# 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 100 g and water of 1 litre 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 21 days, 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 flavors, 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].

Probiotics have been used in fermented food products for centuries. However, nowadays it has been claimed that probiotics can serve a dual function by their potentially importing health benefits. The health benefit of fermented foods may be further enhanced by supplementation of Lactobacillus and Bifidobacterium species [3]. L. acidophilus, Bifidobacterium spp. and L. casei speciesare the most used probiotic cultures with established human health in dairy products, whereas the yeast Saccharomyces cerevisiae and some E. coli and Bacillus species are also used as probiotics [4].

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 [5].

All over the world, diarrhoea is a serious health problem especially in children [6]. 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 100 g and water of 1 litre 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 72 hours. 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. 100 g of Acha grains in 400 ml 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 15 minutes. 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 72 hours. 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 (5 ml each) sterile MRS Broth (pH 5.5) and incubated at 45$^{o}$C for 48 hours under microaerophilic conditions. This culture was centrifuged at 10000 g for 15 minutes. The pellet was rinsed out three times with 10 ml 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 1 ml of the stock culture of each isolate into 9 ml sterile distilled water in test tubes to give a dilution of $10^{-1}$. Using a fresh pipette, 1 ml of $10^{-1}$ was pipetted into another test tube containing 9 ml 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 48 hours. The total number of colonies were then counted and recorded.

**2.5 Evaluation of the effect of Acha fermented samples on albino rats**

**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 [7].

***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, 0.1 ml 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 0.5 ml of $10^{-5}$ cfu/ml. For *Shigella dysenteria*; SA, SB, SC, SD and SCTrl infected with 0.2 ml 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 2 hours, 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 [8].

### 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.*as 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 [9].

### 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.

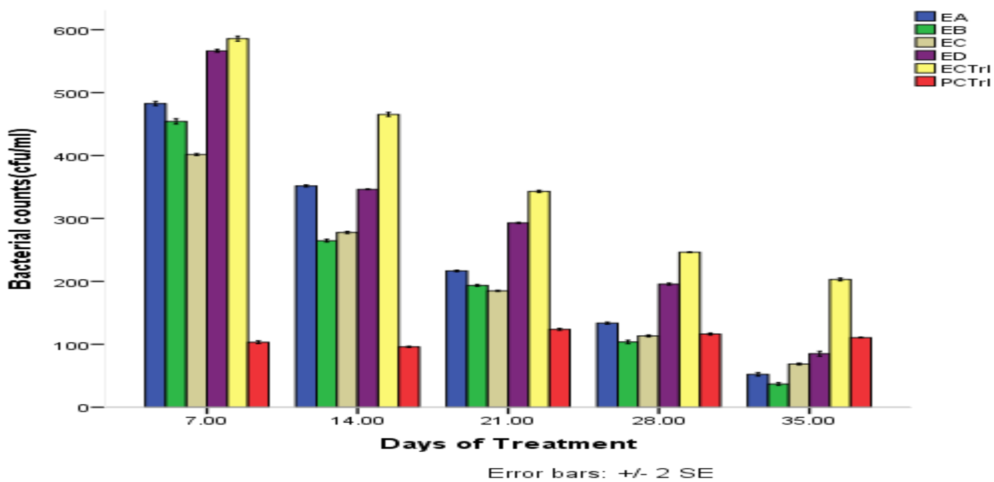



**Figure 1: Bacterial Count of Feacal Samples of Rats Infected with *E. coli* during Treatment**

**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

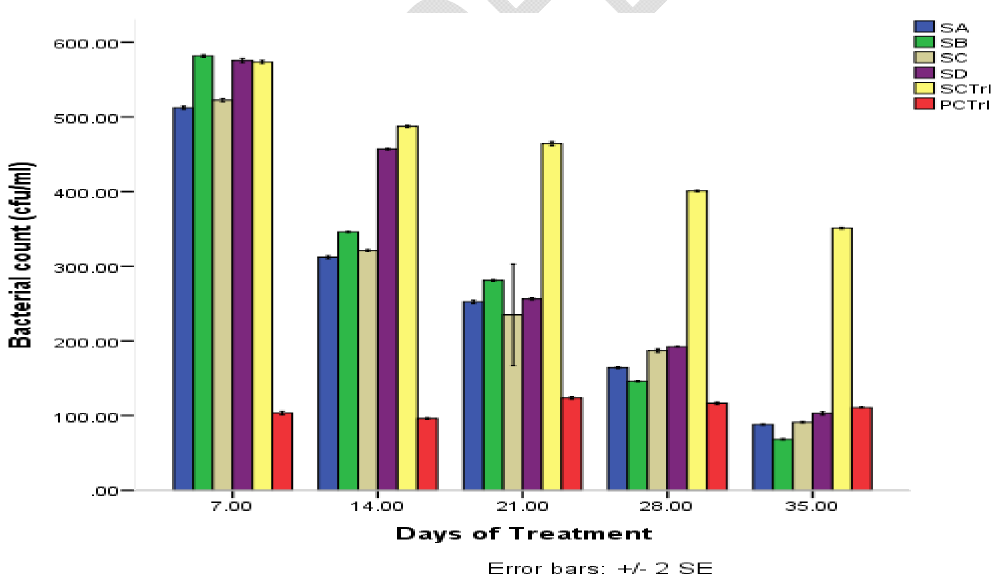



**Figure 2: Bacterial Count of Feacal Samples of Rats Infected with *Shigella dysenteria* during Treatment**

**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

Plate 1 to 4 show the Feacal samples of rat infected with *S. dysenteria,* while the feacal sample of recovered rat infected with *S. dysenteria,* Feacal sample of a rat infected with *E. coli* and the feacal sample of a recovered rat infected with *E. coli.*

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 [10; 11]. 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.

**Plate 1**

**Plate 2**

**Plate 3**

**Plate 4**

**Legend**

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

Plate 2: Feacal sample of a recovered rat infected with *S. dysenteriae* (Black, short and hard) during *in vivo* feeding trial

Plate 3: Feacal sample of a rat infected with *E. coli* (Brown, Long and Moist) during *in vivo* feeding trial

Plate 4: Feacal sample of a recovered rat infected with *E. coli* (Brown and Hard) during *in vivo* feeding trial

Table 1 also shows the colour changes and the features in the feaces of the experimental rats. 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.

**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.

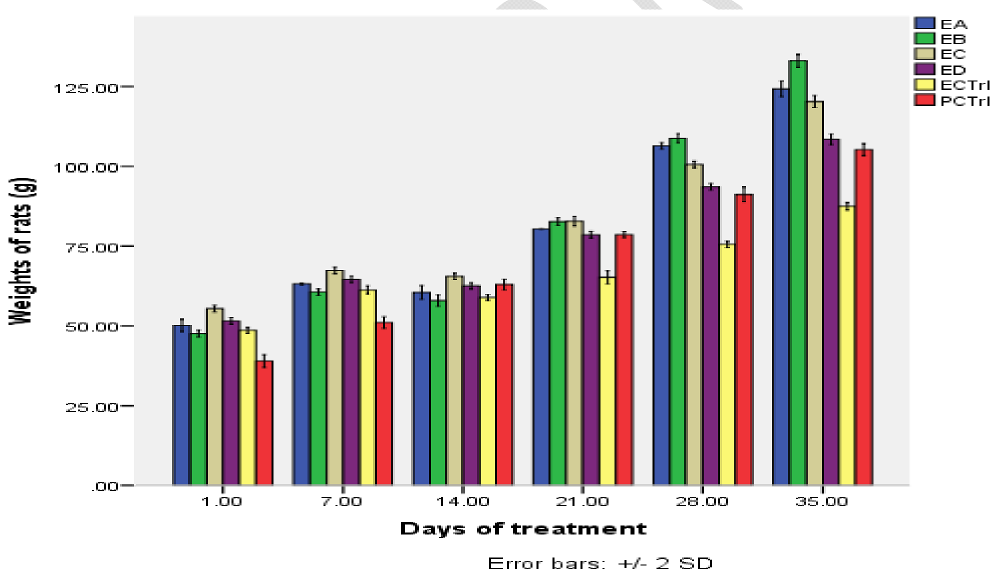



**Figure 3: Weights of the Experimental Animals infected with *E. coli* during *in vivo* Feeding Trials**
**Legend: EA-** rat infected with *E.coli* and treated with Acha fermented with *L. casei,* **EB-** rat infected with *E.coli* and treated with Acha fermented with *L. acidophillus,* **EC-** rat 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 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. This is probably due to improved nourishment of the rat by fermented Acha.

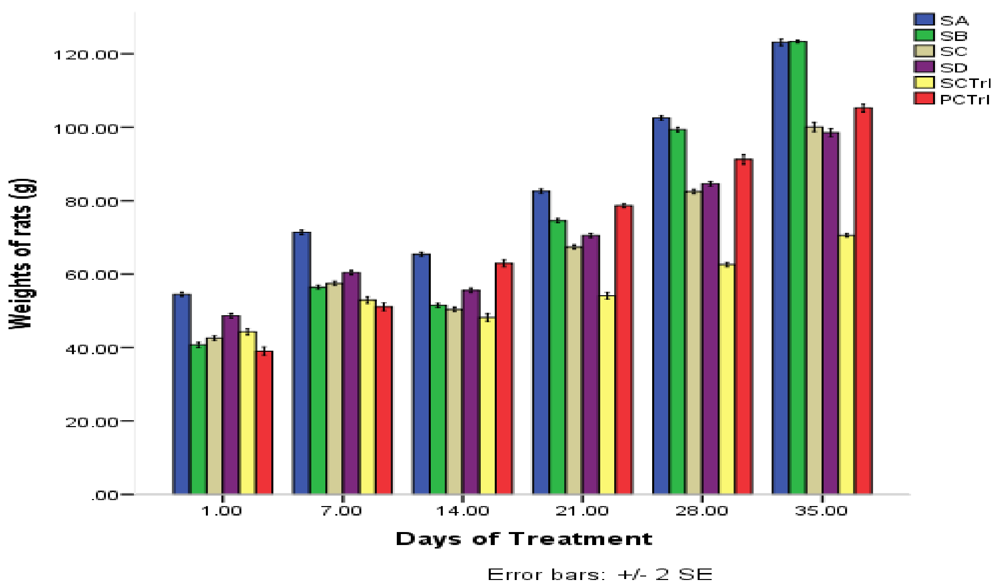



**Figure 4: Weights of the Experimental Animals Infected with *Shigella dysenteriae* during *invivo* Feeding Trials**

**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 [12; 13 and 14].

**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 5- 26 show the histological examination of the small and large intestine of the experimental rats infected with *E. coli*, *S. dysenteriae* and the assigned treatments (rats fed 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 [15].

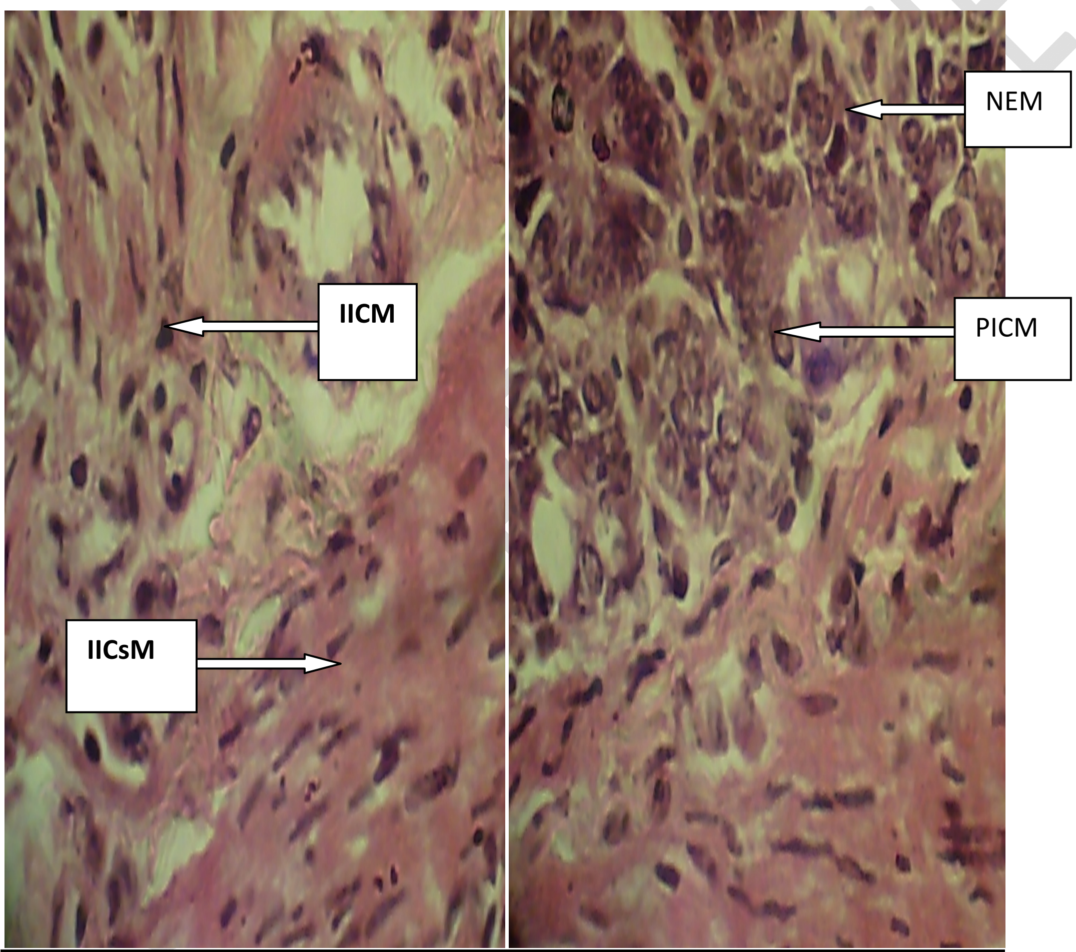
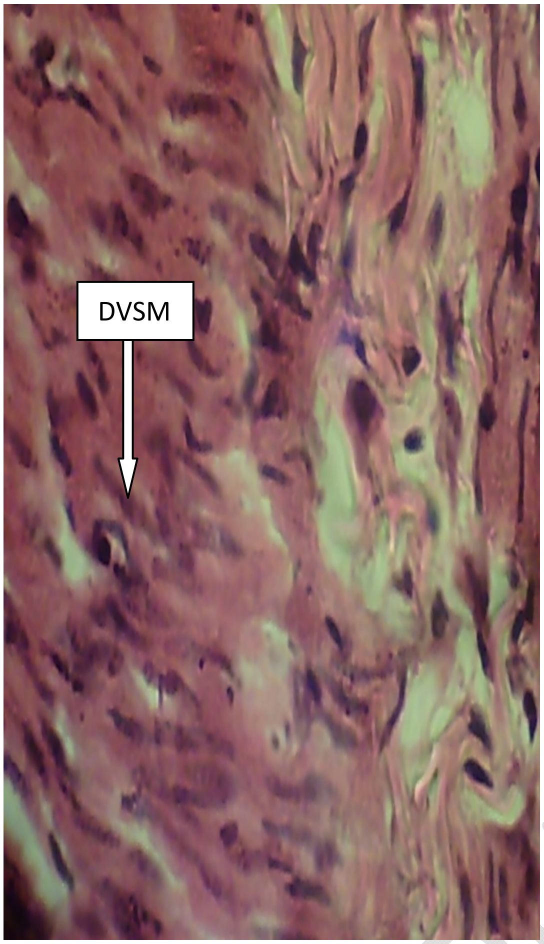



**Plate 5** **Plate 6**

**Plate 5:** Increased inflammatory cell of the mucosa (IICM), increased inflammatory cell of the submucosa (IICsM)

**Plate 6:** Necrotic effect of cells at the mucosa (NEM), Populated inflammatory cell at the mucosa (PICM)



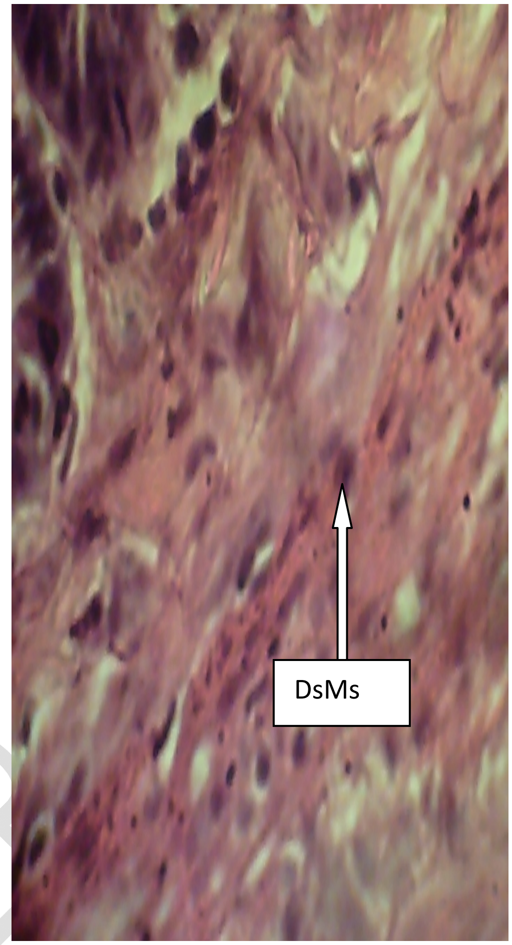
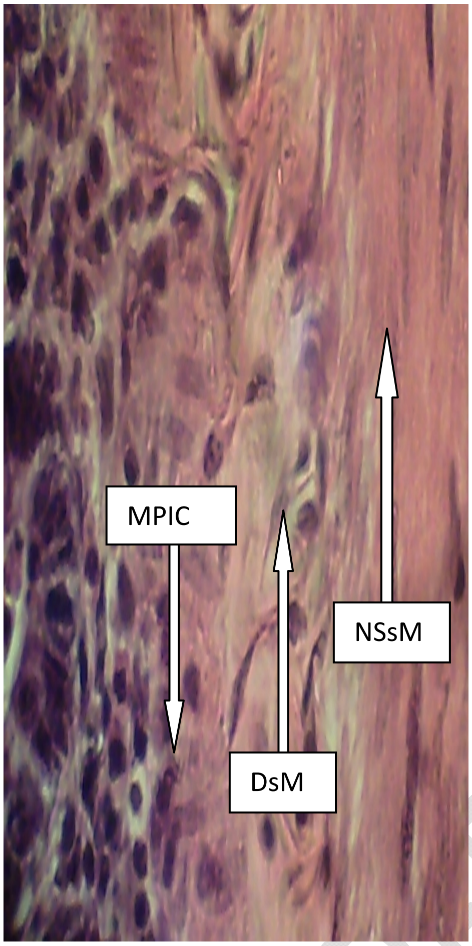




**Plate 7** **Plate 8**

**Plate 7:** Distorted villi structure of the mucosa (DVSM)

**Plate 8:** Distorted submucosa structure of the intestinal wall (DsMS)



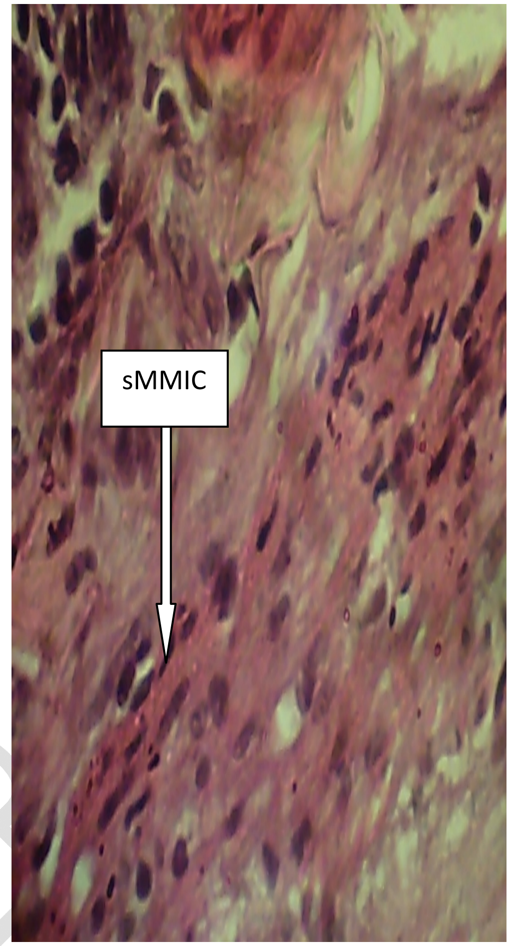
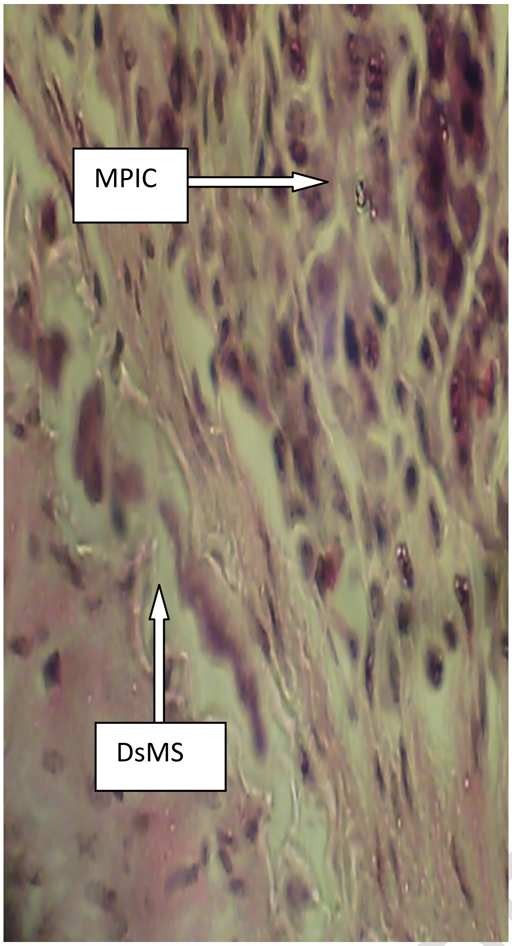




**Plate 9** **Plate 10**

**Plate 9:** Mucosa with populated inflammatory cells (MIC), Normal structure of the submucosa (NSsM)

**Plate 10:** Submucosa with mild inflammatory cells (sMMIC)



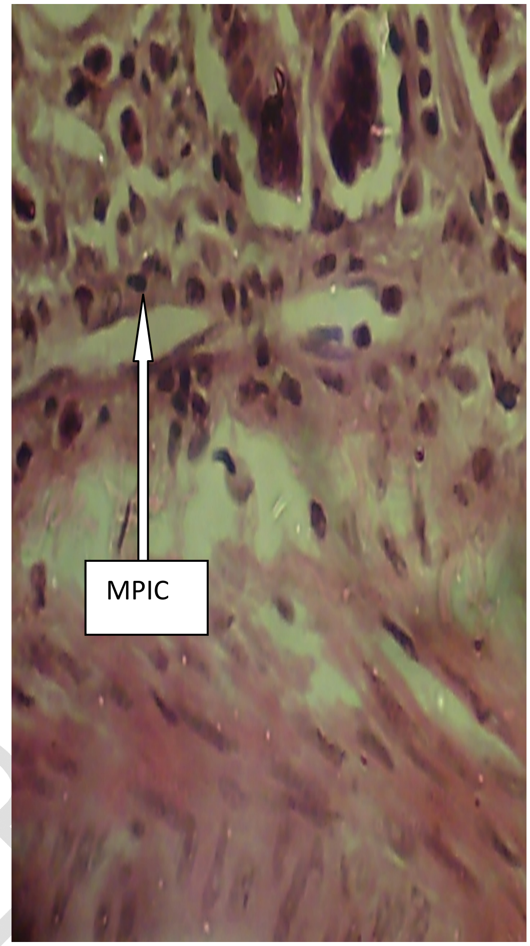
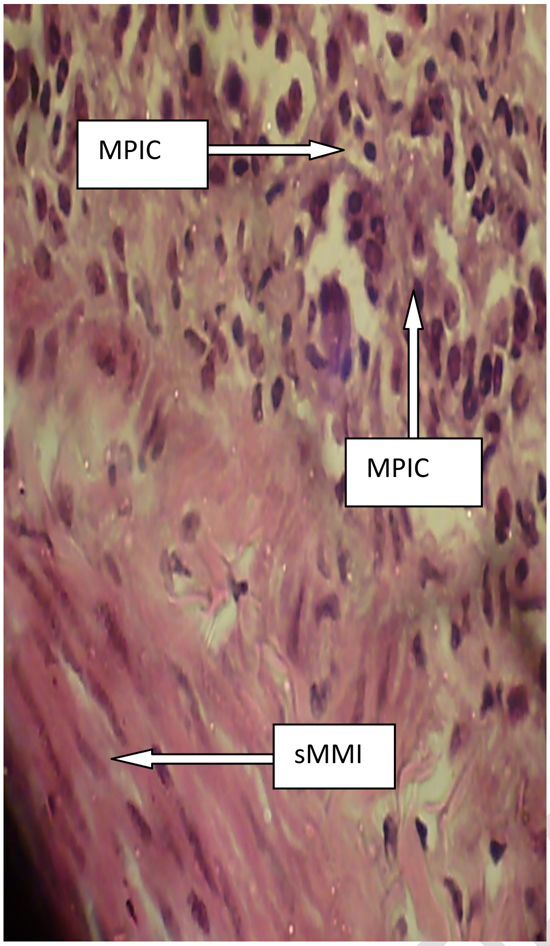




**Plate 11** **Plate 12**

**Plate 11:** Mucosa with populated inflammatory cell (MPIC), distorted submucosa structure (DsMS)

**Plate 12:** Submucosa with mild inflammatory cells (sMMIC)



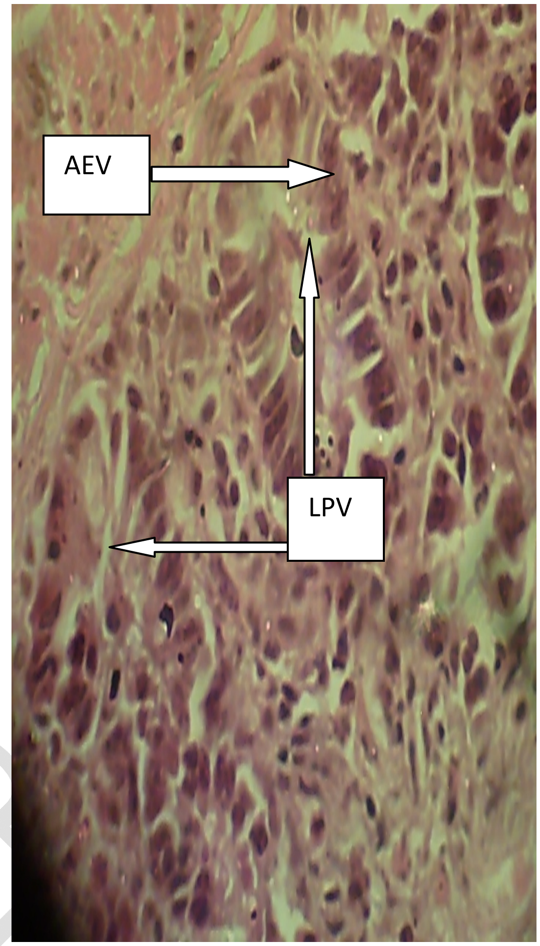
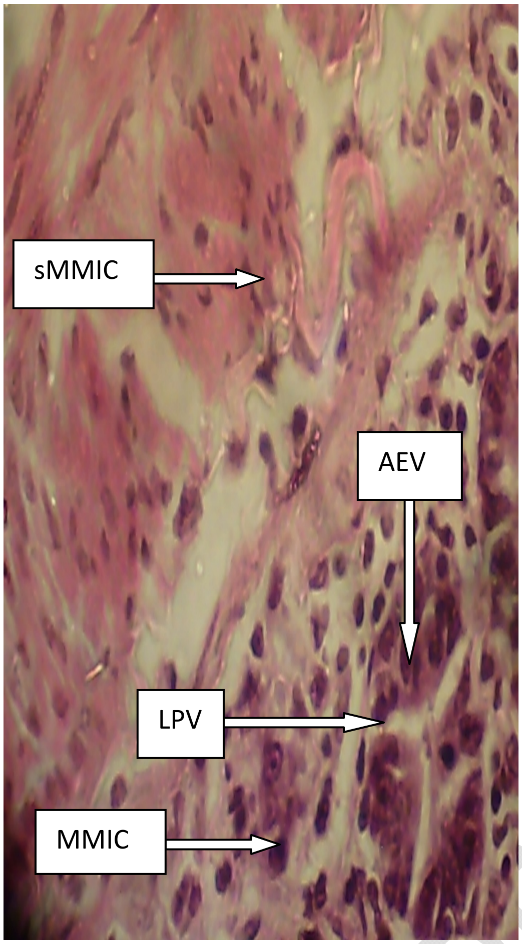




**Plate 13** **Plate 14**

**Plate 13:** Mucosa with populated inflammatory cell (MPIC), submucosa with mild inflammatory cell (sMMIC)

**Plate 14:** Absorptive epithelium of the villus (AEV), Lamina propria of the villus (LPV)



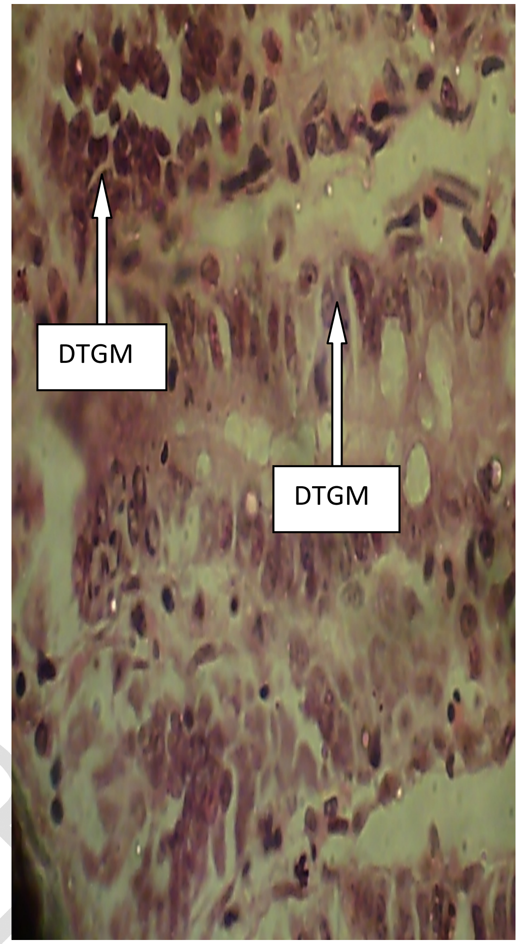




**Plate 15** **Plate 16**

**Plate 15:** 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 16:** Distorted tubular gland of the mucosa (DTGM)

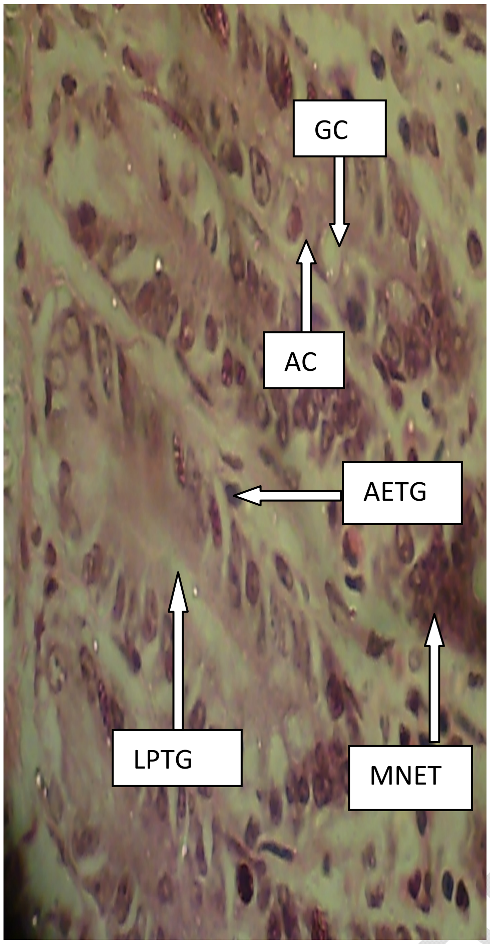



**Plate 17**

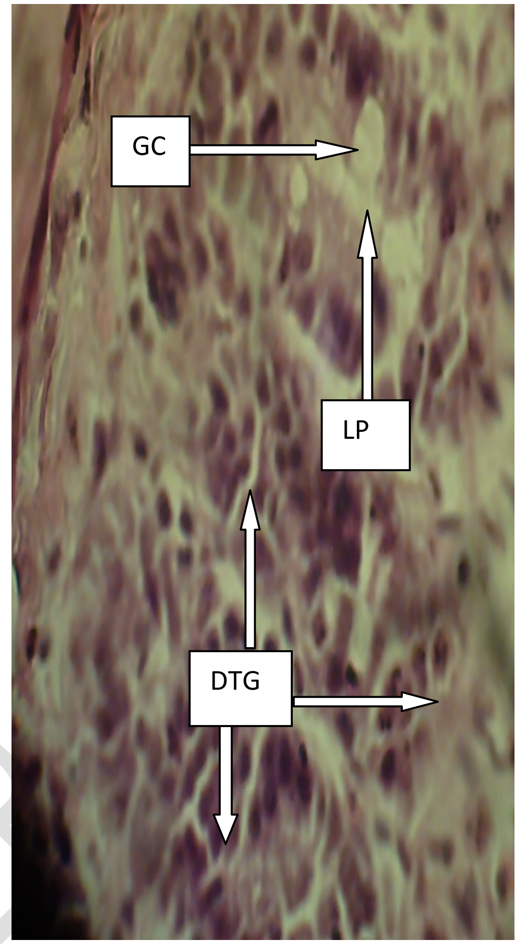
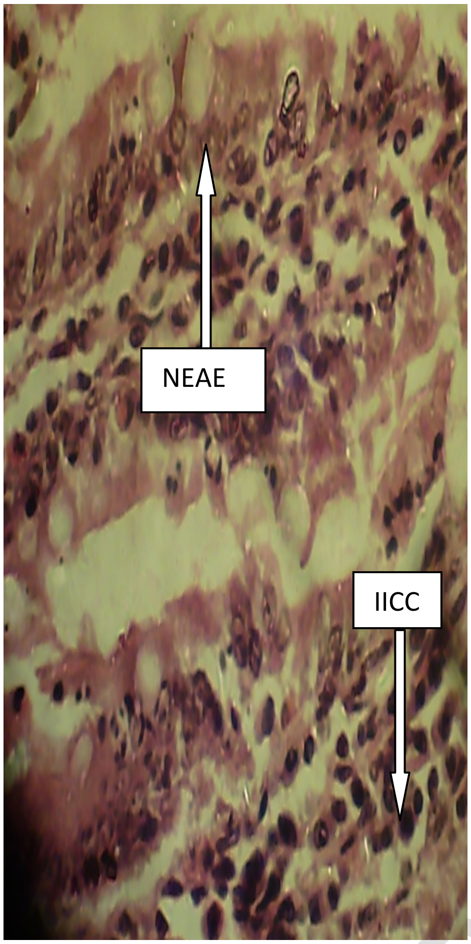



**Plate 18**

**Plate 17:** 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 18:** Goblet cell (GC), Lamina propria (LP), Distorted tubular gland (DTG)



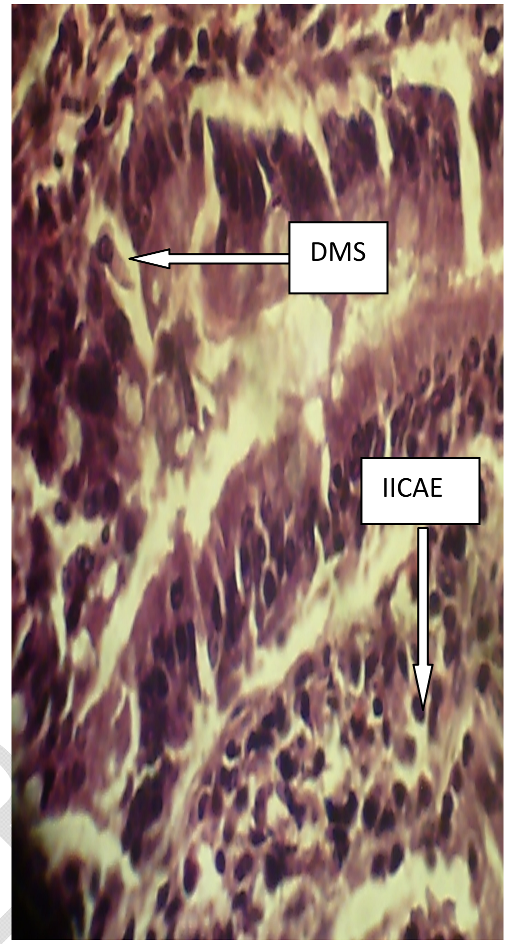
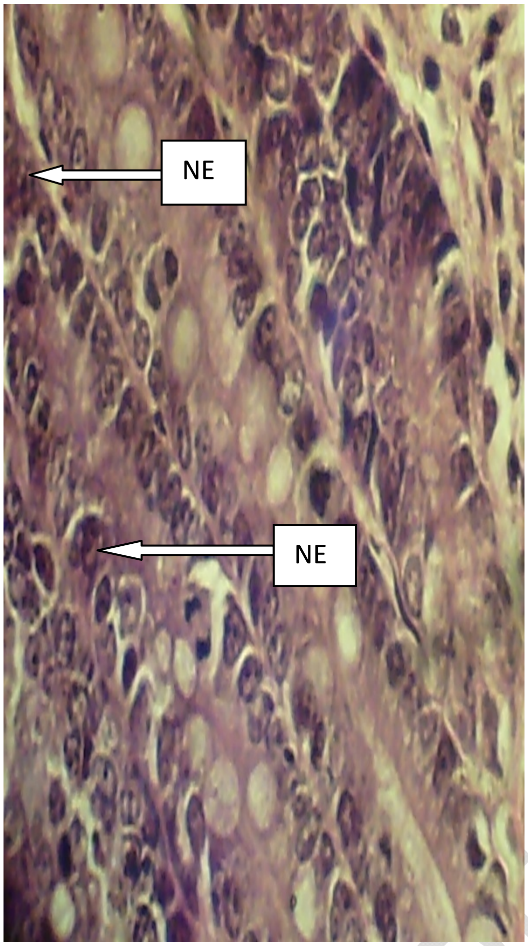




**Plate 19** **Plate 20**

**Plate 19:** Necrotic effect on the absorptive epithelium of the tubular gland (NEAE), Increased inflammatory cells of the crypt (IICC)

**Plate 10:** Distorted mucosa structure (DMS), Increased inflammatory cells of the absorptive epithelium (IICAE)



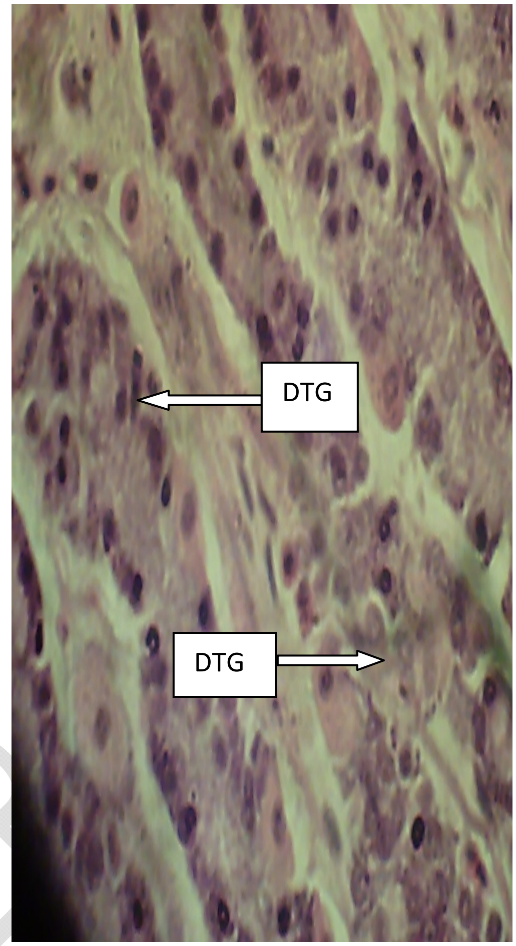




**Plate 21** **Plate 22**

**Plate 21:** Necrotic effect on the absorptive epithelium of the tubular gland (NE)

**Plate 22:** Distorted tubular gland (DTG)

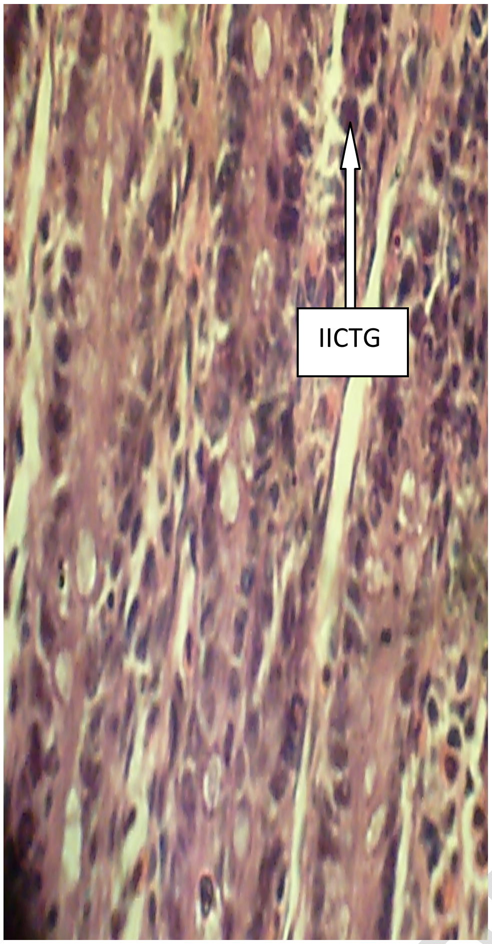
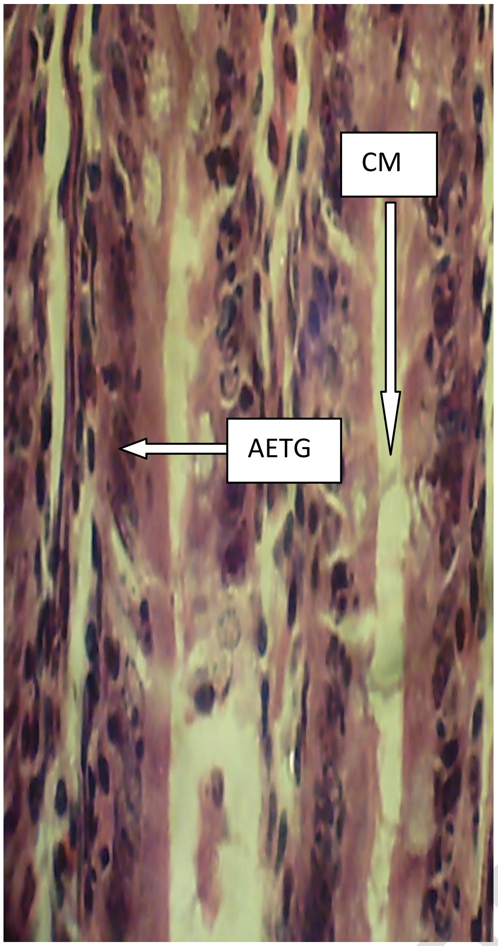




**Plate 23** **Plate 24**

**Plate 23:** Increased inflammatory cell of the tubular gland (IICTG)

**Plate 24:** Mild necrotic effect (MNE) on the absorptive epithelium of the tubular gland



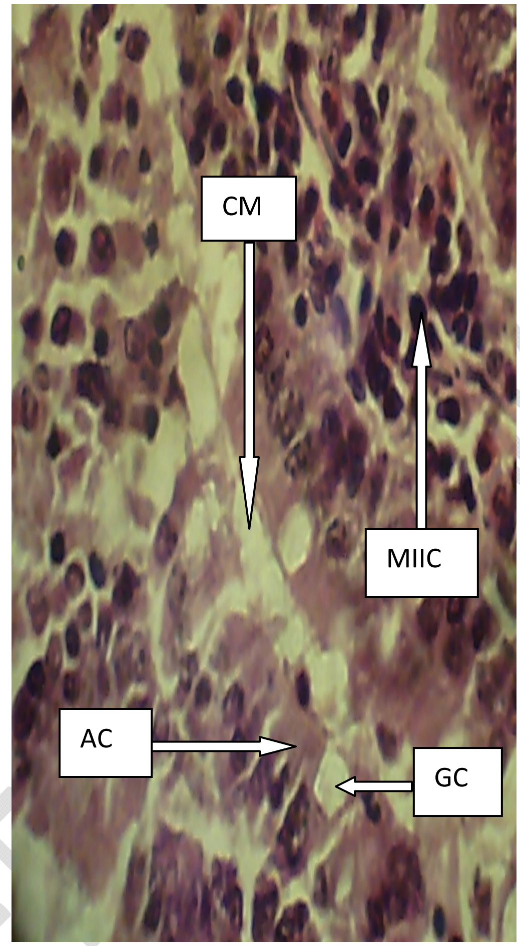




**Plate 25** **Plate 26**

**Plate 25:** Crypt of the mucosa (CM), Absorptive epithelium of the tubular gland (AETG)

**Plate 26:** 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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