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Effects of High - Protein, Low - Carbohydrate and Fat, Nigerian - like Diet on Biochemical Indices in Rabbits

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Abstract: This study aimed to assess the effects of high protein, low carbohydrate and fat (HP/LCF) Nigerian-like diet. Twelve rabbits were randomly divided into two groups of six rabbits per group. The groups were; Group I, which was fed the control diet and the second group II, received the high protein, low carbohydrate and fat diet, containing 65% protein, 28% carbohydrate and 5% Palm oil. The diets were administered for 30 days. At the end of the feeding periods, biochemical analyses were done on the blood samples of rabbits. Results showed that the total protein, albumin and globulin were significantly ($P<0.05$) increased for the rabbits fed a HP/LCF diet. AST (Aspartate transaminase) and ALT (Alanine transaminase) levels increased significantly ($P<0.05$) while ALP (Alkaline phosphatase) decreased significantly ($P<0.05$). The value obtained for serum electrolyte concentrations show a significant ($P<0.05$) increase in sodium and chloride ions when compared with control. Although, potassium increased significantly, the increase was lower than that observed for sodium and chloride ions. Bicarbonate ions showed significant increase ($P<0.05$). Urea values showed significant ($P<0.05$) increased levels. However, Creatinine levels did not show a significant ($P<0.05$) increase. Total cholesterol levels were significantly ($P<0.05$) reduced. The lipoprotein fractions showed significant ($P<0.05$) elevated HDL levels. Triacylglycerol levels and LDL-Cholesterol levels showed no significant change when compared with controls. From the results, the HP/LCF Nigerian diet promotes low total cholesterol levels while increasing the protective HDL-Cholesterol levels. The LDL-Cholesterol and triacylglycerol levels did not change significantly. The increased serum urea levels, AST and ALT levels are indicative of dysfunctional liver and kidney.

Key words: High-protein low carbohydrate, fat diet, serum lipid, serum enzymes, electrolytes

Introduction

The typical Nigerian diet consists of low protein and high carbohydrate levels. However, in recent times, the Atkins Diet in which carbohydrate is restricted but protein is increased, leads to weight loss (Anderson *et al*., 2000), has been proposed to help people maintain desirable body weight (Jeor *et al*., 2001) and induce feeding-suppressive effects (Bensaid *et al*., 2002). The increased incidence of overweight and obesity in the middle class in Nigeria has made this diet popular as a strategy to achieve efficient and long term weight loss.

The middle class Nigerian diet is characterized by elevated intake of red meat and saturated fat. Protein cannot be stored, they must be produced adequately in diet and therefore excess is unnecessary, as it is metabolized to energy.

The positive effects of replacing dietary protein for carbohydrate includes, improvement in the cardiovascular disease risk profile and insulin sensitivity in type 2 diabetes (Parker *et al*., 2002) lowering of white adipose tissue and reduced lipogenesis.(Pichon *et al*., 2006). Johnston *et al*. (2002), reported increases in total energy expenditure by means of increased thermogenesis. This is associated with protein digestion.

Despite the benefits of the high protein diet, there are concerns about the safety of the diet. The diet is linked to detrimental renal and hepatic function (Hammond and Janes, 1998, Morens *et al*., 2000), increase of urinary calcium excretion and a possible bone resorption (Kerstetter *et al*., 1999, Licata *et al*., 1981), an enhancement of oxidative stress (Petzke *et al*., 2000) and a modification of detoxification enzymes (Rao, 1996).

A suitable diet should promote good health and satisfy the needs of the individuals. The present study aims to examine the effect of consuming a high-protein, low-carbohydrate and fat Nigerian-like diet on biochemical indices in rabbits.

Materials and Methods

Animals and management: 3 months old New Zealand white rabbits (Initial mean weight 1.75Kg) were used in the present study.

The rabbits were housed in individual stainless steel animal cages with wire mesh floors to prevent coprography. Light was a 12hr-light and 12hr dark cycle and the temperature was uniform. The animals were acclimatized on growers mash (Bendel feed and Flour Mills (BFFM) Limited, Ewu, Nigeria) for two weeks. Prior

Table 1: Composition of Experimental Diets

	Diet type	
Dietary Component	Control	HP/LCF
Garri	60.00g	28.40g
Fish	15.40g	65.00g
*Mineral and vitamin mix (*OPIMIX PREMIX)	1.00g	1.00g
Palm oil	18.00g	5.00g
Methionine	0.60g	0.60g

Note: Garri is a cassava based meal commonly consumed in Nigeria and contributed to the fiber in the Nigerian- like diet.

*OPIMIX PREMIX

Vitamin A	8,000,000IU	Copper	5.0gm
Vitamin D	1,600,000IU	Iron	20.0gm
Vitamin E	5,000IU	Iodine	1.2gm
Vitamin K	2,000mg	Selenium	200.0mg
ThiamineB_1	1,500mg	Cobalt	200.0mg
Riboflavin B_2	4,000mg	Cholin chloride	200.0gm
Pyridoxine-B_6	1,500mg	Anti oxidant	125.0gm
Niacin	15,000mg	Manganese	80.0gm
Vitamin B_{12}	10mg	Zinc	50.0gm
Pathothenic acid	5,000mg	Biotin	20.0mg
Folic acid	5,000mg		

to the study, food and water was given *ad libitum*. The rabbits were divided into two groups of six rabbits per group, according to body weight similar after the adaptation period. One group was fed the control diet and the second group was fed the High protein, low fat and carbohydrate (HP/LCF) diet for a total period of 30 days.

The composition of both diets is shown in Table 1. Fresh feed was provided on daily basis while stale remnants were discarded after weighing. On the average each rabbit received about 150g/feed/day. Clean drinking water was provided *ad lib*. During this period, feed intake, water intake and dry fecal output were measured daily. Weight gain was recorded weekly. Animal management and experimental procedures were performed in strict accordance with the requirements of the National Research Council's Guide for the use of Laboratory Animals (NRC, 1985).

Blood samples, collection and analysis: The animals were fasted for 18hr and baseline blood samples were drawn from the rabbit ear veins using 21-gauge syringes. At the end of the feeding period, the rabbits were anesthetized with pentobarbital (60mg/Kg body weight). Insertion was made into the heart region for collection with the use of a needle and syringe. The blood samples were collected into labeled bijou bottles and were allowed to clot at room temperature for 45 minutes before being centrifuged (3,000 x g for 10 minutes), to obtain the serum. The serum samples were stored in the biofreezer at -10^{0}C until analyzed. Duplicate serum samples for each animal group were analyzed for Total proteins (Brown, 1976), albumin, globulins (Baertl *et al.*, 1974) electrolytes (Na^+, K^+, HCO_3^-, Cl^-) (Kinsley and Schaffert, 1953), lipid (Anderson, 1971), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT) (Moss *et al.*, 1971) urea and creatinine (Carr, 1959) were measured using commercial kits (Boehringer Mannheim).

Table 2: Weight gain, feed intake, water intake, feed efficiency and dry fecal output of rabbits in the control and experimental groups

Group	Control diet	Experimental diet
Weight gain (g/rabbit)	650±8.0^a	150±6.0^b
Feed intake (g/rabbit/day)	53.8	66.5
Water intake(ml/rabbit/day)	20.50±2.0^a	14.50±7.9^b
Feed efficiency (g/body weight/g feed)	12.08	2.25
Dry fecal output (g/rabbit/day)	5.24±1.14^a	3.60±1.3^b

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly (P<0.05)

Statistical analysis: Data were expressed as mean ± Standard error of the mean (SEM) for each group of rabbits. Comparison between the control and experimental set of data was analyzed by the student's t-test and P values < 0.05 were indicative of significance. The statistical analyses were done with INSTAT statistical package.

Results

Table 1 shows the Composition of control and experimental diet fed to the rabbits. The weight gain, feed and water intake, feed efficiency and dry fecal output of the rabbits in the control and experimental groups are presented in Table 2. Statistical analysis showed that there was a significant decrease in weight gain, the mean body weights were 650±8.0g with animals fed the control diet and 350±9.0g with animals fed the experimental diet. Feed and water intake and Dry fecal output in the experimental diet were also significantly reduced when compared with the control diet.

The values observed for total protein, albumin and globulin are given in Table 3. The values obtained for the control samples, is total proteins (1.28±0.41mg/dl), albumin (0.26±0.15mg/dl) and globulin (1.75±0.52mg/dl).These values were significantly (P <0.05) elevated in rabbits fed the experimental diet.

The values obtained for AST, ALT, ALP is shown in Table 4. The AST and ALT levels significantly (P<0.05) increased while ALP levels significantly (P<0.05) decreased, when compared to the values obtained from rabbits fed the experimental diet.

Table 5 shows the level of electrolytes, creatinine and urea in rabbits. The rabbits fed the HP/LCF diet had a significant increase (P<0.05) in sodium and chloride ions content when compared with the control.

Potassium and bicarbonate ions increased significantly content when compared with the control (P<0.05).The creatinine did not change significantly (P<0.05) while serum urea concentration were 34.9±0.86mg/dl after feeding with the high protein diet, this value increased

Table 3: Mean concentrations of total proteins, albumin and globulins of rabbits on the control and experimental diet

Parameters	Control diet	Experimental diet
Total Protein	1.28±0.41[a]	7.46±0.31[b]
Albumin	0.26±0.15[a]	5.03±0.57[b]
Globulin	1.75±0.52[a]	2.86±1.18[b]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly (P<0.05)

Table 4: Mean concentrations of serum enzymes and of rabbits on the control and experimental diet

Parameters	Control diet	Experimental diet
AST(IU/dl)	2.90±0.07[a]	17.5±0.86[b]
ALT(IU/dl)	7.65±0.47[a]	95.6±0.68[b]
ALP(IU/dl)	32.5±16.48[a]	8.9±0.48[b]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly (P<0.05)

significantly (P<0.05) when compared with control diet. Table 6 shows the values of serum lipids of rabbits fed the control and experimental diet. Serum cholesterol levels were significantly (P<0.05) reduced. The lipoprotein fractions were also significantly altered. HDL-Cholesterol levels were significantly elevated (P<0.05) to 22.67±3.54mg/dl when compared with the control value of 6.5±0.96mg/dl. LDL-cholesterol and triacylglycerol concentration were not significantly altered (P<0.05) in the experimental groups when compared with the control diet.

Discussion

The aim of this study was to determine the consequences of a long term high protein and low carbohydrate and fat Nigerian-like diet in Rabbits. The results show that, the high protein diet increased food intake. There was also, a significant reduction in body weight. This finding is in agreement with the previous studies in rats fed a high protein diet for 6 months. (Lacroix *et al.*, 2004). The source of protein consumed could affect the results obtained for the experimental diet. The source of the protein is fish. He *et al.* (2004), reported that increase intake of fish as a protein source had beneficial effects. Compared with red meat, the white meat from fish contains similar amount of protein and has substantially less saturated fat and cholesterol (Hu, 2005).

The mean concentration of total proteins, albumin and globulins of rabbits were significantly elevated. This showed that the high protein diet favored protein synthesis of new cells and tissues. The high albumin values, is a predictor of future development of clinical renal disease.

The liver enzymes AST and ALT were elevated significantly, in animals fed the high protein diet. The roles of the liver and kidney in amino acid and nitrogen

Table 5: Mean concentrations of electrolytes, creatinine and urea of rabbits on the control and experimental diet

Parameters	Control diet	Experimental diet
Potassium(mM/L)	0.47±0.11[a]	4.23±0.112[b]
Sodium(mM/L)	17.0±2.55[a]	147.3±0.89[b]
Bicarbonate(mM/L)	3.67±0.58[a]	15.34±0.37[a]
Chloride(mM/L)	12.00±0.58[a]	110.0±1.14[b]
Creatinine(mg/dl)	0.46±0.06[a]	0.66±1.71[a]
Urea(mg/dl)	8.33±3.69[a]	34.9±0.86[b]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly (P<0.05)

Table 6: Mean concentrations of plasma lipids on rabbits fed the control and experimental diet

Parameters	Control diet	Experimental diet
Total Cholesterol(mg/dl)	50.18±7.75[a]	40.16±0.87[b]
HDL-Cholesterol(mg/dl)	6.5±0.96[a]	22.67±3.54[b]
LDL-Cholesterol(mg/dl)	32.18±7.22[a]	32.50±5.23[a]
Triacylgl;ycerol(mg/dl)	10.5±7.59[a]	11.6±4.18[a]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly (P<0.05).

metabolism had led to the suspicions of a high protein diet having potential deleterious effects on the structure and function of the organs. (Jean *et al.*, 2001). The high protein diet led to a significantly increase in serum urea levels, this observation in the experimental animals could be due to the inability of the liver to mobilize urea, leading to a build up. A similar observation has been reported by Jenkins *et al.* (2001).

Serum creatinine increased slightly, but not significantly, this suggests that fish protein had no major adverse effects on renal function in the short term. The effects of the long term are unknown. Street (2001), reported no dysfunction in the kidney of healthy individuals consuming an *ad lib* high protein diet. This study is in agreement with the observation.

Creatinine remains significantly unchanged. This is an indicator of normal kidney function. However, the significantly increased levels of the serum electrolytes namely sodium, potassium and chloride ions, could be a pointer to a reduction in renal functioning capacity, due to the inability of the kidney to regulate the levels of these electrolytes. Sodium ion and chloride ion is distributed principally in the extracellular fluid and so associated with its retention.

Wachman and Bernstein (1968) reported dietary protein (animal based) results in acidification of urine. In order to buffer the urine and maintain acid-base homeostasis, bicarbonate is increased. This was observed in this study. The production of endogenous acids may result in the mobilization of calcium from the skeleton resulting in a net calciuria. This results in reduced bone mineral density and increasing the risk of fractures/ osteoporosis

In this present study, the significantly reduced ALP levels observed could be due to the irreversible inhibition of

ALP activity by high urea concentrations (Fleisher *et al.*, 1977). Bone isoenzyme is most susceptible to urea inhibition. This could be linked to the risk of bone loss or osteoporosis. Although, the literature has conflicting reports as to the effect of high protein diet on bone density (Reddy *et al.*, 2002, Bowen *et al.*, 2004). The recent review by (Bonjour, 2005) reported that there is no convincing published data showing that, a high protein diet, consumed for a prolonged period of time under strictly controlled dietary conditions could lead to osteoporosis.

The high fish protein diet resulted in a significantly reduced total cholesterol levels while, HDL increased significantly. Scott *et al.* (1991), reported that a predominantly fish based diet lead to a 40% reduction of plasma lipoproteins-an independent cardiovascular risk factor.

The significantly higher HDL-Cholesterol concentration is also protective. Hu *et al.* (1999a), reported that high protein intake reduces the risk of ischemic heart disease.

This is in agreement with studies performed in humans (Farmsworth *et al.*, 2003; Layman *et al.*, 2003). There was a stabilization of Triacylglycerol and LDL-Cholesterol. This may reduce cardiovascular disease risk. The high protein diet was associated with a reduced risk of cardiovascular disease (Hu *et al.*, 1999b)

Conclusion: The increased substitution of fish for carbohydrate may have health benefits which includes weight control and prevention of cardiovascular disease. Although, Kidney function is not affected, there are indications that declining function may be possible. The potential negative side effects of increased protein, in this study, showed elevated liver enzymes and the apparent inability to mobilize urea which could lead to a build up of intermediary metabolites which could be toxic, indicating liver dysfunction.

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