	.09183	6.0177	6.6023	6.06	6.50
FemaleRBC	*L.fermentum F-6*	4	6.5650	.11446	.05723	6.3829	6.7471	6.44	6.71
	L.fermentum CECT 5716	4	6.1200	.15232	.07616	5.8776	6.3624	5.96	6.32
	L.fermentum cc IMAU:80780	4	6.0000	.18991	.09496	5.6978	6.3022	5.82	6.26
	Total	24	5.9275	.52413	.10699	5.7062	6.1488	4.92	6.71
	C	4	32.3500	2.39096	1.19548	28.5455	36.1545	30.00	35.20
	CM	4	32.3500	1.74069	.87034	29.5802	35.1198	29.80	33.60
	L.fermentum strain MGB 32-1	4	40.6000	2.88791	1.44395	36.0047	45.1953	36.60	43.20
FemalePCV	*L.fermentum F-6*	4	43.9000	1.06145	.53072	42.2110	45.5890	42.70	45.20
	L.fermentum CECT 5716	4	39.0500	2.15174	1.07587	35.6261	42.4739	36.40	41.50
	L.fermentum cc IMAU:80780	4	38.3250	2.26918	1.13459	34.7142	41.9358	35.60	41.10
	Total	24	37.7625	4.70484	.96037	35.7758	39.7492	29.80	45.20
FemaleMCV	*C*	4	62.2000	1.37840	.68920	60.0067	64.3933	61.00	63.80
	CM	4	60.2000	2.30507	1.15253	56.5321	63.8679	56.80	61.90

	L.fermentum strain MGB 32-1	4	64.3000	2.81069	1.40535	59.8276	68.7724	60.40	66.50
	L.fermentum F-6	4	66.8750	.47871	.23936	66.1133	67.6367	66.30	67.40
	L.fermentum CECT 5716	4	63.3500	2.78388	1.39194	58.9202	67.7798	59.40	65.70
	L.fermentum cc IMAU:80780	4	63.4250	2.70724	1.35362	59.1172	67.7328	59.50	65.70
	Total	24	63.3917	2.87068	.58597	62.1795	64.6038	56.80	67.40
	C	4	18.8000	.53541	.26771	17.9480	19.6520	18.30	19.40
	CM	4	18.9000	.21602	.10801	18.5563	19.2437	18.70	19.20
	L.fermentum strain MGB 32-1	4	20.5250	.20616	.10308	20.1970	20.8530	20.30	20.70
FemaleMCH	*L.fermentum F-6*	4	20.8750	.12583	.06292	20.6748	21.0752	20.70	21.00
	L.fermentum CECT 5716	4	20.1750	.22174	.11087	19.8222	20.5278	19.90	20.40
	L.fermentum cc IMAU:80780	4	20.0000	.29439	.14720	19.5316	20.4684	19.70	20.40
	Total	24	19.8792	.83717	.17089	19.5257	20.2327	18.30	21.00
	C	4	30.2500	.23805	.11902	29.8712	30.6288	30.00	30.50
	CM	4	31.4250	1.20381	.60191	29.5095	33.3405	30.50	33.10
	L.fermentum strain MGB 32-1	4	32.0000	1.16333	.58166	30.1489	33.8511	31.20	33.70
FemaleMCHC	*L.fermentum F-6*	4	31.1750	.09574	.04787	31.0227	31.3273	31.10	31.30
	L.fermentum CECT 5716	4	31.9000	1.15181	.57591	30.0672	33.7328	31.10	33.60
	L.fermentum cc IMAU:80780	4	31.5500	1.03441	.51720	29.9040	33.1960	31.00	33.10
	Total	24	31.3833	1.01753	.20770	30.9537	31.8130	30.00	33.70
	C	4	415.5000	38.38837	19.19418	354.4155	476.5845	378.00	452.00
	CM	4	432.0000	58.79909	29.39955	338.4375	525.5625	367.00	497.00
	L.fermentum strain MGB 32-1	4	453.5000	53.56927	26.78463	368.2593	538.7407	402.00	506.00
FemalePLTS	*L.fermentum F-6*	4	448.2500	45.75569	22.87785	375.4425	521.0575	393.00	502.00
	L.fermentum CECT 5716	4	464.7500	32.42813	16.21406	413.1496	516.3504	430.00	498.00
	L.fermentum cc IMAU:80780	4	434.5000	42.78240	21.39120	366.4237	502.5763	386.00	482.00
	Total	24	441.4167	43.99102	8.97963	422.8409	459.9924	367.00	506.00
	C	4	6.9425	.74271	.37136	5.7607	8.1243	6.14	7.82
FemaleWBC	*CM*	4	6.5675	.40393	.20196	5.9248	7.2102	6.22	7.15
	L.fermentum strain MGB 32-1	4	6.4750	.49749	.24875	5.6834	7.2666	6.01	6.92

	L.fermentum F-6	4	6.4575	.36409	.18204	5.8782	7.0368	6.02	6.85
	L.fermentum CECT 5716	4	6.7025	.29613	.14806	6.2313	7.1737	6.35	7.05
	L.fermentum cc IMAU:80780	4	6.6250	.36810	.18405	6.0393	7.2107	6.22	6.98
	Total	24	6.6283	.44705	.09125	6.4396	6.8171	6.01	7.82

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
FemaleGDL	Between Groups	48.457	5	9.691	37.167	.000
	Within Groups	4.694	18	.261		
	Total	53.150	23			
FemaleRBC	Between Groups	5.744	5	1.149	35.983	.000
	Within Groups	.575	18	.032		
	Total	6.318	23			
FemalePCV	Between Groups	425.139	5	85.028	18.225	.000
	Within Groups	83.978	18	4.665		
	Total	509.116	23			
FemaleMCV	Between Groups	98.273	5	19.655	3.876	.015
	Within Groups	91.265	18	5.070		
	Total	189.538	23			
FemaleMCH	Between Groups	14.537	5	2.907	33.070	.000
	Within Groups	1.582	18	.088		
	Total	16.120	23			
FemaleMCHC	Between Groups	8.018	5	1.604	1.828	.158
	Within Groups	15.795	18	.878		
	Total	23.813	23			
FemalePLTS	Between Groups	6181.333	5	1236.267	.581	.714
	Within Groups	38328.500	18	2129.361		
	Total	44509.833	23			
FemaleWBC	Between Groups	.642	5	.128	.585	.711
	Within Groups	3.954	18	.220		
	Total	4.597	23			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) GengerOrgan	(J) GengerOrgan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
FemaleHGB	*C*	*CM*	-.36250	.36107	.329	-1.1211	.3961
		L.fermentum strain MGB 32-1	-3.19000*	.36107	.000	-3.9486	-2.4314
		L.fermentum F-6	-3.91250*	.36107	.000	-4.6711	-3.1539
		L.fermentum CECT 5716	-2.57000*	.36107	.000	-3.3286	-1.8114
		L.fermentum cc IMAU:80780	-2.21000*	.36107	.000	-2.9686	-1.4514
	CM	*C*	.36250	.36107	.329	-.3961	1.1211
		L.fermentum strain MGB 32-1	-2.82750*	.36107	.000	-3.5861	-2.0689
		L.fermentum F-6	-3.55000*	.36107	.000	-4.3086	-2.7914
		L.fermentum CECT 5716	-2.20750*	.36107	.000	-2.9661	-1.4489
		L.fermentum cc IMAU:80780	-1.84750*	.36107	.000	-2.6061	-1.0889
	L.fermentum strain MGB 32-1	*C*	3.19000*	.36107	.000	2.4314	3.9486
		CM	2.82750*	.36107	.000	2.0689	3.5861
		L.fermentum F-6	-.72250	.36107	.061	-1.4811	.0361
		L.fermentum CECT 5716	.62000	.36107	.103	-.1386	1.3786
		L.fermentum cc IMAU:80780	.98000*	.36107	.014	.2214	1.7386
	L.fermentum F-6	*C*	3.91250*	.36107	.000	3.1539	4.6711
		CM	3.55000*	.36107	.000	2.7914	4.3086
		L.fermentum strain MGB 32-1	.72250	.36107	.061	-.0361	1.4811
		L.fermentum CECT 5716	1.34250*	.36107	.002	.5839	2.1011
		L.fermentum cc IMAU:80780	1.70250*	.36107	.000	.9439	2.4611

FemaleRBC	*L.fermentum CECT 5716*	*C*	2.57000*	.36107	.000	1.8114	3.3286
		CM	2.20750*	.36107	.000	1.4489	2.9661
		L.fermentum strain MGB 32-1	-.62000	.36107	.103	-1.3786	.1386
		L.fermentum F-6	-1.34250*	.36107	.002	-2.1011	-.5839
		L.fermentum cc IMAU:80780	.36000	.36107	.332	-.3986	1.1186
	L.fermentum cc IMAU:80780	*C*	2.21000*	.36107	.000	1.4514	2.9686
		CM	1.84750*	.36107	.000	1.0889	2.6061
		L.fermentum strain MGB 32-1	-.98000*	.36107	.014	-1.7386	-.2214
		L.fermentum F-6	-1.70250*	.36107	.000	-2.4611	-.9439
	C	*L.fermentum CECT 5716*	-.36000	.36107	.332	-1.1186	.3986
		CM	-.17500	.12634	.183	-.4404	.0904
		L.fermentum strain MGB 32-1	-1.11250*	.12634	.000	-1.3779	-.8471
		L.fermentum F-6	-1.36750*	.12634	.000	-1.6329	-1.1021
		L.fermentum CECT 5716	-.92250*	.12634	.000	-1.1879	-.6571
		L.fermentum cc IMAU:80780	-.80250*	.12634	.000	-1.0679	-.5371
	CM	*C*	.17500	.12634	.183	-.0904	.4404
		L.fermentum strain MGB 32-1	-.93750*	.12634	.000	-1.2029	-.6721
		L.fermentum F-6	-1.19250*	.12634	.000	-1.4579	-.9271
		L.fermentum CECT 5716	-.74750*	.12634	.000	-1.0129	-.4821
		L.fermentum cc IMAU:80780	-.62750*	.12634	.000	-.8929	-.3621
	L.fermentum strain MGB 32-1	*C*	1.11250*	.12634	.000	.8471	1.3779
		CM	.93750*	.12634	.000	.6721	1.2029
		L.fermentum F-6	-.25500	.12634	.059	-.5204	.0104
		L.fermentum CECT 5716	.19000	.12634	.150	-.0754	.4554
		L.fermentum cc IMAU:80780	.31000*	.12634	.025	.0446	.5754
	L.fermentum F-6	*C*	1.36750*	.12634	.000	1.1021	1.6329
		CM	1.19250*	.12634	.000	.9271	1.4579
		L.fermentum strain MGB 32-1	.25500	.12634	.059	-.0104	.5204

		L.fermentum CECT 5716	.44500*	.12634	.002	.1796	.7104
		L.fermentum cc IMAU:80780	.56500*	.12634	.000	.2996	.8304
		C	.92250*	.12634	.000	.6571	1.1879
		CM	.74750*	.12634	.000	.4821	1.0129
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.19000	.12634	.150	-.4554	.0754
		L.fermentum F-6	-.44500*	.12634	.002	-.7104	-.1796
		L.fermentum cc IMAU:80780	.12000	.12634	.355	-.1454	.3854
		C	.80250*	.12634	.000	.5371	1.0679
		CM	.62750*	.12634	.000	.3621	.8929
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.31000*	.12634	.025	-.5754	-.0446
		L.fermentum F-6	-.56500*	.12634	.000	-.8304	-.2996
		L.fermentum CECT 5716	-.12000	.12634	.355	-.3854	.1454
		CM	.00000	1.52732	1.000	-3.2088	3.2088
		L.fermentum strain MGB 32-1	-8.25000*	1.52732	.000	-11.4588	-5.0412
	C	*L.fermentum F-6*	-11.55000*	1.52732	.000	-14.7588	-8.3412
		L.fermentum CECT 5716	-6.70000*	1.52732	.000	-9.9088	-3.4912
		L.fermentum cc IMAU:80780	-5.97500*	1.52732	.001	-9.1838	-2.7662
		C	.00000	1.52732	1.000	-3.2088	3.2088
		L.fermentum strain MGB 32-1	-8.25000*	1.52732	.000	-11.4588	-5.0412
	CM	*L.fermentum F-6*	-11.55000*	1.52732	.000	-14.7588	-8.3412
FemalePCV		*L.fermentum CECT 5716*	-6.70000*	1.52732	.000	-9.9088	-3.4912
		L.fermentum cc IMAU:80780	-5.97500*	1.52732	.001	-9.1838	-2.7662
		C	8.25000*	1.52732	.000	5.0412	11.4588
		CM	8.25000*	1.52732	.000	5.0412	11.4588
	L.fermentum strain MGB 32-1	*L.fermentum F-6*	-3.30000*	1.52732	.044	-6.5088	-.0912
		L.fermentum CECT 5716	1.55000	1.52732	.324	-1.6588	4.7588
		L.fermentum cc IMAU:80780	2.27500	1.52732	.154	-.9338	5.4838
	L.fermentum F-6	*C*	11.55000*	1.52732	.000	8.3412	14.7588
		CM	11.55000*	1.52732	.000	8.3412	14.7588

		L.fermentum strain MGB 32-1	3.30000*	1.52732	.044	.0912	6.5088
		L.fermentum CECT 5716	4.85000*	1.52732	.005	1.6412	8.0588
		L.fermentum cc IMAU:80780	5.57500*	1.52732	.002	2.3662	8.7838
		C	6.70000*	1.52732	.000	3.4912	9.9088
		CM	6.70000*	1.52732	.000	3.4912	9.9088
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-1.55000	1.52732	.324	-4.7588	1.6588
		L.fermentum F-6	-4.85000*	1.52732	.005	-8.0588	-1.6412
		L.fermentum cc IMAU:80780	.72500	1.52732	.641	-2.4838	3.9338
		C	5.97500*	1.52732	.001	2.7662	9.1838
		CM	5.97500*	1.52732	.001	2.7662	9.1838
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-2.27500	1.52732	.154	-5.4838	.9338
		L.fermentum F-6	-5.57500*	1.52732	.002	-8.7838	-2.3662
		L.fermentum CECT 5716	-.72500	1.52732	.641	-3.9338	2.4838
		CM	2.00000	1.59221	.225	-1.3451	5.3451
	C	*L.fermentum strain MGB 32-1*	-2.10000	1.59221	.204	-5.4451	1.2451
		L.fermentum F-6	-4.67500*	1.59221	.009	-8.0201	-1.3299
		L.fermentum CECT 5716	-1.15000	1.59221	.479	-4.4951	2.1951
		L.fermentum cc IMAU:80780	-1.22500	1.59221	.452	-4.5701	2.1201
		C	-2.00000	1.59221	.225	-5.3451	1.3451
	CM	*L.fermentum strain MGB 32-1*	-4.10000*	1.59221	.019	-7.4451	-.7549
FemaleMCV		*L.fermentum F-6*	-6.67500*	1.59221	.001	-10.0201	-3.3299
		L.fermentum CECT 5716	-3.15000	1.59221	.063	-6.4951	.1951
		L.fermentum cc IMAU:80780	-3.22500	1.59221	.058	-6.5701	.1201
		C	2.10000	1.59221	.204	-1.2451	5.4451
		CM	4.10000*	1.59221	.019	.7549	7.4451
	L.fermentum strain MGB 32-1	*L.fermentum F-6*	-2.57500	1.59221	.123	-5.9201	.7701
		L.fermentum CECT 5716	.95000	1.59221	.558	-2.3951	4.2951
		L.fermentum cc IMAU:80780	.87500	1.59221	.589	-2.4701	4.2201

		C	4.67500*	1.59221	.009	1.3299	8.0201
		CM	6.67500*	1.59221	.001	3.3299	10.0201
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	2.57500	1.59221	.123	-.7701	5.9201
		L.fermentum CECT 5716	3.52500*	1.59221	.040	.1799	6.8701
		L.fermentum cc IMAU:80780	3.45000*	1.59221	.044	.1049	6.7951
		C	1.15000	1.59221	.479	-2.1951	4.4951
		CM	3.15000	1.59221	.063	-.1951	6.4951
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.95000	1.59221	.558	-4.2951	2.3951
		L.fermentum F-6	-3.52500*	1.59221	.040	-6.8701	-.1799
		L.fermentum cc IMAU:80780	-.07500	1.59221	.963	-3.4201	3.2701
		C	1.22500	1.59221	.452	-2.1201	4.5701
		CM	3.22500	1.59221	.058	-.1201	6.5701
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.87500	1.59221	.589	-4.2201	2.4701
		L.fermentum F-6	-3.45000*	1.59221	.044	-6.7951	-.1049
		L.fermentum CECT 5716	.07500	1.59221	.963	-3.2701	3.4201
		CM	-.10000	.20966	.639	-.5405	.3405
	C	*L.fermentum strain MGB 32-1*	-1.72500*	.20966	.000	-2.1655	-1.2845
		L.fermentum F-6	-2.07500*	.20966	.000	-2.5155	-1.6345
		L.fermentum CECT 5716	-1.37500*	.20966	.000	-1.8155	-.9345
		L.fermentum cc IMAU:80780	-1.20000*	.20966	.000	-1.6405	-.7595
		C	.10000	.20966	.639	-.3405	.5405
FemaleMCH	*CM*	*L.fermentum strain MGB 32-1*	-1.62500*	.20966	.000	-2.0655	-1.1845
		L.fermentum F-6	-1.97500*	.20966	.000	-2.4155	-1.5345
		L.fermentum CECT 5716	-1.27500*	.20966	.000	-1.7155	-.8345
		L.fermentum cc IMAU:80780	-1.10000*	.20966	.000	-1.5405	-.6595
		C	1.72500*	.20966	.000	1.2845	2.1655
	L.fermentum strain MGB 32-1	*CM*	1.62500*	.20966	.000	1.1845	2.0655
		L.fermentum F-6	-.35000	.20966	.112	-.7905	.0905
		L.fermentum CECT 5716	.35000	.20966	.112	-.0905	.7905

		L.fermentum cc IMAU:80780	.52500*	.20966	.022	.0845	.9655
		C	2.07500*	.20966	.000	1.6345	2.5155
		CM	1.97500*	.20966	.000	1.5345	2.4155
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	.35000	.20966	.112	-.0905	.7905
		L.fermentum CECT 5716	.70000*	.20966	.004	.2595	1.1405
		L.fermentum cc IMAU:80780	.87500*	.20966	.001	.4345	1.3155
		C	1.37500*	.20966	.000	.9345	1.8155
		CM	1.27500*	.20966	.000	.8345	1.7155
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.35000	.20966	.112	-.7905	.0905
		L.fermentum F-6	-.70000*	.20966	.004	-1.1405	-.2595
		L.fermentum cc IMAU:80780	.17500	.20966	.415	-.2655	.6155
		C	1.20000*	.20966	.000	.7595	1.6405
		CM	1.10000*	.20966	.000	.6595	1.5405
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.52500*	.20966	.022	-.9655	-.0845
		L.fermentum F-6	-.87500*	.20966	.001	-1.3155	-.4345
		L.fermentum CECT 5716	-.17500	.20966	.415	-.6155	.2655
		CM	-1.17500	.66238	.093	-2.5666	.2166
		L.fermentum strain MGB 32-1	-1.75000*	.66238	.017	-3.1416	-.3584
	C	*L.fermentum F-6*	-.92500	.66238	.180	-2.3166	.4666
		L.fermentum CECT 5716	-1.65000*	.66238	.023	-3.0416	-.2584
		L.fermentum cc IMAU:80780	-1.30000	.66238	.065	-2.6916	.0916
		C	1.17500	.66238	.093	-.2166	2.5666
FemaleMCHC		*L.fermentum strain MGB 32-1*	-.57500	.66238	.397	-1.9666	.8166
	CM	*L.fermentum F-6*	.25000	.66238	.710	-1.1416	1.6416
		L.fermentum CECT 5716	-.47500	.66238	.483	-1.8666	.9166
		L.fermentum cc IMAU:80780	-.12500	.66238	.852	-1.5166	1.2666
	L.fermentum strain MGB 32-1	*C*	1.75000*	.66238	.017	.3584	3.1416
		CM	.57500	.66238	.397	-.8166	1.9666
		L.fermentum F-6	.82500	.66238	.229	-.5666	2.2166

		L.fermentum CECT 5716	.10000	.66238	.882	-1.2916	1.4916
		L.fermentum cc IMAU:80780	.45000	.66238	.506	-.9416	1.8416
		C	.92500	.66238	.180	-.4666	2.3166
		CM	-.25000	.66238	.710	-1.6416	1.1416
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	-.82500	.66238	.229	-2.2166	.5666
		L.fermentum CECT 5716	-.72500	.66238	.288	-2.1166	.6666
		L.fermentum cc IMAU:80780	-.37500	.66238	.578	-1.7666	1.0166
		C	1.65000*	.66238	.023	.2584	3.0416
		CM	.47500	.66238	.483	-.9166	1.8666
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.10000	.66238	.882	-1.4916	1.2916
		L.fermentum F-6	.72500	.66238	.288	-.6666	2.1166
		L.fermentum cc IMAU:80780	.35000	.66238	.604	-1.0416	1.7416
		C	1.30000	.66238	.065	-.0916	2.6916
		CM	.12500	.66238	.852	-1.2666	1.5166
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.45000	.66238	.506	-1.8416	.9416
		L.fermentum F-6	.37500	.66238	.578	-1.0166	1.7666
		L.fermentum CECT 5716	-.35000	.66238	.604	-1.7416	1.0416
		CM	-16.50000	32.62944	.619	-85.0519	52.0519
		L.fermentum strain MGB 32-1	-38.00000	32.62944	.259	-106.5519	30.5519
	C	*L.fermentum F-6*	-32.75000	32.62944	.329	-101.3019	35.8019
		L.fermentum CECT 5716	-49.25000	32.62944	.149	-117.8019	19.3019
		L.fermentum cc IMAU:80780	-19.00000	32.62944	.568	-87.5519	49.5519
FemalePLTS		*C*	16.50000	32.62944	.619	-52.0519	85.0519
		L.fermentum strain MGB 32-1	-21.50000	32.62944	.518	-90.0519	47.0519
	CM	*L.fermentum F-6*	-16.25000	32.62944	.625	-84.8019	52.3019
		L.fermentum CECT 5716	-32.75000	32.62944	.329	-101.3019	35.8019
		L.fermentum cc IMAU:80780	-2.50000	32.62944	.940	-71.0519	66.0519
	L.fermentum	*C*	38.00000	32.62944	.259	-30.5519	106.5519

	strain MGB 32-1	*CM*	21.50000	32.62944	.518	-47.0519	90.0519
		L.fermentum F-6	5.25000	32.62944	.874	-63.3019	73.8019
		L.fermentum CECT 5716	-11.25000	32.62944	.734	-79.8019	57.3019
		L.fermentum cc IMAU:80780	19.00000	32.62944	.568	-49.5519	87.5519
		C	32.75000	32.62944	.329	-35.8019	101.3019
	L.fermentum F-6	*CM*	16.25000	32.62944	.625	-52.3019	84.8019
		L.fermentum strain MGB 32-1	-5.25000	32.62944	.874	-73.8019	63.3019
		L.fermentum CECT 5716	-16.50000	32.62944	.619	-85.0519	52.0519
		L.fermentum cc IMAU:80780	13.75000	32.62944	.678	-54.8019	82.3019
		C	49.25000	32.62944	.149	-19.3019	117.8019
	L.fermentum CECT 5716	*CM*	32.75000	32.62944	.329	-35.8019	101.3019
		L.fermentum strain MGB 32-1	11.25000	32.62944	.734	-57.3019	79.8019
		L.fermentum F-6	16.50000	32.62944	.619	-52.0519	85.0519
		L.fermentum cc IMAU:80780	30.25000	32.62944	.366	-38.3019	98.8019
		C	19.00000	32.62944	.568	-49.5519	87.5519
	L.fermentum cc IMAU:80780	*CM*	2.50000	32.62944	.940	-66.0519	71.0519
		L.fermentum strain MGB 32-1	-19.00000	32.62944	.568	-87.5519	49.5519
		L.fermentum F-6	-13.75000	32.62944	.678	-82.3019	54.8019
		L.fermentum CECT 5716	-30.25000	32.62944	.366	-98.8019	38.3019
	C	*CM*	.37500	.33142	.273	-.3213	1.0713
		L.fermentum strain MGB 32-1	.46750	.33142	.175	-.2288	1.1638
		L.fermentum F-6	.48500	.33142	.161	-.2113	1.1813
		L.fermentum CECT 5716	.24000	.33142	.478	-.4563	.9363
FemaleWBC		*L.fermentum cc IMAU:80780*	.31750	.33142	.351	-.3788	1.0138
		C	-.37500	.33142	.273	-1.0713	.3213
	CM	*L.fermentum strain MGB 32-1*	.09250	.33142	.783	-.6038	.7888
		L.fermentum F-6	.11000	.33142	.744	-.5863	.8063
		L.fermentum CECT 5716	-.13500	.33142	.689	-.8313	.5613

		L.fermentum cc IMAU:80780	-.05750	.33142	.864	-.7538	.6388
		C	-.46750	.33142	.175	-1.1638	.2288
		CM	-.09250	.33142	.783	-.7888	.6038
	L.fermentum strain MGB 32-1	*L.fermentum F-6*	.01750	.33142	.958	-.6788	.7138
		L.fermentum CECT 5716	-.22750	.33142	.501	-.9238	.4688
		L.fermentum cc IMAU:80780	-.15000	.33142	.656	-.8463	.5463
		C	-.48500	.33142	.161	-1.1813	.2113
		CM	-.11000	.33142	.744	-.8063	.5863
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	-.01750	.33142	.958	-.7138	.6788
		L.fermentum CECT 5716	-.24500	.33142	.469	-.9413	.4513
		L.fermentum cc IMAU:80780	-.16750	.33142	.619	-.8638	.5288
		C	-.24000	.33142	.478	-.9363	.4563
		CM	.13500	.33142	.689	-.5613	.8313
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	.22750	.33142	.501	-.4688	.9238
		L.fermentum F-6	.24500	.33142	.469	-.4513	.9413
		L.fermentum cc IMAU:80780	.07750	.33142	.818	-.6188	.7738
		C	-.31750	.33142	.351	-1.0138	.3788
		CM	.05750	.33142	.864	-.6388	.7538
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	.15000	.33142	.656	-.5463	.8463
		L.fermentum F-6	.16750	.33142	.619	-.5288	.8638
		L.fermentum CECT 5716	-.07750	.33142	.818	-.7738	.6188

*. The mean difference is significant at the 0.05 level.

Appendix xvi: Effect of 13 weeks consumption of 1ml of fermented milk on the relative organ weights ofmale albino rats.

Descriptive Information for LRW Male Rats

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	2.45925	.011177	.005588	2.44147	2.47703	2.448	2.474
CM	4	2.45300	.030518	.015259	2.40444	2.50156	2.416	2.487
L. fermentum MGB 32-1	4	2.45325	.067801	.033900	2.34536	2.56114	2.352	2.496
L. fermentum F-6	4	2.43800	.018385	.009192	2.40875	2.46725	2.420	2.458
L. fermentum CECT 5716	4	2.45125	.014431	.007215	2.42829	2.47421	2.435	2.467
L. fermentum cc IMAU:80780	4	2.43750	.016663	.008332	2.41098	2.46402	2.417	2.457
Total	24	2.44871	.030230	.006171	2.43594	2.46147	2.352	2.496

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	5	.000	.294	.910
Within Groups	.019	18	.001		
Total	.021	23			

Descriptive Information for BRWMale Rats

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	.54600	.013880	.006940	.52391	.56809	.534	.566
CM	4	.55025	.012685	.006343	.53006	.57044	.542	.569
L. fermentum MGB 32-1	4	.54725	.010243	.005121	.53095	.56355	.536	.560
L. fermentum F-6	4	.53675	.010996	.005498	.51925	.55425	.525	.548

L. fermentum CECT 5716	4	.54950	.013026	.006513	.52877	.57023	.533	.564
L. fermentum cc IMAU:80780	4	.54100	.008524	.004262	.52744	.55456	.534	.553
Total	24	.54513	.011452	.002338	.54029	.54996	.525	.569

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	5	.000	.805	.561
Within Groups	.002	18	.000		
Total	.003	23			

Descriptive Information for HRWMale Rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	.35275	.021329	.010664	.31881	.38669	.329	.378
CM	4	.34750	.007550	.003775	.33549	.35951	.339	.357
L. fermentum MGB 32-1	4	.34625	.003096	.001548	.34132	.35118	.342	.349
L. fermentum F-6	4	.34125	.003862	.001931	.33510	.34740	.337	.345
L. fermentum CECT 5716	4	.34575	.003775	.001887	.33974	.35176	.343	.351
L. fermentum cc IMAU:80780	4	.34175	.003096	.001548	.33682	.34668	.339	.346
Total	24	.34588	.009405	.001920	.34190	.34985	.329	.378

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	5	.000	0.758	0.348
Within Groups	.002	18	.000		
Total	.002	23			

Descriptive Information for KRW Male Rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	.67475	.022081	.011041	.63961	.70989	.646	.694
CM	4	.67850	.002082	.001041	.67519	.68181	.676	.681
L. fermentum MGB 32-1	4	.66825	.003594	.001797	.66253	.67397	.663	.671
L. fermentum F-6	4	.66450	.004203	.002102	.65781	.67119	.660	.670

L. fermentum CECT 5716	4	.67250	.003697	.001848	.66662	.67838	.670	.678
L. fermentum cc IMAU:80780	4	.66625	.003594	.001797	.66053	.67197	.663	.671
Total	24	.67079	.009838	.002008	.66664	.67495	.646	.694

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	5	.000	1.265	0.427
Within Groups	.002	18	.000		
Total	.002	23			

Descriptive Information for SRW Male Rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	.18875	.004272	.002136	.18195	.19555	.184	.194
CM	4	.18875	.004500	.002250	.18159	.19591	.185	.195
L. fermentum MGB 32-1	4	.18425	.000957	.000479	.18273	.18577	.183	.185
L. fermentum F-6	4	.18400	.001155	.000577	.18216	.18584	.183	.185
L. fermentum CECT 5716	4	.18600	.007746	.003873	.17367	.19833	.175	.193
L. fermentum cc IMAU:80780	4	.18425	.000500	.000250	.18345	.18505	.184	.185
Total	24	.18600	.004191	.000856	.18423	.18777	.175	.195

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	5	.000	1.200	.349
Within Groups	.000	18	.000		
Total	.000	23			

Appendix xvii: Effect of 13 weeks consumption of 1ml of fermented milk on the relative organ weights of female albino rats.

Descriptive Information for LRW Female Rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	2.56000	.088811	.044405	2.41868	2.70132	2.474	2.663
CM	4	2.46025	.059606	.029803	2.36540	2.55510	2.407	2.520
L. fermentum MGB 32-1	4	2.54775	.070201	.035101	2.43604	2.65946	2.484	2.610
L. fermentum F-6	4	2.51925	.087698	.043849	2.37970	2.65880	2.403	2.596
L. fermentum CECT 5716	4	2.51875	.086129	.043065	2.38170	2.65580	2.454	2.644
L. fermentum cc IMAU:80780	4	2.49325	.061824	.030912	2.39487	2.59163	2.432	2.564
Total	24	2.51654	.075827	.015478	2.48452	2.54856	2.403	2.663

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.026	5	.005	.896	.505
Within Groups	.106	18	.006		
Total	.132	23			

Descriptive Information for BRW Female Rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	.96325	.026550	.013275	.92100	1.00550	.939	1.000
CM	4	.95525	.006551	.003276	.94483	.96567	.947	.961
L. fermentum MGB 32-1	4	.94875	.012258	.006129	.92925	.96825	.940	.966
L. fermentum F-6	4	.94775	.001500	.000750	.94536	.95014	.946	.949
L. fermentum CECT 5716	4	.95125	.008655	.004328	.93748	.96502	.940	.961
L. fermentum cc IMAU:80780	4	.94775	.004992	.002496	.93981	.95569	.943	.953
Total	24	.95233	.012744	.002601	.94695	.95771	.939	1.000

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	5	.000	.882	.513
Within Groups	.003	18	.000		
Total	.004	23			

Descriptive Information for HRW Female Rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	.37400	.008756	.004378	.36007	.38793	.366	.385
CM	4	.36950	.007234	.003617	.35799	.38101	.361	.376
L. fermentum MGB 32-1	4	.38100	.008124	.004062	.36807	.39393	.375	.393
L. fermentum F-6	4	.36500	.003162	.001581	.35997	.37003	.361	.368
L. fermentum CECT 5716	4	.37175	.005965	.002983	.36226	.38124	.363	.376
L. fermentum cc IMAU:80780	4	.36850	.006245	.003122	.35856	.37844	.361	.376
Total	24	.37163	.007928	.001618	.36828	.37497	.361	.393

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	5	.000	2.605	.061
Within Groups	.001	18	.000		
Total	.001	23			

Descriptive Information for KRW Female Rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	.85050	.003416	.001708	.84506	.85594	.846	.854
CM	4	.84800	.012728	.006364	.82775	.86825	.829	.856
L. fermentum MGB 32-1	4	.84025	.006076	.003038	.83058	.84992	.835	.849
L. fermentum F-6	4	.83850	.016135	.008067	.81283	.86417	.827	.862
L. fermentum CECT 5716	4	.83625	.008539	.004270	.82266	.84984	.828	.847
L. fermentum cc IMAU:80780	4	.83750	.007853	.003926	.82500	.85000	.829	.848
Total	24	.84183	.010474	.002138	.83741	.84626	.827	.862

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	5	.000	1.401	.271
Within Groups	.002	18	.000		
Total	.003	23			

Descriptive Information for SRW Female Rats

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C	4	.25750	.011705	.005852	.23888	.27612	.243	.271
CM	4	.25875	.002062	.001031	.25547	.26203	.256	.261
L. fermentum MGB 32-1	4	.25125	.003775	.001887	.24524	.25726	.246	.255
L. fermentum F-6	4	.25475	.003500	.001750	.24918	.26032	.251	.259
L. fermentum CECT 5716	4	.26075	.005737	.002869	.25162	.26988	.254	.268
L. fermentum cc IMAU:80780	4	.25475	.002500	.001250	.25077	.25873	.252	.258
Total	24	.25629	.006083	.001242	.25372	.25886	.243	.271

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	5	.000	1.335	.294
Within Groups	.001	18	.000		
Total	.001	23			

Appendix xviii: Effect of 13 weeks consumption of 1ml of fermented milk on the serum cholesterol level ofmale and female albino rats.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
CholeMale	*C*	4	1.8450	.09574	.04787	1.6927	1.9973	1.71	1.93
	CM	4	1.8775	.08995	.04498	1.7344	2.0206	1.75	1.95
	L.fermentum strain MGB 32-1	4	1.7025	.08261	.04131	1.5710	1.8340	1.64	1.82
	L.fermentum F-6	4	1.2275	.06994	.03497	1.1162	1.3388	1.16	1.32
	L.fermentum CECT 5716	4	1.7200	.07165	.03582	1.6060	1.8340	1.64	1.79
	L.fermentum cc IMAU:80780	4	1.2825	.07932	.03966	1.1563	1.4087	1.18	1.35
	Total	24	1.6092	.27388	.05590	1.4935	1.7248	1.16	1.95
Chole Female	*C*	4	1.7900	.09416	.04708	1.6402	1.9398	1.70	1.92
	CM	4	1.8225	.09777	.04888	1.6669	1.9781	1.73	1.96
	L.fermentum strain MGB 32-1	4	1.6825	.07500	.03750	1.5632	1.8018	1.60	1.76
	L.fermentum F-6	4	1.1925	.08221	.04110	1.0617	1.3233	1.10	1.28
	L.fermentum CECT 5716	4	1.6525	.07932	.03966	1.5263	1.7787	1.60	1.77
	L.fermentum cc IMAU:80780	4	1.2500	.05292	.02646	1.1658	1.3342	1.20	1.32
	Total	24	1.5650	.26582	.05426	1.4528	1.6772	1.10	1.96

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CholeMale	Between Groups	1.604	5	.321	47.643	.000
	Within Groups	.121	18	.007		
	Total	1.725	23			
CholeFemale	Between Groups	1.505	5	.301	45.278	.000
	Within Groups	.120	18	.007		
	Total	1.625	23			

Post Hoc Tests

Multiple Comparisons

LSD

Dependent Variable	(I) ORGANICHOLESTE13WEEKS	(J) ORGANICHOLESTE13WEEKS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
CholeMale	*C*	*CM*	-.03250	.05802	.582	-.1544	.0894
		L.fermentum strain MGB 32-1	.14250*	.05802	.024	.0206	.2644
		L.fermentum F-6	.61750*	.05802	.000	.4956	.7394
		L.fermentum CECT 5716	.12500*	.05802	.045	.0031	.2469
		L.fermentum cc IMAU:80780	.56250*	.05802	.000	.4406	.6844
	CM	*C*	.03250	.05802	.582	-.0894	.1544
		L.fermentum strain MGB 32-1	.17500*	.05802	.007	.0531	.2969
		L.fermentum F-6	.65000*	.05802	.000	.5281	.7719
		L.fermentum CECT 5716	.15750*	.05802	.014	.0356	.2794
		L.fermentum cc IMAU:80780	.59500*	.05802	.000	.4731	.7169
	L.fermentum strain MGB 32-1	*C*	-.14250*	.05802	.024	-.2644	-.0206
		CM	-.17500*	.05802	.007	-.2969	-.0531
		L.fermentum F-6	.47500*	.05802	.000	.3531	.5969
		L.fermentum CECT 5716	-.01750	.05802	.766	-.1394	.1044
		L.fermentum cc IMAU:80780	.42000*	.05802	.000	.2981	.5419
	L.fermentum F-6	*C*	-.61750*	.05802	.000	-.7394	-.4956
		CM	-.65000*	.05802	.000	-.7719	-.5281
		L.fermentum strain MGB 32-1	-.47500*	.05802	.000	-.5969	-.3531
		L.fermentum CECT 5716	-.49250*	.05802	.000	-.6144	-.3706
		L.fermentum cc IMAU:80780	-.05500	.05802	.356	-.1769	.0669

CholeFemale	*L.fermentum CECT 5716*	*C*	-.12500*	.05802	.045	-.2469	-.0031
		CM	-.15750*	.05802	.014	-.2794	-.0356
		L.fermentum strain MGB 32-1	.01750	.05802	.766	-.1044	.1394
		L.fermentum F-6	.49250*	.05802	.000	.3706	.6144
		L.fermentum cc IMAU:80780	.43750*	.05802	.000	.3156	.5594
	L.fermentum cc IMAU:80780	*C*	-.56250*	.05802	.000	-.6844	-.4406
		CM	-.59500*	.05802	.000	-.7169	-.4731
		L.fermentum strain MGB 32-1	-.42000*	.05802	.000	-.5419	-.2981
		L.fermentum F-6	.05500	.05802	.356	-.0669	.1769
		L.fermentum CECT 5716	-.43750*	.05802	.000	-.5594	-.3156
	C	*CM*	-.03250	.05766	.580	-.1536	.0886
		L.fermentum strain MGB 32-1	.10750	.05766	.079	-.0136	.2286
		L.fermentum F-6	.59750*	.05766	.000	.4764	.7186
		L.fermentum CECT 5716	.13750*	.05766	.028	.0164	.2586
		L.fermentum cc IMAU:80780	.54000*	.05766	.000	.4189	.6611
	CM	*C*	.03250	.05766	.580	-.0886	.1536
		L.fermentum strain MGB 32-1	.14000*	.05766	.026	.0189	.2611
		L.fermentum F-6	.63000*	.05766	.000	.5089	.7511
		L.fermentum CECT 5716	.17000*	.05766	.009	.0489	.2911
		L.fermentum cc IMAU:80780	.57250*	.05766	.000	.4514	.6936
	L.fermentum strain MGB 32-1	*C*	-.10750	.05766	.079	-.2286	.0136
		CM	-.14000*	.05766	.026	-.2611	-.0189
		L.fermentum F-6	.49000*	.05766	.000	.3689	.6111
		L.fermentum CECT 5716	.03000	.05766	.609	-.0911	.1511
		L.fermentum cc IMAU:80780	.43250*	.05766	.000	.3114	.5536
	L.fermentum F-6	*C*	-.59750*	.05766	.000	-.7186	-.4764
		CM	-.63000*	.05766	.000	-.7511	-.5089

		L.fermentum strain MGB 32-1	-.49000*	.05766	.000	-.6111	-.3689
		L.fermentum CECT 5716	-.46000*	.05766	.000	-.5811	-.3389
		L.fermentum cc IMAU:80780	-.05750	.05766	.332	-.1786	.0636
		C	-.13750*	.05766	.028	-.2586	-.0164
		CM	-.17000*	.05766	.009	-.2911	-.0489
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	-.03000	.05766	.609	-.1511	.0911
		L.fermentum F-6	.46000*	.05766	.000	.3389	.5811
		L.fermentum cc IMAU:80780	.40250*	.05766	.000	.2814	.5236
		C	-.54000*	.05766	.000	-.6611	-.4189
		CM	-.57250*	.05766	.000	-.6936	-.4514
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	-.43250*	.05766	.000	-.5536	-.3114
		L.fermentum F-6	.05750	.05766	.332	-.0636	.1786
		L.fermentum CECT 5716	-.40250*	.05766	.000	-.5236	-.2814

*. The mean difference is significant at the 0.05 level.

Group Statistics

	GENDERCHOL13WEEKS	N	Mean	Std. Deviation	Std. Error Mean
CHOLE13WEEKS	MALE	24	1.6092	.27388	.05590
	FEMALE	24	1.5650	.26582	.05426

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
CHOLE13WEEKS	Equal variances assumed	.035	.852	.567	46	.574	.04417	.07791	-.11265	.20099
	Equal variances not assumed			.567	45.959	.574	.04417	.07791	-.11266	.20099

Appendix xix: Sensory evaluation of the fermented milk samples fermented by strains of *Lactobacillus fermentum*

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Taste	*L.fermentum strain MGB 32-1*	12	6.5833	.79296	.22891	6.0795	7.0872	5.00	8.00
	L.fermentum F-6	12	6.4167	.51493	.14865	6.0895	6.7438	6.00	7.00
	L.fermentum CECT 5716	12	6.5000	.52223	.15076	6.1682	6.8318	6.00	7.00
	L.fermentum cc IMAU:80780	12	6.3333	.65134	.18803	5.9195	6.7472	6.00	8.00
	SF	12	7.0000	.73855	.21320	6.5307	7.4693	6.00	8.00
	Total	60	6.5667	.67313	.08690	6.3928	6.7406	5.00	8.00
Aroma	*L.fermentum strain MGB 32-1*	12	6.5833	.66856	.19300	6.1586	7.0081	6.00	8.00
	L.fermentum F-6	12	6.5000	.67420	.19462	6.0716	6.9284	5.00	7.00
	L.fermentum CECT 5716	12	6.7500	.45227	.13056	6.4626	7.0374	6.00	7.00
	L.fermentum cc IMAU:80780	12	6.6667	.49237	.14213	6.3538	6.9795	6.00	7.00
	SF	12	5.8333	.38925	.11237	5.5860	6.0807	5.00	6.00
	Total	60	6.4667	.62346	.08049	6.3056	6.6277	5.00	8.00
texture	*L.fermentum strain MGB 32-1*	12	7.5000	.52223	.15076	7.1682	7.8318	7.00	8.00
	L.fermentum F-6	12	7.6667	.49237	.14213	7.3538	7.9795	7.00	8.00
	L.fermentum CECT 5716	12	7.5833	.51493	.14865	7.2562	7.9105	7.00	8.00
	L.fermentum cc IMAU:80780	12	7.7500	.62158	.17944	7.3551	8.1449	7.00	9.00
	SF	12	6.6667	.49237	.14213	6.3538	6.9795	6.00	7.00
	Total	60	7.4333	.64746	.08359	7.2661	7.6006	6.00	9.00
Colour	*L.fermentum strain MGB 32-1*	12	7.9167	.66856	.19300	7.4919	8.3414	7.00	9.00
	L.fermentum F-6	12	7.8333	.71774	.20719	7.3773	8.2894	7.00	9.00
	L.fermentum CECT 5716	12	8.0833	.79296	.22891	7.5795	8.5872	7.00	9.00

acceptability	*L.fermentum cc IMAU:80780*	12	8.0000	.73855	.21320	7.5307	8.4693	7.00	9.00
	SF	12	6.8333	.83485	.24100	6.3029	7.3638	6.00	9.00
	Total	60	7.7333	.86095	.11115	7.5109	7.9557	6.00	9.00
	L.fermentum strain MGB 32-1	12	7.1667	.38925	.11237	6.9193	7.4140	7.00	8.00
	L.fermentum F-6	12	7.3333	.49237	.14213	7.0205	7.6462	7.00	8.00
	L.fermentum CECT 5716	12	7.2500	.45227	.13056	6.9626	7.5374	7.00	8.00
	L.fermentum cc IMAU:80780	12	7.4167	.51493	.14865	7.0895	7.7438	7.00	8.00
	SF	12	6.5833	.51493	.14865	6.2562	6.9105	6.00	7.00
	Total	60	7.1500	.54695	.07061	7.0087	7.2913	6.00	8.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Taste	Between Groups	3.233	4	.808	1.892	.125
	Within Groups	23.500	55	.427		
	Total	26.733	59			
Aroma	Between Groups	6.433	4	1.608	5.361	.001
	Within Groups	16.500	55	.300		
	Total	22.933	59			
texture	Between Groups	9.233	4	2.308	8.191	.000
	Within Groups	15.500	55	.282		
	Total	24.733	59			
Colour	Between Groups	12.567	4	3.142	5.544	.001
	Within Groups	31.167	55	.567		
	Total	43.733	59			
acceptability	Between Groups	5.233	4	1.308	5.795	.001
	Within Groups	12.417	55	.226		
	Total	17.650	59			

Multiple Comparisons

LSD

Dependent Variable	(I) sensory	(J) sensory	Mean Differenc	Std. Error	Sig.	95% Confidence Interval

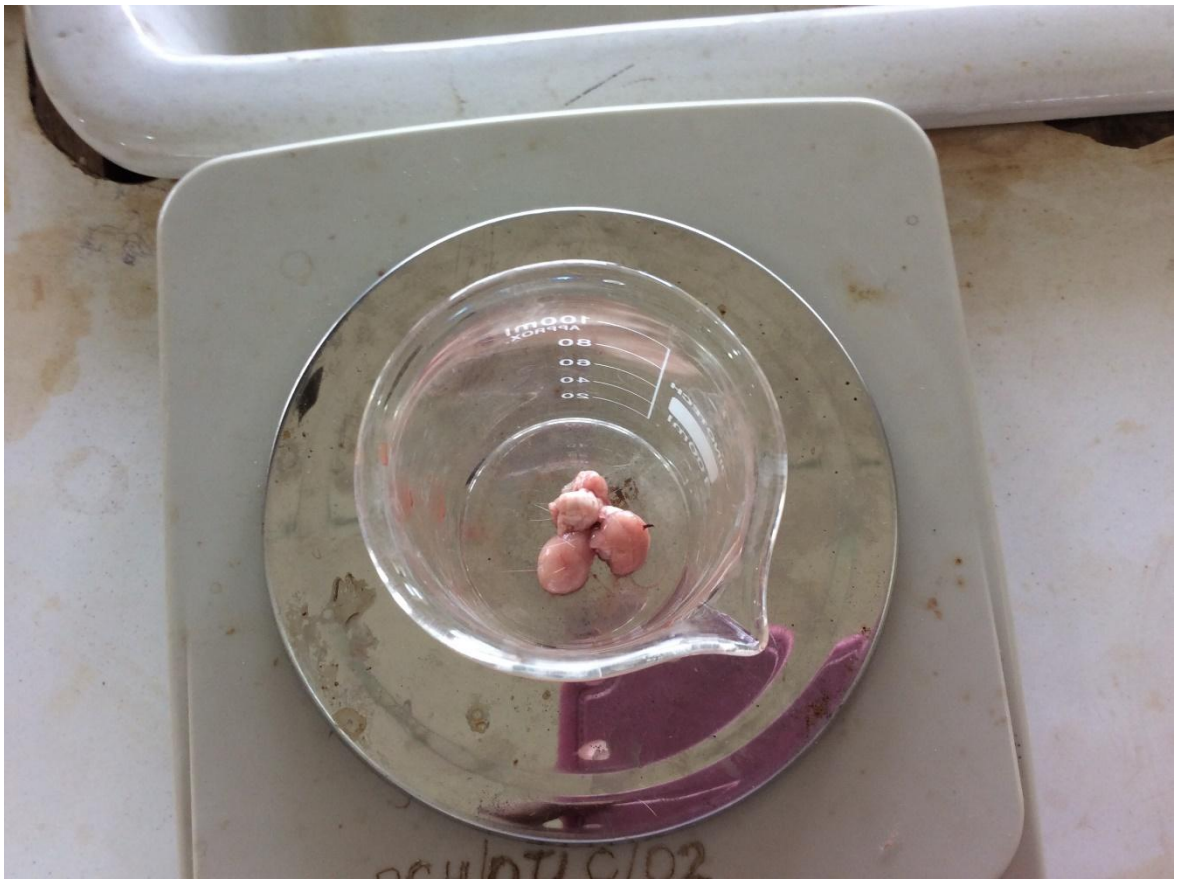
			e (I-J)			Lower Bound	Upper Bound
Aroma	*L.fermentum strain MGB 32-1*	*L.fermentum F-6*	.08333	.22361	.711	-.3648	.5315
		L.fermentum CECT 5716	-.16667	.22361	.459	-.6148	.2815
		L.fermentum cc IMAU:80780	-.08333	.22361	.711	-.5315	.3648
		SFN	.75000*	.22361	.001	.3019	1.1981
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	-.08333	.22361	.711	-.5315	.3648
		L.fermentum CECT 5716	-.25000	.22361	.268	-.6981	.1981
		L.fermentum cc IMAU:80780	-.16667	.22361	.459	-.6148	.2815
		SFN	.66667*	.22361	.004	.2185	1.1148
	L.fermentum CECT 5716	*L.fermentum strain MGB 32-1*	.16667	.22361	.459	-.2815	.6148
		L.fermentum F-6	.25000	.22361	.268	-.1981	.6981
		L.fermentum cc IMAU:80780	.08333	.22361	.711	-.3648	.5315
		SFN	.91667*	.22361	.000	.4685	1.3648
	L.fermentum cc IMAU:80780	*L.fermentum strain MGB 32-1*	.08333	.22361	.711	-.3648	.5315
		L.fermentum F-6	.16667	.22361	.459	-.2815	.6148
		L.fermentum CECT 5716	-.08333	.22361	.711	-.5315	.3648
		SFN	.83333*	.22361	.000	.3852	1.2815
	SFN	*L.fermentum strain MGB 32-1*	-.75000*	.22361	.001	-1.1981	-.3019
		L.fermentum F-6	-.66667*	.22361	.004	-1.1148	-.2185
		L.fermentum CECT 5716	-.91667*	.22361	.000	-1.3648	-.4685
		L.fermentum cc IMAU:80780	-.83333*	.22361	.000	-1.2815	-.3852
texture	*L.fermentum strain MGB 32-1*	*L.fermentum F-6*	-.16667	.21672	.445	-.6010	.2677
		L.fermentum CECT 5716	-.08333	.21672	.702	-.5177	.3510
		L.fermentum cc IMAU:80780	-.25000	.21672	.254	-.6843	.1843
		SFN	.83333*	.21672	.000	.3990	1.2677
	L.fermentum F-6	*L.fermentum strain MGB 32-1*	.16667	.21672	.445	-.2677	.6010

		L.fermentum CECT 5716	.08333	.21672	.702	-.3510	.5177
		L.fermentum cc IMAU:80780	-.08333	.21672	.702	-.5177	.3510
		SFN	1.00000*	.21672	.000	.5657	1.4343
		L.fermentum strain MGB 32-1	.08333	.21672	.702	-.3510	.5177
	L.fermentum CECT 5716	*L.fermentum F-6*	-.08333	.21672	.702	-.5177	.3510
		L.fermentum cc IMAU:80780	-.16667	.21672	.445	-.6010	.2677
		SFN	.91667*	.21672	.000	.4823	1.3510
		L.fermentum strain MGB 32-1	.25000	.21672	.254	-.1843	.6843
	L.fermentum cc IMAU:80780	*L.fermentum F-6*	.08333	.21672	.702	-.3510	.5177
		L.fermentum CECT 5716	.16667	.21672	.445	-.2677	.6010
		SFN	1.08333*	.21672	.000	.6490	1.5177
		L.fermentum strain MGB 32-1	-.83333*	.21672	.000	-1.2677	-.3990
	SFN	*L.fermentum F-6*	-1.00000*	.21672	.000	-1.4343	-.5657
		L.fermentum CECT 5716	-.91667*	.21672	.000	-1.3510	-.4823
		L.fermentum cc IMAU:80780	-1.08333*	.21672	.000	-1.5177	-.6490
		L.fermentum F-6	.08333	.30732	.787	-.5325	.6992
	L.fermentum strain MGB 32-1	*L.fermentum CECT 5716*	-.16667	.30732	.590	-.7825	.4492
		L.fermentum cc IMAU:80780	-.08333	.30732	.787	-.6992	.5325
		SFN	1.08333*	.30732	.001	.4675	1.6992
		L.fermentum strain MGB 32-1	-.08333	.30732	.787	-.6992	.5325
Colour	*L.fermentum F-6*	*L.fermentum CECT 5716*	-.25000	.30732	.419	-.8659	.3659
		L.fermentum cc IMAU:80780	-.16667	.30732	.590	-.7825	.4492
		SFN	1.00000*	.30732	.002	.3841	1.6159
		L.fermentum strain MGB 32-1	.16667	.30732	.590	-.4492	.7825
	L.fermentum CECT 5716	*L.fermentum F-6*	.25000	.30732	.419	-.3659	.8659
		L.fermentum cc IMAU:80780	.08333	.30732	.787	-.5325	.6992
		SFN	1.25000*	.30732	.000	.6341	1.8659

		L.fermentum strain MGB 32-1	.08333	.30732	.787	-.5325	.6992
	L.fermentum cc IMAU:80780	*L.fermentum F-6*	.16667	.30732	.590	-.4492	.7825
		L.fermentum CECT 5716	-.08333	.30732	.787	-.6992	.5325
		SFN	1.16667*	.30732	.000	.5508	1.7825
		L.fermentum strain MGB 32-1	-1.08333*	.30732	.001	-1.6992	-.4675
		L.fermentum F-6	-1.00000*	.30732	.002	-1.6159	-.3841
	SFN	*L.fermentum CECT 5716*	-1.25000*	.30732	.000	-1.8659	-.6341
		L.fermentum cc IMAU:80780	-1.16667*	.30732	.000	-1.7825	-.5508
		L.fermentum F-6	-.16667	.19397	.394	-.5554	.2221
	L.fermentum strain MGB 32-1	*L.fermentum CECT 5716*	-.08333	.19397	.669	-.4721	.3054
		L.fermentum cc IMAU:80780	-.25000	.19397	.203	-.6387	.1387
		SFN	.58333*	.19397	.004	.1946	.9721
		L.fermentum strain MGB 32-1	.16667	.19397	.394	-.2221	.5554
	L.fermentum F-6	*L.fermentum CECT 5716*	.08333	.19397	.669	-.3054	.4721
		L.fermentum cc IMAU:80780	-.08333	.19397	.669	-.4721	.3054
		SFN	.75000*	.19397	.000	.3613	1.1387
acceptability		*L.fermentum strain MGB 32-1*	.08333	.19397	.669	-.3054	.4721
	L.fermentum CECT 5716	*L.fermentum F-6*	-.08333	.19397	.669	-.4721	.3054
		L.fermentum cc IMAU:80780	-.16667	.19397	.394	-.5554	.2221
		SFN	.66667*	.19397	.001	.2779	1.0554
		L.fermentum strain MGB 32-1	.25000	.19397	.203	-.1387	.6387
	L.fermentum cc IMAU:80780	*L.fermentum F-6*	.08333	.19397	.669	-.3054	.4721
		L.fermentum CECT 5716	.16667	.19397	.394	-.2221	.5554
		SFN	.83333*	.19397	.000	.4446	1.2221
		L.fermentum strain MGB 32-1	-.58333*	.19397	.004	-.9721	-.1946
	SFN	*L.fermentum F-6*	-.75000*	.19397	.000	-1.1387	-.3613

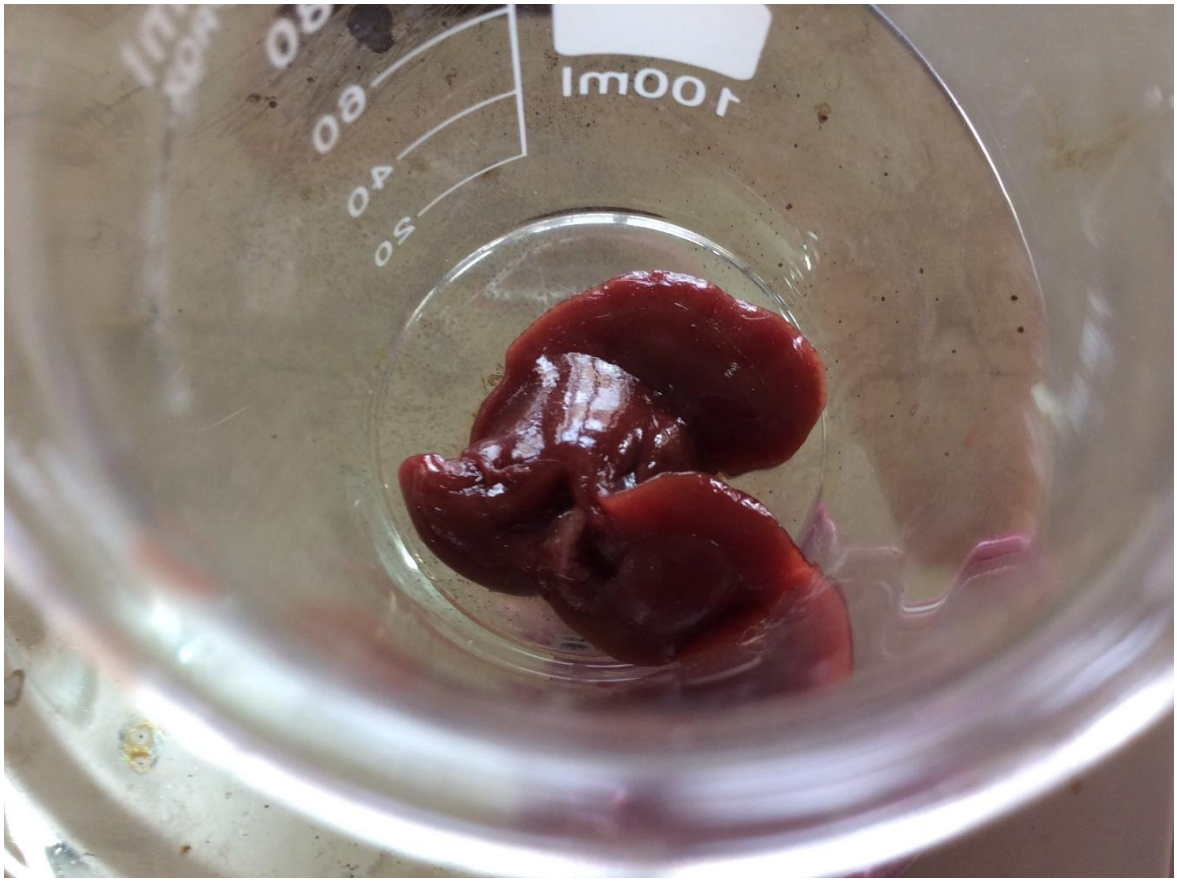
	L.fermentum CECT 5716	-.66667*	.19397	.001	-1.0554	-.2779
	L.fermentum cc IMAU:80780	-.83333*	.19397	.000	-1.2221	-.4446

*. The mean difference is significant at the 0.05 level.



BRAIN

KIDNEYS



LIVER

SPLEEN

HEART