

Effects of Fermented Maize Gruel (*Ogi*) on the Haemato-biochemical Profile of Wistar Albino Rats Challenged with *Shigella Dysenteriae* JBA 010

Aderiye, B. I. & David, O. M.

Department of Microbiology, Ekiti State University, Ado-Ekiti, Nigeria

E-mail: jadesolaaderiye@yahoo.com

Received: Feb. 6, 2013

Accepted: April 12, 2013

Published: June 1, 2013

doi:10.5296/jfs.v2i1.3229

URL: <http://dx.doi.org/10.5296/jfs.v2i1.3229>

Abstract

The ability of fermented foods to inhibit the growth of human pathogenic bacteria has been reported. Effect of *ogi* (fermented maize gruel) against pathogenic strains of *Shigella dysenteriae* was investigated (*in vivo*) using standard chemical and microbiological methods. The proximate composition of commercial feeds showed higher amount of total solid (85.82 %), crude protein (16.31 g/100g) and metabolizable energy (1355.09 KJ) than the *ogi* diet. However, *ogi* had higher concentrations of Fe, Ca and Mg than the commercial feeds. The commercial feed (CF) supported the growth of the animals better than *ogi*. The Packed cell volume (PCV) of all the animals ranged from 20.00 to 26.00 % while the challenged animals had lower WBC count than the infected animals. After 4 days of the experiment there was a decrease in the total bacterial count of the faecal materials of the animals compared to initial count, however, the difference was not significant at $P < 0.05$. Infected animals fed with commercial feeds had the highest microbial load when plated on *Salmonella-Shigella* agar. *Ogi* did not encourage the multiplication of *Shigella dysenteriae* JBA 010 in the animals. Compared to the control and the commercial feed, *ogi* offered a pronounced protection against the infection of the pathogen.

Keywords: Maize gruel, *ogi*, Haemato-biochemical, *Shigella dysenteriae*, Proximate composition, Diarrhoea

Introduction

In the recent times resistance of bacterial pathogens to most antibacterial agents to which they are previously susceptible is on increase (Oli et al., 2012; Hart & Kariuki, 1998). Effective drugs are not within the reach of the common people especially in the resource poor nations of the world. The search for cheap alternative to antimicrobial substances in nature becomes inevitable.

Diarrhoea is the passage of unusually loose or watery stools, usually at least 3 times in a 24 h period (Huppertz, 1986). It is one of the leading causes of death in young children in developing countries; under five years of age (Parashar et al., 2003). Though caused by many other aetiological factors *Shigella* is a major bacterial aetiological agent.

‘*Ogi*’ is a popular fermented product in Western part of Africa. Apart from being a staple food it is used for weaning toddlers (Akinrele, 1990; Odunfa & Adeyele, 1985). Fermentation of *ogi* is usually done by lactic acid bacteria most of which have been reported to possess probiotic properties which when administered in adequate amounts confer a health benefit on the host (Agaliya & Jeevaratnam, 2012; Aderiye & Laleye, 2003; Ogunbanwo et al., 2003; Odunfa, 1985). Pathogenic bacteria have been reported to be inhibited by probiotic organisms. The ability of *ogi* to inhibit the growth of bacterial aetiological agent of diarrhea *in vitro* has been reported (Aderiye & David, 2013) however *in vivo* prebiotic & probiotic activities of *ogi* on *Shigella dysenteriae* were investigated in this study.

Materials and Methods

Preparation of *ogi* slurry and source of commercial feed

Grains of white maize variety (*Zea mays*) were bought at Oja Oba Market in Ado-Ekiti, Nigeria. Grit, dirt and decomposing grains were removed. Two hundred gram of the sorted and washed cereal grains was weighed into sterile small plastic pails with cover containing 300ml water and steeped for 72 h at $28 \pm 2^\circ\text{C}$. After steeping, the water was decanted and the grains were wet-milled. The filtrates were collected into different sterile containers for secondary fermentation for another 72 h by chance inoculants. After the secondary fermentation, the supernatant was decanted and the residue was pressed to further reduce the water contents. The commercial feed used in this study was purchased from Vetromet, a veterinary shop in Ado-Ekiti, Nigeria.

Proximate analyses and mineral composition of feed samples

Ash, crude fat, crude fibre and moisture contents of the samples were determined according to AOAC (2005). Crude protein was determined as described by Pearson (1976) while carbohydrate was determined by difference. All determinations were performed in duplicates.

Determination of Energy Value

The energy values were calculated by adding up the triplicate values obtained for carbohydrates (x 17 kJ), crude protein (x 17 kJ) and crude fat (x 37 kJ) for each of the samples (Kilgour, 1987).

Source of experimental rats

Twenty Albino Wistar male rats were obtained from the Pre-clinical Animal House, University of Ibadan, Nigeria. The animals were acclimatized for five days and fed with grower's mash (TopFeed®) and adequately supplied with distilled water *ad libitum*. The rats were housed in stainless steel cages in a well-ventilated room at about $27 \pm 2^\circ\text{C}$. Lighting regimen was about 13:11 h of light and dark, respectively. The animals were randomly assigned (in cages) into four groups: Commercial feed infected (CFI), Commercial feed non-infected (CFNI), *ogi* infected (*OGI*) and *ogi* non-infected (*OGNI*). All animal management and experimental procedures were performed in strict accordance with the requirements of the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 2011).

Feeding and *Shigella dysenteriae*-challenge experiment

A single *S. dysenteriae* JBA 010 colony from an overnight *Salmonella-Shigella* agar plate culture was inoculated into 50 ml of Nutrient Broth (Oxoid) and the culture was incubated at 37°C for 18 h. The stationary-phase cells were harvested by centrifugation at 2,700 rpm (model TJ-6 centrifuge; Beckman Instruments, Palo Alto, Calif.) for 5 min and washed twice with sterile deionized water. The cell pellet was suspended in distilled water and diluted to give a final concentration of 6.0×10^8 CFU/ml. The organism was orally administered by gavages (100 μl /rat, 4×10^8 cells of *S. dysenteriae* JBA 010) in all four groups of rats to study the survivability and efficacy of *ogi* in protecting the rats against the pathogen.

Determination of the faecal bioburden

The total microbial load, coliform and *S. dysenteriae* was determined in the faeces of both challenged experimental animals and the control using the viable count method. The faeces of the challenged and the control rats were collected, serially diluted and plated on Plate Count, MacConkey and *Salmonella-Shigella* agar media and incubated at 37°C for 24 h.

Determination of haematological parameters

The haematological analyses of the blood samples were carried out using the routinely available clinical methods (Bush, 1975). The haematological indices examined were packed cell volume (PCV), haemoglobin (Hb) and white blood cell (WBC) count (Schalm et al., 1975; Jain, 1986). While the Randox® kits were used to determine the level of calcium and iron in the blood.

Results and Discussion

The proximate composition and mineral analyses of *ogi* and commercial feed is shown in Table 1. Commercial feeds had higher amounts of total solid (85.82 %), crude protein (16.31 g/100g) and metabolizable energy (1355.09 KJ) than the *ogi*. Though *ogi* diet had high amount of carbohydrate (73.07 g/100g), crude fibre was not detected it. *Ogi* was better in Fe, Ca and Mg than the commercial feeds while Na and K values were higher in commercial feed. The Na/K values were 3.36 and 2.01 in commercial feed and *ogi* respectively. The four diet groups produced different performances in the animals as shown in Table 2. Infected animals

gained less weight compared to the uninfected animals. Rats in diet group *OGI* had the least percentage weight gain followed by CFI. Commercial feed (CF) supported the growth of the animals better than *ogi*.

Haematological parameters were used in determining the level of well being of an animal. Harper *et al.* (1979) reported that they are used in feeding trials to establish the effect(s) of the feed component on the blood constituents. The PCV of the experimental animals ranged from 20.00 to 26.00 %. Significant reductions in the level of PCV lead to anaemia (Ajayi & Raji, 2012). In this study it was shown that *Shigella* infection reduced the amount of the RBC in the animals in the challenged groups.

The highest values were observed in the infected animals in CFNI group. Infected animals had lower PCV compared to non-infected animals. Challenged animals had lower WBC count than the infected animals. Animals in diet group *OGI* had the least amount of calcium while those in diet group CFNI had the highest as shown in Table 3. Higher WBC reported in this study was against the report of Akinmutimi (2004) who reported high WBC in infected animals compared to non-infected ones. This showed that the test ingredient could have affected the lymphocytes count, by producing a better health state than the control since abnormal rise in lymphocyte may be as a result of malnutrition among other factors. The pathogens also may prevent proliferation of the WBC. Lymphocytes are important in forming barriers against local disease conditions and may be involved in antibody formation (Ajayi & Raji, 2012).

Figs. 1-3 showed the microbial loads of the faecal materials from the rats. The total bacterial load of the faeces of the experimental rats was monitored for 15 days. There was a decrease in the total bacterial count of the faecal materials collected from the animals after 4 days of feeding. The difference in the total bacterial count however was not significantly different (at $P < 0.05$) using paired T-Test. Diets have been reported to affect the microbial quality and quantity of the faecal materials from the animal (Aderiye & David, 2013). The fermentation of *ogi* is mainly done by lactobacilli, these organisms are known for the production of bacteriocins which are proteinaceous antimicrobial compounds that are inhibitory towards sensitive organisms (Adebayo & Aderiye, 2010; David & Famurewa, 2010; Savadogo *et al.*, 2004; Ogunbanwo *et al.*, 2003). The reduction in the bacterial load may be due to the actions of the probiotic fermenters.

CFI had the highest microbial load among the four diets, when plated on *Salmonella-Shigella* agar. There was decrease in the load of the in the total count (not clear) with the period of feeding. There were no colonies on the plate when the faeces of the rats fed with *OGNI* were plated on the 12 and 15th day of the experiment. The count was more in the faeces of animal fed with non-infected samples compared to the infected ones. There was drastic reduction in the number of coliform in the diets composed from *ogi* (*OGNI* and *OGI*). Oral administration of the pathogen has been reported by David (2010) to affect the appetite of the animals and indirectly affect the haematological parameters. Toward the end of the experiment the faecal materials obtained from the animals in CFI and CFNI diet groups showed increase in count on the MacConkey agar. On the 12 and 15th day of the experiment the coliform loads in CFI

were 2.56 and 2.15 log₁₀ CFU/g of faecal material. These values were higher than those observed in the previous days. *Ogi* did not encourage the multiplication of *Shigella dysenteriae* JBA 010 in the animals; hence offer protection against the infection of the pathogen.

References

Adebayo, C. O., & Aderiyi, B. I. (2010). Antifungal Activity of Bacteriocins of Lactic Acid Bacteria from Some Nigerian Fermented Foods. *Research Journal of Microbiology*, 5(11), 1070-1082. <http://dx.doi.org/10.3923/jm.2010.1070.1082>

Aderiyi, B. I., & David, O. M. (2013). Evaluation of prophylactic and therapeutic properties of *ogi* in rabbits infected with *Salmonella* Typhi. *International Food Research Journal*, 20 (1), 1857-1861.

Aderiyi, B. I., & Laleye, S. A. (2003). Relevance of fermented food products in southwest Nigeria. *Plant Foods for Human Nutrition*, 58, 1-16. <http://dx.doi.org/10.1023/B:QUAL.0000040315.02916.a3>

Agaliya, P. J., & Jeevaratnam, K. (2012). Screening of *Lactobacillus plantarum* isolated from fermented idli batter for probiotic properties. *African Journal of Biotechnology*, 11(65), 12856-12864.

Ajayi, A. F., & Raji, Y. (2012). Haematological and serum biochemical indices of pre-pubertal male rabbits fed with graded level of blood-wild sunflower forage meal mixture. *African Journal of Biotechnology*, 11(35), 8730-8734.

Akinmutimi, A. H. (2004). Evaluation of sword bean (*Canavalia gladiata*) as an alternative feed resource for broiler chickens. Ph.D. Thesis, Michael Okpara University of Agriculture, Umudike, Nigeria.

Akinrele, I. A. (1990). Fermentation studies on maize during the preparation of traditional African starch cake. *Food Journal of Science and Agriculture*, 22, 1001-1008.

AOAC (2005). *Official Methods of Analysis*, 18th ed., Association of Official Analytical Chemists, Washington DC.

Bush, B. M. (1975). *Veterinary Laboratory Manual*. William Heinemann Medical Books Ltd., London. pp: 447.

David, O. M. & Famurewa, O. (2010). Prophylactic and bio-therapeutic benefit of *ogi*: a lactic acid fermented food. *Researcher*, 2(9), 72-77.

David, O. M. (2010). Epidemiological studies of vancomycin-resistant *Enterococcus faecalis* contaminant in the hands of health care workers in selected hospitals in Ekiti, Ondo and Osun States, Nigeria. Ph.D. Thesis, Department of Microbiology, University of Ado-Ekiti. pp: 107.

Harper, A. F., Rodwell, V.W. & Mayes, P. A. (1979). *Review of Physiological Chemistry*. 17th Ed. Lang. Medical, Los Atlos, California. pp. 9422.

- Hart, C. A. & Kariuki, S. (1998). Antimicrobial resistance in developing countries. *British Medical Journal*, 317, 647-650. <http://dx.doi.org/10.1136/bmj.317.7159.647>
- Huppertz, H. I. (1986). An epidemic of bacillary dysentery in Western Rwanda, 1981-1982. *Central Africa Journal of Medicine*, 32, 79-82.
- Jain, N. C. (1986). *Veterinary Haematology*. 4th ed. Lea–Febiger Publishers, Philadelphia, USA pp: 153 – 159.
- Kilgour, O. F. G. (1987). *Mastering Nutrition*. Pp 95-96 Macmillan Education Ltd, London.
- NRC (2011). National Research Council: *Guide for the Care and use of Laboratory Animals*. (8th Edition) Washington DC. The National Academies Press.
- Odunfa, S. A. & Adeyele, S. A. (1985). Microbiological changes during the traditional production of *ogi-baba*, a West African fermented Sorghum gruel. *Journal of Cereal Science*, 3, 173-180. [http://dx.doi.org/10.1016/S0733-5210\(85\)80027-8](http://dx.doi.org/10.1016/S0733-5210(85)80027-8)
- Odunfa, S. A. (1985). African fermented foods. In: *Microbiology of Fermented Foods*, 2. Ed. B. J. B. Wood. Elsevier Applied Science Publication. London. Pp. 155–191.
- Ogunbanwo, S. T., Sanni, A. I., & Onilude, A. A. (2003). Influence of cultural conditions on the production of bacteriocin by *Lactobacillus brevis* OG1. *Africa Journal of Biotechnology*. 2(7), 179-184.
- Oli, A. K., Rajeshwari, R. S., Nagaveni, S., & Kelmani, C. R. (2012). Biofilm formation by Multidrug resistant *Enterococcus faecalis* (MDEF) originated from clinical samples. *Journal of Microbiology and Biotechnology Research*, 2(2), 284-288.
- Parashar, U. D., Bresee, J. S., & Glass, R. I. (2003). The global burden of diarrhoeal disease in children. *Bulletin of the World Health Organization*, 81, 236-241.
- Pearson, D. (1976). *Chemical Analysis of Foods*. 7th ed. Churchill, London. Page 7-11.
- Savadogo, A., Ouattara, C. A. T., Bassole, I. H. N. & Traore, A. S. (2004). Antimicrobial activity of lactic acid bacteria strains isolated from Burkina Faso fermented milk. *Pakistan Journal of Nutrition*, 3(3), 174 -179. <http://dx.doi.org/10.3923/pjn.2004.174.179>
- Schalm, O. W., Jain, N. C. & Carrol, E. (1975). *Veterinary Haematology*. 3rd Edition Lea and Febiger, Philadelphia, USA. pp. 160 – 210.

Table 1. Proximate composition (g/100g), metabolizable energy (KJ) and mineral analyses (mg/100g) of commercial feed and *ogi*

Component	Feeds	
	Commercial feed	<i>Ogi</i>
Moisture	14.18 ± 2.53	23.44±4.01
Total solid	85.82 ± 3.81	76.56±3.88
Ash	2.24 ± 1.57	1.37±0.35
Crude protein	16.31 ± 3.91	1.79±0.21
Crude fat	3.52 ± 1.02	0.34±0.01
Crude fibre	6.83 ± 1.98	N.D
Carbohydrate	56.93 ± 9.01	73.07±5.81
*Metabolizable energy	1355.09	1285.20
Fe	0.20 ± 0.02	15.0±3.81
Ca	25.03 ± 3.81	35.5±3.81
Mg	3.06 ± 0.09	12.7±2.18
K	39.02 ± 4.11	0.74±0.12
Na	31.20 ± 8.28	1.49±0.21
Na/K	3.36	2.01

Data are the means of triplicate determinations

ND; Not detected

*Value was the average of triplicate determinations

 Table 2. Performance of rats fed on commercial feed and *ogi*

Diets		Parameters			
		Initial weight	body weight	Final body weight	% Weight gained
Commercial feed	Non-infected	23.31±2.81		70.66±5.12	67.01
	Infected	26.92±1.72		63.29±4.09	57.47
<i>Ogi</i>	Non-infected	24.07±2.51		66.29±5.12	63.69
	Infected	24.60±2.88		50.02±2.79	50.82

(Data are in grams)

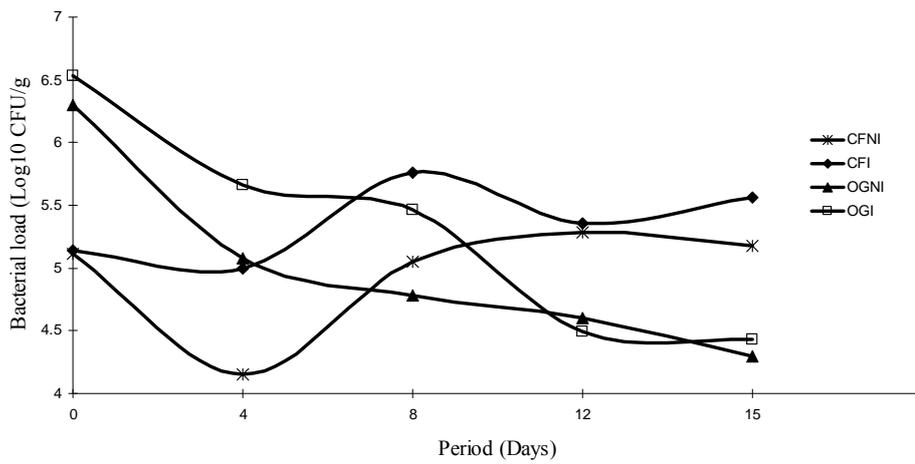


Figure 1. Total bacterial count of commercial and *ogi* diets

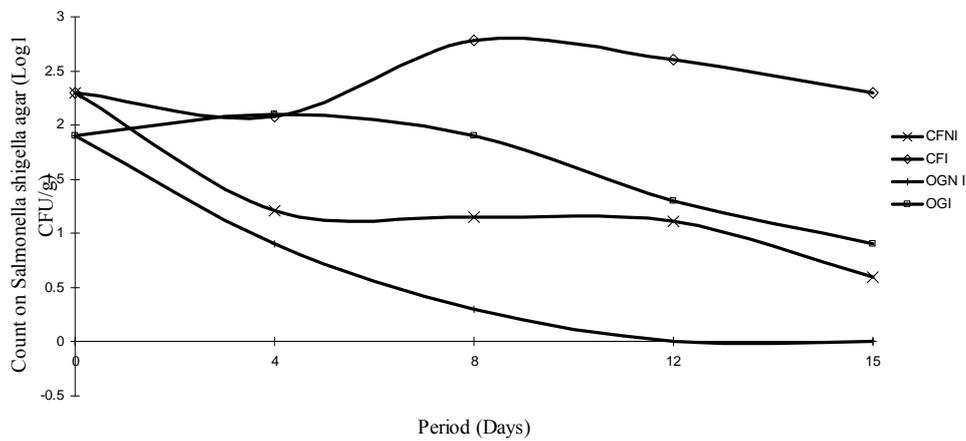


Figure 2. Count on *Salmonella-Shigella* agar of commercial and *ogi* diets

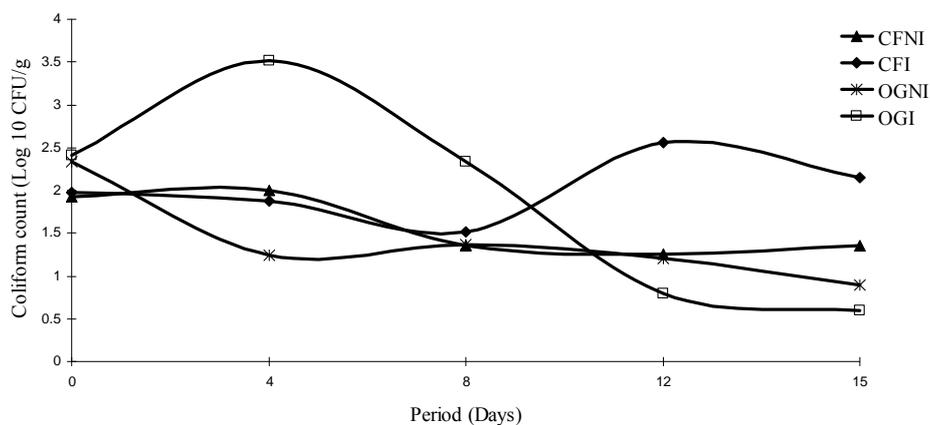


Figure 3. Count on the MacConkey agar of the commercial and *ogi* diets

Table 3. Haematological parameters and mineral contents of experimental animals

Parameter	Commercial feed		<i>Ogi</i>	
	Non-infected (CFNI)	Infected (CFI)	Non-infected (OGNI)	Infected (OGI)
Packed cell volume (%)	26.00	24.00	25.00	20.00
White blood cell (mm ³)	4,900	4,200	7,300	6,300
Haemoglobin (g/dl)	12.99	11.93	8.60	7.95
Calcium (mmol/l)	2.73	2.54	2.66	2.25
Iron (µg/dl)	63.10	58.0	26.50	61.80