EVALUATION OF ACHA (DIGITARIA EXILIS) GRAIN FERMENTED WITH LACTOBACILLUS SPECIES AS A PROBIOTIC FOOD

ABSTRACT

Aims: This study assess the effect of the fermented Acha samples in-vivo using apparently healthy and infected laboratory animals.

Study design: Acha was fermented in two forms (Local fermentation and controlled fermentation).
Place and Duration of Study: Sample: Department of Medicine (Medical Unit IV) and Department of Radiology, Services Institute of Medical Sciences (SIMS), Services Hospital Lahore, between June 2009 and July 2010.
Methodology: Acha was weighed into a fermenting container of 100g and water of 1litre was added to submerge it for 72 hours in the ratio 1:3. Microbial, proximate and mineral analysis was carried on all the samples. For 21days, all fermented samples were used to feed rats infected with *Escherichia coli* and *Shigella dysenteriae* except for the control for *in vivo* study and evaluated for their probiotic potential. Also, hematological study and histopathology analysis were carried out on the small and large intestine of the Albino rats that was fed with the fermented samples. The various fermented samples were freeze dried to retain the organisms used for the fermentation
Results: Haematological study (PCV, WBC, RBC, Platelets, haemoglobin and differential leucocytes) and histopathology analysis (small intestine and large intestine) of rats from all experimental groups showed that Acha fermented with *Lactobacillus acidophilus* was able to rebuild shrinked and ruptured cells on the mucosal lining of the walls of the intestines.
Conclusion: Acha fermented with *Lactobacillus acidophilus* was observed to have the best results on the weight of rats, white blood cell count, red blood cell count and probiotic effect on the intestine of the rats fed with it.

*Keywords: [*Acha, *Lactobacillus acidophilus,* probiotics*,* feacal samples, fermentation*]*

1. INTRODUCTION

Fermented foods are of great significance because they provide and preserve vast quantities of nutritious foods in a wide diversity of flavours, aromas and textures which enrich the human diet [1]. Lactic acid bacteria can be quite beneficial when they are found in the oral cavity, the intestinal tract or

the vagina. The lactic acid bacteria don't just produce acid; they produce a lot of acid - so much acid that it kills or inhibits the growth of other potentially dangerous microbes that could lead to sickness [2].

Eating fermented foods and drinking fermented drinks like Kefir and Kombucha will introduce beneficial bacteria into the digestive system and help the balance of bacteria in the digestive system. Fermented foods are some of the best chelators available. The beneficial bacteria in these foods are highly potent detoxifiers, capable of drawing out a wide range of toxins and heavy metals **[ ] give reference to this paragraph**

Strains of lactic acid bacteria are the most common microbes employed as probiotics, especially *Lactobacillus* and *Bifidobacterium* species, *Lactococci,* enterococci and some streptococci are also included as probiotics. Probiotics have been recommended or suggested for patients receiving radiation treatment, individuals who have recurrent thrush, vaginal yeast infections, or urinary tract infections, persons suffering from irritable bowel syndrome (IBS) or other bowel problems, for travelers abroad to protect against food poisoning and during any period where antibiotics may be taken [3].

All over the world, diarrhoea is a serious health problem especially in children [4]. Although, diarrhoea is self-limiting, but when it is as a result of bacterial infections, antibiotics therapy may be required. However, since most bacteria have become resistant to most antibiotics, the search for alternative therapeutic measures becomes imperative as probiotics serves as an alternative therapy to antibiotics. There's hardly any scientific literature about Acha, so it will be interesting to see if this new study garners attention in the food world and its medical importance.

2. METHODOLOGY

2.1 Source of Materials

Acha was bought from Sabongari market Kano, Kano State, Nigeria.

2.2 Preparation of Acha floury

Acha sample was fermented in two different forms; the local fermentation and controlled fermentation. For the local fermentation, the Acha sample was weighed into a fermenting container of 100g and water of 1litre was added to submerge it for 72 hours in the ratio 1:3. The fermented sample was milled using a sterile milling machine and then lyophilsed. For the controlled fermentation, water was added to a weighed sample and allowed to submerge in ratio 1:6. The sample and water were sterilized at 121^{o}C for 15 minutes. It was allowed to cool and fermented with the 10^{5} cfu/ml of the test isolates under a sterile condition by centrifugation. It was left to ferment for 72hours. The fermented sample was milled using a sterile milling machine and then lyophilsed.

2.3 Fermentation and Storage

Acha grain and distilled water in an amount to adjust moisture content of the mixture to 1:4 (i.e. 100g of Acha grains in 400ml of distilled water) was introduced into seven (7) fermentation jars (A1, A2, B1, B2, C1, C2 and D) which were autoclaved at 121^{o}C for 15minutes. Jars were allowed to cooled after which each jar was innoculated with 10^{5}cfu/ml each of the test isolate *L. casei*, *L. acidophilus and L. debulreki* with A1 and A2 containing *L. casei*, B1 and B2 containing *L. acidophilus*, C1 and C2 containing *L. debulreki* and D was uninnoculated serving as the control. After thorough mixing, the properly corked jars were allowed to ferment for 72hours. After fermentation, jar A1, B1 and C1 were stored at 4±2^{o}C while A2, B2 and C2 were stored at 25±2^{o}C for 14 days respectively. Viable counts of separate LAB in the products were determined during the period of fermentation and after storage.

2.4 Culturing and Harvesting of Lactobacillus Cells

Two loopfuls of each pure culture of isolates A (*Lactobacillus casei*), B (*Lactobacillus acidophilus*), C (*Lactobacillus delbrueckii*) obtained from the traditionally fermented Acha were innoculated into test tubes containing (5ml each) sterile MRS Broth (pH 5.5) and incubated at 45^{o}C for 48hours under microaerophilic conditions. This culture was centrifuged at 10000g for 15minutes. The pellet was rinsed out three times with 10ml phosphate buffer saline (PBS) into sterilized universal bottle and kept in a refrigerator as the stock culture. The total viable cells in the stock were determined by pipetting 1ml of the stock culture of each isolate into 9ml sterile distilled water in test tubes to give a dilution of 10^{-1}. Using a fresh pipette, 1ml of 10^{-1} was pipetted into another test tube containing 9ml sterile distilled water to make a dilution of 10^{-2} and subsequently to dilution 10^{-9}. 0.1ml of 10^{-8}, 10^{-7},10^{-6} and 10^{-5} were pipetted into different plates and cultured respectively at 45^{o}C for 48hours. The total number of colonies were then counted and recorded.

2.5 Evaluation of the effect of Acha fermented samples on albino rats *(mention ethical committee approval with reference number in this section)*

2.5.1 Acclimatization of the rats

Thirty three albino rats aged 6-8 weeks were weighed randomly assigned to eleven groups of three (3) rats each. The rats were housed in stainless steel cages under controlled conditions fed with growers mash and drinking water and observed daily to know if they were healthy before being used for study. After 7 days of acclimatization, all animals were weighed during which fresh fecal samples of the rats were collected for bacterial enumeration using conventional techniques. Wister albino rats of both sexes and weight were used for this experiment [5].

2.5.2 Isolation and enumeration of the feacal microbial flora in the feaces of albino rats

One gram of faeces from experimental animals were taken and weighed aseptically into different test tubes containing 9 ml sterile distilled water and serially diluted to 10^{-10}. From the dilution 10^{-5} and 10^{-6} tube, 0.1ml was taken and pipetted into sterile Petri dishes respectively. Sterile molten MacConkey (For

enumeration of coliforms), Eosin Methylene Blue agar, Samonella-Shigella agar (selective medium for *E. coli* and *Shigella dysenteria* respectively) and Man Rogosa Sharpe agar (for Lactobacillus) at about 50^{o}C was poured and allowed to set. Plates were incubated at 37^{o}C for 24 hours. After incubation, total plates count was done and discrete colonies were subcultured unto new plates of Nutrient agar to obtain pure cultures for identification.

2.5.2.1 Determination of the infectivity dose of *E. coli* and *Shigella dysenteria* in the experimental rats

This was conducted with the stock culture of *E. coil* and *Shigella dysenteria* two loopful of pure culture of the test organism was introduced into the test tubes containing 5 ml each of sterile nutrient broth (pH 5.5) and incubated at 37^{o}C for 24 hours. This was then centrifuged at 10,000 g for 15 minutes. To harvest the cells, the pellets were rinsed out with 9 ml Phosphate Buffer Saline (PBS) into sterilized universal bottles and kept in a refrigerator in the stock culture. From the stock culture, *E.coli* and *Shigella dysenteria* were introduced into the rats at different concentrations of 0.25, 0.5, and 10^{-5}cfu/ml and 10^{-2}cfu/ml respectively.

2.6 Infecting experimental rats with the test organisms

This was administered orally to rats using a feeding loop. Experimental animals were randomly assigned to four treatments designed according to the test organisms. For *E. coli*; EA, EB, EC, ED and ECTrl infected with 0.5ml of 10^{-5}cfu/ml. For *Shigella dysenteria*; SA, SB, SC, SD and SCTrl infected with 0.2ml of 10^{-2}cfu/ml while PCTrl as the positive control which was infected. After post ingestion for a period of 7 days the animals were observed daily for behavioural changes and microbial enumeration of their fresh feacal samples was done. The basal diet was supplemented with 20 g of the fermented samples for 21 days (day 35).

2.7 Histopathological Examination

The internal organs of the rats that were used are the small and large intestine. They were removed and preserved in a 10% formalin solution. After this, they were analyzed and further processed for histopathological studies. The small and large intestines were removed and were diced and cut into small sizes of about 3 mm. The cut were then treated with alcohol of different grades (ethanol, methanol and isopanol) and concentration ranging from 50% - 100% for them to be dehydrated. After this, the diced organs were then cleared using xylex for a period of 2hours, the tissues were then impregnated in molten wax. They were further embedded in paraffin wax after which they were left to solidify, marked out with a sharp sterile knife and then hung on a wooden block for sectioning. Sectioning of the organs was done with a microtone at 5 microns and was 5 stained with haematoxylin – eosin. The excess stained was cleared using tap water. It was further cleared in xylene after which it was mounted in Canada balsam. The sectioned organs were spread out in a water bath. The water bath temperature was regulated at 45^{o}C. They were then collected with slides already rubbed with eggs albumen. They were allowed to dry

up in the oven at a temperature of 40^{o}C after which they were examined under the microscope slide using the low and high power objectives [6].

2.8. Statistical Analysis

All results are means of three independent trials ± standard error. Data were subjected to 1-way Analysis of Variance (ANOVA) using SPSS version 16.0. Duncan's multiple range test was used to separate means at 5% level of significance.

3. Results and discussion

3.1 Microorganisms Isolated from Acha grains

Microorganisms isolated from locally fermented Acha were bacteria and fungi. Eight bacteria were isolated from fermented Acha grain. They were *Bacillus* spp*, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii, Staphylococcus aureus, Streptococcus, Aspergillus niger, Aspergillus flavus, Mucor mucedo, Sacharromyces cerevisae,* and *Candida albicans.* This is shown in Plate 1a and b. Majority of the lactic acid bacteria isolated from Acha belongs to the genus *Lactobacillus*. These organisms increased early in the fermentation of Acha grain. The decrease in sugar concentration could be largely due to the activities of these organisms which metabolized and converted sugars into organic acids during Acha fermentation [7].

3.2 Occurrence of microorganisms in the faecal samples of Albino Rats

The microorganisms isolated from the faeces of Albino rats before feeding with fermented Acha are: *E. coli, S. aureus, Enterococcus spp, L. acidophilus, Streptococcus faecalis, and Proteus vulgaricus*.

Figure 1 shows the occurrence of feacal bacterial in rats infected with *E.coli* and the changes in the bacterial counts during the days of treatment. Figure 2 shows the occurrence of feacal bacterial in rats infected with *S. dysenteriae* and the changes in the bacterial count during the days of treatment.

Bacterial count of feacal samples of both infected rat (group infected with *E. coli* and group infected with *Shigella dysenteriae*) during treatment showed a decrease as the days of treatment increased. The trend was the same for feacal sample of the untreated rat although the bacterial counts of feacal sample of untreated rat were the highest throughout the 72 hours period of the research. Since the bacterial counts of feacal sample of both infected and the uninfected followed the same trend, the infections are probably self-limiting.

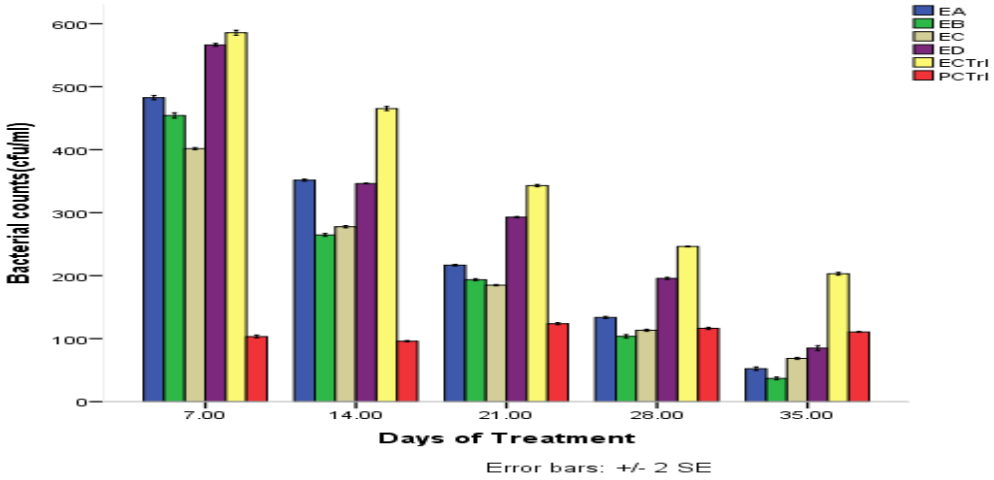


Figure 1: Bacterial Count of Feacal Samples of Rats Infected with *E. coli* during Treatment

Legend: EA- rat infected with *E.coli* and treated withAcha fermented with *L. casei,* **EB-** rat infected with *E.coli*and treated with Acha fermented with *L. acidophillus,* **EC-** rat infected with *E.coli* and treated with Acha fermented *L. delbrueckii,* **ED-** rat infected with *E.coli* and treated with Acha fermented locally, **ECTrl-** rat infected with *E. coli* and without treatment, **PCTrl-** uninfected rat

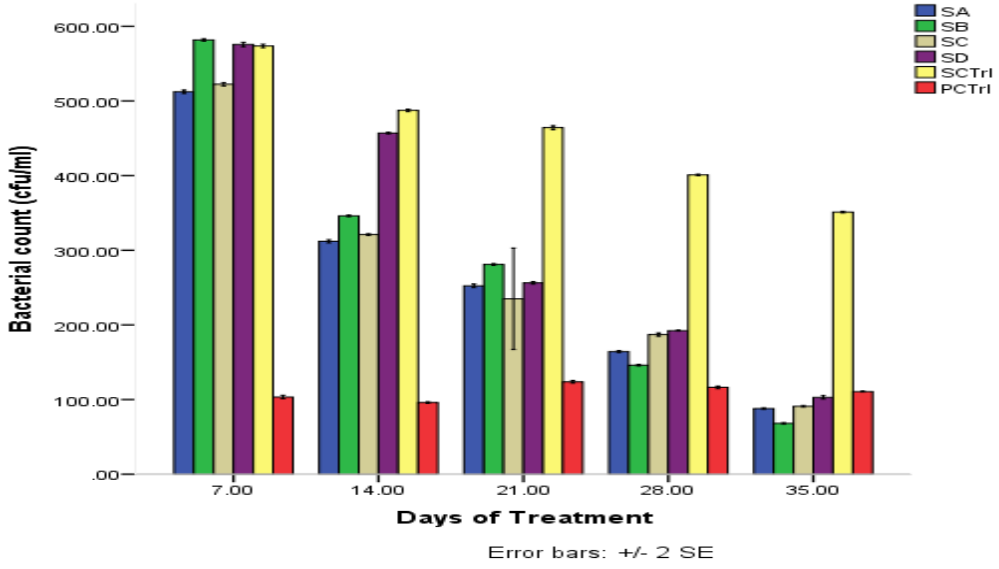


Figure 2: Bacterial Count of Feacal Samples of Rats Infected with *Shigella dysenteria* during Treatment

Legend: SA- rat infected with *S. dysenteria* and treated with Acha fermented with *L. casei,* **SB**- rat infected with *S. dysenteria* and treated with Acha fermented with *L. acidophillus,* **SC**- rat infected with *S.*

dysenteria and treated with Acha fermented *L. delbrueckii,* **SD**- rat infected with *S. dysenteria* and treated with Acha fermented locally, **SCTrl**- rat infected with *S. dysenteria* and without treatment, **PCTrl**- uninfected rat

3.3 Feacal sample observed during *in vivo* feeding trial

Feacal samples of rat infected with *S. dysenteria* were black and blotted while the feacal sample of recovered rat infected with *S. dysenteria* was Black, short and hard. Feacal sample of a rat infected with *E. coli* was brown, long and moist and the feacal sample of a recovered rat infected with *E. coli* was brown and hard. These are shown in plates 1 a, b, c and d. Table 1 also shows the colour changes and the features in the feaces of the experimental rats.

It was observed that the bacterial count of faeces in the gastrointestinal tract (GIT) during *invivo* feeding trial reduces as the day increases. The initial high bacteria counts could alter the microbiota balance in the GIT, which could in turn affect the overall health of the rat [8; 9]. The bacterial counts of GIT of rat treated with Acha from inoculated fermentation were mostly lower than those from GIT of rat treated with Acha fermented locally. Acha from inoculated fermentation would be effective in treating GIT microbiota related problems with further studies.

Feacal sample of the rat infected with *S. dysenteriae* was black and blotted while the feacal sample of recovered rat infected with *S. dysenteriae* was black, short and hard. Feacal sample of the rat infected with *E. coli* was brown, long and moist and the feacal sample of recovered rat infected with *E. coli* was brown and hard. The feacal samples of the two recovered rat (recovered rat infected with *S. dysenteriae* and recovered rat infected with *E. coli*) showed positive effect of the feeding trial on the gastrointestinal tract of the infected rats.

Plate 1a

Plate 1b

Plate 1c **Plate 1d**

Legend

Plate 1a: Feacal sample of a rat infected with *S. dysenteriae* (Black and Blotted) during *in vivo* feeding trial

Plate 1b: Feacal sample of a recovered rat infected with *S. dysenteriae* (Black, short and hard) during *in vivo* feeding trial

Plate 1c: Feacal sample of a rat infected with *E. coli* (Brown, Long and Moist) during *in vivo* feeding trial

Plate 1d: Feacal sample of a recovered rat infected with *E. coli* (Brown and Hard) during *in vivo* feeding trial

Table 1: Colour changes and the observed features in feaces of experimental rats during *in vivo* feeding trials

DAYS	EA	EB	EC	ED	ECTrl	SA	SB	SC	SD	SCTrl	PCTrl
7	Br/H	Br/M	Br/L	Br/H	Br/H	Br/M	Br/H	Bl/M	Bl/M	Bl/H	Bl/H
14	Br/M	Br/B	Br/M	Br/B	Br/B	Bl/M	Bl/B	Bl/M	Bl/B	Bl/S	Br/H
21	Br/M	Br/M	Br/M	Br/M	Br/B	Bl/S	Bl/M	Br/M	Bl/S	Bl/B	Br/L
28	Br/L	Br/S	Br/L	Br/S	Br/M	Bl/S	Bl/M	Bl/L	Bl/B	Bl/B	Br/M
35	Br/H/L	Br/H	Br/M	Br/S	Br/M	Br/S	Br/H	Bl/M	Bl/S	Bl/M	Bl/L

Legend

EA- rat infected with *E.coli* and treated with Acha fermented with *L. casei,* **EB-** rat infected with *E.coli* and treated with Acha fermented with *L. acidophillus,* **EC-** rat infected with *E.coli* and treated with Acha fermented *L. delbrueckii,* **ED-** rat infected with *E.coli* and treated with Acha fermented locally, **ECTrl-** rat infected with *E. coli* and without treatment, **PCTrl-** uninfected rat.

SA- rat infected with *S. dysenteriae* and treated with Acha fermented with *L. casei,* **SB-** rat infected with *S. dysenteriae* and treated with Acha fermented with *L. acidophillus,* **SC-** rat infected with *S. dysenteriae*

and treated with Acha fermented *L. delbrueckii,* **SD-** rat infected with *S. dysenteriae* and treated with Acha fermented locally, **SCTrl-** rat infected with *S. dysenteriae* and without treatment.
Br- Brown feaces, **H**-hard feaces, **M**- Moist feaces, **L**-Long feaces, **B**- Blotted feaces, **S**- Short feaces, **Bl**-Black feaces.

3.4 Changes in the weight of experimental rats during *in vivo* feeding trials

Fig 3, there were increases in weight between Day1 to Day7 for EA, EB, EC, ED, ECTrl and PCTrl respectively. After infecting with *E. coli* the mean weight of the experimental rats was observed to reduce. After infection, feeding was dominated by the fermented Acha samples and the weight increased between Day 21 and Day 35 for EA, EB, EC, ED, ECTrl and PCTrl respectively.

Figure 4 shows the mean weights of rats infected with *S. dysenteriae.* Before infection, weights increased in Day 1 and Day7 for SA, SB, SC, SD, SCTrl and PCTrl respectively. After infecting with *S. dysenteriae,* there was decrease in Day 14. Increase in the weight was observed in Days 21 to Day 35 for SA, SB, SC, SD, SCTrl and PCTrl. The weight of both groups of rats (*S. dysenteriae* infected group and *E. coli* infected group) showed improvement in weight after been fed with Acha fermented for longer hours/days (Figure 3 and 4). This is probably due to improved nourishment of the rat by fermented Acha.

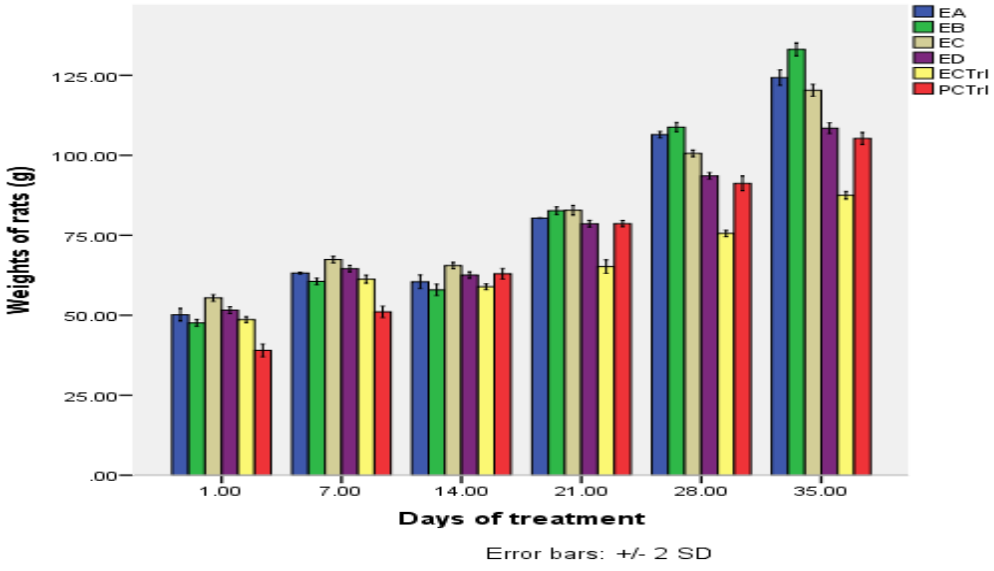


Figure 3: Weights of the Experimental Animals infected with *E. coli* during *in vivo* Feeding Trials
Legend: EA- rat infected with *E.coli* and treated with Acha fermented with *L. casei,* **EB-** rat infected with *E.coli* and treated with Acha fermented with *L. acidophillus,* **EC-** rat infected with *E.coli* and treated with Acha fermented *L. delbrueckii,* **ED-** rat infected with *E.coli* and treated with Acha fermented locally, **ECTrl-** rat infected with *E. coli* and without treatment, **PCTrl-** uninfected rat

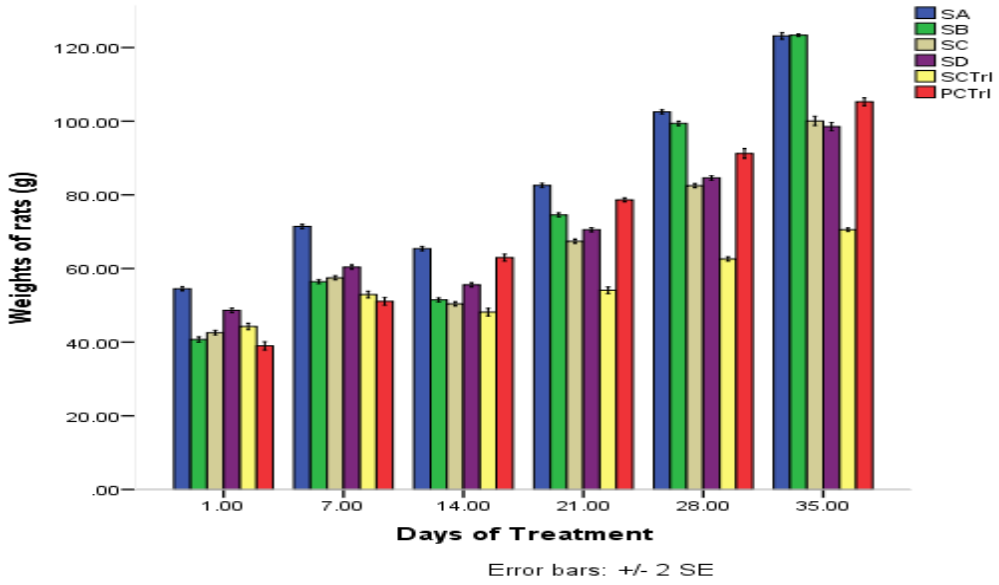


Figure 4: Weights of the Experimental Animals Infected with *Shigella dysenteriae* during *invivo* Feeding Trials

Legend: SA- rat infected with *S. dysenteriae* and treated with Acha fermented with *L. casei,* **SB-** rat infected with *S. dysenteriae* and treated with Acha fermented with *L. acidophillus,* **SC-** rat infected with *S. dysenteriae* and treated with Acha fermented with *L. delbrueckii,* **SD-** rat infected with *S. dysenteriae* and treated with Acha fermented locally, **SCTrl-** rat infected with *S. dysenteriae* and without treatment, **PCTrl-** uninfected rat

3.5 Analysis of the Blood Samples of the Experimental Rats

Table 2 shows that the packed cells volume and red blood cells of the blood samples were highest in ED and SD in each of the groups of infected rats. The white Blood cells were highest in ECTrl and SCTrl as 453 and 451 respectively. The lymphocytes level also was increased in the group of rat infected with *S. dysenteriae* for SA, SB, SC, SD, and SCTrl respectively compared to rats infected with *E.coli* (64, 65, 66, 65, 68 for EA, EB, EC, ED, and ECTrl respectively.

The haematological results revealed that blood samples from the randomly selected rats from each group were less influenced by the different fermented Acha used to feed the rats (Table 2). The differences in the haematological parameters could be due to the fermented Acha, which had less effect on the haematogical components of the tested rats. Although, the neutrophils showed moderate differences, this could be attributed to not only the fermented Acha but other influences. Since neutrophils are one of the first set of white blood cell differential respond to inflammation thus their differences with difference feed type. Inflammation can be caused by bacteria infection, environmental condition, cancer

which can result in chemical signals such as interleukin-8, leukotriene B4, interferon gamma which the body responds to by recruiting immune cells such as neutrophils [10; 11 and 12].

Table 2: Haematological Analysis of Blood Samples of Experimental Rats

S/N	ESR	PCV	RBC	WBC	Hb	LYM	NEU	MON	EOS	BAS
EA	0.5	45	1374	427	15.0	64	27	6	2	1
EB	0.5	46	1416	412	15.3	65	27	5	2	1
EC	0.5	44	1376	443	14.7	66	23	8	2	1
ED	0.5	47	1489	417	15.7	65	26	6	2	1
ECTrl	0.5	43	1314	453	14.3	68	22	7	2	1
SA	0.5	44	1387	422	14.7	65	24	8	2	1
SB	1.0	40	1124	419	13.3	67	23	8	1	1
SC	0.5	47	1506	426	15.7	69	22	6	2	1
SD	0.5	49	1813	438	16.3	70	20	7	2	1
SCTrl	2.0	38	972	451	12.7	68	24	5	2	1
PCTrl	1.0	40	1146	413	13.3	69	21	7	2	1

Legend

EA- rat infected with *E.coli* and treated with Acha fermented with *L. casei,* **EB-** rat infected with *E.coli* and treated with Acha fermented with *L. acidophillus,* **EC-** rat infected with *E.coli* and treated with Acha fermented *L. delbrueckii,* **ED-** rat infected with *E.coli* and treated with Acha fermented locally, **ECTrl-** rat infected with *E. coli*and without treatment,**SA-** rat infected with *S. dysenteriae* and treated withAcha fermented with *L. casei,* **SB-** rat infected with *S. dysenteriae* and treated with Acha fermented with *L. acidophillus,* **SC-** rat infected with *S. dysenteriae* and treated with Acha fermented with *L. delbrueckii,* **SD-** rat infected with *S. dysenteriae* and treated with Acha fermented locally, **SCTrl-** rat infected with *S. dysenteriae* and without treatment,**PCTrl-** uninfected rat

ESR-Erythrocyte Sedimentation Rate**, PCV-**Packed cell volume**, RBC-**Red Blood Cell, **WBC-** White Blood Cell, **Hb-** Hemoglobin**, LYM**-Lymphocytes, **NEU-**Neutrophils,

MON-Mononcyte, **EOS-** Eosinophils**, BAS-**Basophils

3.6 Histological Examination of Small and Large Intestine of the Experimental Rats.

Plate 2a-12b shows histological examination of the small and large intestine of the experimental rats revealing, necrosis, inflammation of the cells of the mucosal lining and also the inflammatory cells in mass, distortion in the mucosa and villi. These are the effects of the infection (with both *E. coli* and *S. dysenteriae)* and the assigned the treatments (feeding the rats with fermented Acha samples).

It was observed that the intestine of the rats exhibited histological alterations such as necrotic effect of intestinal cells, distorted villi structure, distorted structure of the intestinal wall, necrotic effect of the tubular gland and distorted tubular gland. These alterations were mild. The alterations were probably due to the infection [13].

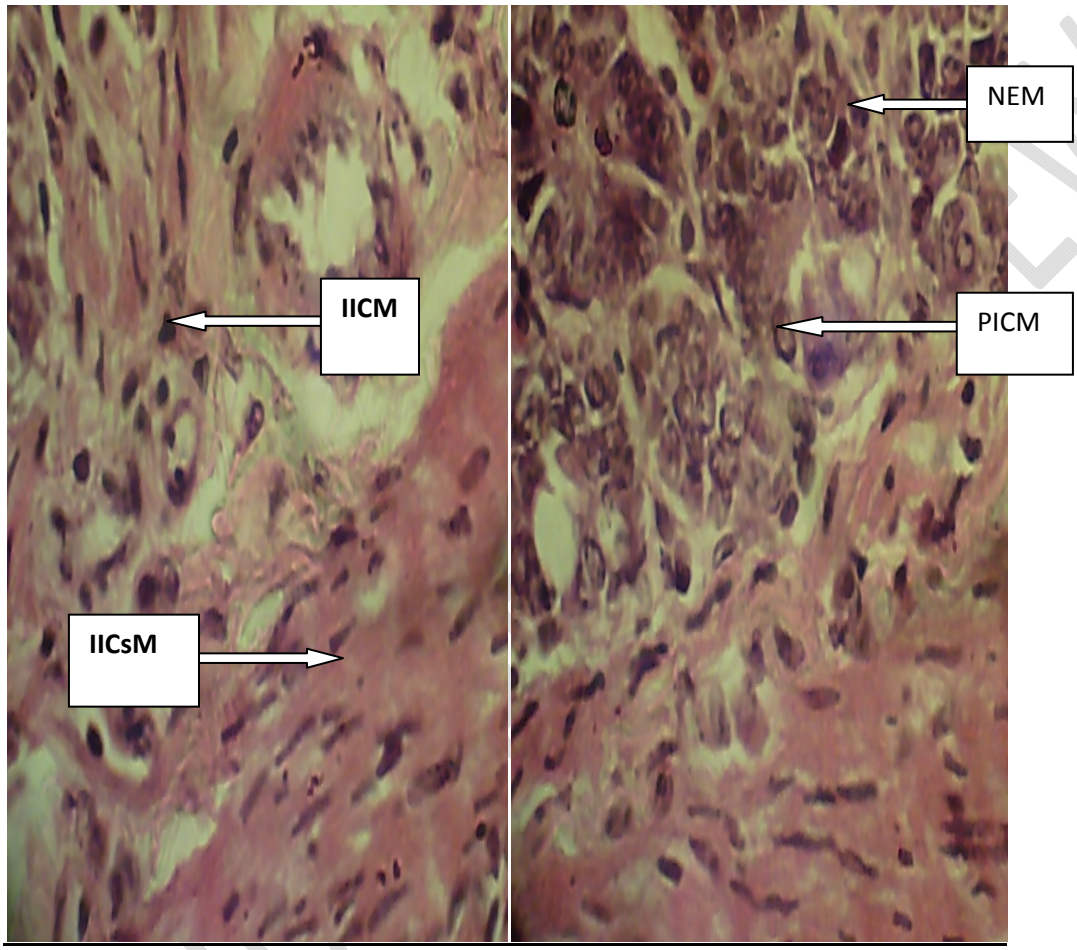
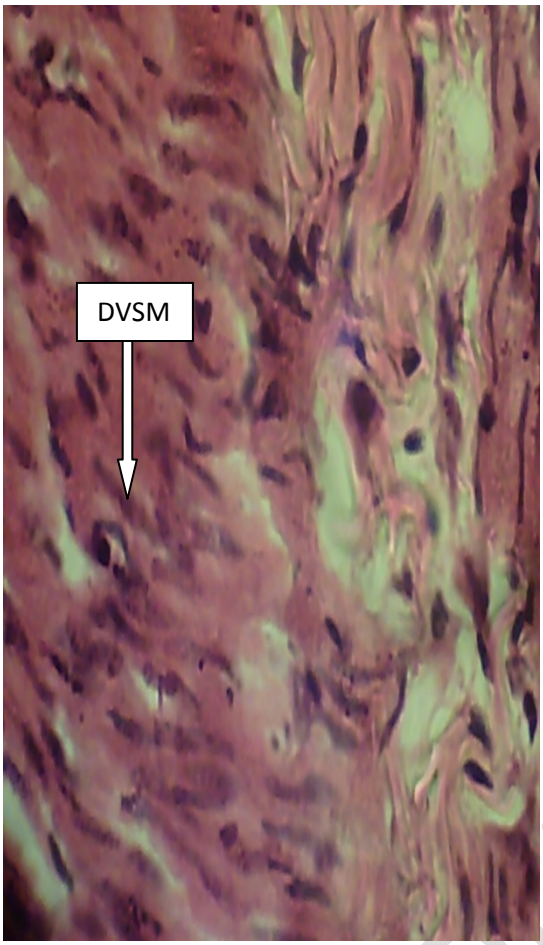


Plate 2a **Plate 2b**

Plate 2a: Increased inflammatory cell of the mucosa (IICM), increased inflammatory cell of the submucosa (IICsM)

Plate 2b: Necrotic effect of cells at the mucosa (NEM), Populated inflammatory cell at the mucosa (PICM)



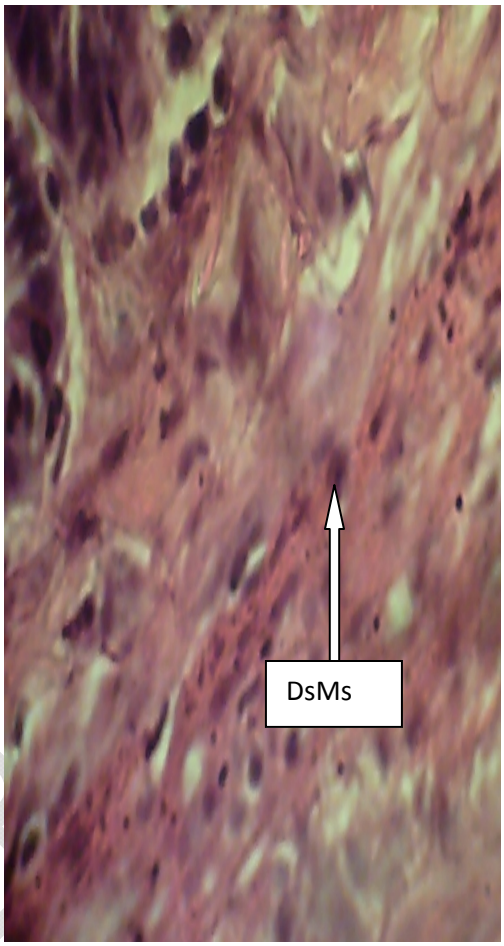
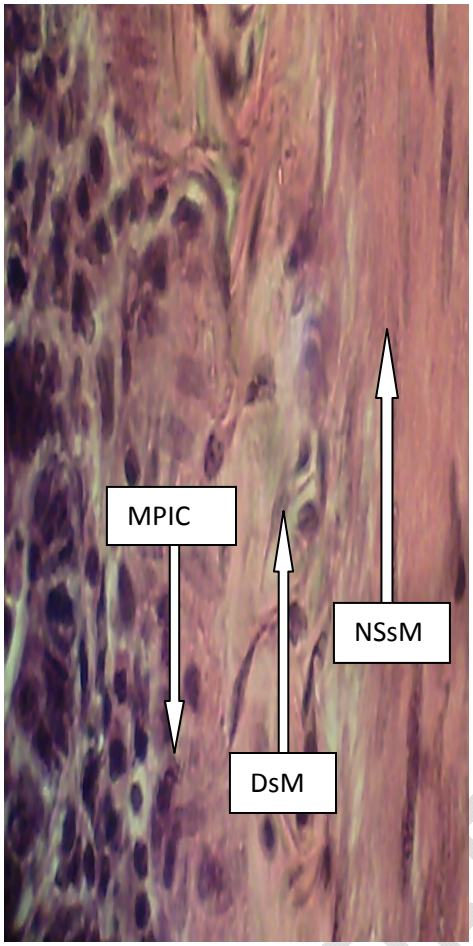


Plate 3a **Plate 3b**

Plate 3a: Distorted villi structure of the mucosa (DVSM)

Plate 3b: Distorted submucosa structure of the intestinal wall (DsMS)



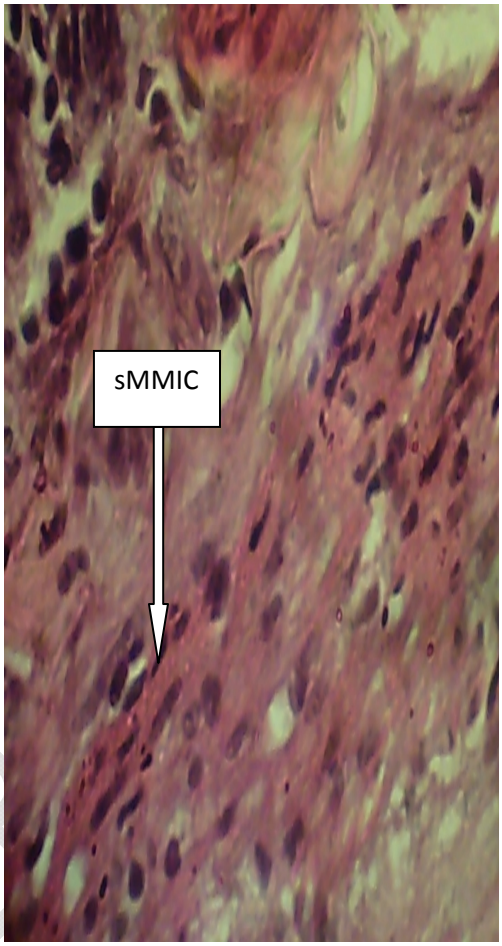
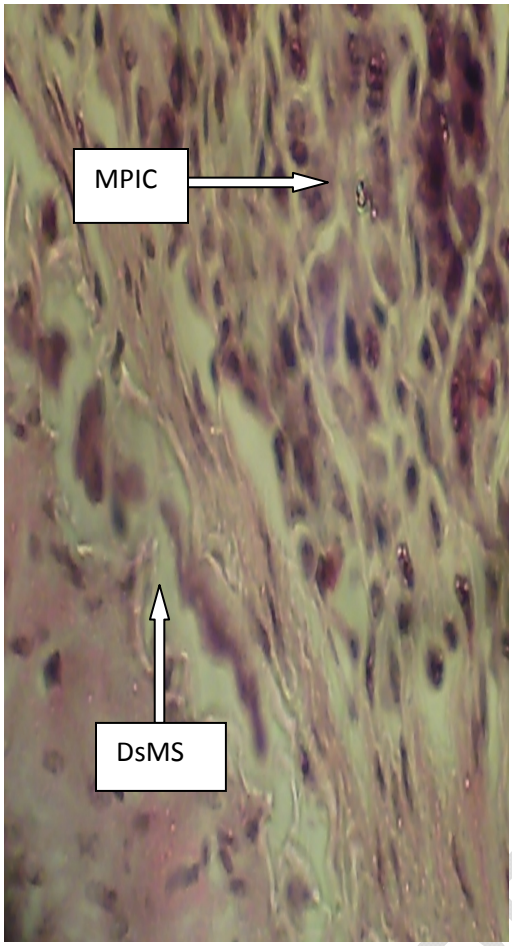


Plate 4a **Plate 4b**

Plate 4a: Mucosa with populated inflammatory cells (MIC), Normal structure of the submucosa (NSsM)

Plate 4b: Submucosa with mild inflammatory cells (sMMIC)



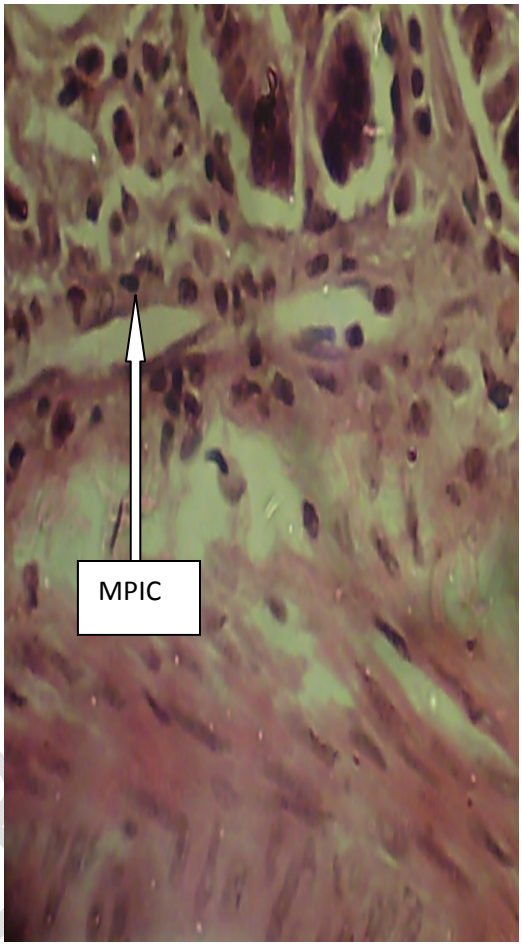
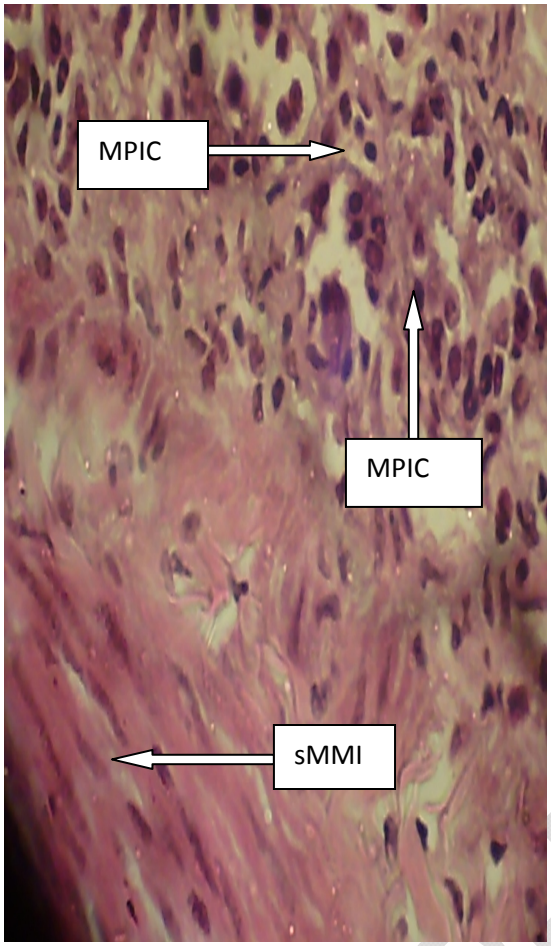


Plate 5a **Plate 5b**

Plate 5a: Mucosa with populated inflammatory cell (MPIC), distorted submucosa structure (DsMS)

Plate 5b: Submucosa with mild inflammatory cells (sMMIC)



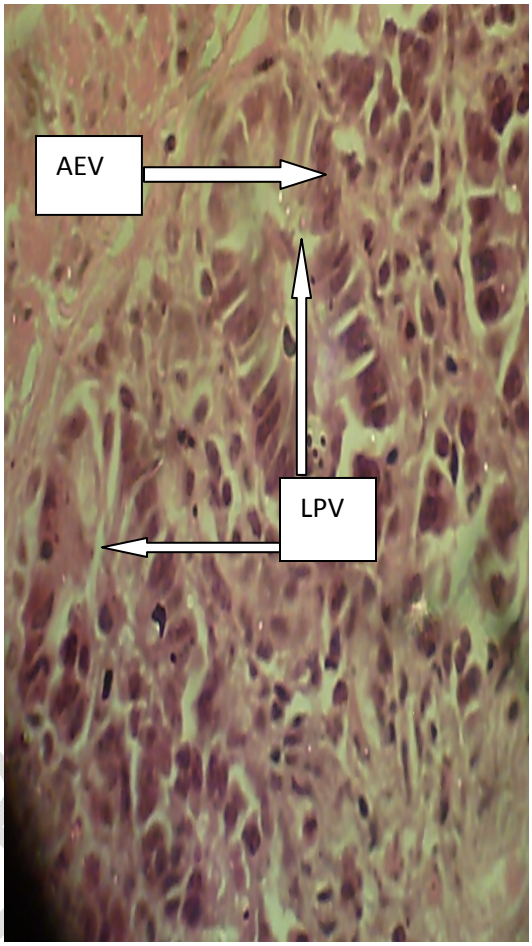
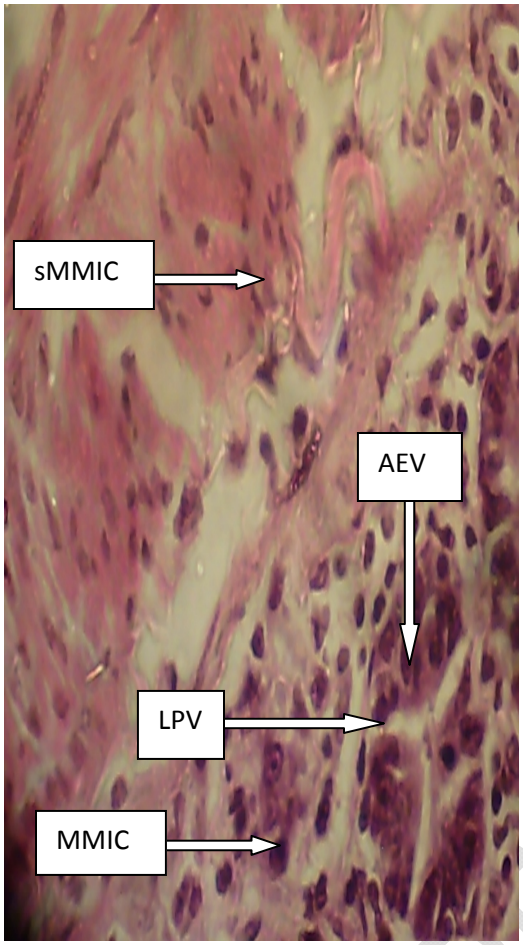


Plate 6a **Plate 6b**

Plate 6a: Mucosa with populated inflammatory cell (MPIC), submucosa with mild inflammatory cell (sMMIC)

Plate 6b: Absorptive epithelium of the villus (AEV), Lamina propria of the villus (LPV)



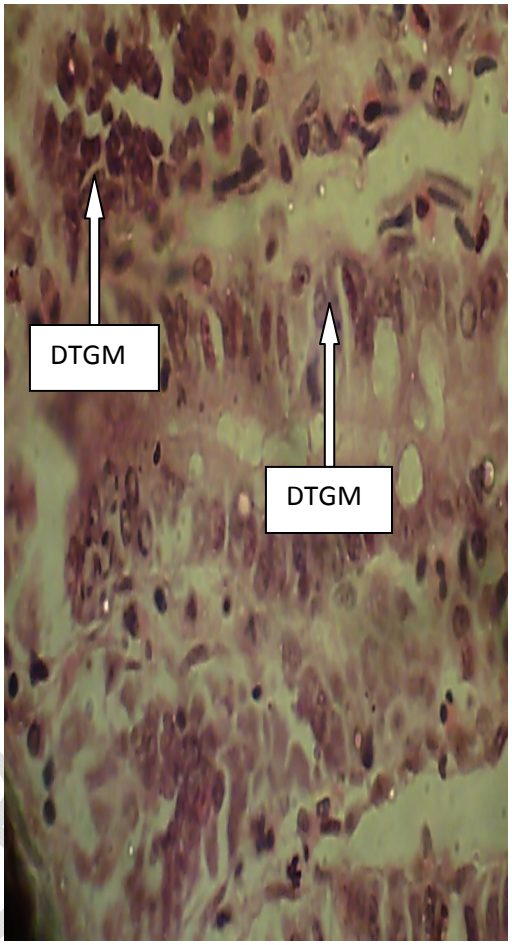
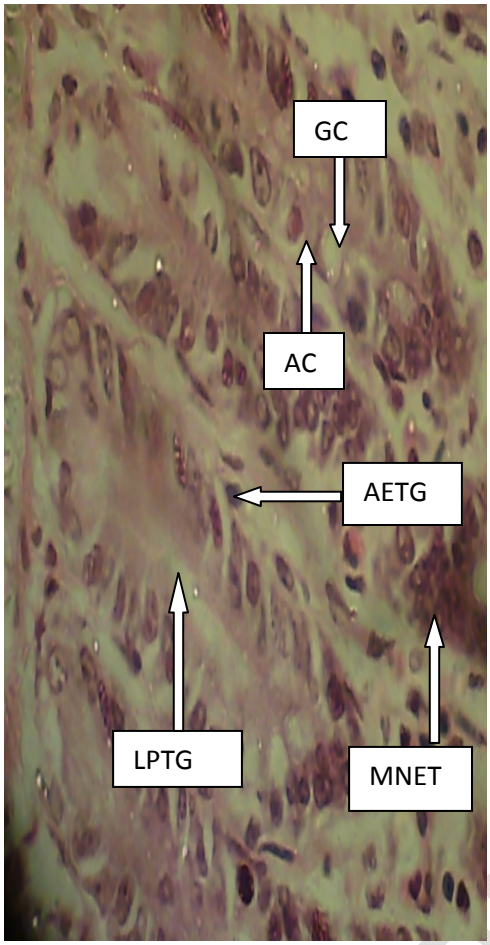


Plate 7a **Plate 7b**

Plate 7a: Submucosa with mild inflammatory cells (sMMIC), Mucosa with mild inflammatory cell (MMIC), Absorptive epithelium of the villus (AEV), Lamina propria of the villus (LPV)

Plate 7b: Distorted tubular gland of the mucosa (DTGM)



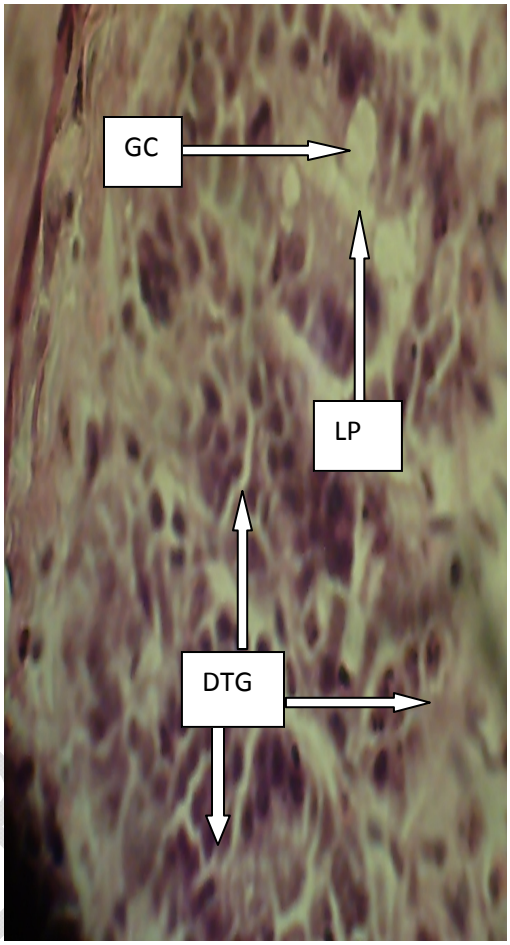
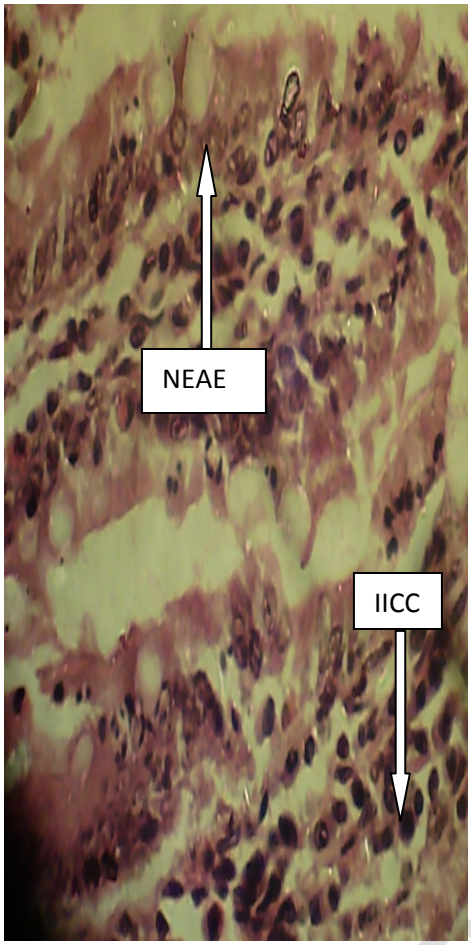


Plate 8a **Plate 8b**

Plate 8a: Goblet cell (GC), Absorptive cell (AC), Absorptive epithelium of the tubular gland (AETG), Lamina propria of the tubular gland (LPTG), Mild necrotic effect of the tubular gland (MNETG)

Plate 8b: Goblet cell (GC), Lamina propria (LP), Distorted tubular gland (DTG)



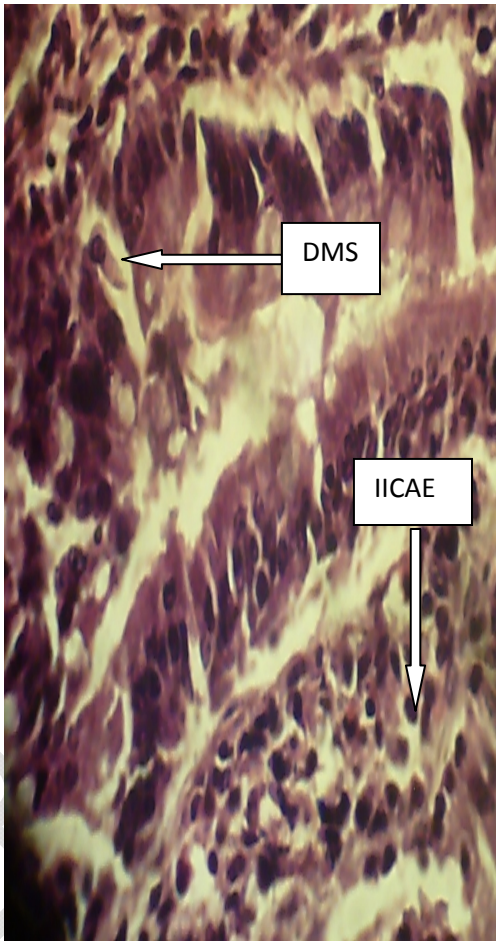
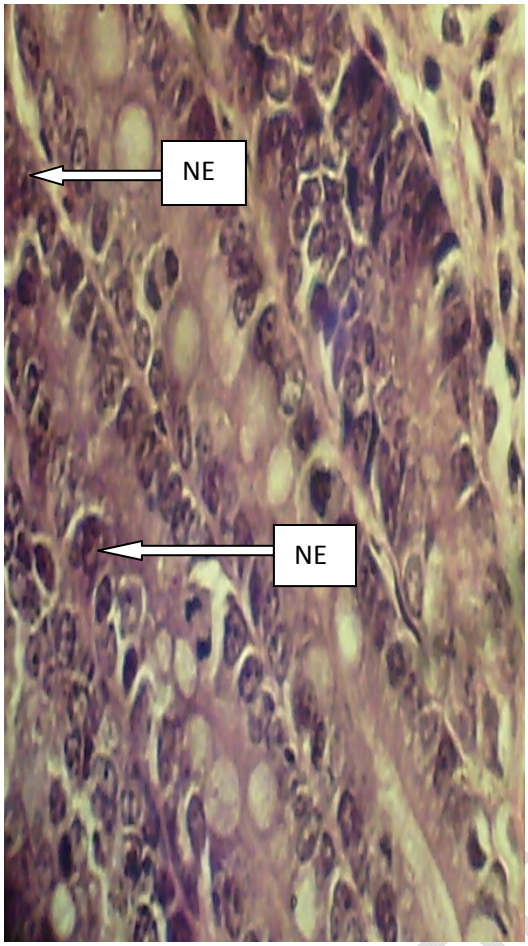


Plate 9a **Plate 9b**

Plate 9a: Necrotic effect on the absorptive epithelium of the tubular gland (NEAE), Increased inflammatory cells of the crypt (IICC)

Plate 9b: Distorted mucosa structure (DMS), Increased inflammatory cells of the absorptive epithelium (IICAE)



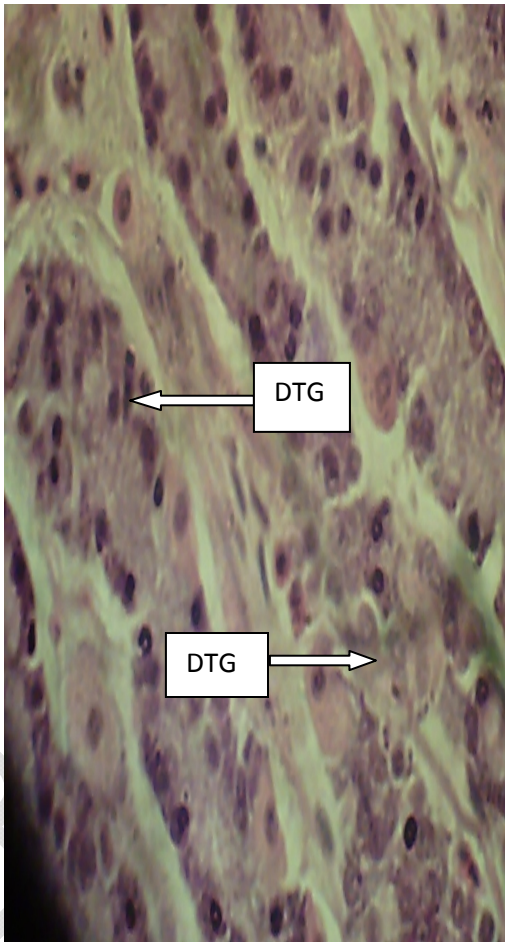


Plate 10a **Plate 10b**

Plate 10a: Necrotic effect on the absorptive epithelium of the tubular gland (NE)

Plate 10b: Distorted tubular gland (DTG)

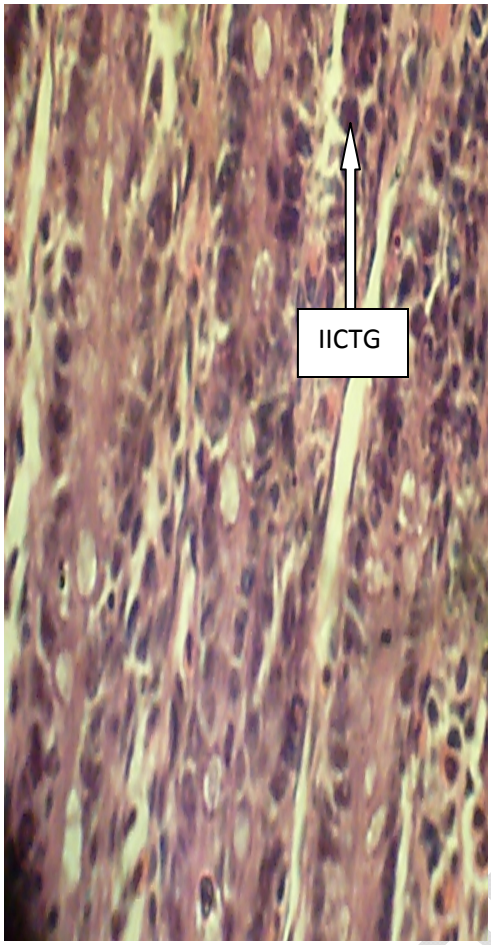
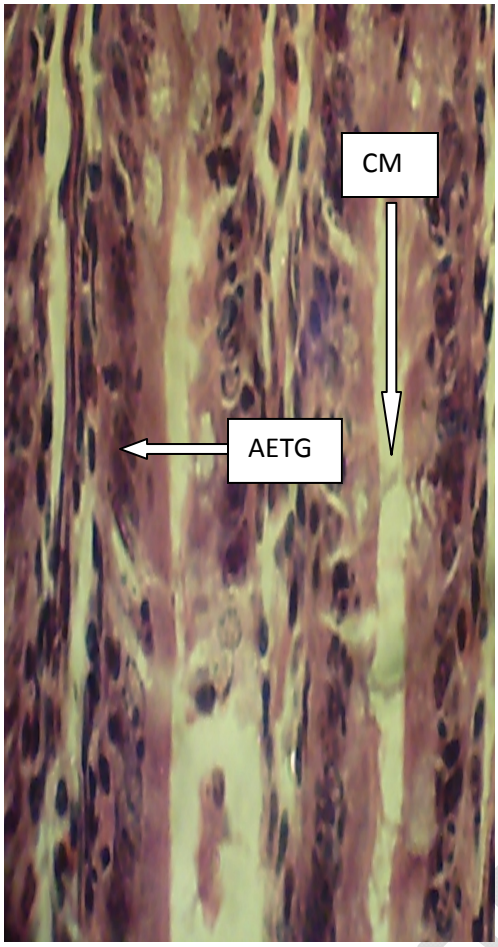


Plate 11a **Plate 11b**

Plate 11a: Increased inflammatory cell of the tubular gland (IICTG)

Plate 11b: Mild necrotic effect (MNE) on the absorptive epithelium of the tubular gland



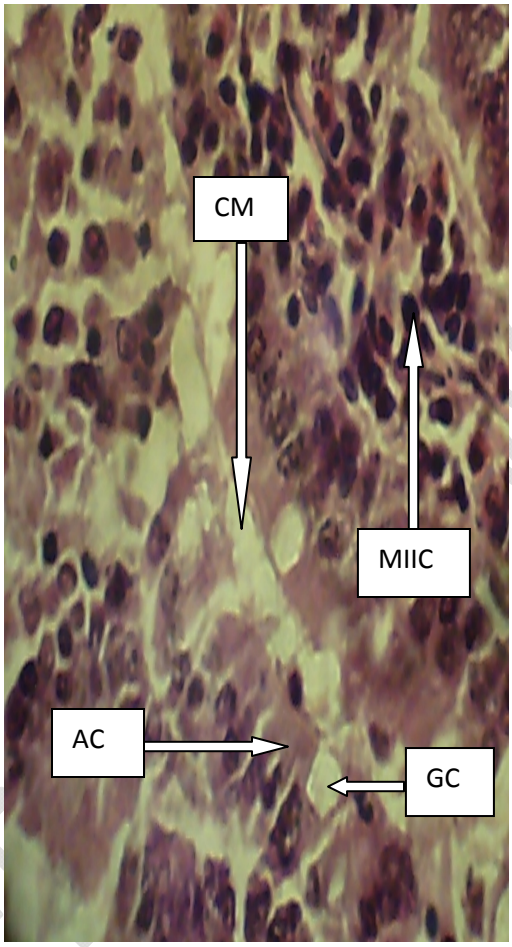


Plate 12a **Plate 12b**

Plate 12a: Crypt of the mucosa (CM), Absorptive epithelium of the tubular gland (AETG)

Plate 12b: Crypt of the mucosa (CM), Mild increased inflammatory cell (MIIC) of the tubular gland, Goblet cell (GC), Absorptive cell (AC)

4.0 Conclusion

This study shows that Acha is a type of food which can be used for probiotic purpose because of the microbial content especially the *Lactobacillus* spp.

The health benefits of wholegrain cereal products are now widely recognized and considered to result from the presence of a range of nutritional components, including dietary fiber and protein. Hence, Acha can help millions in sub-Sahara Africa especially in weaning. Also, Acha can become a staple food because it is rich in carbohydrate and it serves as probiotic when fermented.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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