International Journal of Food Science and Nutrition
ISSN: 2455-4898; Impact Factor: RJIF 5.14
Received: 23-11-2018; Accepted: 26-12-2018
www.foodsciencejournal.com
Volume 4; Issue 1; January 2019; Page No. 62-65

Nutritional composition of Hexa-Whole Grain Diet: its effect on the performance (hematology and blood glucose) of high fat diet induced hyperlipidemic male wistar rat

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Abstract
Nutritional quality of food is a key element in maintaining overall well-being which cushions a sustainable force for health and development. The nutritional composition of whole grain diet comprising guinea corn, maize (white and yellow), millet, wheat and soybeans was investigated, determining the effect of processing on the diets' nutritional quality. The effect of the diet on hematology and blood glucose level in High Fat Diet induced hyperlipidemic rat model was also revealed in a 5 weeks study (n=5). The whole grain diet had an adequate composition of nutrients: a high percentage of protein (25.56%), fibre (7.1%) and fat (20.7%) but a reduced anti-nutritional factor due to processing (boiling).
The hematological parameters and blood glucose of the rats fed whole grain diet showed no significant difference ($p<0.05$) with the control. The whole grain diet with its proper nutrient composition ensured a good hematology profile and glucose level.

Keywords: nutrition, whole grain, high fat diet, hematology, blood glucose

1. Introduction
Good nutrition provided by nutrients in food is a key to mental and physical health, thus, essential for good health and nourishing the body. Food insufficiency and malnutrition have been the major problem of the developing countries, which in extremities leads to death. A poor diet may be challenging causing deficiency diseases such as blindness, anaemia, scurvy, preterm birth, stillbirth and cretinism (Whitney *et al*., 2009) [19]. It can also cause health-threatening conditions like obesity, metabolic syndrome, and osteoporosis amongst others. Currently there is an increasing preference among consumers for foods that contain not only traditional nutrients but also provide other compounds that are beneficial to health and well-being. Therefore, dietary quality of food should be taken into consideration for solving the problem of deep-rooted food insecurity and malnutrition. Grains and grain foods are staples in the diets of culture around the world and have made an important contribution to daily nutrient requirements. They are the leading contributors of key nutrients (fibre, iron, magnesium, iodine, carbohydrate and B vitamins) in the diet of some races especially the Australians. Whole grains consisting of three essential parts (bran, endosperm and germ) provides complex carbohydrates, dietary fiber, minerals, vitamins, phytochemicals and other substances (Jacobs *et al*., 1998) [9]. These nutrients packed in whole grains may prevent diseases, lower blood cholesterol, stabilize blood sugar and improve immune function. (Slavin *et al*., 2013) [14].
Guinea corn (*Sorghum vulgare*) the fifth most important cereal in the world (FAO, 2008) [6], Wheat (*Triticum aestivum*) an excellent health-building food, Millet (*Pennisetum glaucum*), Maize (*Zea mays*) and Soya bean (*Glycine max*) a widely used, inexpensive and nutritional source of dietary protein (Mc Arthur *et al*., 1988) [10], are all nutrient-rich foods that offers a number of nutritional and therapeutic benefits.

Table 1: Review of proximate composition of maize, millet, sorghum, wheat and soya bean

	Protein	Fat	Ash	Moisture	Fibre	Carbohydrate	Reference
Maize	8.58	2.85	1.16	9.19	2.83	75.39	Abdulrahman and Omoniyi (2016) [1]
	8.75	2.40	2.19	7.16	2.40	77.46	Ape *et al*. (2016)
Millet	11.03	2.56	1.67	8.56	3.19	72.99	Abdulrahman and Omoniyi (2016) [1]
Sorghum	10.13	2.70	1.41	8.04	1.86	75.86	Abdulrahman and Omoniyi (2016) [1]
	9.10	3.10	2.07	6.36	2.86	76.51	Ape *et al*. (2016) [1]
Soya beans	37.69	28.2	4.29	8.07	5.44	16.31	Ogbemudia *et al*. (2017)
Wheat	12.39	2.50	1.42	6.90	1.14	75.65	Abdulrahman and Omoniyi (2016) [1]

High-fat diets are known to lead to a positive fat balance and consequently to adipose mass accumulation (Flatt, 1995) [5] which can be directly associated with hyperlipidemia and insulin resistance. Different types of fat in the diet can drive different responses in glucose homeostasis and insulin sensitivity (Tierney and Roche, 2007) [17] and according to Srinivasan *et al*. (2005) [16] prolonged exposure of high-fat diet leads to insulin resistance which can result in mild hyperglycemia. One benefit of whole grain is related to its' ability in decreasing insulin resistance and improve insulin sensitivity possibly due the fact that they have low values of the glycemic index. Whole grains generally have both soluble and insoluble fiber that slows stomach emptying and nutrient absorption, reducing the rise in glucose and insulin to improve blood sugar control.

2. Materials and Methods
2.1 Sample collection and preparation
The grains (millet, maize, guinea corn, wheat), soya beans, were obtained from a market in Ado-Ekiti, Ekiti State,

Nigeria. They were cleaned, boiled, air dried and blended to a fine sample for analysis and diet formulation.

2.2 Chemicals/Reagents
All chemicals and reagents used were obtained commercially and of analytical grade.

2.3 Nutritional analysis
The proximate analysis was carried out using the standard methods of Association of Analytical Chemists (AOAC, 2000), for the determination of moisture content, ash content, crude protein, lipid and fibre. Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition. The mineral elements iron, magnesium and selenium were determined using Atomic Absorption Spectrometry (AAS) machine while K and Na were determined using flame photometry by the method described by Khan and Zeb (2007). Phytate concentration was determined according to the method of Wheeler and Ferrel (1971); oxalate determined according to Day and Underwood (1986).

2.4 Animals and diet
Twenty (20) male Wistar rats weighing (100-150)g were obtained from the Animal House, College of Medicine, Ekiti State University. They were provided rat pellets and water, *ad libitium*, and subjected to standard environmental conditions such as temperature (26-30°c), relative humidity (45-55%) and 12hrs dark/light cycle. The animals were acclimatized for 7 days and randomly divided into four (4) groups: group 1 (fed standard pelletized diet for 5weeks), group 2 (fed whole grain diet for 5weeks), group 3 (fed high fat diet for 2weeks, replaced with whole grain diet for 3weeks) and group 4 (fed high fat diet for 5 weeks).

2.4.1 Diet formulation
The composition of the whole grain diet and high fat diet is as follow:
Whole Grain Diet**:** 12.3% each of guinea corn, yellow maize, white maize, millet, wheat, 24.5% soy beans, 4% vitamin premix and 10% vegetable oil
High Fat Diet: 50% skimmed milk, 16% corn starch, 4% vitamin premix and 30% Lard.

2.5 Biochemical analysis
At the end of five weeks, the animals were fasted overnight, sacrificed under chloroform anaesthesia and blood collected from the jugular vein into heparinized bottle for analysis.
The blood glucose level was determined at the end of 2nd and 5th week using Oncall blood glucose machine and test strips.

2.5.1 Determination of hematology parameters
The packed cell volume (%) and hemoglobin concentration (g/dl) were determined according to the hematocrit method described by Alexander and Griffiths (1993); red blood cells ($\times 10^6$µl) and white blood cells ($\times 10^6$µl) were estimated by visual method using New Improved Neubauer counting chamber. The corpuscular constant were estimated as follows: Mean Corpuscular Volume (MCV in fl) = (PCV % x10)/RBC and Mean Corpuscular Hemoglobin (MCH in P_g) = (HB X 10)/RBC

2.6 Statistical analysis
Data are presented as mean ± standard deviation, analyzed using student's t-test to compare the difference between the control and test groups. P-values less than 0.05 ($p < 0.05$) was considered as indicative of significance. Graph Pad Prism 5.00 was also used for statistical calculations.

3. Results

Table 2: Proximate composition of whole grain diet (%)

Moisture content	**8.2 ± 0.07**
Crude Fibre	7.1 ± 0.06
Crude protein	25.56 ± 0.27
Ash content	3.3 ± 0.01
Crude fat	20.7 ± 0.13
Carbohydrate	35.14 ± 0.41

Values are expressed as Mean ± S.D

Table 3: Mineral and anti-nutritional composition of whole grain diet

Iron (Fe)	**1.76 ± 0.04**
Selenium (Se)	3.89 ± 0.10
Magnesium (Mg)	17.94 ± 0.40
Sodium (Na)	21.37 ± 0.28
Potassium (K)	18.09 ± 0.20
Oxalate	1.667 ± 0.06
Phytate	3.708 ± 0.58

Values are expressed as Mean ± S.D

Table 4: Nutritional comparison between boiled and un-boiled whole grain diet

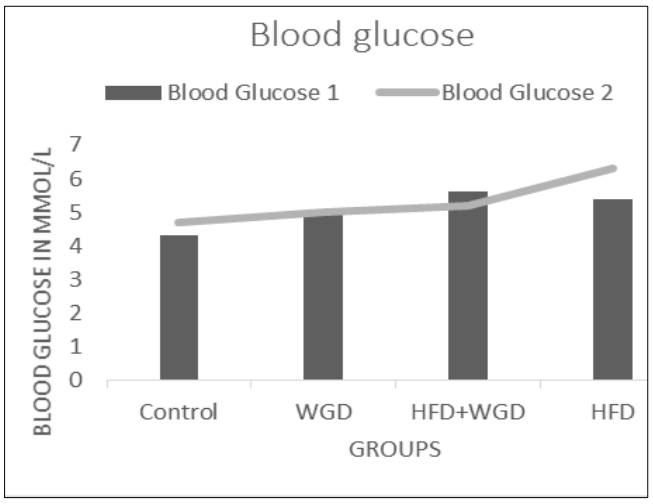
	Boiled	**Raw**
Moisture content	8.2 ± 0.07*	7.2 ± 0.03
Crude Fibre	7.1 ± 0.06	6.7 ± 0.02
Crude protein	25.56 ± 0.27*	29.35 ± 0.25
Ash content	3.3 ± 0.01*	2.9 ± 0.02
Crude fat	20.7 ± 0.13*	17.03 ± 0.25
Carbohydrate	35.14 ± 0.41	36.87 ± 0.02
Iron (Fe)	1.76 ± 0.04	1.1 ± 0.04
Selenium (Se)	3.89 ± 0.10*	5.13 ± 0.08
Magnesium (Mg)	17.94 ± 0.40	23.79 ± 0. 33
Sodium (Na)	21.37 ± 0.28*	29.33 ± 0.53
Potassium (K)	18.09 ± 0.20*	20.98 ± 0.19
Oxalate	1.667 ± 0.06	1.846 ± 0.06
Phytate	3.708 ± 0.58*	24.37 ± 4.49

Values are expressed as Mean ± S.D; significant difference $p < 0.05$
*significant difference compared to raw whole grain diet

Table 5: Hematology profile of rats fed Whole Grain and High Fat Diet

Parameters	**Control**	**WGD**	**HFD+WGD**	**HFD**
PCV (%)	47 ± 0.28	46 ± 0.14	45 ± 0.71c	40 ± 0.57ab
Hb (g/dl)	15.6 ± 0.28	15.3 ± 0.15	15 ± 0.00	13.3 ± 1.70
RBC (×106µl)	5.15 ± 0.04	4.47 ± 0.07	6.05 ± 0.21c	3.68 ± 0.06ab
WBC (×106µl)	76 ± 2.83	60 ± 1.41	64 ± 1.69bc	50 ± 2.12b
MCV (fl)	92.2 ± 0.14	92 ± 0.85	75 ± 0.70abc	100 ± 1.56b
MCH (Pg)	30 ± 1.27	34 ± 0.42	25 ± 1.98c	37 ± 0.71ab

Values are expressed as Mean ± S.D; significant difference $p < 0.05$ as compared to control, b compared to WGD, c compared to HFD
WGD→ Whole Grain Diet
HFD→ High Fat Diet



Blood glucose 1→ at the end of 2nd week
Blood glucose 2→ at the end of 5th week
WGD→ Whole Grain Diet
HFD→ High Fat Diet

Fig 1: fasting blood glucose (mmol/l) of hyperlipidemic rat fed whole grain diet

4. Discussion

Food composition data provides information for epidemiological studies essential for nutritional planning (Ali *et al*., 2008) [9] and a baseline for future policies and interventions. Considering the reviews on nutritional composition of each grains (table 1), it is revealed from the proximate analysis of the whole grain diet (table 2) that the blends of the grains into a diet had more of crude protein (25.56%), fibre (7.1%), crude fat (20.7%) and ash content (3.3%). Investigations have shown that protein content of cereal-legume combination (i.e., two or more plant based food materials) is better than those produced from cereal alone (Solomon, 2005; Wakil and Onilude, 2009) [15, 18], implying that the diet can be useful in reducing the prevalence of malnutrition. The diet which is whole grain can be an important source of fibre which exhibits many therapeutic benefits helping in lowering blood cholesterol levels and reducing the risk of many disease conditions. The ash content 3.3% is indicative that the diet is an important source of minerals as observed in table 3. According to FAO/WHO (2001), minerals such as iron are low in cereals but the addition of legumes can improve the iron content.

Whole grain are good sources of minerals in our diet but their absorption and utilization can be minimal due to the presence of certain naturally occurring constituents in food (anti-nutrients). These anti-nutrients which are chelators of minerals are considered toxic and boiling is known to play a role in there reduction. The effectiveness of boiling the grains (table 4) was revealed in the level of its phytate (3.708) and oxalate (1.667) content when compared to their levels in the raw (24.37 and 1.846 respectively).

The hematology parameters investigated in the whole grain diet fed rats showed no significant difference with reference to the control. The absence of significant difference as shown in table 5 added to the absence of mortality suggested that the whole grain diet is not toxic. The High Fat Diet fed rat showed considerable significant difference in all parameters except hemoglobin when compared to control and whole grain diet fed rats. A change from high fat diet to whole grain diet presents a meaningful significance when compared to group fed high fat di*et al*one. With this, the percentage volume of red blood cells, the oxygen carrying capacity and the immune system capabilities were not altered by the whole grain diet. Moreover, the proper nutrient composition of the diet especially protein and iron necessary for red blood formation (Ayuk and Essien, 2009) [4] enhanced a good hematology parameter as most of these minerals are well-known hematinic and are essential in the formation of red blood cells (Singh *et al*., 2000) [13].

High fat diet have been discovered to indirectly result in mild hyperglycemia due to a reduced sensitivity of insulin (Srinivasan *et al*., 2005) [16]. The result from figure 1 showed a normal range of fasting blood glucose in both the control and Whole Grain Diet groups. However, the continued consumption of High Fat Diet resulted in a significant increase from 5.4mmol/l to 6.3mmol/l with a significant reduction in blood glucose when the diet was changed from High Fat Diet (5.6mmol/l) to Whole Grain Diet (5.2mmol/l). Studies on glycemic index of grains have revealed that whole grains have low glycemic index than dehulled grain (Sheriff *et al*., 2011; Fung *et al*., 2002) [12, 8] and therefore can have some beneficial effect in the glycemic and lipid control of diabetic patients.

5. Conclusion

The knowledge derived from the nutritional composition of the whole grain diet will aid in the formulation and development of new food products with health enhancing characteristics. Whole grains are affordable and can be stored for long periods, therefore choose whole grain foods instead of the refined.

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