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College of Graduate Studies
(SUST)

Assessment of different cereal brans (sorghum, barely and millet) as prebiotics in goat milk fermented with *Bifidbacterium longum BB536*

تقييم نخالة الحبوب المختلفة (الذرة الرفيعة ، الشعير والدخن) كبريبايوتك في لبن الماعز المخمر بواسطة بكتريا *Bifidbacterium longum BB536*

By:

Limia Hashim Mohammed Eltaher

B. Sc of Science (Honors) in Biochemistry and Food Science, University of Khartoum, SUDAN.

M.Sc. in food Science and Technology, College of Agricultural Studies, Sudan University of Science and Technology, SUDAN

Supervisor:

Associate Professor Dr. Barka Mohammed Kabeir Barka

Department of Food Science and Technology

College of Agricultural Studies, SUST

Co- Supervisor:

Assistant Professor Dr. Salma Elghali Mustafa

Department of Food Science and Technology

College of Agricultural Studies, SUST

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(وما أوتيتم من العلم إلا قليلا) .

صدق الله العظيم

سورة الأسراء الآية (85)

Dedication

I dedicated this dissertation

To my great parents

To my husband and my little sons

To my big family for their kind helps and support.

It also goes to teachers, scientists, researchers and all

Seekers for knowledge.

Acknowledgement

Alhamdulillah, I finished my study with help and full support of my lord ALLAH, guidance of my supervisor, cooperation of friends and family.

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Abstract

This study was carried out to explore prebiotic effect of different cereal bran on *Bifidobacterium longum* BB536 growth stimulation for developing functional food. Fermentation medium was formulated from goat milk supplemented with 10% inulin (controlled) or each of the different cereal bran sorghum, barley, and millet. Probiotic strain *B. longum* BB 536 was used for fermentation. Prebiotic effects of different cereal bran were examined. Safety and beneficial effect of fermented goat milk supplemented with different bran on colon bacterial groups of fed rats were evaluated.

Different analyses including proximate, mineral, strain BB536 viable count, physicochemical analysis were conducted. Thirty male Albino rats were acclimatized for two weeks to experimental conditions and randomly assigned into five groups. The control group received a normal rat diet. Other rats groups received fermented goat milk supplemented with each of inulin, sorghum bran, barley bran and millet bran. The experimental trials were extended for 6 weeks. Inulin was an excellent source of fiber, carbohydrate, and minerals (Ca, K, Mg and Na). Among cereal, bran barley contained the highest level of fiber, followed by sorghum and then millet barn. Moreover, cereal bran is a good source of protein, fat and ash. During fermentation, the maximum growths of the strain *BB 536* (12 h incubation) were 7.53±0.16b -8.43±0.03cLog CFU/ml in fermented goat milk supplemented with inulin and different cereal bran. These counts exceed the minimum number (6 log CFU/ml products) required to the presence in probiotic food. Further, rates of strain BB 536 increases induced by different cereal bran (7.65 – 8.43CFU/ml) are comparable to that of the commercial prebiotic inulin (7.53 CFU/ml). Therefore, different types of cereal bran have a prebiotic effect on strain BB 536 growth when supplemented with

goat milk for the development of functional food. Fermentation process was accompanied by significant ($p< 0.05$) changes in physicochemical properties chemical composition. The maximum viable existence of strain BB 536 throughout refrigeration (two weeks) was in fermented goat milk supplemented with millet bran (lowest reduction of 1.48CFU /ml); whereas, the best existence in the first week was in fermented goat milk supplemented with barley bran (0.76 CFU /ml). Therefore, the strain BB 536 existence trend was dependent mainly on both types of fiber sources and the refrigeration period. Hopefully, the final viable count of strain BB536 in all formulated products was above the minimum number required to the presence in probiotic food to exert health benefits upon consumption. During fermentation, significant ($p< 0.05$) increases in acidity and reductions in reducing sugar, TSS, and pH; were revealed due to the strain BB536 enzymatic activities.

In experimental rats, the general health of rats indicated significant ($p< 0.05$) differences in feed intake, weight gain and water consumption between different five groups. However, there were no blood hematology abnormalities and no signs of any moderate and mild deficiencies of nutrients as revealed based on the results of blood biochemistry. Enzymes of the liver such as serum ALT, AST, and ALP of fed rats groups were within the standard normal range of rats. Feeding with fermented goat milk products promoted significant ($p< 0.05$) increases of bifidobacteria and lactobacillus viable count in colon of rats. Besides that, significant ($P<0.05$) decreases in pathogenic bacteria (*Salmonella*, *Staphylococcus*, *Enterococcus* and Enterobacteriaceae) in colon of rats groups were revealed. Therefore, different cereal bran (sorghum, barely and millet) were safe prebiotics. They exerted a prebiotic effect by stimulation growth of comairtial probiotic strain

BB536 (IN *VIVO) and enhanced of beneficial bacteria* and suppression of pathogens in colon of rats (*IN VITRO)*.Thus they are useful for synibiotic (probiotic and prebiotic in same product) application in dairy functional foods.

ملخص البحث

أجريت هذه الدراسة لاستكشاف التأثير التحفيزي لثلاثه انواع مختلفه من نخالة الحبوب تحفيز نمو البكتريا الصديقه (*Bifidobacterium longum BB536*) لتطوير غذاء وظيفي. تم تحضير وسط الخلطه من لبن الماعز المدعم ب 10٪ إينولين (عينة تحكيميه) او واحده من نخالة الحبوب (الذرة الرفيعة ،الشعير والدخن). استخدمت سلالة البروبيوتيك *B. longum BB 536* للتخمير.وقد تم اتبار التأثير التحفيزي للانواع المختلفه لنخالة الحبوب. تم تقييم سلامة لبن الماعز المخمر والمدعم بانواع النخالات المختلفة واثرها المفيد علي مجموعات البكتريا القولونيه في الفئران التي اتغذت عليها . اجريت تحاليل مختلفة شملت العد التقريبي ، المعادن ، العد الحي لسلالة BB536 ، التحليل الفيزيائي والكيميائي. تمت مواءمة ثلاثين من ذكور الفئران الالبينو لمدة أسبوعين تحت ظروف التجربة وتم تقسيمها بشكل عشوائي إلى خمس مجموعات. تلقت المجموعة التحكيميه وجبه غذائيه طبيعيه للفئران. مجموعات الفئران الاخرى تلقت لبن الماعز المخمر والمدعم بكل من الإينولين ونخالة الذرة ونخالة الشعير ونخالة الدخن. استمر التجريب لمدة 6 أسابيع. كان الإنولين مصدرًا ممتازًا للألياف والكربوهيدرات والمعادن (Ca و K و Mg و Na). من بين الحبوب ، إحتوت نخالة الشعير على أعلى مستوى من الألياف ، يليه الذرة الرفيعة ثم الدخن. علاوة على ذلك ، تعتبر نخالة الحبوب مصدرًا جيدًا للبروتين والدهون والرماد. أثناء التخمير كان اعلى نمو لسلالة BB536 (12 ساعة تحضين) 7.53 ± 0.16Log CFU / ml 0.03 ± 8.43 (b) في لبن الماعز المخمر المضاف إليه الإنولين ونخالة الحبوب المختلفة. هذه الأعداد تتجاوز الحد الأدنى للعدد (6 لوغاريثم CFU / مل) في المنتجات المطلوب وجوده في اغذية البكتريا الصديقه. معدل زيادة سلالة BB536 الناتج عن تحفيز نخالة الحبوب

المختلفة (7.65 - 8.43 CFU / ml) مقارنة بتلك الناتجه عن استخدام الأنولين التجاري (7.53 CFU / ml). لذلك فإن أنواعًا مختلفة من نخالة الحبوب لها اثر تحفيزي على سلالة BB536 عند تدعيمها بلبن الماعز لتطوير الغذاء الوظيفي. صوحبت عملية التخمير بتغيرات معنوية ($p < 0.05$) في التركيب الكيميائي للخواص الفيزيائية والكيميائية. كان أعلى وجود حي لسلالة BB536 خلال فترة التبريد في الثلاجه (أسبوعين) في لبن الماعز المخمر المدعم بنخالة الدخن (أقل انخفاض قدره 1.48 CFU / ml) ؛ بينما كان أفضل وجود في الأسبوع الأول في لبن الماعز المخمر المدعم بنخالة الشعير (اقل انخفاض قدره 0.76 CFU / ml). عليه فإن شكل حياة السلاله *BB536* كان معتمدا بصوره رئيسيه علي كل من مصادر الألياف وفترة التبريد. لحسن الحظ العدد النهائي الحي لسلالة *BB536* في جميع المنتجات المخمره أعلى من الحد الأدنى المطلوب للوجود في اغدية البكتريا الصديقه لتعزيز الفوائد الصحية عند الاستهلاك. أثناء التخمير لوحظ زيادة معنويه ($p < 0.05$) في الحموضة وانخفاض في نسبة السكريات المختزله ، والمواد الصلبة الذائبة ، والرقم الهيدوريجيني و يعزي ذلك بسبب للنشاط الأنزيمي لسلالة *BB536*. في تجربة الفئران مؤشرات الصحة العامة اظهرت تباين معنوي ($P < 0.05$) في تناول العلف وزيادة الوزن واستهلاك الماء بين المجموعات الخمس المختلفة. بالرغم من ذلك لم يكن هناك مؤشرات غير طبيعيه لأمراض الدم ولا توجد اي علامات على نقص العناصر الغذائية استناداً علي نتائج الكيمياء الحيوية للدم. كانت إنزيمات الكبد مثل ALT و AST و ALP لمجموعات الفئران ضمن النطاق الطبيعي القياسي للفئران. عززت التغذية بمنتجات لبن الماعز المخمرة زيادة معنوية ($P < 0.05$) في العدد الحي للبكتيريا الصديقة والعصيات اللبنية في القولون لدى الفئران. إلى جانب ذلك تم الكشف عن انخفاض معنوي ($P < 0.05$) في البكتيريا المسببة للأمراض (*Salmonella, Staphylococcus, Enterococcus* and *Enterobacteriaceae*) في القولون لمجموعات الفئران. لذلك كانت نخالة الحبوب

المختلفة (الذرة الرفيعة ، الشعير والدخن) من محفزات البكتريا الصديقه الآمنة. ولها تأثيرًا حيويًا عن طريق تحفيز نمو سلالة البروبيوتيك BB536 وتعزيز البكتيريا المفيدة وكبح مسببات الأمراض في القولون ، وبالتالي فهي مفيدة لتطبيق مبدا التكافلية (بروبيوتيك وبريبايوتيك في نفس المنتج) في الأطعمة الوظيفية لمنتجات الألبان.

CHAPTER ONE

INTRODUCTION

Fermentation prolongs the shelf-life of foods in addition to improving the nutritional value and reducing the risk for food borne illness (Campbell-Platt, 1994). Fermented foods can even have beneficial health effects, when microorganisms used possess probioticis activity. The word probioticis derived from Greek and means “for life” (Metchnikoff, 1907). One of the more detailed current definitions of probiotics is; “a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract”. Mainly specific strains of *lactobacilli*, *Bifidobacterium*, *enterococci* and yeast are today used commercially as probiotics (Naidu *et al*., 1999; Saxelin *et al*., 2005).

Bifidobacteria considered as important probiotics used in the food industry to relieve and treat many intestinal disorders. *Bifidobacterium* exert a range of beneficial health effects, including regulation of intestinal microbial homeostasis, inhibition of pathogens and harmful bacteria that colonize and/ or infect the gut mucosa, modulation of local and systemic immune responses, repression of pro-carcinogenic enzymatic activities within the microbiota, production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules (Mayo and Van Sinderen, 2010).

Bifidobacterium longum is one of the bifidobacteria species found mainly in human faeces and it may be considered as the most common species of

bifidobacteria, being found both in infant and adult. Potential benefits from consumption of B. *longum* include: antagonistic action toward intestinal pathogens, improved lactose utilization, anticarcinogenic action and control of serum cholesterol levels. Scientific studies showed the benefits offered by *Bifidobacterium longum BB536* (Kojima *et al*., 1996; Namba *et al*., 2003). Thus there is considerable interest in incorporating these healths promoting bifidobacteria into food. Nevertheless, probiotic strains, particularly bifidobacteria are rarely used outside the dairy based industry. The scarcity of animal milk in many countries makes it difficult to provide an adequate bifidobacteria intake

However, most human origin probiotics are fastidious when used alone, they are characterized by low growth capability in food media including the dairy, the main recommended carrier of probiotics to human (FAO/WHO, 2001). Particularly in developing countries, where refrigeration is not always an option, the fermentation process is widely used. Usually, freeze drying (in vacuum) is employed. The maximum level of activity of the valuable microbes is maintained. In-air process can involve elevated temperatures which are usually intended for achieving a high rate of drying problem statement (Chen and Liu; 1994).

Milk fermentation is one of the oldest known uses of biotechnology. All over the world, fermented foods continue to constitute an important part of our diet and together with beverages are estimated to present some 20 - 40% of our food supply world-wide (Campbell-Platt, 1994).

Although milk fermentation process is very common in Sudan, it does not go beyond preparation of fermented milk using conventional lactic acid bacteria starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*).

Formulation of milk into probiotic product has not much investigated and to our knowledge no much reported *Bifidobacterium* probiotic fermented milk has been formulated in Sudan. In addition, prebiotic effect of different fiber with probiotic (synbiotic) report is lacking.

General objective:

To formulate the fresh milk into symbiotic with probiotics and different cereal fibers as functional food

Specific objectives :

1- To analyze the proximate composition, sugars, and mineral contents of different sources of milk (cow, goat and camel) and fiber (inulin, sorghum bran, millet bran and barley brain).

2- To assess prebiotic effect of different cereal brans on goat milk using probiotic *Bifidobacterium longum BB536.*

3- To evaluate the physicochemical changes (pH, total soluble solids, titerable acidity) and chemical composition of fermented goat milk products.

4- To study the existence of strain BB536 and physiochemical properties during refrigeration of fermented goat milk products.

5- To examine safety and health benefit of probiotic fermented goat milk supplemented with different cereal brans on fed experimentally rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Functional foods

2.1.1. History of functional foods

The term functional foods was initially presented in Japan in the mid-1980s and refer to processed foods containing ingredients that aid specific bodily functions in addition to being nutritious. To date, Japan is the only country that has formulated a specific regulatory approval process for functional foods, known as Foods for Specified Health Use (FOSHU); these foods are eligible to bear a seal of approval from the Japanese Ministry of Health (Arai, 1996). The Institute of Medicine's Food and Nutrition Board IOM/FNB in (1994) found 100 products are licensed as FOSHU foods in Japan. In the United States, the functional foods category is not recognized legally. Irrespective of this, many organizations have proposed definitions for this new and emerging area of the food and nutrition sciences. The Institute of Medicine's Food and Nutrition Board (IOM/FNB, 1994) defined functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains." Health-conscious baby boomers have made functional foods, the leading trend in the U.S food industry (Meyer, 1998). However, the magnitude of this market varies significantly, as there is no consensus on what constitutes a functional food. Decision resources; estimate the market value of functional foods at $28.9 billion. More significant, maybe, is the potential of functional foods to mitigate disease, promote health, and reduce health care costs (Waltham, 1998).

2.1.2 Definition of functional foods

Functional foods are normal foods and parts of the daily diet, but they contain a component that benefits some particular physiological function and reduce the risk of diseases (Salovaaro, 1999). The wide applications of functional food are in form containing probiotics and non-digestible carbohydrate known as prebiotics (Fuller and Gibson, 1997).

2.1.3 Categories of functional foods

2.1.3.1 Natural foods

It is still a matter of scientific controversy whether natural foods may be regarded as functional foods and whether it would be feasible to use functional or health claims for natural foods. The FUFOSE project (Diplock *et al.* 1999) and Health Canada (1998) have acknowledged that natural foods may be functional foods and this is also the case in the Japanese foods for specified health use (FOSHU) regulations. However, in the US and in several national codes of conduct in European countries, natural foods are not considered as functional foods.

The definition of functional foods is linked to the scientific proof of a functional effect, which goes beyond those of traditional nutrients. Thus if it is, for example, scientifically proven that the flavonoids in apples decrease the risk of a specific disease an apple may be a functional food, in accordance with the saying "an apple a day keeps the doctor away".

2.1.3.2 Processed foods, GM foods

For processed foods various ways of making them functional are considered. Addition of functional ingredients or enrichment by food technology processes seems to be the most obvious way. Another possibility could be the removal of compounds with negative effects, e.g. the removal of phytate

to increase trace element bioavailability. Technological treatment to increase the bioavailability of functional ingredients of foods also offers great potential and new technologies to achieve this goal have been developed in recent years. In some countries, particularly in the US, the addition of synthetic (pharmaceutical) bioactive compounds to foods is also considered. However, in Europe and Germany such a food would very likely be considered to be a medicinal product and would not be marketable as a food. One way of altering food products to enhance their nutritional value is by using genetic modification. Although hardly any genetically-modified functional foods have entered the market yet, the following are examples for ongoing research in this area.

2.1.4Classification of functional foods

Functional food can be classified according to several principles, namely the food group it belongs to (e.g. dairy products, beverages, cereal products, confectionary, oils and fats); the diseases it is expected to prevent or alleviate (e.g. diabetes, osteoporosis, colon cancer); its physiological effects (e.g. immunology, digestibility, anti-tumour activity); the category of its specific biologically active ingredients (e.g. minerals, antioxidants, lipids, probiotics); its physico-chemical and organoleptic properties (e.g. color, solubility, texture), or the processes that are used in its production (e.g. chromatography, encapsulation, freezing) (Juvan *et al.;* 2005).The second level classification groups the functional food products within each food category by the biologically active ingredient (e.g. phenols, terpenoids, saccharide, lipids, peptides, fibers, plant extracts, bacteria cultures).

2.2.1 General description of milks

Milks contain, with some exceptions, the nutrients required for the growth and development of the neonate. If the development time is short then the

milk is nutrient dense. All milks contain specific proteins, fats designed to be easily digested, most have lactose, minerals, vitamins, and other components which may have important roles. These are organized as follows: lipids in emulsified globules coated with a membrane, proteins in colloidal dispersion as micelles, and most minerals and all lactose in true solution (Jensen *et al*., 1991).

Milk is known as nature's most complete food, and dairy products are considered the most nutritious foods. On the other hand, the traditional view of the role of milk has been greatly expanded in recent years beyond the horizon of nutritional subsistence of infants. Milk is now recognized as more than a source of nutrients to mammalian neonates and for healthy growth of children and nourishment of adult humans. Milk contains biologically active compounds besides its major proteins, casein and whey proteins that have important physiological and biochemical functions with significant impact on human metabolism, nutrition and health. Numerous milk-borne biologically active compounds have been proven to have beneficial effects on human nutrition and health, including antimicrobial, biostatic, antihypertensive, angiotensin-converting enzyme (ACE)-inhibitory, antiadhesion, antidiabetic, anticholesterol ,anticarcinogenic, immunomodulatory, antiobesity, probiotic, and prebiotic activities.

Examples of these compounds include β-lactoglobulin, α-lactalbumin, lactoferrin, immunoglobulins, lysozyme, lactoperoxidase, peptides from caseins and whey proteins, glycomacropeptides, phosphopeptides, oligosaccharides, conjugated linoleic acid, polar lipids, gangliosides, sphingolipids, medium- and short-chain fatty acids, monounsaturated and polyunsaturated fatty acids, triglycerides, milk minerals, growth factors, hormones, vitamins, and nucleotides. Among the many valuable constituents

in milk, the high level of calcium plays a particularly important role in the development, strength and density of bones for children and in the prevention of osteoporosis in older people. In addition, calcium has also been shown to be beneficial in reducing cholesterol absorption, and in controlling body weight and blood pressure (Park and Haenlein, 2013).

2.2.2 Goat and milk production

The goat is probably the first ruminant to be domesticated. Goats originated in Asia and are now spread almost all over the globe. Goats are very hardy animals, and they thrive in areas where other animals have difficulties. There are numerous breeds of goat, and it is difficult to define any particular breed as a dairy breed. However, the Swiss breeds have been very successfully selected and bred for their milk yield. They have been exported all over the world to upgrade the milk yield of local breeds. In Sudan the Nubian goat is adopted to produce milk (Cooke, 1961). Non-dairy breeds which should be mentioned are Cashmere and Angora, well-known for the special wool they produce in a well-managed milk production unit a goat can produce between 400 and 900 liter milk per lactation. The period of lactation varies from 200 to 300 days. For a family-sized goat milking operation, 40 to 120 goats are required to reach an acceptable turnover. An enterprise requires a larger number of animals, e.g. 200 to 1 000 goats. An intensive and feasible production unit, family sized operation or enterprise, however, requires not only appropriate machine milking equipment but also effective management, feeding and breeding programs (Cooke, 1961).

2.2.3 Cow and milk production

A calf needs about 1000 liters of milk for growth, and that is the quantity which the primitive cow produces for each calf. Secretion of milk in the

cow's udder begins shortly before calving, so that the calf can begin to feed almost immediately after birth. The cow then continues to give milk for about 300 days. This period is known as lactation (Cooke, 1961).

One to two months after calving the cow can be serviced again. During the lactation period milk production decreases and after approx. 300 days it may have dropped to some 15 – 25 % of its peak volume. At this stage milking is discontinued to give the cow a non-lactating period of up to 60 days prior to calving again. With the birth of the calf, a new lactation cycle begins. The first milk the cow produces after calving is called colostrums. It differs greatly from normal milk in composition and properties (Cooke, 1961).

2.2.4 Camel milk composition

Kosuspayeva *et al*. (2010) conducted a Meta analysis study and given the means of camel (bactrian and dromedary) milk composition for the period between 1905 and 2006. They concluded that camel milk is an important source of proteins for the people living in the arid lands of the world. Variation in camel milk composition was also observed for camels from the same species (dromedary). Further, seasonal variations were found to play a role in camel milk composition even for camels from the same species (dromedary) and regions (Bakheit *et al*., 2008; Haddadin *et al*., 2008; Shuiep *et al*., 2008). An inverse relationship was found between total solids in camel milk and water intake by camels. Konuspayeva *et al*. (2009) found that all components except lactose reached their maximum in mid-winter and decrease to the lowest in the summer. For example, total solids were 13.9% in December and January, and 10.2% in August which can be related to the availability of drinking water (Haddadin *et al*., 2008). Moreover, the fat content of camel milk decreases from 4.3 to 1.1% due to the increase in

water content of milk produced by thirsty camels (Yagil and Etzion, 1980). The increasing in water content could be attributed to the decrease in total solids produced by the thirsty camels. Alhaj and Al Kanhal (2010) reported that changes in camel milk composition could be due to several factors including analytical measurement procedures, camel diet, climate, water availability, livestock management, and other factors.

2.2.5 Processing of milk

Modern food processing is dependent on a range of preservative technologies to ensure that food is maintained at an acceptable level of quality from the time of manufacture through to the time of consumption. These techniques include drying, concentration, and fermentation.

One of the oldest of these technologies is fermentation, a process dependent on the biological activity of microorganisms for production of a range of metabolites which can suppress the growth and survival of undesirable microflora in foodstuffs. Fermentation as a food preservation technique can be traced back thousands of years, at a time when plants and animals were just being domesticated (Fox, 1993).

The link between fermentation and preservation is biopreservation which refers to the extension of the shelf-life and improvement of the safety of food using microorganisms and/or their metabolites. In this respect, it is well known that starter microorganisms can produce a wide range of antimicrobial compounds and proteinaceous substances which can inhibit or reduce undesirable flora in food products (Holzapfel *et al.,* 1995).

2.2.6 Definition of fermented milk

Fermentation was defined by Gale (1948) as the process leading to anaerobic breakdown of carbohydrates, other major compounds such as organic acids,

proteins, and fats. In broader veiew, fermentation is an energy yielding process (Kosikiowski, 1982).

2.2.7 Types of fermentation

Kosikowski (1982) stated that there are six major fermentation reactions in milk:

1- Lactic acid fermentation.
2- Propionic acid fermentation.
3- Citric acid fermentation.
4- Alcohol fermentation.
5- Butyric fermentation.
6- Coliform gassy fermentation.

Many lactic acid bacteria occur normally in milk and are responsible for its spontaneous souring (Stanier *et al.;* 1957).

2.2.8 Fermented milk products

Fermented milk pre cultured dairy products made from skimmed, whole, or slightly concentrated milk, that require specific lactic acid bacteria, to develop their characteristics flavor and texture. Fermented milks are usually fluid or semi fluid in nature, and all contain lactic acid in varying proportions fermentation in milk modifies its properties resulting in, cultured beverages, such as yoghurt, kefier,etc.(Webb *et al.*,1980).

Fermented milk product are unique in the sense that the required organoleptic properties depends on unique characteristics of certain component of milk e.g. curdling properties of calcium caseinate-phosphate complex, blendness of lactose and flavor characteristics of lipolyzed milk fat (Webb *et al*, 1980).

Fermented milk products, depend on starter culture not only for acid development, but also for accumulation of desirable intermediates for example, volatile acid, action (dimthyl ketol, methyl carbinol) and diacetyl (diketobutan, biacetyl).which act as flavoring agents (Peppler and Robert, 1977).

2.2.9 Starter culture for milk fermentation

Dairy starters are cultures of harmless, active bacteria, grown in milk or whey, which imparts certain characteristics and qualities to various milk products. The culture may be one strain of microorganism species, called a single-strain or a number of strains and/or species called a multi strain or mixed-strain culture (kosikowaski, 1982).

Starter culture in fermented milk products are used not only for acid development, but also to lessen the putrefaction taking place in milk as a result of the presence of spoilage bacteria (Musa, 1994).

2.3 Prebiotics

Our intestinal tract is colonized by a complex ecosystem of microorganisms that increase in numbers from 10^2 to 10^4 per gram of contents in the stomach, to 10^6–10^8 per gram in the small intestine, and 10^{10}–10^{12} per gram in the colon (McCartney and Gibson, 2006). It has also become increasingly clear that these bacteria are not merely commensals, but have coevolved with us in a truly symbiotic relationship. Our intestinal microbes provide us with a barrier to infection by intestinal pathogens (Bourlioux *et al*.; 2003), provide much of the metabolic fuel for our colonic epithelial cells (Topping and Clifton 2001), and contribute to normal immune development and function (Bulm and Schiffrin.; 2003), Tlaskalova-Hogenova *et al*.;

2004).Members of the intestinal microbiota can also be involved in acute and chronic diseases such as antibiotic-associated diarrhea (Cummings and Macfarlane. ; 2003) and inflammatory bowel disease (IBD) (Marteau *et al*., 2003). Undesirable metabolic activity of the intestinal microbiota mayplay a role in the development of colorectal cancer (. Saunier and Dore .; 2002); Guarner and Malagelada. ; 2003). Hence, it is reasonable to hypothesize that modifying the intestinal microbiota to develop, restore or maintain a beneficial balance of microorganisms and microbial activities may improve health. The consumption of probiotics aims to directly supplement the intestinal microbiota with live beneficial organisms. Lactobacilli and bifidobacteria are numerically common members of the human intestinal microbiota, and are nonpathogenic, nonputrefactive, nontoxigenic, saccharolytic organisms that appear from available knowledge to provide little opportunity for deleterious activity in the intestinal tract (Crittenden, 2004); Salminen , Gorbach , *et al*., 2004). As such, they are reasonable candidates to target in terms of restoring a favorable balance of intestinal species.

2.3.1. The prebiotic concept

A number of different strategies can be applied to modify microbial intestinal populations. Antibiotics can be effective in eliminating pathogenic organisms within the intestinal microbiota. However, they carry the risk of side effects and cannot be routinely used for longer periods or prophylactically. Prebiotics supplementation represents strategy to manipulate the intestinal microbiota. Rather than supplying an exogenous source of live bacteria, prebiotics are nondigestible food ingredients that selectively stimulate the proliferation and/or activity of desirable bacterial populations already resident in the consumer’s intestinal tract. Prebiotics

identified so far as nondigestible, fermentable carbohydrates. Intestinal populations of bifidobacteria, in particular, are stimulated to proliferate upon consumption of a range of prebiotics, increasing in numbers by as much as 10–100-fold in feces (Crittenden, 1999; Boehm *et al*, 2003. There is an obvious potential to use prebiotics and probiotics together in a complementary and synergistic manner. Therefore, foods containing both probiotic and prebiotic ingredients have been termed synbiotics (Gibson and Roberfroid, (1995).

2.3.2. Advantages and disadvantages of the prebiotic strategy

The prebiotic strategy offers a number of advantages over modifying the intestinal microbiota using probiotics or antibiotics.

2.3.2.1 Advantages over probiotics:

- Stable in long shelf life foods and beverages.
- Heat and pH stable and can be used in a wide range of processed foods and beverages.
- Have physicochemical properties useful to food taste and texture.
- Resistant to acid, protease, and bile during intestinal passage.
- Stimulate organisms already resident in the host, and so avoid host/strain compatibilities, and the need to compete with an already established microbiota.
- Stimulate fermentative activity of the microbiota and health benefits from short chain fatty acids (SCFA).
- Lower intestinal pH and provide osmotic water retention in the gut.

Advantages over antibiotics

- Safe for long-term consumption and prophylactic approaches.

- Do not stimulates side effects such as antibiotic-associated diarrhea, sensitivity to UV radiation, or liver damage.
- Do not stimulate antimicrobial resistance genes.
- Not allergenic.

2.3.2.2Disadvantages of prebiotics

- Unlike probiotics, over dose can cause intestinal bloating, pain, flatulence, or diarrhea.
- Not as potent as antibiotics in eliminating specific pathogens.
- May exacerbate side effects of simple sugar malabsorption during active diarrhea.

Aconsumed probiotic strain must compete with an already established microbiota, and in most cases they persist only transiently in the intestine (Satokari *et al*.; 2001; Brigidi, *et al*., 2003). Individuals also harbor their own specific combination of species and unique strains within their intestinal bacteria (Zetendal. *et al.*, 1998), Mangin. *et al*., 1999) suggesting that certain host–microbiota compatibilities exist. By targeting those strains that are already resident in the intestinal tract of an individual, the prebiotic strategy overcomes the need for probiotic bacteria to compete with intestinal bacteria that are well established in their niche.

2.3.3. Types of prebiotics

Most identified prebiotics are carbohydrates.Within these, there is a wide diversity of molecular structures. However, these carbohydrates share a number of physiological traits important to their beneficial effects. They are

- Non digestible (or only partially digested).
- Non absorbable in the small intestine.
- Poorly fermented by bacteria in the mouth.

- Well fermented by purportedly beneficial bacteria in the gut.
- Poorly fermented by potentially pathogenic bacteria in the gut.

2.3.4. Prebiotics and symbiotics:

The potential prebiotics of today are mainly non-digestible sugar-based molecules that are not adsorbed in the small intestine, and thus provide nutrients to colonic bacteria. These compounds include polysaccharides like resistant starch, inulin (a polyfructan) or different oligo saccharides such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), trans-galacto-oligosaccharides (TOS), iso-malto-oligosaacharides (IMO), xylo-oligosaccharides, soybean oligo saccharides, or the synthetic lactose derivative, lactulose (Fooks *et al.*, 1999; Sako *et al*., 1999). Many of these prebiotics occur naturally in foods, for example FOS is found in chicory, artichoke, Jerusalem artichoke and onion, and various galacto-oligosaccharides are found in human breast milk. Proposed criteria for a prebiotic are:

- Low-digestibility and poor absorption in the upper gastrointestinal tract
- Selective fermentation by potentially beneficial bacteria in the colon
- Improvement of the composition of the intestinal microflora
- An improved state of host health as a result of consumption

The products into which these substances can be added include drinks, bakery products, dairy foods, confectionery etc. Fructooligosaccharides are by far the best characterised prebiotic both from a technological point of view as well as regarding the intestinal and physiological functions.

Combining probiotic bacteria and prebiotic substrates in a food in order to create a synbiotic product is a relatively new concept although products containing both pro- and prebiotics are appearing on the market. Since in breast-fed infants bifidobacteria typically dominate in the microflora and are

reported to decrease in the middle-aged and elderly, synbiotics combining bifidobacteria and their selective substrates have been proposed for these groups (Fooks *et al*., 1999).

2.3.5 Present probiotic and prebiotic foods

It is understandable that the composition and activities of this microflora can have consequences for the health and well-being of the host. Thus there is a theoretical justification for the intentional manipulation of the microbial composition of the intestine by using either probiotics or prebiotics. The probiotics intended for human consumption consist mainly of lactic acid bacteria and bifidobacteria, although one yeast, *Saccharomyces boulardii*, has been used successfully as a human probiotic (Salminen *et al*., 1998). Lactic acid bacteria and yeasts have a long history in food fermentations, while the use of bifidobacteria for food purposes is a recent innovation. The prebiotics represent various types of carbohydrates or their derivatives (Fooks *et al*., 1999).

2.4 Inulin

This prebiotic, also known as raftiline, is found inseveral foods such as wheat, onion, garlic, bananas, fruits, and vegetables, but industrial production utilizes chicory (white carrot) roots. Inulin is extracted from chicory roots through hot water extraction followed by refining and spray drying into a powder that is composed of a mixture of linear molecules with a basic G-Fn chemical structure. G represents a glucosyl moiety, while Fn are the fructose moieties joined together by b -2,1 glycosidic bonds, with n (degree of polymerization, DP) ranging from 3 to 60 with an average value of 10. Some long-chain inulin products with a DP of about 25 are obtained after lower DP fractions have been physically removed from the initial mixture. Inulin is partially soluble in water where it forms slightly thickened

product that has a bland neutral taste without any of flavor and can contribute to body and mouthfeel of food products. Because of the gelling ability, inulin has the capacity to be used as a fat replacer with excellent mouthfeel characteristics and can act as emulsion and foam stabilizers.

2.5 Probiotics

2.5.1 Definition of probiotics

As defined by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 2002, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host." The word 'probiotic', derived from the Greek language, means 'for life' (Fuller, 1989) and has had many definitions in the past. Definitions such as 'substances produced by protozoa that stimulate the growth of another' or 'organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance' were used. These general definitions were unsatisfactory because 'substances' include chemicals such as antibiotics. The definition of probiotics has since then been expanded to stress the importance of live cells as an essential component of an effective probiotic. Most recently, Huis Veld and Havenaar 1991) broadened the definition of probiotics as being 'a mono- or mixed culture of live microorganisms which, applied to man or animal (e.g. as dried cells or as a fermented product), beneficially affects the host by improving the properties of the indigenous microflora. This definition implies that probiotic products, for example fermented milk, contain live microorganisms and improve the health status of the host by exerting beneficial effects in the gastrointestinal tract.

2.5.2 History of probiotics

The idea that some bacteria contained in our food may have beneficial effects is much older than the term probiotic. At the beginning of the 20th century, the Russian Nobel Prize Laureate Elie Metchnikoff associated the observed longevity of Bulgarian peasants with their high consumption of live microbes in fermented milk products, as he reported in his book The prolongation of life (Metchnikoff, 1907). In1930, the Japanese scientist Minoru Shirota isolated a lactic acid bacterium from the feces of a healthy infant. Five years later, one of the first fermented milk drinks thought to support intestinal health was produced with the strain he developed and was named ,, Yakult,, . The concept of probiotics was already successful in Asia for many years when the first probiotics fermented milk products were eventually introduced in Europe (Metchnikoff, 1907).

2.5.4 Probiotic strains

Probiotic cultures naturally occur in certain fermented foods (Rosander *et al*., 2008). Below is a list of different strains of probiotic bacteria.

- *Bacillus coagulans* GBI-30, 6086
- *Bifidobacterium animalis subscp*. lactis BB-12
- *Bifidobacterium longum subsp*. BB536
- *Lactobacillus acidophilus* NCFM
- *Lactobacillus paracasei* St11
- *Lactobacillus johnsonii* La1
- *Lactobacillus plantarum* 299v
- *Lactobacillus reuteri* ATCC
- *Lactobacillus reuteri Protectis*.

2.5.5 Characteristics of probiotics microorganism

Characteristics of successful probiotics determine their ability to survive the upper digestive tract and to colonize in the intestinal lumen and colon for an undefined time period. Probiotics are safe for human consumption and no reports have found on any harmfulness or production of any specific toxins by these strains (Von Wright and Axelsson 2000; Salminen *et al.*, 1998).In addition, some probiotics could produce antimicrobial substances like bacteriocins. Therefore, the potential health benefit will depend on the characteristic profile of the probiotics. Some probiotic strains can reduce intestinal transit time, improve the quality of migrating motor complexes (Husebye *et al*., 2001) and temporarily increase the rate of mitosis in enterocytes (Banasaz*et al*. , 2002; Halvorsen *et al*., 2000).

The most common probiotics are *Lactobacillus* and *Bifidobacterium*. In general most probiotics are gram-positive, usually catalase-negative, rods with rounded ends, and occur in pairs, short, or long chains (Von Wright and Axelsson ,2000).They are non-flagellated, non-motile and non-spore-forming, and are intolerant to salt. Optimum growth temperature for most probiotics is 37°C but some strains such as *L. casei* prefer 30 °C and the optimum pH for initial growth is 6.5-7.0 (Von Wright and Axelsson, 2000). *L. acidophilus* is microaerophilic with anaerobic referencing and capability of aerobic growth.

Bifidobacteria are anaerobic but some species are aero-tolerant. Most probiotics bacteria are fastidious in their nutritional requirements (Desmazeaud, 1983; Marshall and Law, 1984). With regard to fermentation probiotics are either obligate homofermentative (ex. *L. acidophilus*, *L. helvelicas*), obligate heterofermentative (ex. L. *brevis*, L. *reuteri*), or facultative heterofermentative

(ex. L. *casei*, L. *plantarum*), (Barrangou *et al*.; 2011). Additionally, probiotics produce a variety of beneficial compounds such as antimicrobials, lactic acid, hydrogen peroxide, and variety of bacteriocins (Gorbach ., 2002).

Probiotics should have the ability to interact with the host microflora and competitive with microbial pathogens, bacterial, viral, and fungal (Gorbach, 2002).

2.5.6 Health benefits of probiotic

According to (Kullisaar *et al*., 2002)

1. Vitamin production, availability of minerals and trace elements.
2. Production of important digestive enzymes such as β –galactosidase for alleviation of lactose in tolerance.
3. Barrier, restoration, antagonistic effects against: Infectious diarrhea, Antibiotic – associated diarrhea, irradiation – associated diarrhea.
4. Cholesterol –lowering effect.
5. Stimulation and improvement of the immune system.
6. Enhancement of bowel motility, relief from constipation.
7. Anti-carcinogenic effects in the colon.
8. Maintenance of mucosal integrity.
9. Reduction of inflammatory allergic reactions
10. Adherence and colonization resistance.
11. Antioxidateive activities.

In many cases, the health-promoting mechanisms of probiotic action are not sufficiently known. However, the majority of them are based on the positive effect they exert on the immune response, i.e. on their immunomodulatory activity (Isolauri *et al*., 2002; Biancone *et al*., 2002). In most cases, this is

due to stimulation of natural immunity (Newburg, 2005; Galdeano and Perdigon, 2006). In doing so they modulate primarily the production of cytokines and antimicrobial peptides (Trebichavský and Šplíchal, 2006). This is the mode of action of not just typical sour milk functional foodstuffs, such as sour milk, kefir or yoghurt (Meydani and Ha, 2000; Farnworth, 2005) produced by the food-processing industry, but also that of the diet supplements containing the probiotic bacteria in pure form. However, the latter are the products of pharmaceutical industry and, in contrast to functional foodstuffs, they have a standard composition, and known immunomodulatory characteristics, verified both experimentally and in controlled clinical studies. In terms of their quality and efficiency they are also under regular pharmaceutical control. It will be therefore more precise to call them immunobiotics, in order to distinguish them from classical probiotics in functional foodstuffs (Clancy, 2003).

2.5.7 Criteria of selection of appropriate probiotic

Different aspects have to be considered in probiotic selection .Safety criteria for any successful probiotic have been defined in several reviews (Lee and Salminen, 1995; Donohue and Salminen, 1996; Adams, 1999) include the following specifications:

1. Strains use is preferably of human origin.
2. They are isolated from healthy human GI tract.
3. They have a history of being non-pathogenic.
4. They have no history of association with diseases such as infective endocarditic or GI disorders.

5. They do not deconjugate bile salts (bile salt deconjucation or dehydroxylation would be a negative trait in the small bowel (Marteau *et al*., 1995).

6. They do not carry transmissible antibiotic resistance genes.

While in selecting a preferable probiotic strain several aspects of functionality have to be considered:

1. Acid tolerance and tolerance to human gastric juice.

2. Bile tolerance (an important property for survival in the small bowel).

3. Adherence to epithelial surfaces and persistence in the human GI-tract.

4. Immunostimulation, but no pro-inflammatory effect.

5. Antagonistic activity against pathogens such as *Helicobacter pylori*, *Salmonella sp.*, *Listeria monocytogenes* and *Clostridium difficile*.

6. Antimutagenic and antigarcinogenic properties.

Feeding trials with different probiotic strains have shown that the probiotic strain usually disappears from the GI-tract within a couple of weeks after the ingestion is discontinued (Fukushima *et al.,* 1998; Johansson *et al.,* 1998; Alander *et al.,* 1999; Donnet-Hughes *et al.,* 1999). The role of the probiotic persistence in the human GI-tract has therefore been questioned. However, even temporary persistence, which has been noted for several ingested probiotic strains, may enhance their chances for beneficial functions in the GI-tract, and is therefore considered a desirable trait. Necessary safety and functional criteria the aspects related to probiotic production and processing are also of utmost importance, such as:

1. Good sensory properties.

2. Phage resistance.

3. Viability during processing.

4. Stability in the product and during storage.

Good viability and activity of probiotics are considered prerequisites for optimal functionality. However, several studies have shown that non-viable probiotics can have beneficial effects such as immune modulation and carcinogen binding in the host (Ouwehand and Salminen, 1998; Salminen *et al*.; 1999). Thus, for certain probiotic strains it might be sufficient that they grow well during initial production steps (to obtain high enough numbers in the product) but they do not necessarily need to retain good viability during storage.

2.5.8 Microorganisms used as probiotic

According to the current definition of a probiotic, a large variety of microbial species and genera are considered to have probiotic potential, most of which belong to the lactic acid bacteria (LAB). In the development of human probiotics, strains belonging to the genera *Lactobacillus* and *Bifidobacterium* have been most commonly used, even though some probiotic preparations are based on other LAB or even non-LAB species and yeasts (Holzapfel *et al.*, 1995).

2.5.9 Bifidobacteria

Bifidobacteria spp are high-GC content, Gram-positive bacteria which belong to the Actinobacteria branch and these species naturally colonize the gastrointestinal tract (GIT) of mammals, birds and insects (Ventura *et al*., 2007). Scientists have determined the major probiotic properties of *Bifidobacteria spp* isolated from the human intestine and these properties include the strengthening of the intestinal barrier, modulation of the immune response and antagonism pathogens (Marco *et al*., 2006).

Bifidobacterium spp has been reported to possess various glycosyl hydrolases (GH) and these hydrolases metabolize plant- or milk-derived

oligosaccharides including nondigestible ones such as galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) (schell *et al*., 2002'sela *et al*., 2008). The capability to utilize nondigestible oligosaccharides confers a competitive advantage to *Bifidobacterium spp* in the human gut.

Bifidobacterium longum (B. longum) and various other *bifidobacteria* strains are often added to probiotic products in combination with other lactic acid bacteria (LAB). Through their long and safe history of application, LAB have acquired the status of "Generally Regarded As Safe" (GRAS), but the safety of *bifidobacteria* and other LAB strains selected for probiotics still need to be carefully evaluated.

The key safety aspects for use of *bifidobacteria* and other LAB strains in probiotics include antibiotic resistance, production of harmful metabolites and the potential for virulence. Antibiotic resistance in potential probiotic strains is not considered a risk factor unless resistance is transferred to pathogens or it renders the probiotic untreatable in very rare cases infection (Borriello *et al.,* 2003). Biogenic amines, D-lactic acid, azoreductases and nitroreductases produced by bifidobacteria and other LAB strains are potential health hazards (O'Brien. *et al*., 1999, McBain *et al*., 1997). And the safety of some of these compounds have been evaluated (Ruiz-Moyano *et al.;* 2009).

2.5. 10. *B.longum*BB536

Bifidobacterium longum is one of the *bifidobacteria* species found mainly in human faeces and it may be considered as the most common species of bifidobacteria, being found both in infant and adult. Potential benefits from consumption of *B. longum* include: antagonistic action toward intestinal

pathogens, improved lactose utilization, anticarcinogenic action and control of serum cholesterol levels. Scientific studies showed the benefits offered by *Bifidobacterium longum BB536* (Kojima *et al*., 1996; Namba *et al*., 2003). Thus there is considerable interest in incorporating these healths promoting *bifidobacteria* into food. Nevertheless, probiotic strains, particularly *bifidobacteria* are rarely used outside the diary based industry. The scarcity of animal milk in many countries makes it difficult to provide an adequate *bifidobacteria* intake.

CHAPTER THREE

MATEIALS AND METHODS

3.1. Raw materials

Cow and goat fresh milk was obtained from the animal farm at Department of Animal Production, College of Agricultural Studies (CAS), Sudan University of Science and Technology (SUST), Sudan. While camel milk was purchased from local market in Khartoum State. Different cereal grains (sorghum, barley and millet) were purchased from local cereal market in Bahari. Care was taken to ensure that clean and mold free grains were selected.

Prebiotic inulin was obtained from a natural Product Company in London (UK). Whereas, sorghum bran, barley bran and millet bran were purchased from a local cereal market in Bahri, (Khartoum state, Sudan).

Bifidobacterium longum BB536 strain was obtained from the stock culture of microbiology laboratory (Department of Food Science Technology, CAS, SUST).

Chemicals (analytical grade) were purchased from local chemical suppliers company in Khartoum State.

Experimental rats were obtained from the National Research Center (Khartoum –Sudan).

3.1.1. Preparation of different brans

Selected cereal grains were cleaned and peeled. The obtained bran was ground and sieved using appropriate mesh. The resulting bran powder was stored in a dark polyethylene bag in freezer until used.

3.1.2. Preparation of fermentation inoculums

B.longumBB536 was obtained from the stock culture of microbiology laboratory (Department of Food Science and Technology, College of Agricultural Studies, SUST. The strain was maintained at -20 ˚C in 20% glycerol solution. Stock culture was prepared by activation of the strain in sterlized skim milk, incubated an aerobically at 37 ˚C for 24h. The obtained culture was reactivated again under the same conditions to prepare enough stock for the experiment. The working culture was prepared by twice successive transformation in 10% sterilized skim milk (121˚C for 15 min) and incubation at 37 ˚C for 24h.

3.1.3 Production of fermented product

Fresh raw goat milk was heated to 65ºC for 30 minutes and then cooled to 37 ºC, following this 3% of starter culture *B.longumBB536* was added to the milk and incubated at 37 ºC divided into four parts and treatments with 1% of inuline and different fibers (sorghum bran, barley bran and millet bran) powder. Next, using mixer the resulting fermented products were filled into plastic containers and stored under refrigeration (4˚C) for 3 weeks. Subsequent physicochemical and microbiological analyses at storage were performed at 0, 1, 2 and 3 weeks.

3.2 Experimental rats design

3.2.1 Animals

Six weeks old male albino rats with an average initial weight of 95g were purchased from College of pharmacy, Ahfad University for Women. They were housed six per cage. A 12 h light dark cycle and a controlled atmosphere (22.11±2.36°C) were maintained throughout the study. After fifteen days acclimatization period under experimental condition, rats were randomly

assigned into five different groups (n = 6) at random and treated for thirty days. During the thirty days trial, the rats were offered water and feed *ad libitum*. The bedcovers in cages was changed twice a week. Individual body weight of every rat was measured at ten days intervals.

3.2.2 Experimental design

3.2.2.1 Chemical and apparatus

All chemicals and kits were purchase from Bio systems (Spain) and were provided by local company in Khartoum. Test tubes, (K3 EDTA) and sterile containers were obtained from local company in Khartoum.

3.2.2.2 Experimental design feed

All experimental designs are shown in table 1.

Table 1: The experimental rat groups and their diets

Group	Experimental diets (per 6 rats)
Control (E)	Normal diet + sterile water
Treatment A	Normal diet + sterile water+2.5ml orally dairy goat milk with inulin without added *B. long BB536*
Treatment B	Normal diet + sterile water+2.5ml orally dairy goat milk with sorghum bran supplemented w 2% *BB536*
Treatment C	Normal diet + sterile water+2.5ml orally dairy goat milk with barley bran line supplemented w

	2% *BB536*
Treatment D	Normal diet + sterile water+2.5ml orally dairy goat milk with millet bran

3.2.4 Blood sampling

Blood samples of three rats of each group were collected at the end of the experiment period (30 days)from vein plexus eye in sterile tubes containing EDTA and kept at 4 ºC for hematology analysis, after that the rats were sacrificed under anesthetize and the blood was collected in clean test tubes. Serum was separated by centrifugation at 1500 rpm for 20min to investigate the biochemistry parameters.

3.3 Methods

3.3.1Physico-chemical methods

3.3.1.1 Total soluble solids

The total soluble solids as percent (TSS %) of the different samples were measured as described by Ranganna (2001**)**.

Principle: The index of refraction of a substance is a ratio of light velocity under vacuum to its velocity in the substance which is largely dependent on the composition, concentration and temperature of the sample solution.

Procedure: After the adjustment of the Hand-Refractometer (No.002603, BS-eclipse, UK) with distilled water, the sample was placed on the surface of the refractometer prism, the prism was closed and the reading was recorded to the nearest 0.01 as TSS %.

3.3.1.2 Hydrogen ion concentration

The pH of the different samples was determined as described by Ranganna (2001).

Principle: The pH value of the different samples was measured with pH-meter. After standardization of the pH-meter electrodes with a buffer solution, the reading of the sample is recorded as pH value.

Procedure: After standardization of the pH-meter (model HI 8521 microprocessor bench PH/MV/C° meter. Romania) with buffer solutions (pH 4.01 and 7.0), the electrode of the pH-meter was rinsed with distilled water, immersed in the sample and left to stand until a staple reading was achieved.

3.3.1.3 Titrable acidity

Ten ml of the sample preparation was titrated against 0.1N NaOH using phenolphthalein as indicator. Total acidity (mg/100g) expressed as lactic acid according to Ranganna(1979).

$$\text{Acidity (mg/100g)} = \frac{\text{Titre} * \text{N(NaOH)} * \text{dilution factor} * 100 * \text{eq.wt}}{\text{weight of sample} * 1000 * \text{vol.titre}}$$

Where:

eq.wt = equivalent weight of lactic acid

3.3.2Proximate analyses

3.3.2.1 Moisture content

The moisture content was determined according to the standard methods of the Association of Official Analytical Chemists **(**AOAC, 2003**).**

Principal: The moisture content in, a weighed sample is removed by heating the sample in an oven (under atmospheric pressure) at 105 °C. Then, the difference in weight before and after drying is calculated as a percentage of the initial weight.

Procedure: A sample of 2 g ±1 mg was weighed into a dish. Then, the sample was placed into an oven (No.03-822, FN 400, Turkey) at 105 ± 1 °C until a constant weight was obtained. After drying, the covered sample was transferred to desiccators and cooled to room temperature before reweighing. Triplicate results were obtained for each sample and the mean value was reported to two decimal points according to the following formula:

Calculation:

$$\text{Moisture content (\%)} = \frac{(Ws - Wd)}{\text{Sample weight (g)}} \times 100\%$$

Where:

Ws = weight of sample before drying.

Wd = weight of sample after drying.

3.3.2.2 Ash content

The ash content was determined according to the method described by AOAC (2003).

Principle: The inorganic materials which are varying in concentration and composition are customary determined as a residue after being ignited at a specified heat degree.

Procedure: A sample of 5g ±1 mg was weighed into a pre-heated, cooled,

weighed and tarred porcelain crucible and placed into a Muffle furnace (No.20. 301870, Carbolite, England) at 550°C until a white, gray ash was obtained. The crucible was transferred to desiccators, allowed to cool at room temperature and weighed. After that, the ash content was calculated as a percentage based on the initial weight of the sample.

Calculation:

$$\textbf{Ash (\%)} = \frac{[(\text{Wt of crucible} + \text{Ash}) - (\text{Wt of empty crucible})]}{\text{Initial weight (Wt)}} \times 100\ \%$$

3.3.2.3 Oil content

Oil content was determined according to the official method of AOAC **(**2003).

Principle: The method determines the substances which-are soluble in petroleum ether (65-70 °C) and extractable under the specific conditions of Soxhlet extraction method. Then, the dried ether extract (oil content) is weighed and reported as a percentage based on the initial weight of the sample.

Procedure: A sample of 5g ± l mg was weighed into an extraction thimble and covered with cotton that previously extracted with hexane (No.9-16-24/25-29-51, LOBA Cheme, India). Then, the sample and a pre-dried and weighed extraction flask containing about 100 ml hexanes were attached to the extraction unit(Electrothermal, England) and the extraction process was conducted for 6 hour. At the end of the extraction period, the flask was disconnected from the unit and the solvent was redistilled. Later, the flask with the remaining crude hexane extract was put in an oven at 70 °C for 3 hrs, cooled to room temperature in a desiccators, reweighed and the dried extract was registered as oil content according to the following formula;

Calculation:

$$\text{Oil content (\%)} = \frac{(W2 - W1)}{W3} \times 100\ \%$$

Where:

W_2 =Weight of the flask and hexane extract

W_1 =Weight of the empty flask

W_3=initial weight of the sample

3.3.2.4 Crude protein content

The protein content was determined in all samples by micro-Kjeldhalmethod using a copper sulfate-sodium sulfate catalyst according to the official method of AOAC (2003).

Principle: The method consists of sample oxidation and conversion of its nitrogen to ammonia, which reacts with the excess amount of sulphuric acid forming ammonium sulphate. After that, the solution was made alkaline and the ammonia was distilled into a standard solution of boric acid (2%) to form the ammonia-boric acid complex. Which is titrated against a standard solution of HC1 (0.1N) .The protein content is calculated by multiplying the total N % by 6.25 as a conversion factor for protein.

Procedure: A sample of two grams (2 gm) was accurately weighed and transferred together with, 4g Na_2SO4 of Kjeldhal catalysts (No. 0665, Scharlauchemie, Spain) and 25 m1 of concentrated sulfuric acid (No.0548111, HDWIC, India) into a Kjeldhal digestion flask. After that, the flask was placed into a Kjeldhal digestion unit (No.4071477, type KI 26, Gerhardt, Germany) for about 2 hours until a colourless digest was obtained and the flask was left to cool at room temperature (25˚C).

The distillation of ammonia was carried out into 25m1 boric acid (2%) by using 20 ml sodium hydroxide solution (45%).

Finally, the distillate was titrated with standard solution of HC1 (0.1N) in the presence of 2-3 drops of bromocreasol green and methyl red as an indicator until a brown reddish colour was observed.

Calculation:

$$\text{Crude Protein (\%)} = \frac{(\text{ml Hcl sample} - \text{ml Hcl blank}) \times N \times 14.00 \times F}{\text{Sample weight (g)} \times 1000} \times 100\%$$

Where:

N: normality of HCl.

F: protein conversion factor = 6.25

3.3.2.5 Crude fiber content

The crude fiber was determined according to the official method of AOAC (2003).

Principle: The crude fiber is determined gravimetrically after the sample is being chemically digested in chemical solutions. The weight of the residue after ignition is then corrected for ash content and is considered as a crude fiber.

Procedure: About 2g ± 1 mg of a defatted sample was placed into a conical flask containing 200 m1 of H_2SO_4 (0.26 N). The flask was then, fitted to a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digest was filtered (under vacuum) through a porclain filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiled water followed by boiling in 200 ml NaOH (0.23 N)

solution for 30 minutes under reflux condenser and the precipitate was filtered, rinsed with hot distilled water, 20m1 ethyl alcohol (96%) and 20 ml diethyl ether.

Finally, the crucible was dried at 105 °C (overnight) to a constant weight, cooled , weighed, a shed in a Muffle furnace (No.20. 301870, Carbolite, England) at 550-600 °C until a constant weight was obtained and the difference in weight was considered as crude fiber.

Calculation:

$$\text{Crude fiber (\%)} = \frac{(W1 - W2)}{\text{Sample weight (g)}} \times 100\%$$

Where: W1 = weight of sample before ignition (g).

W2 = weight of sample after ignition (g).

3.3.2.6 Minerals content

Potassium (K), (Na) and calcium (Ca) were determined by flame photometer (Sherwood Flame Photometer i410, Sherwood Scientific Ltd. Cambridge, UK) according to procedure of AOAC (1990). The knob of flame photometer was adjusted to potassium, sodium and calcium respectively and reading was set to zero using deionized water. Blank solution was run and reading was again set to zero. Standard solution of each mineral was run and recorded the reading of flame photometer. The reading of potassium, sodium and calcium in each sample was taken by running the sample one by one. Standard solution was run after every sample .The standard curves (appendix I, II, III) were obtained by plotting absorbance values of standards against appropriate concentrations of these three elements. One gram of dried beverage samples was subjected to wet digestion method as described by Richards (1968). Then analysis was conducted through

absorption spectrophotometer (Varian AA 240, Victoria, Australia) for determination of minerals (Mg) using standard curve. To determine phosphorus content in products samples, colorimetric estimation method was used as described by Kitson and Mellon (1944).

3.4.2.7 Total and reducing sugars

They were determined according to Lane - Eynon methods (AOAC, 1984).

Twenty-five grammes of the pulps were filtered through a Whatman filter paper (No. 4) .Then transferred to a 250 ml volumetric flask. 100 ml of distilled water was carefully added and then neutralized with 1.0 N NaOH to a pH 7.5-8.0. About 2ml of lead acetate were added and the flask was then shake, and left to stand for 10 minutes. Then 2 grams of sodium oxalate were added to remove the excess lead. Distilled water was again added to make the volume to mark (250ml). The solution then filtered and 50 ml of the filtrated were pipatted into a 250ml volumetric flask. 50g citric acid and 50 ml distilled water were added slowly to the new mixture. The contents of the flask were boiled gently for 10 minutes to invert the sucrose, and when cooled a few drops of phenolphthalein were added. In order to neutralize the mixture, a 20% NaOH solution was continuously added until the colour of the mixture disappeared, and the volume was made to mark before titration.

Standard method of titration: Ten ml of a mixed solution of Fehling (A) and (B) were pipatted into a conical flask. A burette was filled with a clarified sugar solution and the whole volume required reducing the Fehling's solution was run so that 0.5-1.0 ml was still required to complete the titration performed. The contents of the flask were mixed and heated to boiling for 2 minutes. Three drops of methylene blue indicator were added. Then the titration was completed until the colour has completely disappeared.

Mg total sugar in 100 ml = $\frac{\text{Factor} \times 100}{\text{Titre}}$

a- Totalsugar%=

$$\frac{\frac{\text{Mg}}{100\text{g}} \times \text{dilution} \times 100}{1000 \times \text{wt. taken.}}$$

The filtrate can be used directly for titration was according to Lane and Eynon (1984) usingthe following equation for calculation:

b- Reducing Sugars:

Reducing sugar % = $\frac{\text{Mg}/100\text{g} \times \text{dilution} \times 100}{1000 \times \text{wt.taken}}$

3.3.2.8 Calculation of carbohydrates

Carbohydrates were calculated by difference according to the following:

Total carbohydrates = 100% - [Moisture (%) + Protein (%) +Fat (%) + fiber (%) and Ash (%)].

3.4 Production of probiotic fermented product

3.4.1 Production of fermented product

Fresh raw goat's milk was heated to 65ºC for 30 minutes and then cooled to 37 ºC, following this 3% of starter culture *B.longumBB536* was added to the milk and incubated at 37 ºC divided in to four parts and treatments with 1% of inuline and different fiber(sorghum bran, barley bran and millet bran) powder. Next, using mixer the result in fermented product were filled in to plastic containers and stored under refrigeration (4˚C) for 3 weeks. Subsequent

physicochemical and microbiological analyses at storage were performed at 0, 1, 2 and 3weeks.

3.5 Chemical analysis of milk and fermented product

3.5.1 Protein content

The crude protein was determined by the micro-Kjeldahl method according to AOAC (2003) as follows:

Digestion

Two gram of sample was weighed and placed in small digestion flask (50ml), about 0.4 gram catalyst mixture (96% anhydrous sodium sulphate and3.5% copper sulphate) was added and 3.5ml of approximately 98%of $H_2SO_4$was added. The contents of the flask were then heated on an electrical heater for 2hours till the colour changed to blue-green. The tubes were then removed from digester and allowed to cool.

Distillation

The digested sample was transferred to the distillation unit and 20ml of 40%sodium hydroxide were added. The result as ammonia was received in 100ml of2%boric acid plus 3-4 drops of methyl red indicator. The distillation was continued until the volume reached 50ml.

Titration

The content of the flask were titrated against 0.02 N HCL. The titration reading was recorded. The crude protein was calculated using the following: equation (calculated on dry matter basis):

$$\mathbf{N\%} = \frac{\boldsymbol{mlHCL \times Normality of HCL(0.1) \times 0.014}}{\textbf{Sampleweight}} \times \mathbf{100}$$

Protein (%) = (N %) × 6.25

Where N = Nitrogen content.

0.014=molecular weight of nitrogen/1000

3.5.2. Determination of fat content

The fat content was determined by Gerber method according to AOAC (2003) as follows:

In a clean dry Gerber tube, 10 ml of sulfuric acid (density 1.815 gm/ml at 20ºC) were poured, and then 10.94 ml of milk sample and three grams of cream cheese samples were added. Amy1 alcohol 1.0 ml (density 0.815) was added to the mixture followed by addition of water to raise the level of fat in the column. The contents were thoroughly mixed till no white particles could be seen. The Gerber tubes were centrifuged at 1100 revolutions per minute (rpm) for 4-5minutes and the tubes were then transferred to a water bath at 65 ˚C for three minutes. The fat percentage was then read out directly from the fat column.

3.5.3. Ash content

The ash content was determined according to the method described in AOAC (2003). Ten millimeters of milk samples and two g of fiber samples were weighed in a suitable crucible and evaporated to dryness on a steam bath. The sample was placed in a muffle furnace (550ºC) for 1.5 hours, then cooled in desiccators and weighed. The ash content was calculated using the following equation:

$$\text{Ash (\%)} = \frac{w1}{w2} \times 100$$

Where:

W1= weight of ash

W2 = weight of sample

3.5.4 Determination of crude fiber

Fiber was determined according to official method of AOAC(2003) .About 2g of a sample was placed into a conical flask containing 200ml of H_2 SO_4(0.26N).

The flask was fitted to a condenser and allowed to boil for 30 minutes. At the end of the digestion period, the flask was removed and the digest was filtered through a proclaim filter crucible (No.3). After that, the precipitate was repeatedly rinsed with distilled boiled water followed by boiling in 200ml NaOH (0.23N) solution for 30 min under reflux condenser and the precipitate was filtered. Rinsed with hot distilled water, 20 ml ethyl alcohol (96%) and 20ml diethyl ether. Finally, the crucible was dried at 105 ˚C until a constant weight was obtained and the difference in weight was considered a crude fiber.

Crude fiber % =

$$\frac{[(\text{Dry residue + crucible(g)- (ignited residue +crucible (g))}]}{\text{Sample weight}} \times 100$$

3.5.5 Calculation of carbohydrates

Carbohydrates were calculated by difference according to the following:

Total carbohydrates = 100% - [Moisture (%) + Protein (%) +Fat (%) and ash (%)].

3.5.6 Determination of pH value

The pH value of the fermented product and milk were determined using a pH-meter (model HI 8521 microprocessor bench PH/MV/C meter. Romania). Two standard buffer solution of pH 4.00 and 7.00 were used for calibration of the pH meter at room temperature. The pH meter was allowed to stabilize for one minute and then the pH of the different cream cheese and milk were directly measured.

3.6 Microbiological analysis of fermented product

3.6.1. Preparation of equipments and media

Glass ware such as test tubes, flasks, Petri dishes and pipettes were sterilized by heating in an oven at 160 ˚C for 2 hours. Media, dilutions (distilled water), and tips were sterilized by autoclaving at 121˚C for 15 minutes. The media were then cooled to 45 ˚C (Barrow and Feltham, 1993).

3.6.2 Preparation of the samples

Different types of fermented product samples stored under similar conditions were taken in sterile plastic containers. Fifty grams of fermented product were taken for microbiological examination. Samples were taken aseptically from containers. Ten grams of fermented product were added to 90 ml of distill water in a flask and shaken well to complete mixing 10^{-1} after that to make 10^{-2} dilution 1 ml from the above mentioned dilution (10^{-1}) was aseptically transferred to 9 ml sterile peptone water. This procedure was repeated to make serial dilutions of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}

from suitable dilutions , 1 ml was transferred to Petri-dishes (duplicate) followed by addition of 18-20 ml culture medium was poured aseptically into each Petri- dish , mixed gently , left to solidity and incubated (in an inverted position) (Houghtb*et al*., 1992).

3.6.3 Enumeration of viable cells of *B. longumBB536*

MRS medium was used to enumerate *B. longumBB536* of different fermented products using the plate count technique. Samples were drawn at one day and every 15days intervals during storages (21 days). One gram of fermented product was diluted in peptone water, followed by plating on Rogosa Sharp agar (MRS)

supplement with 0.05% L- cystiene. The plates were incubated an aerobically at 37 ˚C for 48 h. The growth was calculated as colony forming unit per g (CFU/g).

Colony counters where:

Colony forming units/gm (CFU/gm) = Total number of colonies in the dilution multiplied by the reciprocal of dilution (Houghtb*et al*., 1992).

3.6.4 Total bacterial count (TBC)

Total bacterial count using nutrient agar medium was determined according to the method by Houghtb*et al*. (1993).

3.6.4. 1 Preparation of the medium

The medium was prepared according to manufacturer's instructions by dissolving 23.5 gram of powder in 1000 ml of distilled water, heated to boiling point and then sterilized in an autoclave at 121 ^{o}C for 15 minutes (Frank *et al*., 1993).

3.6.4. 2 Plating

From each selected dilution 1 ml was transferred into sterile Petri dishes (duplicate) followed by addition of 15 – 18 ml melted, cooled (45 ^{o}C) nutrient agar was mixed thoroughly by rotating the dishes first in one direction and then in the opposite direction. When medium was solidified, the dishes were incubated in an inverted position at 35 ± 2 °C for 24 hours.

3.6.4.3 Counting

Plates contain 25 – 250 colony were selected and counted using colony counter. The number of colony forming units (cfu) in the dilution was obtained by multiplying the reciprocal of the dilution.

3.6.5 *E.coli* count

The count was performed according to William and Dennis (1998) using MaConkey agar media and Eosin methylene blue agar (EMB) for identification.

3.6.5.1 Preparation of the medium

The manufacturer's instructions were followed by dissolving 55 grams of powder in 1000 ml of distilled water, heated to boiling point and sterilized in an autoclave at 121 ^{o}C for 15 minutes, (Christen *et al*., 1992).

3.6.5.2 Plating and counting

One ml amounts of each sample dilutions were streaked in dried plate of Maconkey agar media. The culture was incubated at 35 °C for 24 hours, and then colonies were used for further confirmation of the presence of *E. coli* by streaking a loop from each colony on Eosin methylene blue agar (EMB) for identification of colonies which show brilliant green, the characteristic features of growth of *E. Coli* in the medium. The isolates were further characterized by biochemical tests according to Barrow and Feltham (1993) .Special attention was paid to the pattern of reactions of the organism in IMVIC tests, and the positive tests were recorded for a positive *E. coli* count.

3.6.6 *Staphylococcus aureus* count

The count was achieved according to method by Christen *et al*., (1992) using Mannitol salt agar.

3.6.6.1 Preparation of the medium

The manufacturer's instructions were followed by dissolving 111 grams of powder in 1000 ml of distilled water, heated to boiling point and sterilized in an autoclave at 121 ^{o}C for 15 minutes (Christen *et al*., 1992).

3.6.6.2 Plating and counting

One ml quantities of each sample dilutions 10^{2}-10^{6} was transferred into sterile Petri dishes followed by addition of 15 – 18 ml melted media, cooled (45 ^{o}C) Petri dishes with Mannitol salt agar was mixed thoroughly by rotating the dishes first in one direction and then in the opposite direction. When medium was solidified, the culture was incubated at 35 ± 2 °C for 24hours where colonies of *Staphylococcus aureus* were recognized by bright yellow zones formation in Mannitol salt agar (Jawez and Adel, 1990) and then colonies were counted by colony counter.

3.6.7 Yeast and molds count

The yeast and molds count were determined according to Harrigan and McCance (1976).

3.6.7.1Preparation of the medium

Medium were prepared according to manufacture instructions by suspending 39 grams of Potatoes dextrose agar in one liter distilled water and boiled until it dissolved completely, then it was sterilized by autoclaving at 121ºC for 15minuts.

3.6.7.2 Plating and counting

One ml from suitable dilutions was transferred into sterile Petri dishes followed by addition of 15 – 18 ml potato dextrose agar. The plates were incubated at 25°C for up to 72 hours. And then colonies were counted by colony counter.

3.6.8. *Salmonella*

The count was achieved according to method by Liong and Shah, (2006) using Brilliant green agar.

3.6.8.1 Preparation of the medium

Medium were used according to manufacture instructions by suspending 58.09 grams of Brilliant green agar in one of liter distilled water , boiled until dissolved completely and then it was sterilized by autoclaving at 121ºC for 15 minuts.

3.6.8.2 Plating and counting

One ml quantity of each sample dilution was transferred into sterile Petri dishes (duplicate) followed by addition of 15 – 18 ml melted media, cooled to45^{o}C.Petridishes with Brilliant green agar was mixed thoroughly by rotating the dishes first in one direction and then in the opposite direction. Then media was solidified. The culture was incubated at 35 ± 2 °C for 24hours.

3.6.8.3 Counting

Plates contain 30 – 300 colony were selected and counted using colony counter. The number of colony forming units (CFU) in the dilution was obtained by multiplying the reciprocal of the dilution.

3.7. Hematology

The procedure for complete blood profile including: hemoglobin concentration (HGB), red blood cell (RBC), white blood cell (WBC) and their respective differentials were preformed with automated analyzer (Mindry-BC 3000 plus).

3.8. Biochemistry

3.8.1 Serum enzymes and minerals

Serum enzyme [aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP)] activities and total protein (TP), albumin (Alb), globulin (Glob) content and uric acid of the blood were estimated according to methods based on the enzymatic colorimetric test method also Na, ca, k, P were determined using kits method obtained from **Bio Systems (Spain).**

3.8.2 Glucose

Measurement was based on the colorimetric end point test method.

Principle

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine product.

Protocol

	Blank	Standard	Sample
Sample	-	-	10 µL
Standard	-	10 µL	-
Enzyme reagen	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 505 nm. The concentration of the standard was then multiplied by the product of the sample absorbance/standard absorbance.

3.8.3 Direct bilirubin

Principle

Direct bilirubin (conjugated) reacts in acid environment with diazotized sulphalinic acid. The formed colouredazobilirubin is measured photometrically at 546 nm.

Protocol

	Sample	**Blank**
Direct bilirubin reagent	1000 µL	1000 µL
Nitrite reagent	20 µL	-
Sample	50 µL	50 µL

The reaction mixture was mixed well and incubated in the dark at room temperature for 5 minutes. The absorbance of the sample was measured against respective sample blank within 8 minutes at 546 nm. The concentration wascalculated by subtracting the blank absorbance from the sample absorbance and multiplied by the factor.

3.8.4 Urea

Measurement was based on the colorimetric end point test method.

Principle

The Berthelot reaction has long been used for the measurement of urea and ammonia. The present method is a modified Berthelot method. The urea colorimetric procedure is a modification of the Berthelot reaction. Urea is converted to ammonia by the use of urease. Ammonium ions then react with a mixture of salicylate, sodium nitroprusside and hypochlorite to yield a blue-green chromophore. The intensity of the color formed is proportional to the urea concentration in the sample.

Protocol

	Blank	Standard	Sample
Urea buffer	1000 µL	1000 µL	1000 µL
Urea enzy reagent	100 µL	100 µL	100 µL
Standard	-	10 µL	-
Sample	-	-	10 µL
Mixed well and incubated for 5 minutes at 37^{o}C.			
Urea col developer	1000 µL	1000 µL	1000 µL
Mixed well and incubated for 5 minutes at 37^{o}C.			

The absorbance of sample and standard was measured against reagent blank at 578 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance.

3.8.5 Creatinine

Measurement was based on the colorimetric kinetic test method developed by Jaffe reaction.

Principle

Creatinine in alkaline solution reacts with picrate to form a coloured complex which absorbs at 500-520 nm. The amount of complex formed is directly proportional to the creatinine concentration.

Protocol

	Blank	Standard	Sample
Sample	-	-	100 µL
Standard	-	100 µL	-
Picrate and bu reagents	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and after 30 seconds at room temperature the initial absorbance was read and read again after 1 minute. The change in absorbance in a minute of the standard and sample were measured against the reagent blank at 500 nm. The concentration of the standard was then multiplied by the product of sample absorbance/standard absorbance.

3.8.6 Cholesterol

Measurement was based on the enzymatic colorimetric test method.

Principle

Cholesterol esters are hydrolysed to produce cholesterol. Hydrogen peroxide is then produced from oxidation of cholesterol by cholesterol oxidase. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantypyrine in the presence of phenol and peroxide. The absorption of the red quinoneimine dye is proportional to the concentration of cholesterol in the sample.

Protocol

	Blank	Standard	Sample
Sample	-	-	10 μL
Standard	-	10 μL	-
Enzyme reagent	1000 μL	1000 μL	1000 μL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 505 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance.

3.8.7 High density lipoprotein (HDL)

Measurement was based on the CHOD-PAP tests method

Principle

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated by the precipitating reagent. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction remains in the supernatant in this phase and is determined by an enzymatic (CHOD-PAP) method.

Protocol

Precipitation step

Test tubes were contained 500 μL of each sample then added 500 μL Precipitating reagent was mixed and allows standing for 5 minutes. Centrifuge for 10 minutes at 3000 rpm and determining the cholesterol content by the CHOD-PAP method. Only clear supernatant must be used.

	Blank	**Standard**	**Sample**
Sample	-	-	50 μL
Standard	-	50 μL	-
Enzyme reagent	1000 μL	1000 μL	1000 μL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 520 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance and a serum dilution factor.

3. 8.8 Triglycerides

Measurement was based on the enzymatic colorimetric test method

Principle

Triglycerides are determined after enzymatic hydrolysis with lipases. The quinoneimine indicator if formed from hydrogen peroxide, 4-aminophenazone and 4-chorophenol under the catalytic influence of peroxidase.

Protocol

	Blank	Standard	Sample
Sample	-	-	10 µL
Standard	-	10 µL	-
Enzyme reagen	1000 µL	1000 µL	1000 µL

The reaction mixture was mixed well and incubated for 5 minutes at 37^{o}C. The absorbance of sample and standard was measured against reagent blank at 505 nm. The concentration of the standard was then multiplied by the product of sample absorbance and divided by the standard absorbance.

3.9 Bacteriological enumeration in colon of rats fed with fermented goat milk supplemented with inulin and different cereal brans

Three rats from each group were used for bacteriological enumeration in colon content were collected in severe aseptic conditions in sterile bottle to avoid any cross contamination. The content was homogenized inside a cabinet and serially diluted prior to plating on different agar plates.

Media used for total aerobe, total anaerobe and *enterobacteriacae* ,*bifidobacteria* and *salmonella* were according to reported by (Santos *et al*., 2006).*Staphylococcus*, *lactobacillus* and coliform enumerated following Liong and Shah (2006) method. Incubation environment of media used for enumerations are shown in Table (2).

Table 2: Enumeration media and incubation environments of different microbiota groups in colon of rats fed with different products.

Bacterial group	Type of media	Incubation
Total aerobe*	Nutrient agar	Aerobic
Total anaerobe**	Brain heart infusion agar	Anaerobic****
Coliform**	Macconky agar	Anaerobic****
*Salmonella***	Brilliant green agar	Aerobic
*Staphylococcus***	Mannitol salt agar	Aerobic
*Lactobacillus***	De Man Rogosa Sharpe agar	Anaerobic****
*Bifidobacteria***	De Man Rogosa Sharpe ag L- cystiene	Anaerobic****

[a]All samples were incubated at 37 ºC.

* Incubation for one day.

** Incubation for two days

** Incubation for three days

****Anaerobic condition was created in anaerobic jars

3.10 Statistical analysis

One- way ANOVA and two sample paired test were performed to examine significant differences between normally distributed data of replicated measurement. Probability level of less than 0.05 was considered significant ($p<0.05$). All data were analyzed using vision 17MINITAB statistical software for windows (2006).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate composition of different cereal brans (sorghum, barley and millet)

Proximate composition of different cereal brans (sorghum, barley and millet) and inulin are shown in Table3. The composition of inulin including: moisture, protein, fat, ash, fiber and carbohydrates were 3.00%, nil, nil, nil, 89.00% and 8.00%, respectively. Results of inulin showed a low content of water and highest fiber level as compared to tested types of bran. Similarly, low lipid and ash levels of inulin were documented by (Ayadi *et al*., 2009). These differences in components levels of inulin might be due to the diversity between commercial varieties. Also significant differences ($P< 0.05$) in chemical components levels between cereals bran types were assured (Table 3). In fact inulin was the richest in fiber and carbohydrate from among cereal bran types. While millet bran was the highest in moisture and fat levels as presented in Table 3. Whereas, barley bran was the highest source of protein and ash refer to Table 3. The moisture (6.34%), ash (2.52%) and fiber (2.80%) values in barely grain were comparable to those stated by Badahdah *et al*., (2019). The values of moisture (8.47%) and protein (12.93 %) in millet bran were close by to that reported by Liang *et al*. (2010).

Table 3: Proximate composition of Inulin and different cereal brans *

Components (%)	Inulin	Types of bran		
		Sorghum	Barley	Millet
Moisture	3.00 ± 0.00	68.48± 0.33	50.15 ±0.156	68.71 ± 0.290
Fat	ND	3.13 ± 1.04	4.935 ±0.679	5.935 ± 0.0212
Proteins	ND	12.60 ± 2.97	13.915±1.110	11.375 ± 1.237
Fiber	89.00 ± 0.00	6.49 ± 0.29	19.685±0.870	4.635 ± 0.346
Ash	ND	2.43 ± 0.021	8.660±0.0141	3.190 ± 0.283
Carbohydrates	8.00± 0.00	6.87 ± 0.35	2.640±0.156	6.140 ± 0.0849

*Values are mean ± SD for replicate independent analysis

ND= Not determined

4.2 Minerals content

Table 4 shows minerals content (Ca, K, Mg, Na, and P) in inulin and different cereal brans (sorghum, barley and millet). Chemical analysis exposed significant (P< 0.05) differences in different components. Inulin recorded the highest levels of Ca, K, Mg and Na; except P. Inulin contained the lowest P as compared to its level in different cereal brans. All cereal brans contain small amount of Ca, K, Mg and Na (Table 4). Moreover, there was no significant (P< 0.05) difference in Ca, K and P between different types of bran. While no significant (P< 0.05) difference in Mg level between sorghum and barely brans. A predominance of potassium and calcium was observed and low levels of sodium and magnesium was reported by Femenia *et al*, (1997).

Table 4 Mineral content (mg/100g) of inulin and different cereal bran *

Elements (mg/100g)	Inulin	Types of bran		
		Sorghum	**Barley**	**Millet**
Ca	1.62±0.03[a]	0.08±0.01[b]	0.08±0.00[b]	0.08±0.00[b]
K	0.76±0.01[a]	0.11±0.01[b]	0.10±0.00[b]	0.09±0.01[b]
Mg	0.82±0.02[a]	0.03±0.00c	0.02±0.00[c]	0.05±0.21[b]
Na	1.74±0.00[a]	0.11±0.13[b]	0.02±0.06[c]	0.02±0.00[c]
P	0.26±0.00[b]			

*Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw of are significantly different at p<0.05.

4.3. Total sugars, reducing sugar and none reducing sugars

Significant total sugars were presented in Table (5). Values obtained were no significantly (P< 0.05) differences in different types of sugar between bran of sorghum, barely and millet. Barely bran contained the higher total and non reducing sugars levels form among other two types (sorghum, millet). Nevertheless, the highest amount of reducing sugar was found in millet bran.

Table 5: Total, reducing and non reducing sugars (mg/100g) of cereal brans *

Types of sugars (%)	**Types of bran**		
	Sorghum	**Barley**	**Millet**
Total	1.73± 0.00 ^{c}	2.62± 0.00^{a}	1.43±0.01^{d}
Reducing	0.24±0.00 ^{d}	0.30± 0.00^{c}	0.49±0.01^{a}
Non reducing	1.42±0.00 ^{d}	2.20±0.00^{a}	1.99±0.02^{c}

*Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw of are significantly different at p<0.05.

4.4. Physicochemical properties and chemical composition of different fresh milks

Table 6 shows the physicochemical properties and chemical composition of different fresh milks (goat, cow and camel). There were no significant ($P< 0.05$) differences in components and properties between different typs of milks. The highest pH was recorded for goat milk. While cow milk recorded the highest levels of TSS, lactose and protein. Whereas, camel milk contained the highest moisture and ash levels. Moisture content of goat (88.98 %), caw (89.23%) and camel (90.21%) milks were almost similar to the value 87.3% reported by Walstra and Jenness (1984). They also reported 3.7% fat and 4.6% lactose and 3.25% protein which are not comparable to all values in Table 6.

Table6. Physicochemical properties and chemical composition of different fresh milks

Parameter	Types of milk		
	Goat	Cow	Camel
Total solids (%)	10.91 ± 0.01^{b}	11.38 ± 0.01^{b}	9.80 ± 0.02^{b}
Moisture (%)	88.98 ± 0.02^{a}	89.23 ± 0.01^{a}	90.21 ± 0.01^{a}
Ash (%)	0.75 ± 0.01^{g}	0.73 ± 0.02^{g}	0.93 ± 0.02^{g}
Fat (%)	3.21 ± 0.01^{e}	3.12 ± 0.02^{e}	2.34 ± 0.02^{e}
Lactose (%)	4.15 ± 0.01^{d}	4.90 ± 0.02^{d}	4.44 ± 0.04^{d}
Proteins (%)	2.24 ± 0.01^{f}	3.02 ± 0.02^{f}	2.04 ± 0.03^{f}
pH	6.65 ± 0.00^{c}	6.640 ± 0.00^{c}	6.57 ± 0.00^{c}

*Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw of are significantly different at $p<0.05$.

4.5. The growth of *Bifdobacterium longum* BB356 during fermentation of different fresh milks

Growth of *B. longum* ***BB536*** in cow, goat and camel fresh milk is shown in Table (7). There were significant ($p<0.05$) increases in *B longum* BB356 viable count by extended fermentation period. The maximum growth of *B. longum* BB356 was attained at 12h in all types of fermented milk. Goat milk recorded the highest level of *B. longum* BB356 followed by cow milk and then the camel milk. The *B. longum* BB356 increases in the fermented milks were 3.84, 3.07 and 2.90 in fermented goat, cow and camel milks, respectively. The difference in growth of *Bifidobacteria longum* BB356 in the different fermented fresh milks could be attributed to the quality, quantity and availability of nutrients of the tested milk. However, after maximum growth of the strain there was reduction in number of *B. longum* BB536 of each specific types of fermented milk. That may be due to the accumulation of acids or reduction of availability of nutrient required for the growth (Kabeir *et al*., 2005). On the other hand, the viable count of *B. longum* BB536 in all types of fermented milk was above the number required to presence in probiotic which was at least 6 log cfu/ml fermented products (Viderola and Reinheimer; 2000).

Table 7: The viable count CFU/ml of *Bifidobacterium longum* BB536 log (CFU/ml) during fermentation of different types of fresh milk *

Fermentation period (h)	Types of fermented milk		
	Goat	Cow	Camel
0	4.89±0.21a	4.80±0.12[a]	4.56±0.25 [a]
6	6.79 ±0.04[abc]	5.78±0.15[b]	5.40±0.14 [b]
12	8.73±0.01[a]	7.87±0.14[c]	7.46±0.20 [c]
18	7.93±0.06[a]	6.86±0.11[d]	6.55±0.22 [d]
24	6.31±0.04[c]	5.46±0.14[d]	5.46±0.25 [a]

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

4.6. pH changes during fermentation of different fresh milk with *B.longum* BB356

There was significant ($P<0.05$) decrease in pH values of each specific milk type by extended fermentation (Table 8). The pH decreases were 0.37, 0.40, and 0.33 in fermented cow milk, goat milk, and then the camel milk, respectively. Level of acidity increased by extended fermentation period and thus caused reduction in pH. The acid produced is beneficial and reported to have antibacterial such as preventing the proliferation of pathogens (Bullen *et al*., 1976).

4.7. TSS changes during fermentation of different fresh milk with *B. longum* BB536

Table 9 shows changes in TSS during fermentation of different fresh milk with *B. longum* BB356.

There was significant ($P<0.05$) decrease in TSS levels of each specific type of fermented fresh milk by extended fermentation. The TSS was 1.80, 2.25, and 1.95% in fermented cow milk, goat milk, and camel milk, respectively, at maximum growth. However, the level of TSS changes at 18h of fermentation were, 0.64, 0.63, and 0.37% in the fermented goat milk, fermented cow milk, and the fermented camel milk respectively.

Table 8: pH changes during the growth of *Bifidobacterium longum* BB536 **in different types of fresh milk ***

Fermentation periods (h)	Types of fermented milk		
	Goat	Cow	Camel
0	6.31± 0.01[a]	6.81± 0.00[a]	6.32±0.01[a]
6	5.89 ± 0.0141[b]	5.080 ± 0.000[c]	4.88 ±0.01[c]
12	6.51 ± 0.1273[a]	5.41 ± 0.01[b]	5.77±0.014[b]
18	5.65 ± 0.0212[b]	4.67± 0.01[d]	4.18 ±0.014[e]
24	4.84 ± 0.495[c]	3.28 ±0.014[e]	4.50±0.000[d]

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

Table9: TSS (100%) changes during the growth of *Bifidobacterium longum* BB536 **in different types of fresh milk ***

Fermentation periods (h)	Types of fermented milk		
	Goat	Cow	Camel
0	6.21 ± 0.01[a]	1.85 ± 0.08[bc]	6.14 ±0.01[a]
6	4.95 ± 0.00[b]	2.10 ± 0.00[bc]	6.01 ±0.01[b]
12	4.39 ± 0.01[e]	3.21 ± 0.01[ab]	5.65 ±0.07[c]
18	4.81 ± 0.00[c]	1.00 ± 0.000 [c]	5.47 ±0.014[d]
24	4.61 ± 0.00[d]	4.18±0.97[a]	4.50±0.01[e]

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

4.8. Chemical composition of fermented goat milk supplemented with inulin and different brans

There was no significant ($p<0.05$) difference in moisture and carbohydrate content at initial fermentation and maximum strain BB 536 growth of each specific fermented product (Table 10). Whereas, level of fat in inulin and sorghum bran fermented goat milk at initial and maximum growth were significant ($p<0.05$). However, ash content was only significant ($p<0.05$) between the initial and maximum growth in sorghum bran supplemented goat milk fermented with strain BB536. Similarly, fiber content was only significant ($p<0.05$) between the initial and maximum growth in millet bran supplemented goat milk fermented with strain *BB536* (Table 10)

Table 10: Chemical composition of goat milk supplemented with inulin and different brans fermented with *Bifidobacterium longum BB536* *

Component	Fermented goat milk							
	Inulin		Types of bran					
			sorghum		barely		millet	
	Initial	Maximum	Initial	Maximum	Initial	Maximum	Initial	Maximum
Moisture (%)	86.9±0.01[a]	86.77±0.04[a]	88.54±0.01[a]	88.50±0.28[a]	84.55±0.00[a]	86.46±0.00[a]	85.28±0.02[a]	85.79±0.01[a]
Fat content (%)	2.37±0.007[d]	2.42±0.03[e]	1.97±0.01[e]	2.54±0.03[c]	1.840±0.00[e]	2.015±0.00[e]	2.32±0.028[e]	2.44±0.02[e]
Protein content (%)	2.30±0.00[e]	2.56±0.02[d]	2.59±0.00[d]	2.66±0.01[c]	2.62±0.00[c]	2.75±0.00[c]	2.52±0.00[c]	2.74±0.02[d]
Ash content (%)	1.44±0.02[f]	1.84±0.02[f]	1.54±0.01[f]	1.76±0.00[d]	1.55±0.00[f]	1.64±0.01[f]	1.77±0.01[f]	1.85±0.01[f]
Carbohydrates (%)	13.06±0.01[b]	13.23±0.04[b]	11.46±0.01[b]	11.51±0.28[b]	15.46±0.00[b]	13.56±0.01[b]	14.73±0.02[b]	14.21±0.01[b]
Fiber (%)	3.86±0.01[c]	3.71±0.01[c]	2.97±0.01[c]	2.87±0.04[c]	2.40±0.00[d]	2.02±0.01[d]	2.62±0.02[d]	2.60±0.00[c]

*Values are mean ± SD for replicate independent runs.

**Value of each component that bear different superscript letter of each supplement type is significantly different at $p<0.05$.

4.9. Justification of using goat milk for formulation of fermented product

The maximum growth of *B. longum* BB356 was attained at 12h in all types of fermented milk. Goat milk recorded the highest level of *B. longum* BB356 followed by cow milk and then the camel milk. Nevertheless, the *B. longum* BB356 increases were 3.84, 3.07 and 2.90 log cfu/ml in fermented goat, cow and camel milk; respectively.

Goat milk differs from cow or human milk by having better digestibility, higher alkalinity, increased buffering capacity, and certain therapeutic effects that may be useful in medicine and human nutrition. The good acceptability and digestibility of goat milk are important beneficial factors for its inclusion in formulated diets prescribed for children and convalescent people. In many cases, goat milk may be successfully used as substitute for cow milk in the regular diet of allergic individuals (Haenlein, 2003).

4.10. The growth of *Bifidobacterium. longum BB536* during fermentation of goat milk supplemented with inulin and different cereal brans

Comparative growth of *B. longum BB536* cultured in goat milk supplemented with inulin and different bran (sorghum, barley and millet) is shown in (Table 11).

There were significant ($p<0.05$) increases in strain *B. longum BB536* viable count by extended fermentation period in all types of formulated goat milk supplemented with inulin and different brans, as compared to strain level at beginning of fermentation. The maximum growth of *B. longum BB536* in all types of fermented products was attained at 12h incubation. These high viable count of strain *BB536* (7.53±0.16b -8.43±0.03cLog CFU/ml) in all fermented goat milk products exceed the minimum number (6 log CFU/ml fermented products) required to be present in probiotic food (Viderola and Reinheimer, 2000). After the maximum growth the strain declining was observed in all types of fermented goat milks supplemented with inulin and goat milk with different brans (sorghum, barley and millet) (Table 11)

The *B. longum BB536* increases in different fermented goat milk supplemented with inulin and goat milk with different bran were 2.57, 2.31, 1.93 and 1.18 CFU/ml in fermented goat milk with sorghum bran, goat milk with inulin, goat milk with millet bran and goat milk with barely bran, respectively as compared to strain initial level at the beginning of fermentation. These increases induced by different cereal bran are comparable to the prebitication (support growth of strain *BB 536*) with the commercial prebiotic. Therefore, tested cereal bran might have prebiotic effect on strain BB536. On the other hand, the variations in growth rate of

strain *BB536* could be attributed to variances in availability of nutrients required for growth in the different formulated products. In fact, goat milk contains almost the essential nutrient for strain growth. Together the different fiber combinations could complement the nutrient component demand for strain BB 536 growth in formulated goat milk medium. However, after maximum growth (12 h) the strain started to decline in all types of fermented goat milk products (Table 6). The decline of the strain might be due to the accumulation of acids or reduction of availability of nutrient required for the growth as stated by Kabeir *et al*. (2005) during fermentation of Sudanese thin porridge Medida. In spite of the continuous declining in viable count of strain BB536 in all types of fermented goat milk up to 24h of incubation, the remained viable counts still above the number required to presence in probiotic food which is at least 6 log CFU/ml fermented product (Viderola and Reinheimer, 2000).

Table 11: The viable count of *Bifidobacterium longum* BB536 log (CFU/ml) during fermentation of goat milk supplemented with inulin and different brans *

Fermentation time(h)	Fermented goat milk supplemented with different types of fiber			
	inulin	sorghum bran	barley bran	millet bran
0	5.52 ± 0.318^{a}	5.86 ± 0.06^{a}	6.07 ± 0.0283^{a}	6.03 ± 0.1061^{a}
6	7.33 ± 0.0070^{a}	6.07 ± 0.099^{c}	6.99 ± 0.00707^{ab}	6.31 ± 0.417^{bc}
12	7.53 ± 0.163^{b}	8.43 ± 0.0283^{c}	7.65 ± 0.0636^{b}	7.96 ± 0.0212^{b}
18	7.39 ± 0.00707^{b}	7.25 ± 0.0636^{b}	7.84 ± 0.0778^{a}	7.25 ± 0.219^{b}
24	7.30 ± 0.0636^{a}	6.93 ± 0.170^{ab}	6.79 ± 0.0636^{b}	6.83 ± 0.148^{b}

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

4.11. pH changes during fermentation of goat milk supplemented with inulin and different cereal brans with *Bifidobacterium longum BB536*

During fermentation process with strain *B. longum BB536* there were significant ($P<0.05$) decrease in pH levels in all types of goat milk supplemented with inulin and different bran (sorghum, barley and millet) by extended fermentation period to 24h (Table 12).The decreases in pH are due to increased acids production during fermentation process as a result of fermented sugar by *B. longum BB536*, which produced acetic and lactic acid as reported by De Vries *et al.* (1967). Moreover, the accumulated acids produced by bifidobacterium strain, reported to have antibacterial activity such as prevention of the proliferation of pathogens (Bullen *et al*., 1976). The pH decreases at maximum growth at (12h incubation) of strain *B. longum BB536* were1.82, 1.07, 0.67 and 0.49pH in fermented goat milk supplemented with barely bran, inulin , millet bran and sorghum bran, respectively. Level of acidity increased by extended fermentation period and thus caused reduction in pH.

Table 12: pH changes during the growth of *Bifidobacterium longum* BB536 **in goat milk supplemented with inulin and different brans ***

Fermentation time (h)	Fermented goat milk supplemented with different types of fiber			
	Inulin	Sorghum bran	Barley bran	Millet bran
0	6.18 ± 0.00^{c}	6.15 ± 0.00707^{d}	6.22 ± 0.00707^{b}	5.06 ± 0.0141^{e}
6	6.04 ± 0.0141^{b}	6.01 ± 0.0141^{b}	4.95 ± 0.00^{c}	5.15 ± 0.0141^{c}
12	5.11 ± 0.000^{c}	5.65 ± 0.0707^{b}	4.40 ± 0.00707^{d}	4.45 ± 0.0141^{d}
18	5.01 ± 0.0141^{c}	5.47 ± 0.0141^{b}	4.81 ± 0.000^{d}	3.79 ± 0.0212^{e}
24	4.87 ± 0.00707^{a}	4.51 ± 0.00707^{c}	4.61 ± 0.000^{b}	3.73 ± 0.0283^{d}

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

4.12. TSS changes during fermentation of goat milk supplemented with inulin and different brans with *Bifidobacterium longum BB536*

Table 13 shows changes in total soluble solids (TSS) during fermentation of formulated goat milk supplemented with inulin and different brans (sorghum, barley and millet) with *Bifidobacterium. longum BB536*.There were significant ($P<0.05$) decrease in TSS levels in all types of fermented goat milk product by extended fermentation period to 24h. The TSS decreases at maximum strain *BB536* growth were 1.7, 1.25, .95 and 0.75% in fermented goat milk supplemented with barely bran, sorghum bran, millet bran and inulin, respectively. Enzymatic activity of the strain plays a vital role in TSS reduction. The strain utilized soluble solids for energy source, particularly reducing sugar, the main components of TSS. Reductions in TSS by fermentation with *B. longum* BB536 and other probiotic strains were reported by Ibrahima *et al*. (2015), Kabeir *et al*. (2005), Badahdah *et al*. (2019) and Muyanj *et al*. (2010).

Table 13: TSS changes during fermentation of goat milk supplemented with inulin and different brans with *Bifidobacterium longum BB536* *

Fermentation time (h)	Fermented goat milk supplemented with different fiber			
	Inulin	Sorghum bran	Barley bran	Millet bran
0	8.30±0.21[a]	7.45±0.07[a]	8.20± 0.14[a]	9.55± 0.07[b]
6	8.25±0.42[a]	6.45±0.07[b]	7.50± 0.00[b]	9.05± 0.00[a]
12	7.55±0.07[a]	6.20±0.14[b]	6.50± 0.00[c]	8.60±0.14[c]
18	6.45±0.07[b]	5.60 ±0.014[c]	5.20± 0.00[d]	7.35±0.07[d]
24	5.90±0.00[b]	4.45±0.21[d]	4.99± 0.16[d]	6.25±0.07[e]

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$.

4.13. Titratable acidity during fermentation of goat milk supplemented with inulin and different brans with *Bifidobacterium longum BB536*

Referring to the result in Table 14, there were significant ($p<0.05$) increases in titratable acidity of different goat milk formulations by extended fermented period to 24h. The increases were 0.56, 0.49, 0.46 and 0.2% at maximum growth of strain *B. longum BB536* (12h) in fermented goat milk supplemented with barely bran, millet bran, inulin and sorghum bran, respectively. The increased acidity is explained by accumulation of acetic, lactic acid and other organic acids produced during fermentation of the formulated products (Sefa *et al*., 2003). Similarly, acid increase due to fermentation was reported by many authors as a result of sugar fermentation (Ibrahim *et al*., 2015); Kabeir *et al*., 2005)

Table 14: Titrable acidity (%) during fermentation of goat milk supplemented with inulin and different brans with *Bifidobacterium longum BB536* *****

Fermentation time (h)	Fermented goat milk supplemented with inulin and different fiber			
	Inulin	Sorghum bran	Barley bran	Millet bran
0	0.19± 0.00^{d}	0.24±0.071^{d}	0.26±0.00^{e}	0.44± 0.01^{c}
6	0.22±0.01^{d}	0.20±0.01^{e}	0.75± 0.00^{d}	0.95± 0.01^{b}
12	0.65±0.01^{c}	0.44±0.01^{c}	0.82± 0.01^{c}	0.93± 0.01^{b}
18	0.76± 0.01^{b}	0.62±0.01^{b}	0.85±0.01^{b}	0.94± 0.02^{b}
24	0.81±0.01^{a}	0.73±0.01^{a}	0.92± 0.01^{a}	1.02± 0.01^{a}

* Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same Column are significantly different at $p<0.05$

4.14. Mineral contents of goat milk supplemented with inulin and different types of cereal bran fermented with *Bifidobacterium longum BB536*

Table 15 displays the minerals content (Ca, K, Mg, Na and P) of fermented goat milk supplemented with inulin and different brans (sorghum, barley and millet) at initial (0h) and at maximum growth time (12h). There were no significant ($p<0.05$) difference in Na of all fermented milk. On the other hand there was significant an increase in Ca and K at maximum viable count of strain BB536 in all fermented milk as compared to their levels at initial fermentation. In contrast Mg decreased at maximum strain count in all fermented products.

Table 15: Mineral contents (100%) of goat milk supplemented with inulin and different brans fermented with *Bifidobacterium longum BB536* *

Minerals	Fermented Goat milk supplemented with different fiber							
	Inulin		sorghum		barley		millet	
	Initial	Maximum	Initial	Maximum	Initial	Maximum	Initial	Maximum
Ca	2.01±0.01^{c}	3.00±0.00^{c}	1.40±0.00^{c}	3.00±0.00^{c}	1.00±0.00^{c}	2.05±0.07^{d}	1.10±0.14^{c}	1.82±0.02^{d}
K	1.52±0.01^{d}	3.08±0.01^{b}	1.03±0.01^{d}	3.85±0.01^{b}	1.04±0.0212^{c}	3.56±0.03^{b}	0.51±0.01^{d}	2.56±0.00^{b}
Mg	3.10±0.14^{b}	2.60±0.01^{d}	3.15±0.07^{b}	2.00±0.00^{d}	3.61±0.01^{b}	3.15±0.21^{c}	2.55±0.07^{b}	2.15±0.07^{c}
Na	7.84±0.02^{a}	6.97±0.02^{a}	10.44±0.01^{a}	6.94±0.01^{a}	6.95±0.01^{a}	7.84±0.02^{a}	6.96±0.00^{a}	7.82±0.00^{a}
P	0.76±0.01^{e}	0.63±0.01^{e}	0.81±0.01^{e}	0.82±0.00^{e}	0.91±0.01^{d}	0.81±0.01^{e}	0.46±0.01^{d}	0.62±0.02^{e}

*Values are mean ± SD for replicate independent runs.

** Values that bear different superscript letter in the same row are significantly different at $p<0.05$

4.15. The viable counts of *Bifidobacterium longum* BB536 log (CFU/ ml) during refrigeration storage of fermented goat milk supplemented with inulin and different cereal brans

Table 16 shows the viable counts of *B. longum BB536* during refrigeration storage of different formulated goat milk supplemented with inulin and different cereal brans (Sorghum, Barely and Millet). The survival of probiotic bacteria in fermented dairy bio-products depends on such varied factors as the strains used, interaction between species present, culture conditions, chemical composition of the fermentation medium (e.g. carbohydrate source), final acidity, milk solids content, availability of nutrients, growth promoters and inhibitors, concentration of sugars (osmotic pressure), dissolved oxygen (especially for Bifidobacterium sp.), level of inoculation, incubation temperature, fermentation time and storage temperature. Referring to the result in Table 16, there were significant ($p<0.05$) reductions in *B. longum BB536* viable count in all fermented samples at refrigeration. With regard to total reduction of strain BB536throughout the storage period (two weeks), the highest value was in goat milk supplemented with barley bran (1.92 CFU /ml), followed by sorghum bran(1.83 CFU /ml), inulin (1.81 CFU /ml)and then millet bran (1.24 CFU /ml) in descending order (Table 3). The maximum rate of reduction in the first week of the refrigeration storage was in in fermented goat milk supplemented with millet bran (1.48CFU /ml); while the lowest was in in fermented goat milk supplemented with barely bran (0.76 CFU /ml). Nevertheless, the trend of reduction based on fiber source differed in the second week. It revealed highest strain *B. longum BB536* reduction in goat milk supplemented with sorghum bran (1.57CFU /ml), but the lowest reduction was in goat milk supplemented with millet bran (0.44CFU /ml) as

presented in Table 3. Therefore, the trend of strain BB 536 reduction throughout the refrigeration period in fermented goat milk supplemented with different fiber was dependent mainly on its source type. For instant, the highest total reduction was in fermented goat milk supplemented with barley bran; whereas, the maximum reductions in the first and second week refrigeration were in fermented goat milk supplemented with millet bran and sorghum bran, respectively. Hopefully, the final viable count of strain *B. longum BB536* in fermented goat milk after two weeks refrigeration storage was above the minimum number required to presence in probiotic to exert health benefits upon consumption, which was at least 6 log CFU/ml. Nevertheless, Kabeir *et al.* (2005) reported that survivability of *B. longum BB536* under refrigeration storage of fermented Sudanese Medida (Sudanes cereal thin porridge) beverages was not affected for a period of 2 week. While Akalin *et al*. (2004) noted a significant reduction on B.longum BB46 in yogurt after 1 week refrigeration. Lactate and acetate accumulation caused limitation on growth and survival of *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Bifidobacterium longum* cultivated in milk (Desjardins *et al*., 1990). This indicates that the viability of Bifidobacterium in fermented products was dependent on the carrier type and pH of the fermented products during the storage. Overall, most strains of Bifidobacterium are sensitive to pH values below 4.6. Therefore, for practical application, a pH value of the final product must be maintained above 4.6 to prevent the decline of Bifidobacterium populations (Tamime and Robinson, 1985; Modler *et al*., 1990; Laroia and Martin, 1991). The variances in survival were interpreted by the metabolic activity of *Bifidobacterium* in different fermented products; which might be affected by

the composition and availability of nitrogen and carbon sources in growth media as stated by Chou and Hou (2000).

Table 16: The viable counts of *Bifidobacteriumlongum BB536***log (CFU/ ml) during refrigeration storage of fermented goat milk supplemented with inulin and different cereal brans***

Type of supplementation	Storage period (weeks)		
	O (Initial)	One	Two
Inulin	7.83 ±0.16[a]	6.98 ± 1.54	6.02 ± 0.08
Sorghum bran	8.43 ±0.03[a]	7.03 ± 0.01[b]	6.60 ± 0.49[c]
Barley bran	7.25 ±0.06[a]	6.49 ± 0.64[b]	6.01 ± 0.04[c]
Millet bran	7.96 ±0.02[a]	6.48 ± 0.68[b]	6.04 ± 0.15[b]

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

4.16. Reducing sugars during refrigeration storage of fermented goat milk supplemented with inulin and different brans

Reducing sugars levels of the different fermented goat milks supplemented with inulin and different cereal brans (Sorghum, Barely and Millet) during refrigeration storage was presented in Table 17. The reductions in sugar were significant ($p<0.05$) in all fermented goat milk products. The amount of sugar decreases in the first week of refrigeration storage were 0.73,0.50,0.29 and 0.03% in fermented goat milk supplemented with barely bran, sorghum bran, millet bran and inulin, respectively. On the contrary, reductions in the second week were not following similar trend of the first recorded values of 0.28, 0.21, 0.19, and 0.11% in fermented goat milk supplemented with inulin, sorghum bran, millet bran and barely bran, respectively. These rates of sugar reduction were well correlated with the values of pH present in table 18.

Table 17: Reducing sugar during refrigeration storage of fermented goat milk supplemented with inulin and different cereal brans*

Type of supplementation	Storage period (weeks)		
	O (Initial)	One	Two
Inulin	1.35 ± 0.01^{a}	1.32 ± 0.01^{a}	1.04 ± 0.00^{b}
Sorghum bran	1.30 ± 0.01^{a}	0.80 ± 0.00^{b}	0.59 ± 0.00^{c}
Barley bran	1.31 ± 0.00^{a}	0.59 ± 0.01^{b}	0.48 ± 0.01^{b}
Millet bran	0.87 ± 0.00^{a}	0.59 ± 0.01^{b}	0.40 ± 0.00^{b}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

4.17. Reduction of pH during refrigeration storage of fermented goat milk supplemented with inulin and different brans

Table 18 shows, the pH measurement of different fermented goat milks supplemented with inulin and cereal brans (Sorghum, Barely and Millet) during refrigeration storage. There was significant ($p<0.05$) reduction in pH due to supplementation with inulin and different cereal brans at two weeks of refrigeration (Table 18). The highest pH reduction in the first week was in fermented goat milk supplemented with sorghum bran (1.39), while the lowest reduction was in that supplemented with barely bran (0.09). While the reductions recorded in the second week of refrigeration were 0.98, 0.62, 0.38 and 0.13 in fermented goat milk supplemented with inulin, barely bran, sorghum bran and millet bran, respectively. In fact, reduction of pH is mainly due to the fermentation of sugars (Table 17) and accumulation of acid shown in Table 8. The created condition maintained a relatively acid pH even in large intestine, thus preventing the proliferation of pathogens causing unfavorable disorders. Nevertheless, it was reported that low pH and storage temperature are the most important determinations in *Bifidobacterium* mortality during storage (Sakai *et al.,* 1987; Shah (1995, 2000) also found similar decreases in pH values during storage of commercial yoghurts containing *L. acidophilus* and *B. bifidum.*

Table 18: pH during refrigeration storage of fermented goat milk supplemented with inulin and different cereal brans*

Type of supplementation	Storage period (weeks)		
	O (Initial)	One	Two
Inulin	5.11 ± 0.00^{a}	4.78 ± 0.01^{a}	3.81 ± 0.01^{b}
Sorghum bran	5.65 ± 0.01^{a}	4.27 ± 0.01^{b}	3.88 ± 0.01^{c}
Barley bran	4.40 ± 0.01^{a}	4.31 ± 0.01^{a}	3.69 ± 0.00^{b}
Millet bran	4.45 ± 0.03^{c}	3.97 ± 0.02^{b}	3.84 ± 0.01^{ab}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same row are significantly different at $p<0.05$.

4.18. Changes in TSS during the storage of fermented goat milk supplemented with inulin and different cereal brans *

The effect of inulin and cereal bran (Sorghum, Barely and Millet) supplemented in fermented goat milk on TSS during refrigeration was obvious in Table 19. There were significant ($p<0.05$) increases in TSS of all types of fermented samples under refrigerated storage in the first week, then decreased in the second week; except for product supplemented with sorghum bran (Table 19) . The amount of increases in the first week of refrigerated storage were 0.53, 0.23, and 0.0.02% in fermented goat milk with barely bran, millet bran and inulin, respectively. The increases could be attributed to the breakdown of macro-components to simple soluble ones. While in the second weeks they decreased (1.58, 1.82, and 1.64%) in fermented goat milk supplemented with barely bran, millet bran and inulin, respectively. The decreases might be due to slight fermentation of soluble sugars by strain BB536 together with the increases in moisture as a dilution factor (Table 20).

4.19. Changes in moisture content during the storage of fermented goat milk supplemented with inulin and different cereal brans *

By extend storage period moisture content of fermented goat milk supplemented with inulin and different cereal bran (Sorghum, Barely and Millet) was slightly increased (Table 20); due to the reduction in TSS by strain BB536 activity (Table 19). The slight increase in moisture might indicate slow enzymatic activity that break down the macro component into simple and releases of some water. Thus, over all levels of moisture after two weeks refrigeration storage of fermented samples increased as compared to their initial levels at the beginning of the storage (Table 20). This increase in moisture might indicate high enzymatic activity that break down the macro component into simple and to the release of water.

Table 19: TSS during refrigeration storage of fermented goat milk supplemented with inulin and different cereal brans *

Type of supplementation	Storage period (weeks)		
	O (Initial)	One	Two
Inulin	13.23± 0.04[a]	13.25 ± 0.01[a]	11.61± 0.01[b]
Sorghum bran	11.70 ± 0.01[a]	10.33 ± 0.02[b]	11.94 ± 0.02[a]
Barley bran	13.56 ± 0.01[a]	14.09 ± 0.01[a]	12.51 ± 0.01[b]
Millet bran	14.21± 0.01[a]	14.44 ± 0.01[a]	12.62 ± 0.22[b]

*Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

***Initial storage = 12h.

Table 20: Moisture during refrigeration storage of fermented goat milk supplemented with inulin and different cereal brans*

Type of supplementation	Storage period (weeks)		
	O (Initial)	One	Two
Inulin	86.77± 0.04[b]	87.75 ± 0.01[a]	88.40 ± 0.07[a]
Sorghum bran	88.50 ± 0.28[a]	88.68 ± 0.02[a]	89.07 ± 0.02[a]
Barley bran	86.45 ± 0.71[a]	86.92 ± 0.07[a]	87.50± 0.01[a]
Millet bran	85.79 ± 0.01[b]	86.57 ± 0.07[a]	87.24 ± 0.00[a]

*Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

4.20. Changes in titratable acidity during the storage of fermented goat milk supplemented with inulin and different bran*

Table 8 shows the titratable acidity of different fermented goat milk supplemented with inulin and different cereal bran (Sorghum, Barely and Millet). Titratable acidity of the different fermented samples significant ($p<0.05$) increased by extended storage period for the two weeks correlating well with the reduction in pH (Table 18) .The rates of titratable acidity increases in the first week refrigeration were 0.37, 0.14, 0.04 and 0.02% in fermented goat milk supplemented with sorghum bran, barely bran, inulin and millet bran, respectively. In the second week the titratable acidity increases were even higher recording values of 0.88, 0.75, 0.28 and 0.25% in fermented goat milk supplemented with millet bran, barely bran, inulin and sorghum bran, respectively. Strain BB536 as well as other probiotic *Bifidobacterium* produces lactic acid, acetic acid, hydrogen peroxide, and bactericides are known to inhibit the development of pathogenic bacteria It was also reported that lactic acid and acetic acid in fermented dairy product have antibacterial effect (Bullen *et al*, 1976).

Table 21: Changes in titratable acidity during the storage of fermented goat milk supplemented with inulin and different bran *

Type of supplementation	Storage period (weeks)		
	O (Initial)	One	Two
Inulin	0.65 ± 0.00^{c}	0.69 ± 0.07^{b}	0.96 ± 0.01^{a}
Sorghum bran	0.44 ± 0.07^{c}	0.80 ± 0.00^{b}	1.06 ± 0.00^{a}
Barley bran	0.82 ± 0.00^{c}	0.96 ± 0.00^{b}	1.71 ± 0.07^{a}
Millet bran	0.93 ± 0.00^{d}	0.95 ± 0.00^{d}	1.82 ± 0.71^{a}

*Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw are significantly different at $p<0.05$.

4.21. Changes in minerals content during the storage of fermented goat milk supplemented with inulin and different brans*

Tables 22 and 23 showed significant ($p<0.05$) differences in mineral content in all fermented goat milk supplemented with inulin and different bran (Sorghum, Barely and Millet) except in Na. Ca increased in all fermented goat milk except the beverage supplemented with sorghum bran. Whereas, decreases in K, Mg and P were recorded in all fermented goat milk beverages supplemented with inulin and different cereal bran.

Table 22: Mineral contents of fermented goat milk supplemented with inulin and sorghum bran during refrigeration storage*

Mineral	Goat milk with inulin			Goat milk with sorghum bran		
	Initial storage***	After 1 week	After 2 week	Initial storage***	After 1 week	After 2 week
Ca	3.00±0.00^{c}	3.55±0.08^{b}	3.60±0.00^{b}	3.00±0.00^{c}	2.40±0.00^{b}	1.83±0.05^{d}
K	3.08±0.02^{b}	3.07±0.01^{e}	3.07±0.00^{c}	3.85±0.01^{b}	2.04±0.02^{c}	2.55±0.017^{c}
Mg	2.59±0.01^{d}	3.20±0.00^{c}	0.41±0.017^{e}	2.00±0.00^{d}	1.60±0.00^{d}	4.00±0.00^{b}
Na	6.63±0.01^{a}	6.13±0.00^{a}	7.83±0.022^{a}	6.94±0.01^{a}	4.34±0.00^{a}	6.96±0.01^{a}
P	6.97±0.02^{e}	0.98±0.00^{e}	0.49±0.00^{d}	0.82±0.00^{e}	0.55±0.02^{e}	0.60±0.00^{e}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw of each specific products are significantly different at $p<0.05$.

***Initial storage = 12h.

Table 23: Mineral contents of the fermented goat milk supplemented with barley and millet brans during refrigeration storage*

Mineral	Goat milk with barley bran			Goat milk with millet bran		
	Initial storage***	After 1 week	After 2 week	Initial storage***	After 1 week	After 2 week
Ca	2.05 ± 0.07^{d}	2.41 ± 0.01^{c}	3.00 ± 0.00^{b}	1.82 ± 0.02^{d}	2.00 ± 0.00^{c}	2.00 ± 0.00^{c}
K	3.56 ± 0.03^{b}	2.56 ± 0.00^{b}	2.57 ± 0.01^{c}	2.56 ± 0.01^{b}	3.07 ± 0.00^{b}	2.56 ± 0.01^{b}
Mg	3.15 ± 0.21^{c}	1.22 ± 0.02^{d}	0.40 ± 0.00^{e}	2.15 ± 0.07^{c}	2.00 ± 0.00^{c}	1.21 ± 0.01^{d}
Na	7.84 ± 0.02^{a}	4.34 ± 0.00^{a}	6.08 ± 0.00^{a}	7.82 ± 0.00^{a}	7.84 ± 0.02^{a}	5.21 ± 0.00^{a}
P	0.81 ± 0.01^{e}	0.40 ± 0.00^{e}	0.53 ± 0.02^{d}	0.62 ± 0.02^{e}	0.81 ± 0.01^{d}	0.51 ± 0.01^{e}

* Values are mean ± SD for replicate independent runs.

**Values that bear different superscript letter in the same raw of each specific products are significantly different at $p<0.05$.

***Initial storage = 12h.

4.22. Effect of oral feeding with strain BB536 of fermented goat milk supplemented with different prebiotics on body weights of rat

Table 24 presented the weight of rats during the experimental period of 30 days. The initial weight was measured before starting treatment and there was significant difference between the five groups of rats. The average of initial body weight recorded a range of 95.00-113.00g for groups. The body weight increased gradually in all groups throughout the study period, recording values of 167.67g in the control group157.67g in (inulin), and 168.17g for (sorghum bran), 162.00g for (barely bran) and 181.67g for (millet bran) group. Rats fed formulated fermented goat milk supplemented with millet bran gained the highest weights as compared to other groups. This increased weight might be caused by the high antioxidant properties of millet bran. Generally, all groups of rats have showed an equivalent growth pattern with variances in weight gain at the end of experimental treatment period. There is significant ($p< 0.05$) difference between different groups of rats in weight at initial but not at final (Table 24).

Group fed with goat milk supplemented with millet bran recorded the highest weight gain, while inulin supplemented resulted in the lowest weight gain. In fact as in (Table 24) all groups resaved cereal bran supplemented goat milk show high weight gain than the inulin clouding the control (received sterile water). Therefore, the variation in weight gain of groups of rat in this study is types of supplement dependant and that inulin was the best control weight.

Therefore, these results point toward the safe profiles of goat milk supplemented with different bran of cereal fed to rats. Generally in animal

model studies, loss of weight is sign of negative health effects. The losses may take place as a result of toxicity or illnesses (Abdo *et al.*, 2001).

Table (24): Weight (g) of different groups of rats orally fed with goat milk supplemented with different prebiotics for 30 days

Weight	Rats groups orally feed goat milk supplemented with different prebiotics				
	Control	Inulin	Sorghum bran	Barely bran	Millet bran
Initial body weight(g)	95.00± 3.61 [b]	113.00±7.94 [a]	103.67±4.04[ab]	94.00± 4.58 [b]	105.00±10.44[ab]
Final body weight(g)	167.67±12.50[a]	157.67±11.02[a]	166.33±10.02 [a]	162.00±12.53[a]	181.67± 10.26 [a]
Weight gained	72.67± 8.96[a]	44.67±12.66 [a]	59.33±3.79 [a]	68.00±15.72 [a]	76.67± 16.80 [a]

Values are means ±SD (n=6); means with different superscripts in the same row are significantly different ($p< 0.05$).

All groups feed on normal diets orally feed goat milk supplemented with different prebiotics

Control group: orally fed sterile water

4.23. Feed intake and water consumption of rats orally fed with different treatments

The results of average feed and water consumption in control and experimental groups of rats during the 30 days of study period are showed in Table (25). The average feed intake of rat groups (control, inulin, sorghum, barely and millet bran) ranged between 91.33 and 100.33 g at the end of the first week. Also, the results in Table (25) presented that there were significant differences ($p< 0.05$) between five different rats groups in feed consumption. The lowest feed consumption was of the barely bran group similarly, Al-rewashdeh (2009) reported that rat groups fed barley containing diets had lower daily food intake than those fed with the control diet. Moreover, the highest feed consumption was recorded for group the orally fed formulate fermented goat milk with inulin. This group consumed the highest feed, but recorded the lowest weight gain (table 25). This lower fed intake was reflected by a significantly lower mean body weight gain. modulation of gut microbiota by probiotic treatment or dietary intervention lead to beneficial effects on body weight, influencing on glucose and fat metabolism, improving insulin sensitivity and reducing chronic systemic inflammation (Tennyson and Friedman, 2008). Water is significant to the health and makes up approximately two-thirds of the body by weight. In order to maintain physical equilibrium in the body, it is necessary to coordinate and integrated link between the various organs. Control group consumed the highest water followed by sorghum bran supplemented groups finally inulin supplemented one. Al-Rewashdeh (2009) found that rats showed to eat less after feeding high satiety diets (fiber containing diets) than after feeding low satiety diet (fiber free

diet).Therefore consumption vary depending on types and levels of fibers in supplemented feeds. Dietary fiber has a high water holding capacity (Eastwood, *et al*. ,1973) and it is likely that rats fed fibers, in spite of the lower energy in their diet compared to those fed fiber- free diet, did not eat more because the fiber is swelling in their intestine and gives satiety sense. As a result rats fed fiber diets utilized more fats from their food, while the control rats deposited it in their tissues.

Table (25): Feed intake and water consumption of rats orally fed with different prebiotics

Parameter	Rats groups orally feed goat milk supplemented with different prebiotics				
	control	Inulin	Sorghum bran	Barely bran	Millet bran
Feed Intake(g/day	91.33± 3.06^{ab}	100.33±2.52^{a}	85.67± 6.03^{b}	82.67± 5.51^{b}	93.00± 4.58^{ab}
Water consumption(ml/d)	192.00± 2.00^{a}	164.33±5.03^{b}	181.00±15.72^{ab}	185.33±10.26^{ab}	175.33±3.21^{ab}

Values are means ±SD (n=6); means with different superscripts in the same row are significantly different ($p<0.05$).

4.24. Haematological parameters of rats orally fed with BB536 fermented goat milk supplemented with different prebiotics

Haematological parameters of the rats fed with fermented goat milk products were shown in Table (26). There was no indication of any health problems or serious illness symptoms on rats group fed with the fermented product. In addition no signs of any deficiencies in CBC parameters were recorded. Additionally there were considerable signs of positive health effect on red blood cells (RBC), haemoglobin (HGB) when compared with the control rat group. Red blood cells (RBC), 14.85g/dl, was the highest in rat groups fed with fermented goat milk supplemented with inulin followed by group fed fermented goat milk supplemented with sorghum bran (14.65g/dl). HCT, ,MCH/pg, PLT levels, MPV, PCT, Monocyte, Eosinophil and Bosinoph of rat groups were within the standard range and were not significant ($p < 0.05$) between all groups of rats.While there were significant differences ($P<0.05$) in MCV, RDW-SD%, and Lymph of treated rats groups. Neutrophil and Eosinophil count decreased in all groups compared with control.Variation in CBC parameters may be associated with each individual rat adaptability to environmental conditions as stated by Koubkova *et al*. (2002). *Bifidobacteriumlongum BB536* oral feeding did not produce any abnormal change effect on haematological parameters. Therefore, increased of lymphocytes count (lymphocytosis) is an indication that fermented oral feeding led to improved immune system. While the reduced monocytes suggest absence of contamination. In general there are good indications of inflammation and toxicity absence in rat groups. This result is in agreement with previous reported by Yiming *et al*. (2012) on lymphocyte proliferation and immune modulators properties of the spices.

Furthermore, all the changes that observed in hematological parameters were within normal standard ranges of rats.

Table 26: Hematology parameters of rats orally fed with goat milk supplemented with different prebiotics for 30 days

Parameter	Rats groups orally fed with goat milk supplemented with different prebiotics				
	control	inulin	Sorghum bran	Barely bran	Millet bran
WBCs /L	14.35±0.64 [a]	7.40± 3.68 [a]	8.80± 4.38 [a]	10.40± 5.52 [a]	9.00± 3.82 [a]
HGB g/dl	14.45±0.21 [a]	14.85± 0.21 [a]	14.65± 0.35 [a]	14.45± 1.77 [a]	14.55± 0.50 [a]
RBCs/L	8.70± 0.09 [a]	8.04± 0.10 [a]	8.09± 0.03 [a]	7.93± 0.76 [a]	8.52± 0.36 [a]
HCT %	44.40±0.28 [a]	46.05± 1.34 [a]	44.40± 1.70 [a]	44.00± 5.37 [a]	45.85± 0.64 [a]
MCV/fl	51.10±0.85 [b]	57.30± 0.99 [ab]	54.95± 1.91 [ab]	55.50± 1.56 [ab]	53.95± 3.04 [ab]
MCH/pg	16.60±0.42 [a]	18.40± 0.00 [a]	18.05± 0.35 [a]	18.15± 0.50 [a]	17.05± 1.34 [a]
MCHC/g/dl	32.50±0.28 [a]	32.20± 0.42 [a]	32.95± 0.50 [a]	32.80± 0.00 [a]	31.65± 0.64 [a]
RWD Cv/%	17.10±0.28 [a]	16.05± 0.50 [ab]	14.40± 1.27 [b]	15.30± 0.42 [ab]	16.00± 0.57 [ab]
RDW-SD/%	30.95±0.64 [ab]	33.10± 0.00 [a]	30.50± 1.27 [b]	30.95± 0.64 [ab]	31.40± 0.00 [ab]

PLT/L	666.0±35.4 [a]	639.0± 283 [a]	676.0± 114.6 [a]	755.0± 45.3 [a]	675.5± 17.7 [a]
MPV/fl	6.15±0.07 [a]	7.05± 0.35 [a]	6.35± 0.50 [a]	6.35± 0.07 [a]	6.35± 0.21 [a]
PDW/fl	14.80± 0.14 [a]	15.75± 0.07 [a]	15.70± 0.57 [a]	15.50± 0.28 [a]	15.15± 0.64 [a]
PCT%	0.41± 0.02 [a]	0.46± 0.22 [a]	0.43± 0.11 [a]	0.48± 0.02 [a]	0.43± 0.03 [a]
Neutr%	55.50± 10.61 [a]	33.50± 6.36 [a]	45.0± 15.6 [a]	40.00± 0.00 [a]	52.50± 0.71 [a]
Lymph%	30.50± 0.71 [b]	62.00± 5.66 [a]	50.00± 14.10 [ab]	53.00± 4.24 [ab]	40.00± 2.83 [ab]
Monocy%	3.50± 3.54 [a]	2.00± 0.00 [a]	1.00± 0.00 [a]	2.50± 0.71 [a]	0.50± 0.71 [a]
Eosinoph%	9.50± 6.36 [a]	2.50± 0.71 [a]	3.50± 2.12 [a]	4.00± 2.83 [a]	5.50± 0.71 [a]
Bosinoph%	1.00± 1.41 [a]	0.00± 0.00 [a]	0.50± 0.71 [a]	0.50± 0.71 [a]	1.50± 0.71 [a]

Values are means ±SD (n=6); means with different superscripts in the same row are significantly different ($p < 0.05$).

All groups feed on normal diets feed and oral of 2.5 ml of different diets treatment

Control group: orally fed sterile water

4.25. Blood biochemistry parameters of rats orally fed with goat milk supplemented with different prebiotics

The result of feeding different fermented gat milk supplemented with different prebitics on blood biochemistry parameters of rats is presented in Table (28). The glucose levels of rats orally fed the formulated fermented products were lower than that of the control rats without fermented product except group (millet bran). It might be due to the different bran contents of fiber which has various physiological effects on regulating blood glucose level and insulin response among diabetics. It was reported by Behall et *al.* (2005) that oat and barley can reduce glycemic responses, decrease glucose level and insulin responses as a result of the high soluble fiber content which considered the major factor. Total protein level was higher in (sorghum bran and millet bran) followed by barely bran, inulin and then control rat groups. The concentration of albumin and globulin were significantly ($p<0.05$) different between rats groups. Electrolytes play basic role in keeping body fluid pH and the dynamic balance of water (Radostits *et al.*, 2007). Sodium, potassium, calcium, phosphate concentrations in the blood of treated rats groups were significantly ($P<0.05$) different. Also, creatinine, uric acid and blood urea were significant ($p<0.05$) between all groups of treated rats. The creatinine, potassium ion and sodium high level in blood indicated kidney damage. In this study, serum electrolytes in all treated rats group were significantly ($P< 0.05$) differences as compared with the control group. Nevertheless, all values were within the normal ranges of rats. Estimation of liver functions is very important in toxicity assessment because organs are necessary for the endurance and welfare of an organism. Referring to Table (26), AST, ALT and D. Billiand ALP showed significant ($P< 0.05$)

difference as compared with the control. However, any decreasing in the level of serum ALT, AST, and ALP activity are indication of hepatic disease (Crawford and Henry, 2003). In this study all levels of blood enzymes were within the normal standard range of rats. All groups orally treated with fermented products showed best serum activity, due to their high phenolic compounds and beta glucan content. The results of the biological assay in Table (28) show the changes in plasma HDL, LDL, cholesterol and triglyceride levels at the end of experiment. HDL and LDL (A, B, C and D) in orally fed rat reduced serum triglyceride, total cholesterol, as compared with the rat control group. The rat groups fed the different fermented products recorded the lowest level in cholesterol, and triglyceride as compared with the control group. These findings were dependable with those of several studies reported such as, Jue *et al.*(2004), who reported that the barley diet significantly decreased total cholesterol, triacylglycerol, and LDL-cholesterol. However, Sindhu and Khetarpaul, (2003) found that the rats group fed by probiotic fermented diet resulted significant decreases in total serum cholesterol and triglycerides, whereas, HDL cholesterol increased compared to control group diets. On the other hand, Abd El-Gawad *et al.* (2005) reported that consumption of *bifidobacterium* fermented soymilk significantly reduced plasma cholesterol VLDL+LDL-cholesterol and increased the HDL-cholesterol level in animals fed cholesterol-enriched diet. Several mechanisms are suggested for cholesterol reducing activity of probiotics. One of them is deconjugating bile acids through bile salt hydrolase catalysis. Since, cholesterol is the precursor for the synthesis of new bile acids the use of cholesterol, to synthesize new bile would lead to decreasing concentration of cholesterol (Lye and Looi 2010).

Table 28: Biochemistry parameters of rats orally with fermented goat milk supplemented with different prebiotics for ±30 days

Parameter	Rats groups orally with fermented goat milk supplemented with different prebiotics				
	control	inulin	Sorghum bran	Barely bran	Millet bran
Glucose(mg/dl)	84.30± 0.42 b	72.05± 0.07 d	67.00± 0.00 e	82.25± 0.21 c	90.05± 0.07 a
T. protein(g/dl)	5.89± 0.02 c	5.81± 0.01 c	6.40± 0.14 a	6.05± 0.07 bc	6.30± 0.00 ab
Albumin. (g/dl)	3.11± 0.01 b	3.05± 0.07 b	3.10± 0.00 b	3.05± 0.07 b	3.61± 0.01 a
Globulin.(g/dl)	2.80± 0.00 c	2.82± 0.03 c	3.21± 0.01 ab	3.05± 0.07 b	2.72± 0.01 c
Na(mmol /L)	119.50±0.71 c	120.50 ± 0.71 bc	117.50± 0.71 cd	123.00± 1.41 b	126.50±0.71 a
K/mmol/l	3.63± 0.03 d	4.05± 0.07 bc	3.82± 0.02 cd	4.44± 0.02 a	4.10± 0.14 b

Ca/mmol/l	6.00± 0.00 abc	6.50± 0.71 abc	7.50± 0.71 ab	8.50± 0.71 a	4.50± 0.71 c
phos/mmol/l	3.31±0.01 a	3.61± 0.01 a	3.81± 0.01 a	4.12± 0.01 a	3.45± 0.64 a
blood urea/mg/	37.00± 1.41 d	40.00± 0.00 cd	49.50± 0.71 b	65.00± 1.41 a	41.50± 0.71 c
creat/mg/dl	1.05± 0.07 bc	1.10± 0.14 b	0.81± 0.01 c	1.12± 0.01 b	2.22± 0.02 a
uric acid/mg/dl	1.43± 0.04 e	3.05± 0.07 a	2.31± 0.01 c	2.80± 0.00 b	2.05± 0.07 d
D.Billi/mg/dl	0.23± 0.03 e	0.33± 0.02 d	0.42± 0.03 c	0.61± 0.00 b	0.86± 0.01 a
AST/U/L	6.00± 0.00 b	10.50± 0.71 a	7.00± 1.41 b	7.00±0.00 b	5.50±0.71 b
ALT/U/L	0.51± 0.01 c	2.05± 0.07 a	1.94± 0.06 a	1.61± 0.01 b	1.66±0.01 b
ALP/U/L	28.00± 0.00 a	22.50± 0.71 c	27.00± 0.00 b	27.00± 1.41 b	12.50±0.71 d
HDL/mg/dl	36.00± 1.41 b	28.50± 0.71 c	41.50± 2.12 a	40.50± 0.71 ab	29.00±1.41 c
LDL/L/mg/dl	10.50± 0.71 c	19.00± 0.00 a	12.50± 0.71 bc	16.00± 1.41 ab	13.00±0.00 b c
Cholesterol /mg/dl	72.20± 0.28 d	61.45± 0.64 a	62.05± 0.07 a	58.00± 0.00 b	51.15±0.21 c

Triglyceride/ml	99.00± 1.41 [d]	151.50 ± 0.71 [a]	143.00± 1.41 [b]	138.00± 0.00 [c]	93.00±0.00 [e]
VLDL	24.97±0.042 [a]	12.20± 0.28 [e]	21.15± 0.07 [b]	15.99± 0.01 [d]	19.05±0.07 [c]

Values are means ±SD (n=6); means with different superscripts in the same row are significantly different ($p<0.05$).

4.26. Different microbial groups in colon of rats orally fed with fermented goat milk supplemented with different prebiotics

Based on feeding trials, changes in colonic microbial groups of the rats are ensured. Different microbial groups in colon of rats as presented in Table (28).

Total aerobes in the large bowel changed little due to the ingestion of different products compared with the control.

Total aerobes of colon significant different increased in all groups of rats received the different products as compared to the control. Group (sorghum bran) showed the highest increase in total aerobes in colon (7.92log CFU/ g). However, (inulin) group showed lower count of 7.72log CFU/ g that might be due to activity of the viable fiber supplements. In general, total anaerobes were also higher for rats fed with fermented goat milk supplemented with sorghum bran compared with the other groups and control. Although prebiotics offer one rational approach to the probiotic concept, the health consequences have been defined. In hypothesis, a number of potential benefits may arise (Gibson, 1999). However, it might enhance resistance to pathogens possibil. The bifidobacteruim and lactic acid bacteria of the gastrointestinal tract are thought to play a important role in enhanced colonization resistance.

Feeding with fermented different products promoted the highest lactobacillus increase in colon compared with control group. Bifidobacteria displayed the highest increased among all microbiota communities in colon as fine. However, the increases were significant (P <0.05) in all groups fed different fermented prouducts with prebiotics Table (28) showed the highest bifidobacteria increases were in group inulin (7.80) followed sorghum bran (7.77) ,barely bran (7.39)and millet bran (7.17 CFU/g). Increased bifidobacterial numbers in the contributes towards enhanced competitive exclusion of pathogens. (Shiba *et al*., 2003). Lactobacillus and Bifidobacterium are non-pathogenic bacteria of the bowel tract. These micro-organisms increases resistance to diseases by reducing the growth of pathogenic and putrefactive bacteria via production of inhibitory substances, competing directly for substrates and mucosal attachment sites (Collado, *et al*., 2009).

The harmful pathogens of gut microbiota contain species of Coliform Salmonella and Staphylococcus. In Table (28) Coliform and Salmonella groups showed significantly (p<0.05) lower level in all groups treated with prebiotics as compared with control. The results also showed that different prebiotics might vary in the effected on the growth of the harmful pathogens. Schoster *et al.*, (2013) reported that probiotics effectively inhibited the development of pathogenic bacteria, such as *Clostridium perfringens*, *Campylobacter jejuni*, *Salmonella Enteritidis*, *Escherichia coli*, various species of *Shigella*, *Staphylococcus*, and *Yersinia*, so prevent us from food poisiong .

Both prebiotics sorghum bran and millet bran were effective against salmonella and did not show any viable count. In fact symbiotic effect of strain BB536 and prebiotic(cereal bran) are increase beneficial bacteria and decrease the pathogens in fed rats. However there are symbiotic effects dependent on composition and types of fiber used.

Table 28: Different microbial count in colon of rats orally fed with fermented goat milk supplemented with different prebiotics

Bacterial groups (log CFU/g)	Rats group orally fed of goat milk supplemented with different prebiotics				
	control	inulin	Sorghum bran	Barely bran	Millet bran
Total anaerobe	7.03 ± 0.71^{ab}	7.69 ± 0.29^{a}	7.88 ± 0.02^{a}	7.07 ± 0.04^{b}	6.93 ± 0.85^{b}
Total aerobe	7.05 ± 0.71^{ab}	7.72 ± 0.29^{a}	7.92 ± 0.02^{a}	7.07 ± 0.04^{b}	7.08 ± 0.20^{b}
Lactobacillus	6.14 ± 0.06^{c}	7.65 ± 0.11^{a}	7.64 ± 0.17^{a}	7.63 ± 0.13^{a}	6.93 ± 0.06^{b}
Bifidobacteria	7.09 ± 0.08^{a}	7.80 ± 0.11^{a}	7.77 ± 0.16^{a}	7.39 ± 0.50^{a}	7.17 ± 0.04^{a}
Coliform	4.22 ± 0.03^{a}	3.81 ± 0.05^{b}	3.80 ± 0.01^{b}	3.84 ± 0.03^{b}	3.41 ± 0.03^{c}
Salmonella	4.83 ± 0.05^{ab}	3.71 ± 0.03^{b}	0.00 ± 0.00^{c}	3.90 ± 0.12^{b}	0.00 ± 0.00^{c}
Staphylococcus	4.02 ± 0.06^{ab}	4.26 ± 0.48^{ab}	4.69 ± 0.15^{a}	4.42 ± 0.47^{ab}	3.71 ± 0.27^{b}

Values are means ±SD (n=6); means with different superscripts in the same row are significantly different ($p< 0.05$).

All groups feed on normal diets feed and oral of 2.5 ml of different diets treatment

Control group: orally fed sterile water

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

1. The chemical composition of different cereal brans revealed high levels of fiber, protein, fat that could contribute to improve the nutritional value of food.

2. Goat milk was nutritionally higher as compared to cow and camel milk. Cereal brans could produce complementary product provide both the nutritional and benefits to consumers.

3. Inulin was superior source of fiber, carbohydrate and minerals as compared to tested cereal brans. By fermentation and supplementation with inulin and different cereal brans, maximum growth of the strain BB 536 that fulfills the number required to presence in probiotic food was attained. Therefore, cereal bran could have prebiotic effect on strain BB 536 in goat milk medium for development of fermented functional food.

4. Oral feeding of goat milk supplemented with cereal bran to rats exerted a positive health effects i.e weight were not retarded and no signs of toxicity or pathogenicity were revealed on blood hematology and biochemistry analysis of fed rats.

5. Products effectively inhibited development of *Sallmonela* in colon via enhanced of beneficial *Bifidobacterium* and *lactobacillus* levels in colon of fed rats.

5.2. Recommendations

1. More research should be conducted on cereal bran effects on growth and survival of probiotic strains.
2. Improve the acceptability and sensory characteristics of different probiotic fermented products.
3. More research to be conducted on nutritional values and functional properties of the developed probiotic fermented product to explore it is health benefits.

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