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Evaluation of prophylactic and therapeutic properties of *ogi* in rabbits infected with *Salmonella typhi*

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Abstract

The therapeutic and prophylactic effects of fermented maize gruel (*ogi*) in protecting against and reducing the concentration of *Salmonella typhi* in rabbits were determined using standard methods. All the experimental animals were fed with commercial diet during the first feed trial. The control group was maintained only on the diet while the first treatment group fed with the commercial diet was later substituted with ogi in the third phase of the trial. The second treatment group was fed with ogi in both the second and third phases of the study. In the second feed trial, the treatment groups were challenged with *Salmonella typhi*. Infected rabbits that were fed with commercial diets showed high load of *Salmonella typhi* in the faeces and blood compared to those fed with ogi. The weights gained and food intakes of the animals in the control group were higher than the experimental group. The animals in the first treatment group recorded the least food intake while there was weight loss in the second treatment group. Bacteremia was established in the treatment groups. The level of white blood cells (WBCs) in the rabbits infected with *Salmonella typhi* but later maintained on ogi, decreased from 4900 mm^3 to 4200 mm^3. Similarly the WBC count decreased from 7300 mm^3 to 6300 mm^3 in rabbits that were initially infected (but fed on commercial diet) and later fed with ogi. It could be concluded that ogi could be potent in preventing Salmonella infection and the reduction of microbial load of *Salmonella typhi* in the blood of the mammals as shown by the microbial and haematological indices.

Introduction

Fermented foods are mostly consumed in Africa where they constitute the main diet (Aderiye and Laleye, 2003). They had undergone a desirable change due to the action of the invading microorganisms or their metabolic products. Fermented foods are prepared from tubers, cereals, oil rich seeds and other animal products. *Ogi* is the most popular cereal meal consumed in the western part of Nigeria (Odunfa and Adeyele, 1985; Adeyemi and Beckley, 1986; Akingbala *et al*., 1987; Adeyemi, 1988; Johansson *et al*., 1989; Adewusi *et al*., 1991). Ogi serves as a major source of macro- and micronutrients for humans (Asiedu *et al*., 1992).

Ogi has been recognized as the most popular traditional health-sustaining fermented food in Western Nigeria. It is prepared from cereal: white maize (*Zea mays*), yellow maize (*Z. mays*, yellow variety), red guinea corn (*Sorghum vulgare*), white guinea corn (*Sorghum bicolor*) and millet (*Pennisetum typhoideum*) but not from rice and wheat (Adebolu *et al*., 2007). Aderiye and Ajibade (2007) reported that the growth of *Salmonella typhi* in different forms of processed *ogi* was very poor. The raw uncooked *ogi* sample showed a tremendous inhibitory effect on the microbes, reducing its initial concentration to 4.57 x 10^3 CFU/ml within 5h of incubation.

In some communities in southwestern Nigeria, *ogi* is normally administered to people having gastroenteritis to reduce/minimize discomforts (Aderiye and Laleye, 2003; David and Famurewa, 2010) but the empirical/scientific proofs for this claim is lacking. The nutritional benefits of *ogi* have been investigated extensively unlike the therapeutic and preventive effects of *ogi* which form the bases for this study.

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Materials and Methods

Preparation of ogi slurry

Maize grains (*Zea mays*) were bought at Oja Oba, an open market in Ado-Ekiti, Nigeria. The grains were sorted to remove grit, dirt and decomposing ones and later washed. Two hundred gram of the grains was weighed and steeped in plastic container containing 300ml clean water. The container was covered and the grains fermented for 72h at 28 ± 2°C. After steeping, the water was decanted and the grains were wet-milled. The resulting pastes were sieved using sterile muslin cloth (Adebayo and Aderiye, 2007). The filtrates were collected into different sterile containers and allowed to settle for 3 days during which fermentation took place by the natural flora of the grains.

Source of experimental animals

Fifteen (15) crossbred rabbits *Leporida* sp. (Dutch, New Zealand White) of mixed sexes with age ranging from 5 to 7 weeks were purchased from Ondo State Ministry of Agriculture, Akure, Nigeria and randomly grouped into three of four rabbits each, with the three rabbits serving as control. Each rabbit was housed individually in a cage and supplied daily with the experimental diets. Clean drinking water was also provided ad libitum throughout the experimental period. The rabbits were fed with the commercial mash obtained from Bendel Feed and Flour Mill Limited, Benin City, Nigeria. The rabbits were kept in stainless steel cages in a well-ventilated room at about 27±2°C. Lighting regimen was about 13 hr: 11 hr of light and dark. Animal management and experimental procedures were performed strictly in accordance with the requirements of the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 1985).

Feed formulation and feeding experiment

As shown in Table 1, the composition of the diets was the same except *ogi* which was not added to the control diet and maize and wheat offal which were substituted with ogi in the experimental diet. The experimental animals (rabbits) used for this study were subjected to fasting a day prior to the introduction of the diets. Rabbits in the first treatment group (OgSC) were fed with diet containing *ogi* and later challenged with *Salmonella typhi* while those in second treatment group (CCOg) were fed with the commercial diet. *Salmonella typhi* was orally administered to the animals. The animals in second treatment group (CCOg) were finally fed with diets containing *ogi*. The control group was only fed with the commercial diets and not challenged with the pathogen.

Table 1. Percentage composition of diets

Component	Diets (g/100g)	
	Commercial feed	Diets containing *ogi*
Maize	28.5	--
Wheat offal	30.0	--
Ogi	--	58.5
Fish meal	12.5	12.5
Bone meal	12.5	12.5
Oyster shell	1	1
Mineral (salt)	0.25	0.25
Vitamin (premix)	15	15
Soya meal	15	15
Total	**100**	**100**

Table 2. Administration of diets at different feeding phases

Phase	Control	Experimental group	
		*Og*SC	CC*Og*
A	Commercial diet	Commercial diet	Commercial diet
B	Commercial diet	Diets containing *ogi*	Commercial diet
C	Commercial diet	Diets containing *ogi*	Diets containing *ogi*

At the beginning of the experiments all the animals were fed with commercial diet during the first feed trial (phase 1). At the second phase of the experiment, the commercial diet was substituted for diet containing *ogi* in the first treatment group (OgSC). In the third phase of the experiment, the second treatment group (CCOg) was fed with diet containing *ogi* while OgSC group was maintained on diet containing *ogi* as shown in Table 2. At the end of the first feeding phase, the treatment groups were challenged with *Salmonella typhi*.

Preparation of Salmonella typhi inoculum for challenge test

An overnight culture of the pathogen, *Salmonella typhi* was grown in peptone water. The culture was concentrated by centrifugation at 14,636g (for 15 min at 26°C). The bacterial pellet was suspended in 2 ml of normal saline, to a final concentration of 10^8 CFU/ml. The bacterial suspension was diluted to 5 x 10^5 CFU/ml and 100µl was administered orally into the experimental animals by intubation. The number of bacteria injected per rabbit was confirmed by broth dilution of the inoculum and viable plate counts.

Establishment of Salmonella typhi colonization

The presence of *Salmonella typhi* was determined in both faeces and blood of the challenged experimental animals using the viable count method. The faeces and blood of the *Salmonella typhi* challenged rabbits were collected, serially diluted and plated on *Salmonella*-shigella agar (Oxoid) and incubated at 37°C for 24h.

Collection of Blood

At the end of the experiment, blood samples were collected from the rabbits in each treatment

for determination of the haematological and serum biochemical indices. Blood samples were collected from the ear vein of the rabbits by venipuncture using disposable needle 25 gauge needle and syringes. The rabbits were fasted overnight (12h) and normally bled in the morning to avoid excessive bleeding (Gambo *et al*., 2011).

The collection site was cleaned with 70% alcohol to sterilize the site and xylene to dilate the veins. Sterile cotton was used to cover the punctured vein after collection. The blood samples were collected in sample bottles containing dipotassium salt of ethylene diamine–tetra acetic acid which served as an anticoagulant (Cheesbrough, 2006).

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 (RANDOX Laboratories Ltd, Crumlin, United Kingdom) were used to determine the level of calcium and iron in the blood.

Results and Discussion

Salmonella typhi was not detected in the blood of the animals at the onset of the experiment. After the inoculation the pathogen load reached detectable level in the blood and faeces of the animals in the treatment group (Table 3). At the end of third phase of experiment, the load of the pathogen in the blood and the faeces decreased compared to the second phase of the experiment. This shows that raw *ogi* has bioactive components, which had inhibitory activity on these organisms. One of such could be bacteriocins which are proteinaceous antimicrobial compounds that are inhibitory towards sensitive organisms (Ogunbanwo *et al*., 2003) and which act by destroying the bacterial membrane (Savadogo *et al*., 2004). This could be a possibility because lactobacilli which play a principal role in the production of *ogi* and have been reported by Lindgren and Dobregosz (1990) and Brink *et al*. (1994) to produce various compounds which including organic acids, diacetyl, hydrogen peroxide and bacteriocin during lactic acid fermentation. These compounds had actually been detected in the liquors (Adebayo and Aderiye, 2007).

The performance of the animals in the groups is represented in Table 4. The control group had the highest weight gained (0.81g) and food intake (91.5g). There was weight loss in the CCOg group while the food intake in the OgSC group recorded the least value (78.8g). The rabbits in the treatment groups were depressed and less active compared to those in the control group. The weight gained in the animal control group (0.18kg) was higher than the values recorded for those in the animal treatment groups (OgSC and CCOg). There was loss in the weight (0.9kg) of rabbits in the experimental group fed initially with the commercial diet and later substituted with *ogi* (OgSC). The low performance of the animals in the experimental group may be due to the effect of the pathogen in their system. Oral administration of the pathogen is known to indirectly affect the haematological parameters by reducing the appetite of the animals (David, 2010). Infection of the animals by the pathogen makes the nutrients unavailable and could also affect haemopoesis (Xing *et al*., 1995).

Table 3. *Salmonella typhi* load (CFU/ml) in the faeces and the blood of the experimental rabbits

Experimental phases	Samples	Control	Treatment Group	
			*Og*SC	CC*Og*
	Blood	ND	ND	ND
A	Faeces	2.3×10^1	2.6×10^1	1.9×10^1
B	Blood	ND	3.0×10^2	5.7×10^2
	Faeces	3.7×10^1	2.9×10^3	4.8×10^3
C	Blood	ND	1.2×10^2	1.6×10^2
	Faeces	2.0×10^1	2.6×10^2	3.6×10^2

Table 4. Performance of rabbits fed with *ogi* and commercial diets (weight in gram)

Parameter	Control	Treatment group	
		*Og*SC	CC*Og*
Final weight	1.93±0.21	1.94±0.21	1.74±0.29
Initial weight	1.75±0.17	1.79±0.61	1.83±0.31
Weight gain	0.18±0.03	0.15±0.08	(-0.9±0.13)*
Food intake	91.5±7.89	78.8±4.92	83.5±9.21

*weight loss

As shown in Table 5, the level of white blood cells (WBCs) in the rabbits in group OgSC increased following the challenge with the pathogen. The WBC count rose from 4200 mm^3 to 4850 mm^3. Similarly the WBC decreased from 7300 mm^3 to 6300 mm^3 for rabbits in group CCOg but the value decreased after substituting the commercial diet with ogi. The PCV unlike the WBC count was lower in the experimental animals compared to the control group in the second phase of the experiment. The platelet counts showed a drastic reduction in the experimental group compared to the controls. In the CCOg group, the WBC count was reduced initially and later increased. The calcium levels of rabbits in the experimental groups were lower than those of the control group. In the second phase the least value was recorded in the OgSC. The values further decreased after infection with *Salmonella typhi*. The average iron level in the blood of rabbits in the experimental groups was lower than the control group.

The improvement of Hb and PCV values among

Table 5. Haematological changes in rabbits during *Salmonella* infection

Parameters	Control			Treatment groups					
				Ogi(*Og*SC)			CC*Og*		
	Phase A	Phase B	Phase C	Phase A	Phase B	Phase C	Phase A	Phase B	Phase C
PCV (%)	28.67	33.00	34.00	28.25	24.00	34.00	28.00	30.00	36.00
	±3.83	±7.23	±1.45	±2.28	±2.11	±3.61	±2.90	±2.22	±4.72
WBC (mm^3)	4366	4500	4200	4200	4850	3500	4300	5050	4100
	±85.53	±23.81	±38.83	±87.29	±92.20	±91.41	±0.28.89	±200.48	±112.60
Haemoglobin (g/dl)	7.80	11.20	10.04	8.64	9.87	7.93	7.63	9.09	10.95
	±1.21	±2.14	±2.01	±2.09	±3.01	±0.29	±1.27	±2.92	±2.91
Calcium (mmol/l)	3.70	3.70	3.90	3.40	3.10	2.70	3.90	3.60	2.70
	±1.82	±0.99	±0.30	±0.64	±0.12	±0.01	±2.93	±0.62	±0.91
Iron (µg/dl)	247.1	229.4	230.6	234.95	126.1	196.0	243.8	126.9	136.8
	±39.81	±19.24	±34.51	±51.92	±19.34	±23.52	±0.12.80	±29.03	±36.82

Data are represented in the means ±SD of triplicate determinations

the treated rabbits in this study is an indication that the *ogi* is useful for treatment of gastroenteritis in mammals, hence the justification for its use as prophylactic medication in southwest Nigeria (Aderiye and Laleye, 2003). The administration of the ogi stimulated decreased production of WBC in the animal treatment groups. This could be as a result of possible stimulation of immune system (Kashinath, 1990; Gambo *et al*., 2011) and clearance of the pathogen. Furthermore, reports have shown that persistent antigen load in the body results in lymphocytosis (Schalm *et al*., 1975; Anon, 1980). The result for the haematological and serum biochemical indices is shown in Table 4. The administration of *Salmonella typhi* did not affect PCV values. The values obtained in this study were similar to those reported by other workers (Dairo *et al*., 2005; Mohammed *et al*., 2005).

From this study, it is clearly noted that ogi offered a protective measure against *Salmonella typhi* infection. There was low load of *Salmonella typhi* in both faeces and blood of the experimental animals fed with *ogi*. The commercial diet fed animals recorded high load of *Salmonella typhi* in the blood and faeces of the animals, however the load was lowered after the feed was substituted with *ogi*. This study has been able to confirm that uncooked *ogi* has anti-salmonella property. It is, therefore, suggested that people living in typhoid fever endemic area and/or where access to medical attention is poor should constantly take raw *ogi* liquor as prophylactic and therapeutic measures against *Salmonella typhi*.

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