International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 2 (4): 393-397, 2007
ISSN 1811-9743

Hypocholesterolemic Activity of *Nono* in Albino Rats

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Abstract: The effect of *Nono* on induced hypercholesterolemia in Albino rats was investigated. Induction was by feeding a high lipid cholesterol diet to the animals for two weeks. Following hypercholesterolemia, diet (HLCF) supplemented with *Nono* was administered to the rats for another eight weeks. The control group was fed normal diet only. The high density lipoprotein cholesterol (HDLC) value in rats fed with HLCF only or with supplement increased slightly above those recorded for the control. The values are significantly different at $p \leq 0.05$. The rat groups whose diets were supplemented with *Nono* had lower values for serum total cholesterol, triglyceride, low density lipoproteins and liver enzymes (Alanine and Aspartate aminotransferases) activity. These results demonstrate that increased consumption of *Nono* can be recommended for hypercholesterolemic individuals with a view to reducing the incidence of coronary heart diseases in Nigeria.

Key words: *Nono*, hypercholesterolemia, cholesterol, lipoproteins, triglycerides, enzyme activity

INTRODUCTION

Cholesterol is essential for the biosynthesis of several hormones as well as bile acids in animal and human cells. Individuals acquire cholesterol from two major sources namely that synthesized by the body and dietary intake (Hilsden and Shaffer, 2005). Hypercholesterolemia refers to high level of cholesterol in the blood especially the Low Density Lipoprotein (LDL), which has been implicated as a major risk factor associated with coronary heart disease. The ability to maintain serum cholesterol at a desirable level is one of the major preventive strategies for this disease (Abd El-Gawad *et al*., 2005).

Mann and Spoerry (1974) pioneered research into the use of fermented foods as a means of lowering serum cholesterol. As a result much attention has been given to the relationship between diet and serum cholesterol levels in animal models (Usman and Hosono, 2000) and humans (Kawase *et al*., 2001). Fermented milk products such as yoghurts and acidophilus yoghurts have been recommended as dietary supplement in hypercholesterolemic animals (Parvez *et al*., 2006).

Nono is a highly soured product obtained from fermented cow milk which is widely consumed in northern Nigeria. Lactic acid bacteria have been reported to be involved in the fermentation (Akinyanju, 1989; Savadogo *et al*. (2005). There is a growing interest in the therapeutic role of some Nigerian fermented foods. Aderiye and Laleye (2003) reported the consumption of some of these foods e.g., *Fufu*, *Gari* and *Ogi* as an alternative way of preventing/inhibiting severe occurrence of diarrhea or dysentery and common stomach upsets before such individuals seek professional medical attention.

There is very little information available in Nigeria on the ability of *Nono* to influence hypercholesterolemia in rats. This study was therefore initiated to investigate the effect of consumption of *Nono* on serum cholesterol levels and the activity of some metabolic enzymes in hypercholesterolemic rats.

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MATERIALS AND METHODS

Source of Materials

Nono samples used in this study were purchased from Oja-Oba market, Ikare-Akoko, Ondo State, Nigeria. Samples of this food product were obtained from only one producer/retailer throughout the course of the study.

The commercial feed (Purina Chow) was also purchased from Oja-Oba market in Ikare-Akoko and this served as the main diet in the control group.

The Albino rats (*Rattus nervigicus*) were obtained from the animal house of Institute of Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria.

Experimental Design

Thirty-two post weaning apparently healthy albino rats were divided into four different groups. Each group consists of eight rats. The type of diet administered to each group is described below:

A: Normal diet
B: Normal diet + *Nono* as supplement
C: High lipid + 1% cholesterol feed (HLCF)
D: High lipid + 1% cholesterol feed (HLCF) + *Nono* as supplement

Diet Formulation and Feeding

The lipid content of the feed was increased by adding a known quantity of pork to give 20% lipid composition for the high lipid feed. Later, 1% cholesterol (Sigma) was added to this feed, designated HLCF.

Rats in each group were fed with 35-45 g of the diet thrice daily. Nono was administered as supplement by oral intubations two times daily at a ratio of 1:20 (volume of fermented food to animal body weight). The animals were starved for 24 h and then fed on the high lipid cholesterol feed for 2 weeks to induce hypercholesterolemia. *Nono* was also administered to the rats after hypercholesterolemia and feeding continued for another 8 weeks. The weights of the rats were taken fortnightly and the mean value for each group was determined

Blood Sample Collection

The method of Usman and Hosono (2000) was employed in the analyses of Total Cholesterol (TC), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL), Triglycerides and metabolic enzymes, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT). These parameters were determined using enzymatic reagent kit (Biosystem S.A Spain).

Statistical Analysis

Data obtained were evaluated by analysis of variance (ANOVA) to study differences between means at $\alpha = 0.05$. Multiple comparison of the mean was done using Turkey's test. The data obtained in some cases were presented as means±SEM (Standard Error of Mean).

RESULTS AND DISCUSSION

Generally, there was an increase in rats' body weights after 8 weeks of feeding, with 82.4 and 73.5% weight gain in rats fed on HLCF and normal diets respectively (Table 1). With *Nono* as supplement, the percent weight gain in the rats was lower (51.86 and 41.9%, respectively), an indication that the fermented milk product did not encourage much weight gain within 8 weeks.

All the rat groups fed high lipid cholesterol feed (HLCF) showed high level of serum cholesterol after 2 weeks (Table 2). This may be as a result of the increased lipid content of the food by 20% and

Table 1: Body weight[1] changes of rats fed high cholesterol diet supplemented with *Nono*

		Weeks			
Group	Initial* Weight (g)	2	4	6	8
Normal feed	84.30±2.01	9.70±2.90	108.80±2.11	136.80±5.22	146.30±1.41
Normal feed +*Nono*	84.70±0.02	95.30±2.79	98.30±3.26	140.00±5.18	128.63±4.03
HLCF	83.50±0.05	91.60±3.86	113.47±3.58	126.74±4.02	152.34±2.05
HLCF + *Nono*	8590±0.50	95.03±1.45	109.30±1.02	124.20±1.15	125.90±3.07

[1]Values (g) recorded are means of 6 rats in a group±SEM; *Weight of rats after hypercholesterolemia

Table 2: Changes in Cholesterol levels in serum of rats on high cholesterol diet supplemented with *Nono*

		Weeks			
Group	Initial*	2	4	6	8
Normal feed	53	56	57	51	54
Normal feed + *Nono*	53	58	45	41	37
HLCF	110^{a}	180^{b}	192^{bc}	216^{de}	200^{cd}
HLCF + *Nono*	110^{ab}	170^{cd}	140^{bc}	80^{a}	56^{a}

Values with different notations are significantly different ($p \leq 0.05$); *Value after hypercholesterolemia has been established

Table 3: Changes in low density lipoprotein cholesterol levels in serum of rats on high cholesterol diet supplemented with *Nono*

		Weeks			
Group	Initial*	2	4	6	8
Normal feed	120^{a}	160^{ab}	130^{a}	130^{a}	130^{a}
Normal feed + *Nono*	120	100	140	120	140
HLCF	190	180	190	190	170
HLCF + *Nono*	190^{bc}	160^{ab}	140^{a}	110^{a}	110^{a}

Values with different notations are significantly different ($p \leq 0.05$); *Value after hypercholesterolemia has been established

Table 4: Changes in Triglyceride levels in serum of rats on high cholesterol diet supplemented with *Nono*

		Weeks			
Group	Initial*	2	4	6	8
Normal feed	40	30	40	30	50
Normal feed + *Nono*	40^{a}	90^{b}	50^{a}	30^{a}	30^{a}
HLCF	73^{a}	85^{a}	110^{bc}	105^{ab}	120^{cd}
HLCF + *Nono*	73^{bc}	50^{a}	30^{a}	40^{a}	70^{ab}

Values with different notations are significantly different ($p \leq 0.05$)' *Value after hypercholesterolemia has been established

the incorporation of cholesterol in the diet. The most common cause of elevated serum cholesterol is the eating of foods that are rich in saturated fats and contains high level of cholesterol (American Heart Association, 2005). These dietary lipids are absorbed through the gut, assembled into special packets called chylomicrons and delivered through the blood stream to the liver, where they are processed (Rich, 2001).

The serum Low Density Lipoprotein Cholesterol (LDLC) (Table 3) and triglyceride (Table 4) contents of the rats were reduced considerably in all the groups fed with diets supplemented with the fermented food. This was expected since triglycerides have been reported to influence the composition of LDLC, its physical properties and the cell specific binding factor of microbial cells (Castelli, 1996). These reductions may also be as a result of the fact that the supplement (*Nono*) does not in any way increase the saturated fat content of the diet.

There was an increase in the High Density Lipoprotein Cholesterol (HDLC) value in all the rat groups except in those fed on HLCF only (Table 5). High HDLC levels in animal and human blood protect against heart attack since the lipoprotein has been reported to carry cholesterol away from the arteries back to the liver where it is excreted in the bile as free cholesterol or as bile salts following conversion to bile acids (AHA, 2005). HDLC has also been reported to increase triglyceride catabolism (Tietz, 1986).

Table 5: Changes in High Density Lipoprotein levels in serum of rats on high cholesterol diet supplemented with *Nono*

		Weeks			
Group	Initial*	2	4	6	8
Normal feed	55^{ab}	50^{a}	52^{a}	45^{a}	45^{a}
Normal feed + *Nono*	55^{ab}	48^{a}	45^{a}	43^{a}	43^{a}
HLCF	30^{bc}	28^{ab}	32^{cd}	22^{a}	16^{a}
HLCF + *Nono*	30^{a}	32^{a}	35^{a}	40^{a}	42^{ab}

Values with different notations are significantly different ($p \leq 0.05$); *Value after hypercholesterolemia has been established

Table 6: Changes in Aspartate Aminotransferase level in serum of rats on high cholesterol diet supplemented with *Nono*

		Weeks			
Group	Initial*	2	4	6	8
Normal feed	33	65	80	90	65
Normal feed + *Nono*	33	55	70	90	70
HLCF	90^{a}	170^{a}	270^{b}	310^{bc}	320^{cd}
HLCF + *Nono*	90^{a}	220^{cd}	210^{cd}	200^{bc}	180^{b}

Values with different notations are significantly different ($p \leq 0.05$); *Value after hypercholesterolemia has been established

Table 7: Changes in Alanine Aminotransferase levels in serum of rats on high cholesterol diet supplemented with *Nono*

		Weeks			
Group	Initial*	2	4	6	8
Normal feed	90^{a}	150^{a}	110^{a}	210^{bc}	200^{ab}
Normal feed + *Nono*	90^{a}	130^{a}	110^{a}	200^{ab}	200^{bc}
HLCF	280^{a}	340^{a}	380^{ab}	360^{a}	400^{bc}
HLCF + *Nono*	280^{d}	190^{bc}	160^{b}	70^{a}	140^{a}

Values with different notations are significantly different ($p \leq 0.05$); *Value after hypercholesterolemia has been established

The presence of Alanine and Aspartate aminotransferases, in animal or human blood, are indicators of damage or injury to organs (Worobetz *et al*., 2005). These enzymes are normally found in diversity of tissues including the liver, heart, kidney and brain (MedicineNet, 2005). They are found in the cells of these organs and may leak out to the blood when the cells are injured. The results from this work showed an initial increase and then a decrease in the values of the two enzymes (Table 6 and 7).

The increase in the concentration of these enzymes may be as a result of an increase in the fat component of the diet fed to the rats. These fatty molecules may have accumulated on the walls of the tissues which may lead to eventual damage of the cells and leakage of the enzyme into the blood. Diseases such as hypercholesterolemia, diabetes mellitus and chronic hepatitis have been reported to increase the concentrations of these enzymes in the liver (MedicineNet, 2005).

The reduction in activity of these enzymes during feeding with *Nono* indicates that there may have been an improvement in the recovery of the organ from injury. This may be as a result of the ability of lactic acid bacteria present in most fermented foods to produce polyamines from amino acids. Polyamines are important mediators of cell growth and differentiation (McCormack and Johanson, 1991). Present finding agrees with the report of Adawi *et al.* (1997) that supplementation of the diet with *Lactobacillus plantarum* and arginine reduced hepatocellular necrosis and inflammatory cell infiltration in the liver.

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