

ASSESSMENT OF THE FUNCTIONAL QUALITY AND SAFETY OF YOGHURTS PRODUCED WITH STARTER CULTURES OBTAINED FROM SELECTED COMMERCIALY SOLD YOGHURTS

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ABSTRACT

The study focused on the examination of laboratory prepared yoghurts which were fermented with selected starter cultures from commercially sold yoghurt. The starter cultures were molecularly identified (16s rRNA) as *Enterococcus lactis*, *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Pediococcus pentosaceus* and *Enterococcus durans*. The isolates were examined for bile tolerance as an indicator of their ability to survive in the gut. The starter cultures were used to produce different yoghurts in the following order: *Enterococcus lactis* produced yoghurt, *L. plantarum* and *L. pentosus* produced yoghurt, *Pediococcus pentosaceus* produced yoghurt, *E. durans* produced yoghurt and yoghurt produced with all starter cultures. All yoghurts were examined for nutritional quality (vitamin A, B12 and C content, soluble and casein bound magnesium and calcium and proximate nutrient composition). At $p \leq 0.05$, there was statistical significant difference in the nutritional content with *P. pentosaceus* contained yoghurt, *E. durans* contained yoghurt and yoghurt produced with a combination of all isolates recording the highest nutritional values and the lowest was observed with the control. Safety tests such as haematology and histology were carried out on wistar rats. After 7 days of feeding the rats in groups with the different yoghurts and a control without yoghurt, there were marked improvements in the red blood cell counts, white blood cell counts but no significant difference in the differentials at $p \leq 0.05$. The isolates were also observed to have no disruptive effect on the morphology and structure of the small intestine. Overall, the use of these lactic acid bacteria strains showed immense benefits in their use as starter cultures and the study demonstrated safety of the final products for consumption.

Keywords: bile tolerance; nutritional quality; haematology; histology

INTRODUCTION

Yoghurt is a fermented milk product obtained from the milk or the milk products by the lactic acid fermentation through the action of *Streptococcus salivarius* subsp. *Thermophilus*, *Lactobacillus delbrueckii* subsp. *Bulgaricus* (FAO/WHO, 1977). When a sufficient quantity of lactic acid is produced then the milk coagulates and this coagulated milk is called yoghurt. The probiotic yoghurt, having probiotic effect is a fermented milk product with adjuvant microorganisms. There are numerous advantages of consuming fermented dairy products containing probiotic bacteria. A high population of probiotic organisms in the colon contributes to good intestinal health. Consequently, consumption of products such as yoghurt containing viable probiotic organisms adds benefit to human gut health. Moreover, yoghurt supplies good quality proteins, also an excellent source of calcium, phosphorus, potassium and contains significant quantities

of general vitamins. Yoghurt could be used for feeding, owing to its higher Ca/Na ratio (Demott, 1985).

Yoghurts vary in appearance, flavor and ingredients. The quality and composition of yoghurt of applied bacterial cultures affects the quality of the yoghurt obtained as the result of the milk fermentation processes. There is a symbiotic relationship between the two species of bacteria i.e. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* that is why there is more rapid acid development than in the single strain culture (Rasic et al., 1978; Tamime et al., 1980).

Various combinations of starter cultures are selected during manufacturing of yoghurt to achieve desirable characteristics of product and also to provide the consumers with a wide choice of therapeutic benefits. Depending on its activity, manufacturer usually adds 2 – 4% yoghurt starter culture. These days, there has been increasing trends to fortify the dairy product with fruits (natural fruit juice, pulp, dry fruits) (Desai et al., 1994;

Ghadge et al., 2008). Aesthetic value of new product can be increased by using fruit juice as a functional pigment in fermented milks with array of colors and flavor properties. Coissonet al., (2005) used *Euterpeoleracea* juice as functional pigment for yoghurt, which is dark purple in color having high anthocyanin and phenolic content.

Yoghurt is a functional food. The functional food includes probiotics, prebiotics and synbiotics. Probiotics can be defined as "live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance" (Champagne et al., 2005).

Prebiotics is defined as "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". Symbiotic is a combination of probiotics and prebiotics that "beneficially affects the host by improving the survival and the implantation of live microbial dietary supplements in the gastro-intestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria" (Di Rienzo et al., 2000).

Yoghurt is one of the oldest fermented milk products known. Fermentation of milk involves the action of microorganisms, principally the lactic acid bacteria. These microorganisms sour the milk by converting the milk sugar lactose to lactic acid (Kagan, 1985). Yogurt gels are built of clusters of aggregated casein particles formed as a result of gradual fermentation of lactose by lactic acid bacteria (Horne, 1993). The Food and Drug Administration (FDA, 2008) standard of identity for yogurt drinks specifies >8.25% milk solids-not-fat and fat levels to satisfy nonfat yogurt (<0.5%), low-fat yogurt (2%), or yogurt (>3.25%) before the addition of other ingredients (Chandan et al., 2006). Yoghurt is among the most common dairy products consumed around the world (Saint-Eve et al., 2006).

As the popularity of yogurt products continues to grow, manufacturers are continuously investigating value-added ingredients to entice health-conscious consumers (Allgeyer et al., 2010).

Scientific hypothesis

The present study investigates the functional quality and safety of yoghurts produced from starter cultures obtained from randomly sampled commercial yoghurts and conducted with the following objectives:

1. Molecular identification of starter cultures Yoghurt production with identified starter cultures
2. Evaluation of physicochemical and nutritional properties that contribute to final product quality such as pH, titratable acidity, reducing sugar concentration, vitamin content, mineral composition and proximate nutrient composition
3. Assessment of probiotic potential of the yoghurts with antibacterial assay and safety to the intestine

MATERIAL AND METHODS

The media that were used in this work include nutrient agar, Macconkey agar, Eosinmethyleneblue agar (EMB), peptone water. The composition of the media, their method

of production and the list of other equipment and reagents used in this work are presented in the appendix.

Sample Collection

Six different brands of bottle packaged yoghurt were brought from hawkers and beverage stores in Ikeji Arakeji, Osun State. Isolates from each brand was used and the brands were designated A, B, C, D and E. The samples were brought to the laboratory, stored in the refrigerator and analyzed within 6 hours of collection.

Analysis of Sample

Each sample was serially diluted using sterile distilled water as diluents according to (Prescott et al., 2002) and 1 milliliter of 10^{-3} sample was plated in duplicate using the pour plate method on nutrient agar media. The plates were incubated at 37 °C for 24hrs. After incubation the colonies developed on the nutrient agar plates were counted and used to determine the total bacterial count of the yoghurt samples (CFU.ml⁻¹). The representative colonies on the plates were sub-cultured on fresh nutrient agar to obtain pure cultures of the isolates. The pure cultures were then transferred into nutrient agar slants for molecular characterization.

Identification of Isolates

Molecular identification of the lactic acid bacteria isolate involved Denaturing Gradient Gel Electrophoresis, Polymerase Chain reaction and pure sequencing of 16s rRNA (bacteria) genes as described by Akabanda et al. (2013) and Cocolin et al. (2000).

Inoculum Preparation

Pure cultures of the bacterial isolates were inoculated in sterilized lactose broth and incubated at 37 °C for 18 hours

Preparation of Yoghurt

One hundred milliliter of whole milk collected from lactating cow was poured into a 500 mL beaker; was brought to boiling point without being allowed to boil and immediately cooled to 60 °C. 3.5 grams of non fat dry milk was added to the milk and stirred vigorously with a glass rod to dissolve the powder. The mixture was allowed to cool to 45 °C. 10 milliliter of 18 hour culture of the lactic acid bacteria was added in single, double and allied fermentation trials. This was done separately for the purpose of comparison. The beakers were covered with aluminium foil and incubated for 6 hours at 45 °C until it becomes firm (Fassara, 2010).

Determination of Physicochemical parameter

The determination of physico-chemical parameters such as pH, total acidity, reducing sugar and optical density were determined according to the Association of Analytical Chemists, (2000).

Proximate Analysis and Analysis of Nutrient Composition

The proximate analysis and determination of nutrient composition of the prepared yoghurt were determined according to A.O.A.C (2000) and ASEAN (2011).

Determination of Calcium and Magnesium

The analytical method used for the analysis of heavy metal concentration was the Atomic Absorption Spectroscopy (AAS) using the calibration plot method. For each element, the instrument was auto-zeroed using the blank (distilled water) after which the standard was aspirated into the flame from the lowest to the highest concentration. The corresponding absorbance was obtained by the instrument and the graph of absorbance against concentration was plotted. The samples were analyzed in duplicates with the concentration of the metals present being displayed in parts per million (ppm) after extrapolation from the standard curve (Greenberg et al., 1985).

In-vitro antibacterial activity

The in-vitro antibacterial activity of the yoghurts was carried out by agar well diffusing assay with Mueller-Hinton agar (Lab M) (Clinical and Laboratory Standards 2013). The yoghurts were centrifuged and the supernatant was tested against *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 29929 and *Staphylococcus aureus* ATCC 29293.

Biological material

This was done by modifying the methodology of Hounkpatin et al. (2013). The animal material was composed of 14 male albino Wistar rats weighing about 250 grams. These rats were purchased from a local rat farmer in Ado-Ekiti, Nigeria and were acclimated for 3 days before the experiments. They were placed in designed sterile polypropylene cages at room temperature (25 to 30 °C). The cages were illuminated with a sequence of 12 h light and 12h darkness. The rats had free access to water and food.

Treatment of Wistar rats with the yoghurts

The wistar rats in duplicates were fed differently with the different yoghurts twice daily and supplemented with grains while the control was fed only with grains and water. The animals were fed for a period of 7 days after which they were sacrificed for haematological and histological assays.

Blood collection and haematological analysis

This was done by modifying the methodology of Hounkpatin et al. (2013).

After 7 days of treatment, rats were fasted overnight. They were weighed before the collection of blood and sacrifice. All samples were taken between 7 and 9 am to avoid variations due to circadian rhythm. Whole blood was obtained from a puncture of the retro-orbital sinus by the conventional method (Van Herckel et al., 1992). Blood samples collected in ethylene diamine tetra-acetic acid (EDTA) anticoagulant tubes (8.5%) was quickly returned by mixing with anticoagulant in the tube. All blood samples were labeled and immediately conveyed to the laboratory for analysis. Hematological parameters were analyzed: Packed Cell Volume (PCV), white blood cell count (WBC) and its differentials such as: Leukocyte, Eosinophil, Neutrophil, Basophils and Monocyte counts. All hematological parameters were analyzed in the "Haematology Unit, Federal Polytechnic Ado-Ekiti, Ekiti

State, Nigeria Medical Laboratory using the automated method with the automatic analyzer "Haematology auto analyzer Sysmex KX-21N".

Histology

The tissue were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The deparaffinised sections were stained routinely with haematoxylin and eosin. Photomicrographs of the desired results were obtained using digital research photographic microscope in the laboratory (Eweka and Om'Iniabohs, 2011).

Analysis of data

The results were presented as the mean standard values of three replicates. A one-way analysis of variance (ANOVA) was carried out using SPSS 16.0. Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

The commercial yoghurts used in the study are presented as a random sampling of five different brands. All of five samples were used as starter cultures for the production of yoghurts in the laboratory. An assessment of the physical properties of the commercial yoghurt showed that sample A and B were thick and sour, sample C was very thick and very sour while sample D and E were slightly thick and sour. Samples A, B and E showed smooth consistency while samples C and D were coarsely smooth. The isolates were identified through the characterization of the 16S rRNA sequence and were analysed with the Basic Local Alignment Search Tool for sequence alignment (Table 1) while figure 1.0 shows the phylogenetic relationship between the organisms. The lactic acid bacterial isolates used in the study were isolated from the commercial yoghurts and their cell morphology and Gram stain reaction were observed. All isolates were observed to be Gram positive short rods and coccobacilli. The bacterial isolates were tested for their ability to survive and grow on bile salts and tested positive. The isolates used for the laboratory production of yoghurt in this study are lactic acid bacteria which have been reported as probiotic in functional dairy. *Enterococci* are lactic acid bacteria in large numbers and are naturally present in vegetables, plant material and food stuff; especially those of animal origin such as dairy products (Girraffa, 2003); Katie and Carol, 2009). Because they produce bacteriocins, *Enterococcus* species have been used widely over the last decade in the food industry as probiotics or as starter cultures (Foulquie, et al., 2006). Studies on the microbiota of traditional cheeses of Mediterranean countries produced mainly from raw milk of sheep, goats and cows indicate that *Enterococci* are a relevant component of the natural cultures involved in fermentation and play important role in the final quality of the product. They are also used to extend product shelf life and improve the hygienic safety of foodstuffs because they produce antimicrobial substances such as lactic acid, hydrogen peroxide, and bacteriocins (enteroxins) (Franz et al., 2007).

Table 1 Molecularly identified Lactic Acid bacteria isolates.

ISOLATE/CODE	Name of Isolate	ACCESSION NUMBER
A1	<i>Enterococcus lactis</i>	LC318433.1
B1	<i>Lactobacillus planetarium</i>	MF942369.1
B2	<i>Lactobacillus Pentosus</i>	CP022130.1
C1	<i>Pediococcus pentosaceus</i>	MF942373.1
D1	<i>Pediococcus pentosaceus</i>	MF942373.1
D2	<i>Enterococcus durans</i>	MF628998.1
E1	<i>Enterococcus durans</i>	MF628998.1
E2	<i>Enterococcus durans</i>	MF583025.1

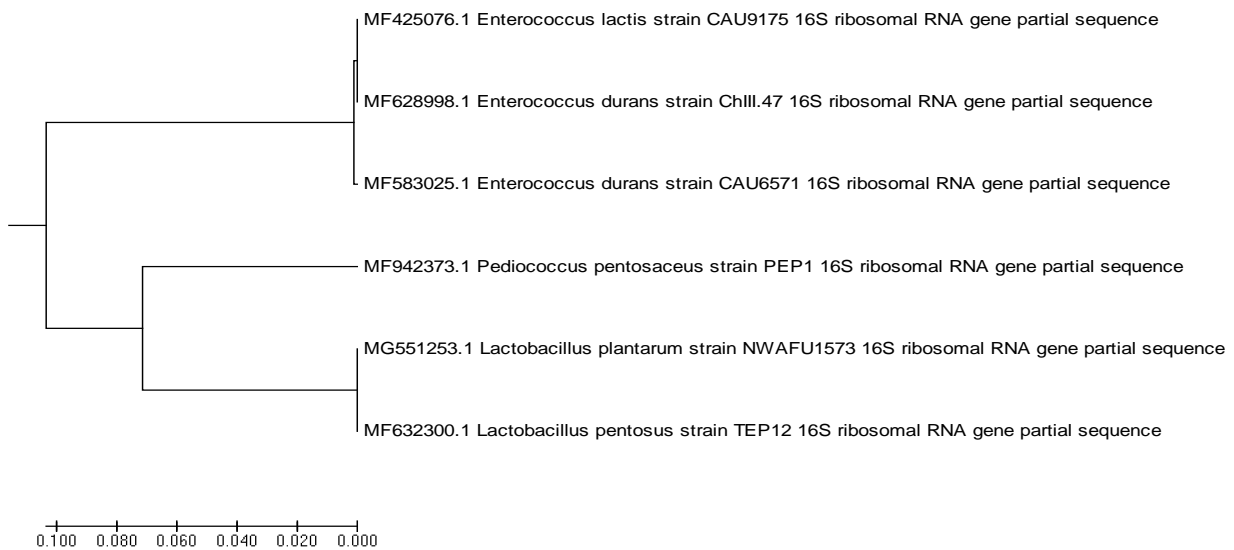


Figure 1 Phylogenetic tree of the lactic acid bacteria isolates.

According to **Franz et al. (2007)** and **Naoualet al. (2010)**, *Enterococcus durans* and *Enterococcus lactis* are reported as non pathogenic. **Fabianova, (2010)** reported the presence of members of the enterobacteriaceae in raw cow milk and their presence may be as a result of their presence in the raw milk used for the production of the yoghurts. **Kumar et al. (2016)** reported the use of *Lactobacillus plantarium* in yoghurt fermentation properties and was said to give excellent antimicrobial and final product quality. **Swain et al. (2014)** and **Lilis (2015)** described *Pediococcus pentosaceus* and *Pediococcus pentosaceus* to be of high probiotic quality and were isolated from Indonesian fermented foods in a bid to review health promoting lactic acid bacteria.

After 24 hours of incubation at 37 °C on MacConkey agar with bile salt, all isolates were observed to survive the growth condition. After 18 hours of incubation of the laboratory prepared yoghurts at 37 °C, physical parameters such as texture, taste, consistency and colour were determined while taste was determined as the only organoleptic property. Yoghurt produced with *Enterococcus lactis* (L.A) and yoghurt produced with *Lactobacillus plantarum* and *L. pentosus* (L.B) as starter cultures were thick and sour; yoghurt produced with *Pediococcus pentosaceus* (L.C) as starter culture was slightly thick and very sour while yoghurt produced with *Pediococcus pentosaceus* and *E. durans* as starter cultures (L.D) and yoghurt produced with *E. durans* as starter

culture (L.E) were slightly thick and sour. They all showed a smooth consistency with a creamy colouration. The pH of the yoghurts (Table 2) after 8 hours of fermentation was found to be within the range of 5.51 in *Pediococcus pentosaceus* produced yoghurt and 5.76 in *Enterococcus durans* produced yoghurt. This finding corresponds with the report of **Obi et al. (2016)** who reported pH 4 – 5.5 and 5.5 as optimum. The study is also in agreement with the report of **Charles et al. (2016)**, who reported titratable acidity between 0.02 – 0.06%. The control revealed the progress of fermentation and decrease in pH of the samples inoculated with the lactic acid bacteria as starter cultures. The study also revealed increasing optical density in all samples and decreasing trend in the reducing sugars as a result of the fermentative activities of the starter cultures and this is supported by the report of **Shao-Chi et al. (2007)**.

The vitamin content (A, B₁₂ and C) as determined in all the yoghurts had varying differences ($p \leq 0.05$) which was as a result of different starter cultures and combinations of the starter cultures (Table 3). The vitamin A and B₁₂ content of yoghurt produced with *Pediococcus pentosaceus*, *Enterococcus durans* and a combination of all the isolates resulted in higher vitamin A content when compared with others. The vitamin A and B₁₂ content recorded in this study is higher than the record found in the report of **Ihemeje et al. (2015)**.

Table 2 Physicochemical assessment of the laboratory prepared yoghurts.

Yoghurt code	Total			
	pH ±SD	titratable acidity (moles) ±SD	Optical density ±SD	Reducing sugar (mg/g) ±SD
L.A	5.52 ±0.005 ^b	0.050 ±0.000 ^b	1.32 ±0.005 ^b	15.1 ±0.294 ^a
L.B	5.67 ±0.005 ^d	0.053 ±0.000 ^c	1.69 ±0.005 ^f	16.5 ±0.005 ^b
L.C	5.41 ±0.005 ^a	0.056 ±0.000 ^e	1.42 ±0.005 ^d	18.7 ±0.005 ^d
L.D	5.61 ±0.005 ^c	0.055 ±0.000 ^d	1.40 ±0.005 ^c	15.1 ±0.005 ^a
L.E	5.76 ±0.005 ^e	0.060 ±0.000 ^f	1.60 ±0.005 ^e	16.5 ±0.010 ^b
L.ABCDE	5.42 ±0.005 ^a	0.059 ±0.000 ^e	1.80 ±0.005 ^g	17.52 ±0.010 ^c
Control	7.05 ±0.005 ^f	0.000 ±0.000 ^a	0.60 ±0.005 ^a	25.2 ±0.050 ^e

Note: SD: Standard deviation, L.A: Yoghurt produced with *Enterococcus lactis* as starter culture, L.B: Yoghurt produced with *Lactobacillus plantarum* and *Lactobacillus pentosus* as starter cultures, L.C: Yoghurt produced with *Pediococcus pentosaceus* as starter culture, L.D: Yoghurt produced with *Pediococcus pentosaceus* and *Enterococcus durans* as yoghurt starter cultures, L.E: Yoghurt produced with two isolates of *Enterococcus durans* as starter cultures and ABCDE: Yoghurt produced with all isolates as starter cultures.

Table 3 Vitamin content of the laboratory prepared yoghurts.

Yoghurtcode	Vitamin A (IU/100G) ±SD	Vitamin B12 (µg/100) ±SD	Vitamin C (mg/100g) ±SD
L.A	297.20 ±0.007 ^b	1.95 ±0.007 ^b	2.90 ±0.007 ^d
L.B	258.50 ±0.353 ^a	1.84 ±0.007 ^a	2.71 ±0.007 ^c
L.C	401.80 ±0.007 ^e	3.40 ±0.007 ^f	3.50 ±0.007 ^g
L.D	370.00 ±0.007 ^c	3.20 ±0.007 ^e	2.30 ±0.007 ^a
L.E	396.50 ±0.007 ^d	2.55 ±0.007 ^d	2.33 ±0.007 ^b
L.ABCDE	612.00 ±0.014 ^g	3.82 ±0.007 ^g	3.88 ±0.007 ^f
RAW	479.00 ±0.007 ^f	2.46 ±0.007 ^c	3.64 ±0.007 ^e

Note: ND: Not Determined, L.A: Yoghurt produced with *Enterococcus lactis* as starter culture, L.B: Yoghurt produced with *Lactobacillus plantarum* and *Lactobacillus pentosus* as starter cultures, L.C: Yoghurt produced with *Pediococcus pentosaceus* as starter culture, L.D: Yoghurt produced with *Pediococcus pentosaceus* and *Enterococcus durans* as yoghurt starter cultures, L.E: Yoghurt produced with two isolates of *Enterococcus durans* as starter cultures and ABCDE: Yoghurt produced with all isolates as starter cultures.

Table 4 Determination of soluble and casein bound magnesium and calcium.

Yoghurtcode	Soluble Magnesium(ppm)	Soluble calcium	Caseinbound Magnesium	Caseinbound calcium
L.A	4.147 ±0.001 ^a	48.600 ±0.007 ^c	4.250 ±0.001 ^a	48.550 ±0.007 ^b
L.B	5.756 ±0.000 ^f	39.800 ±0.007 ^b	4.260 ±0.001 ^b	66.200 ±0.007 ^d
L.C	5.555 ±0.007 ^b	62.500 ±0.007 ^f	5.080 ±0.003 ^e	75.600 ±0.007 ^e
L.D	5.567 ±0.001 ^d	59.800 ±0.007 ^d	4.510 ±0.003 ^d	85.400 ±0.007 ^f
L.E	5.713 ±0.002 ^e	60.100 ±0.007 ^e	4.960 ±0.001 ^f	58.100 ±0.007 ^c
L.ABCDE	4.140 ±0.007 ^a	66.500 ±0.007 ^g	4.300 ±0.007 ^c	48.450 ±0.007 ^a
RAW	5.650 ±0.007 ^c	32.400 ±0.007 ^a	ND	ND

Note: ND: Not Determined, Superscript a-g denotes statistical difference in mean at P≤0.01. L.A: Yoghurt produced with *Enterococcus lactis* as starter culture, L.B: Yoghurt produced with *Lactobacillus plantarum* and *Lactobacillus pentosus* as starter cultures, L.C: Yoghurt produced with *Pediococcus pentosaceus* as starter culture, L.D: Yoghurt produced with *Pediococcus pentosaceus* and *Enterococcus durans* as yoghurt starter cultures, L.E: Yoghurt produced with two isolates of *Enterococcus durans* as starter cultures and ABCDE: Yoghurt produced with all isolates as starter cultures.

Table 5 Proximate analysis of the laboratory prepared yoghurts.

SAMPLE	MOISTURE (%) ±SD	ASH (%) ±SD	FAT (%) ±SD	CRUDE FIBRE (%)	PROTEIN (%) ±SD	CARBOHYDRATE (%) ±SD
RAW MILK	81.94 ±0.05 ^g	0.68 ±0.01 ^c	0.00 ±0.00 ^a	ND	16.91 ±0.01 ^g	0.48 ±0.02 ^a
ABCDE	79.59 ±0.02 ^f	0.29 ±0.01 ^a	0.02 ±0.01 ^b	ND	5.19 ±0.03 ^a	14.93 ±0.04 ^c
A	78.01 ±0.01 ^e	1.14 ±0.01 ^f	0.02 ±0.01 ^b	ND	9.4 ±0.01 ^f	11.43 ±0.05 ^b
B	71.71 ±0.01 ^d	0.95 ±0.05 ^d	0.06 ±0.04 ^e	ND	8.39 ±0.00 ^e	18.9 ±0.04 ^d
C	61.73 ±0.01 ^a	0.96 ±0.05 ^e	0.55 ±0.01 ^d	ND	7.81 ±0.01 ^d	28.96 ±0.60 ^g
D	67.31 ±0.00 ^c	0.54 ±0.05 ^b	0.03 ±0.01 ^c	ND	7.51 ±0.01 ^c	24.62 ±0.02 ^e
E	64.36 ±0.00 ^b	1.03 ±0.05	0.03 ±0.01 ^c	ND	6.87 ±0.01 ^b	27.71 ±0.02 ^f

Note: SD: Standard Deviation, ND: Not Determined, Superscript a-g denotes statistical difference in mean at $p \leq 0.01$. A: *Enterococcus lactis* as yoghurt starter culture. B: *Lactobacillus plantarum* and *Lactobacillus pentosus* as yoghurt starter culture. C: *Pediococcus pentosaceus* as yoghurt starter culture. D: *Pediococcus pentosaceus* and *Enterococcus durans* as yoghurt starter culture. E: Two isolates of *Enterococcus durans* as yoghurt starter culture. ABCDE: All isolates

Table 6 Antibacterial assay of the laboratory prepared yoghurts on selected clinical isolates using well agar diffusion.

Clinical isolates/ Test organisms	Zones of inhibition (mm)					
	A	B	C	D	E	ABCDE
<i>Staphylococcus aureus</i>	9	7	7	6	7	12
<i>Escherichia coli</i>	4	7	5	4	5	6
<i>Salmonella typhi</i>	4	3	3	3	3	3

Note: A: *Enterococcus lactis* as yoghurt starter culture, B: *Lactobacillus plantarum* and *Lactobacillus pentosus* as yoghurt starter culture. C: *Pediococcus pentosaceus* as yoghurt starter culture. D: *Pediococcus pentosaceus* and *Enterococcus durans* as yoghurt starter culture. E: Two isolates of *Enterococcus durans* as yoghurt starter culture, ABCDE: All isolates.

Table 7 Hematological evaluation of Wistar rats treated with the prepared yoghurts.

SAMPLE	DIFFERENTIAL COUNT (dl)						
	PCV (%)	TOTAL WBC	NEUTROPHIL	LEUCOCYTES	EOSINOPHILS	MONOCYTES	BASOPHILS
A	38	11.8 X 10 ⁹	52	42	1	5	0
B	30	11.5 X 10 ⁹	53	42	1	4	0
C	32	10.9 X 10 ⁹	51	43	1	5	0
D	36	11.3 X 10 ⁹	51	41	1	7	0
E	32	10.7 X 10 ⁹	52	41	1	6	0
ABCDE	40	11.2 X 10 ⁹	53	42	1	4	0
CONTROL	24	12.3 X 10 ⁹	52	42	1	5	0

Note: A: *Enterococcus lactis* as yoghurt starter culture. B: *Lactobacillus plantarum* and *Lactobacillus pentosus* as yoghurt starter culture. C: *Pediococcus pentosaceus* as yoghurt starter culture. D: *Pediococcus pentosaceus* and *Enterococcus durans* as yoghurt starter culture. E: Two isolates of *Enterococcus durans* as yoghurt starter culture. ABCDE: All isolates.

The combination of all the starter cultures resulted in higher vitamin C content which is lower when compared to the report of **Ihemeje et al. (2015)**. This increase may be as a result of more efficient oxidation of sugar by the consortium of lactic acid bacteria to ascorbic acid in a fermentative pathway of L-galactose to L-ascorbic acid (vitamin C) as reported by **Berry (2002)**. *Pediococcus pentosaceus* used as starter culture also produced yoghurt at a close range with the combined isolates, vitamins are essential organic compounds required in very small amounts to maintain the fundamental functions of the body (**Hassen et al., 2010**).

The soluble and casein bound calcium and magnesium of the yoghurts showed varying concentrations of magnesium

and calcium which showed statistically significant differences ($p \leq 0.05$) (Table 4.0). The soluble magnesium recorded lied between $4.140 \pm 0.007 - 5.713 \pm 0.002$ while the raw milk before fermentation was 5.650 ± 0.007 . Therefore, there was depletion in the soluble magnesium content as it is a mineral requirement of lactic acid bacteria (**Boyaval, 1989**) but yoghurt produced with *Enterococcus durans* as starter culture recorded improved magnesium content and was found to have the highest magnesium content. The highest soluble calcium in the study was observed with yoghurt produced with all the isolates followed by *Enterococcus durans* and *Pediococcus pentosaceus* with relatively high values.

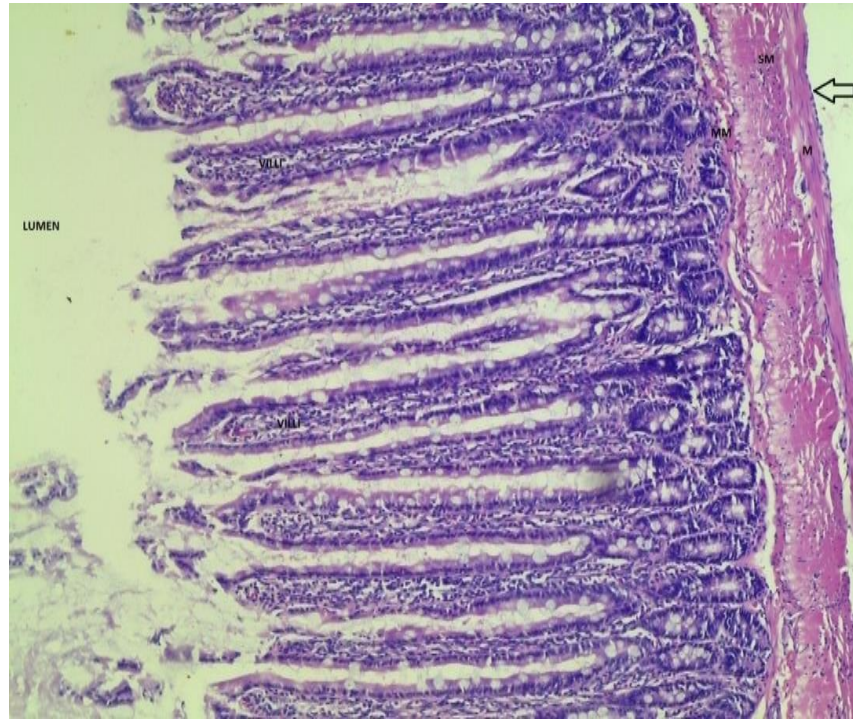


Figure 2 Histology of the small intestine of wistar rat fed without yoghurt (Control) .Note: Control intestine, X100, HE STAIN. HE (Haematoxylin and Eosin stain).

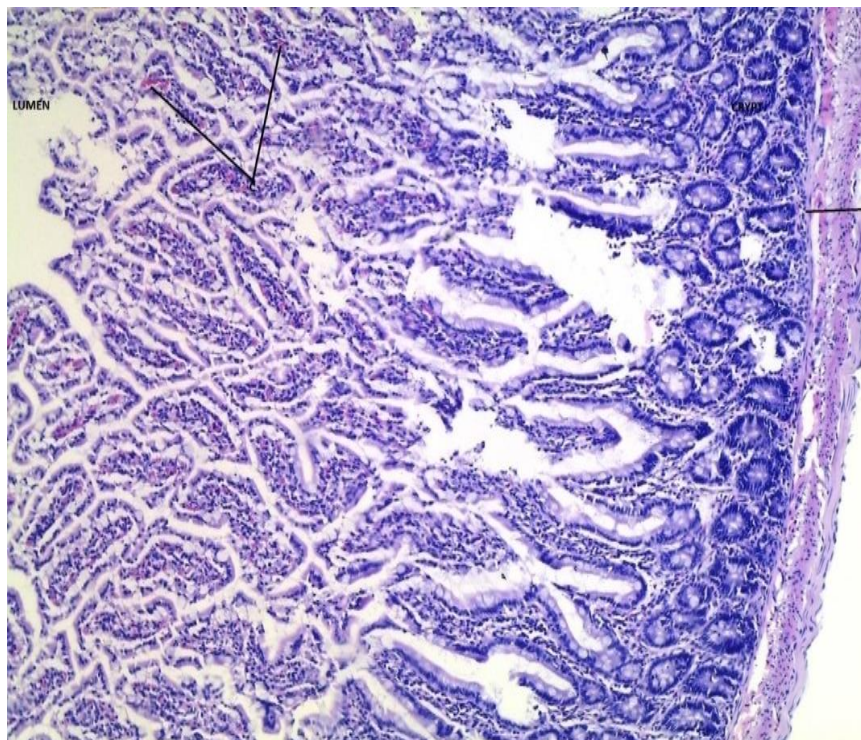


Figure 3 Histology of the small intestine of wistar rat fed with *Enterococcus lactis* containing yoghurt. Intestine, X100, HE STAIN.

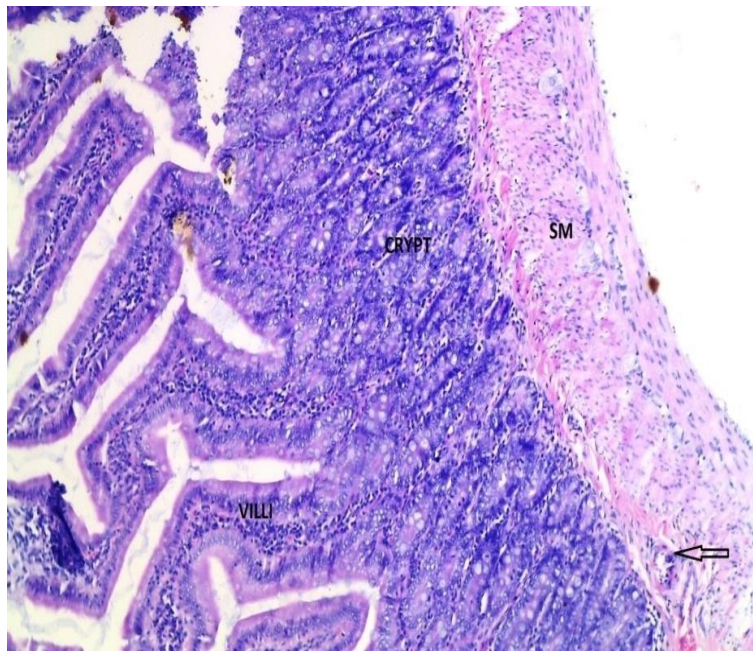


Figure 4 Histology of the small intestine of wistar rat fed with *Lactobacillus plantarum* and *Lactobacillus pentosus* containing yoghurt. Intestine, X100, HE STAIN.

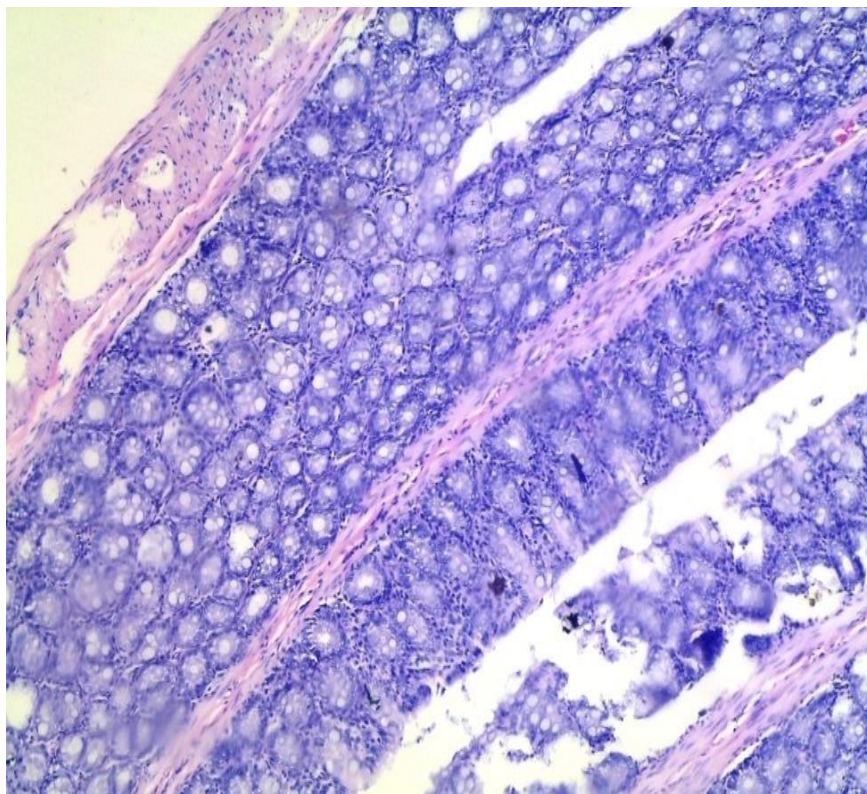


Figure 5 Histology of the small intestine of wistar rat fed with *Pediococcus pentosaceus* containing yoghurt. Intestine, X100, HE STAIN.

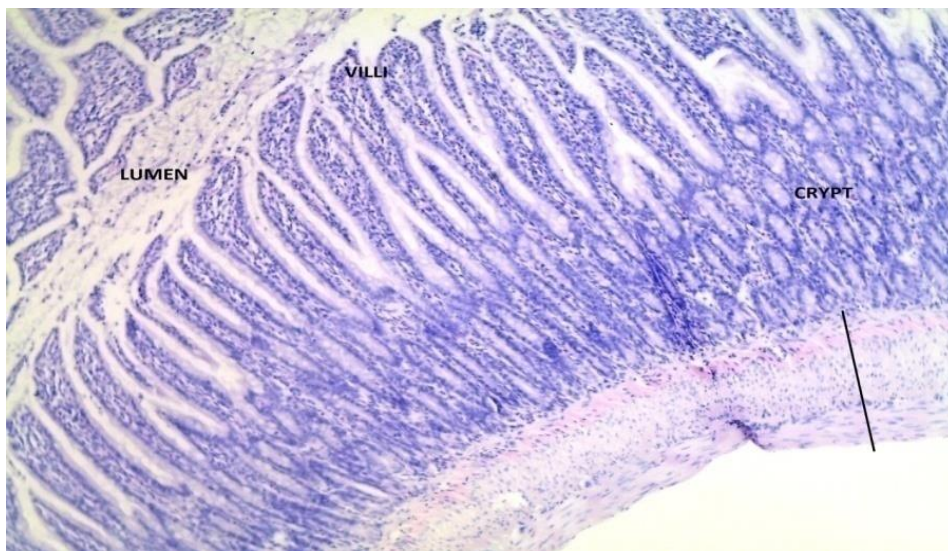


Figure 6 Histology of the small intestine of wistar rat fed with *Pediococcus pentosaceus* and *Enterococcus durans* containing yoghurt. Intestine, X100, HE STAIN.

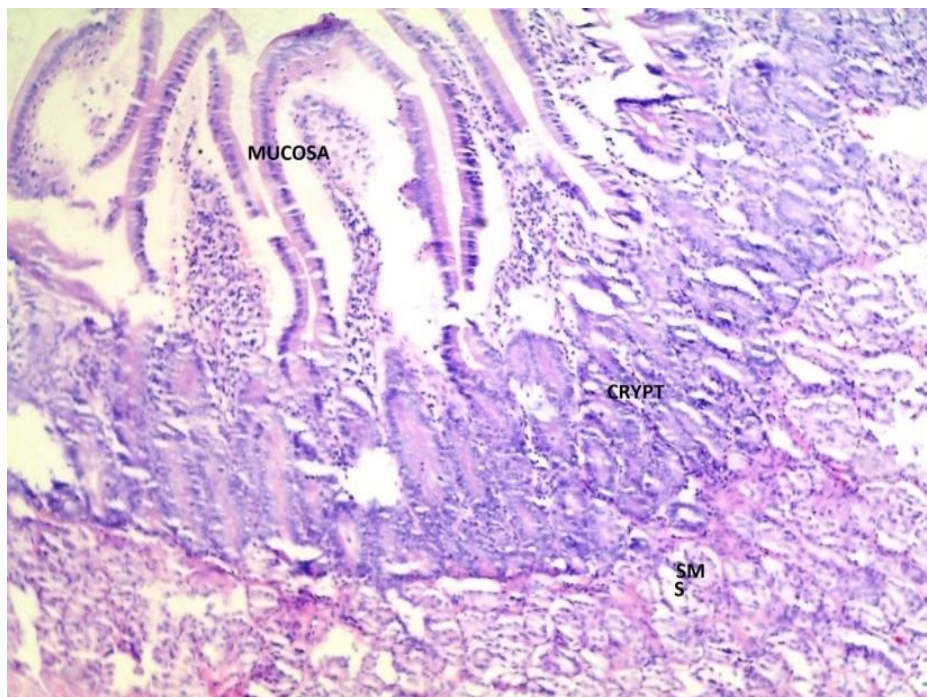


Figure 7 Histology of the small intestine of wistar rat fed with *Enterococcus durans* containing yoghurt. Intestine, X100, HE STAIN.

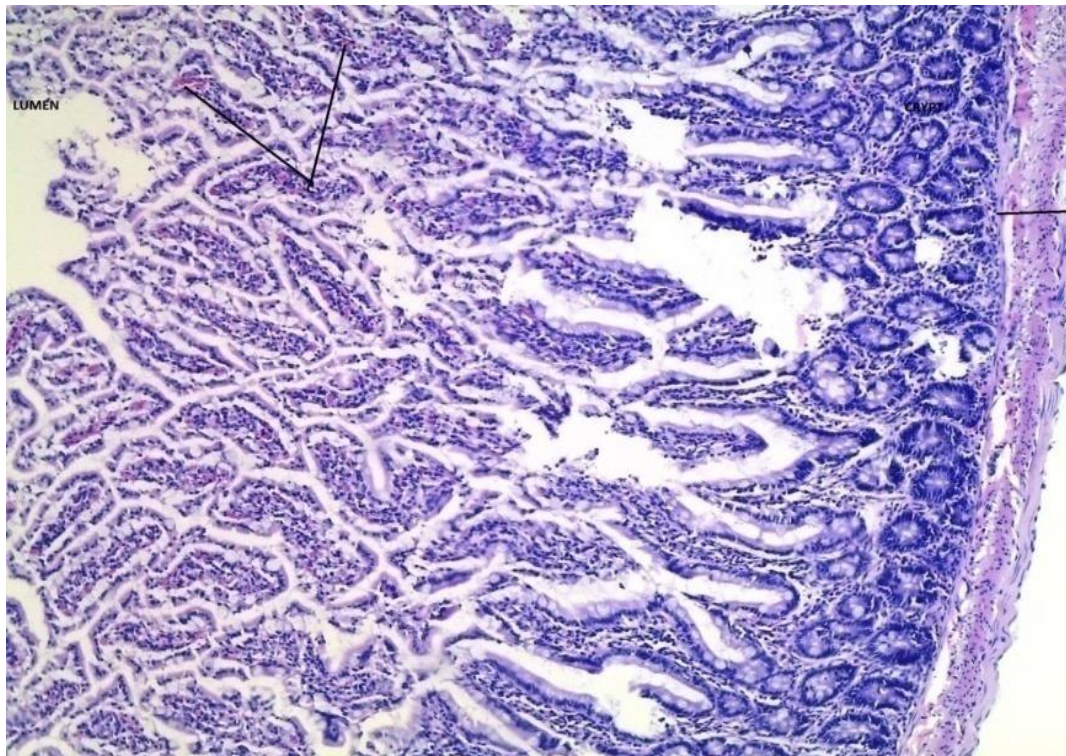


Figure 8 Histology of the small intestine of wistar rat fed with *Enterococcus lactis* containing yoghurt. Intestine, X100, HE STAIN.

The casein bound calcium in this study were relatively higher than the concentrations found in the soluble calcium content in yoghurt which had starter cultures of *Lactobacillus plantarium* and *L. pentosus* as dual starter cultures, *Pediococcus pentosaceus* and *Enterococcus durans* as dual starter culture and *Enterococcus durans* as starter culture. However, *Enterococcus lactis* and the combination of the isolates gave improved magnesium content in the casein bound magnesium. The result of the study in terms of calcium and magnesium content is in contrast with the work of **Gad et al. (2010)** who reported higher calcium and magnesium values.

The report of this study agrees with the work of **Miguel et al. (2003)** who reported such variations between the soluble and casein bound magnesium and calcium.

The proximate composition of the yoghurts as determined in comparison with the raw, recorded a decrease. However, the carbohydrate content increased in all the yoghurts produced. The highest protein content was found in the yoghurt produced with *Enterococcus lactis* while the highest carbohydrate content was observed with the yoghurt produced with *Pediococcus pentosaceus* as starter culture. There was significant difference in all tested parameters of the different yoghurts ($p \leq 0.05$) (Table 5). The fat content of the yoghurts were relatively low. Fat plays an important role in improving the consistency of yoghurt and also provides twice the energy of carbohydrate and protein (**Ehirim and Onyeneke, 2013**). The findings of this study in terms of proximate composition agree with the report of **Igbabul et al. (2014)** and **Dairy Council (2013)**.

The yoghurts were observed to exhibit antibacterial activity against selected bacterial isolates representative of the Gram positives and Gram negatives as in *Staphylococcus aureus* and *Escherichia coli* and *Salmonella typhi* respectively (Table 6). The yoghurts were observed to express antibacterial activity although at minimal levels while the highest inhibitory activity was found in an assay performed using the synergistic potential of all the laboratory prepared yoghurts. This is in agreement with the report of **Hami (2011)** and **Okiki et al. (2018)** who established such antibacterial properties in nono fermented spontaneously by naturally occurring lactic acid bacteria. A number of studies have found probiotic consumption to be useful in the treatment of many types of diarrhea and intestinal disorders (**Isolauro et al., 1991; Oksanen et al., 1990 and Siitonen et al., 1990**). Antimicrobial effects of lactic acid bacteria are formed by producing some substances such as organic acids, carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins (**Ouwehand and Vesterlund, 2004; Romanova and Urminska 2017**).

The examination of the yoghurts influence on haematological parameters using Wistar rats showed improvements in the tested parameters which was demonstrated in the difference between the control and the treated samples (Table 7). The study revealed that combined use of the isolates always yielded better outcomes in terms of parameters examined. In a previous study, **Shu et al. (1999)** reported that 4 weeks of consumption of *Lactobacillus rhamnosus*, *Lactobacillus*

acidophilus, and *Bifidobacterium lactis* had no adverse effect on mice general health status including haematology. In this study there was general increase in total white blood cell, packed cell volume but there was no significant effect on differentials such as the neutrophils, leukocytes, eosinophils, monocytes and basophil count. The report of this study differs from **Hamid et al. (2008)** as the study recorded higher blood cell counts as a result of longer exposure to treatment. A high level of packed cell volume is an indication that the rats are not anemic while a lower level is an indication of anemia (**Aboderin and Oyetayo, 2006**). The white blood cell is important in defending our body against infection (**Aboderin and Oyetayo, 2006**). However, the leukocyte counts cannot give specific information and this necessitated the differential counts. The lymphocytes did not change significantly and this is in agreement with the report **Hamid et al. (2008)**.

Figure 2 shows a section of small intestinal tissue, the submucosa (SM), muscularis mucosa (MM) and serosa arrow. The projection of the intestinal villi into the lumen together with the intestinal glands are lined by columnar epithelium, numerous goblets cells are seen. No sign of intestinal inflammation. Figure 3 shows a section of intestinal tissue with marked disruption of the mucosa layer (line), other layers of the intestine appear normal. Figure 4 shows a section of small intestinal tissue, the submucosa (SM), muscularis mucosa (MM) and serosa. The projection of the intestinal villi into the lumen together with the intestinal glands are lined by columnar epithelium, numerous goblets cells are seen. Arrow shows vascular leucocytes Margination. Figure 5 shows a section of the small intestinal tissue, the submucosa (SM), muscularis mucosa (MM) and serosa arrow. The projection of the intestinal villi into the lumen together with the intestinal glands which are lined by columnar epithelium, numerous goblets cells are seen. No sign of intestinal inflammation. Figure 6 shows a section of intestinal tissue with intestinal villi projection into the lumen, the intestinal crypt and adjoining layers (line) appear essentially normal. Figure 7 shows a section of unremarkable intestinal layers, mucosa, sub-mucosa (SM), muscularis and mucosa appear essentially normal. Figure 8 shows a section of intestinal tissue with some disruption of the mucosa layer (line), other layers of the intestine appeared normal. The projection of the intestinal villi into the lumen together with the intestinal glands is lined by columnar epithelium. The lactic acid bacteria strains were reported to cause no morphological abnormalities to the small intestine. The report of this study is in the affirmative to the reports of **Ramiah et al., (2009)** who reported the safety examination of *Enterococcus mundtii* and *Lactobacillus plantarum* and the organisms were found to have caused no histological abnormalities or inflammation. **Cheng-Chih et al., (2014)** also examined *Lactobacillus acidophilus*, *L. pentosus*, *L. plantarum*, *L. reuteri* and *Enterococcus faecium* and their toxicity. The small intestines of the Wistar rats fed with the different yoghurts which contained starter cultures; *Enterococcus lactis*, *L. pentosaceus*, *L. pentosus*, *L. plantarum* and *E. durans* showed no abnormality, inflammation or damage.

CONCLUSION

As determined in this study, the final product quality of yoghurt or functional dairy is dependent on the selected lactic acid bacteria (LAB) cultures; therefore the choice of organism(s) to drive the fermentation is as important as the product itself. The study also demonstrated the importance of using animal models to determine the safety of the LAB cultures to be employed during yoghurt production. The use of more than one starter culture results in enhanced product quality as use of combined isolates in this study gave better outcomes in terms of nutritional quality. Finally, yoghurt is an important source of nutrients required for the body and also a remedy for gastrointestinal disorders caused by pathogens.

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