Hematological properties, liver function and lipid profile of albino rat fed with acha and moringa seed flour blends

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Abstract

Background: Therapeutic use of leaves of *M. oleifera* has been evaluated in diabetes because of its possible capacity to decrease blood glucose and lipids concentration after ingestion, as result of the polyphenols content and others compounds. Nevertheless most results have been obtain from leaf extract of *Moringa oleifera*, however, this study use seed powder to evaluate its effects on the hematological properties, liver function and lipid profile of the flour fed albino rats.

Objective: The work is aimed at fortifying acha based food with moringa seed paste to boost the immune system of the body. In the present study, the hematological properties, liver function and lipid profile of moringa-acha composite flour fed albino rats was investigated

Methods: This exploratory study was conducted to investigate the effect of *Moringa oleifera* seed meal supplemented diets on the performance of albino rats. Six different graded levels of acha and *Moringa oleifera* flour blends were used as rat feed. Ration was formulated using acha and *Moringa oleifera* seed flour as ingredient for albino rats. Thirty-six rats were randomly allocated to six treatments (100% acha flour, 95% acha+5% moringa, 90% acha+10% moringa, 85% acha+15% moringa, 80% acha+20% moringa and 75% acha+25% moringa) in a Completely Randomized Design: The albino rats were distributed into 6 replicates comprising three (3) rats per replicate and managed under dip litter system for a period of 4 weeks. Water and feed were provided at ad libitum. Feed consumption, weight gain and feed conversion ratio were evaluated for the individual replicate of each dietary treatment. The hematological properties were determined weekly, while liver function and lipid profile were determined at the end of the experiment. Statistical analysis of the data carried out using SPSS version 23.

Results: The added *Moringa oleifera* seed flour significantly, p=0.5, increase Parked Volume(PVC), White Blood Cells (WBC), Red Blood Cells (RBC) and Total Bilmbim significantly, with positive relationship(r=0.68-0.78). The total cholesterol (CHOL) and triglyceride (TAG) increased slightly while there was a decrease in The High Density Lipoprotein (HDP), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL), with increase in added moringa seed flour. The aspartate aminotransferase (AST), alkaline phosphatase (ALP) and assay acid phosphatase (ACP) decrease slightly, while the lymphocyte increased with increase in the percentage of added moringa seed flour (0-25%).

Conclusions: These results help to increase information over the most popular use of *M. oleifera* and its safety. However there are needed more studies over the hypoglycemic mechanisms and effects over intestinal microbiota.

Keywords: Hematological, liver, lipid, properties, acha and *Moringa oleifera* seed flour, albino rat.

INTRODUCTION

Globally, agriculture plays a key role in improving livelihood, especially in rural communities. A prolonged and good-quality food supply is essential for the development of any stable community. Generally it is expected that individual should be able to fulfill their nutritional requirements while consuming vegetables, fruits, cereals, meat, and milk, but many of these products are not affordable as a great number of persons, especially those who are living below the poverty line in the developing nation. Another important factor is the ignorant of the rural dwellers of the nutrient richness of the spectrum of plants around their immediate environment.

The current search for alternative to wheat flour particularly in the developing nations that have been adapted to wheat based baked products has leads to research into some lost crops or underutilized crops. Acha, millet and maize have since attract attention of researchers.

Acha (*Digitaria exillis*) is a cereal grain in the family of gramineae and commonly referred to as fonio or hungry rice (Alamu, 2001; Ayo and Nkama 2004). The major traditional foods from the grain are: thick (tuwo) and thin (kuku), porridge, steamed product (burabusko or couscous), gwete and alcoholic beverages (Jideani and Akingbala, 1993; Jideani, 2012; Ayo and Nkama, 2006). Acha grains may be boiled like rice; flour from acha may be fortified with other cereals flour especially for the production of porridge or pudding (Nzlibe, 1995; Ayo et al., 2009). Nigerian, particularly in Plateau, Bauchi and Kaduna states apart from consumption of the grain also burns the straw to produce ash which is extracted with water and the filtrates used as potash for cooking indigenous delicacies(Ayo et al., 2010; Ayo and Kajo, 2017).

Acha is nutritious and its seed is rich in methionine and cystine, which are vital to human health and deficient to today's major cereals like wheat, rice, maize, sorghum, barley and rye (Jideani and Akingbala, 1993; Ayo et al., 2008). Enrichment of cereal food with other protein sources such as legumes (soybean, bambara groundnut, tiger-nut, etc) has received considerable attention because it's high lysine content (an essential amino acid) which is limiting in most cereals (Alabo, 2001; Ayo et al., 2010; Ayo et al., 2017). However, despite the nutrient quality of acha grain, there is still need to upgrade the quantity of the nutrient using locally available but underutilized crops such as *Moringa oliveria.*

Moringa oliveria, highly nitrous and with adaptability to the diverse and challenging environments, is one of the plants easily grown with little effort and are available within the reach of the people. The nutritional value and composition have been strongly emphasized in the literature for different trees and shrubs to expose their importance for livestock (D'Mello and Devendra, 1995). The recommendation of trees, shrubs, or fodder crops is based on proximate analysis including crude protein, crude fiber, ash mineral contents, etc. Their high protein content is one of the most cited advantages of moringa leaves. For example, they contain 9 times more protein than yoghurt does, as is repeatedly mentioned in the literature (Mathur, 2006).

The leaves of moringa are eaten as vegetables of food ingredient because of the high content of vitamins, antioxidants and macronutrients to improve nutritional deficiencies (Asare et al., 2012). However the study of effect of biological compounds of different part of *Moringa oleifera* plant have brought different action mechanism, functional benefits and toxicity profile that have not yet been elucidated.

Many studies have shown the potential use of different parts of *M. Oleifera* in food applications such as in making soups (Babayeju et al., 2014), weaning foods (Arise et al., 2014), amala, a stiff dough made from yam and plantain flour (Karim et al., 2015: 2013), herbal biscuits (Alam et al., 2014), bread (Chinma et al., 2014) and cake (Kolawole et al., 2013). The use of this nutrient rich plants in fortifying foods is getting more attention. The research work is aimed at evaluating the effect of adding moringa seed flour on the hematological properties, liver function and lipid profile of albino rat fed with acha flour

MATERIALS AND METHODS

Materials

Acha flour: Acha (*Digitaria exilis*), the creamy colored type, locally grown, was purchased at Jos central market, Jos, Nigeria. The acha grain were cleaned (by handpicking the chaff), de-stoned (water sedimentation), dried (at 45˚C in a hot air cabinet Dryer APV-machine Dryer), milled (attrition mill machine-Lister Inc. England), sieved (0.4 mm aperture) and vacuum packaged (Phlico Vacum sealer, Hongkong) in polyethylene.

Moringa seed flour: The *Moringa oleiferal* seeds was purchased from Wukari New market, Wukari, Nigeria, washed (using tap water), dried in hot air dryer (at 50-55°C), milled (Attrition mill) sieved (0.4 mm), dry (50˚C for 30 mins) and vacuum packed in colored poly-ethylene and stored at 4-6˚C until usage.

Acha-moringa flour blend: Moringa seed flour was substituted into acha flour at 5, 10, 15, 20 and 25% and mixing thoroughly using Kenwood mixer to produced acha and moringa flour blend.

Experimental design: Study was conducted to investigate the effect of *Moringa oleifera* seed meal

supplemented diets on the performance of albino rats. Ration was formulated using acha and *Moringa oleifera* seed flour as ingredient for albino rats. Thirty-six rats were randomly allocated to six treatments: i. 100% acha flour, ii. 95% acha+5% moringa, iii. 90% acha+10% moringa, iv. 85% acha+15% moringa, v. 80% acha+20% moringa and vi. 75% acha+25% *moringa* in a Completely Randomized Design. The albino rats were distributed into 6 replicates comprising 3 rats per replicate and managed under dip litter system for a period of 4 weeks. Water and feed were provided at *ad libitum*. The hematological parameters were determined weekly while the liver function and lipid profile were determined at the end of the feeding period on the slaughtered rats.

METHODS

Determination of hematological properties: The blood samples were collected from the rat tail veins by ocular method. Packed Cell Volume (PCV) was determined by the Microhaematocrit method (Ochei and Kolhatkar, 2008), White Blood Cell, Red Blood Cell, Lymphocyte, hemoglobinof fed animal (rabit) were determined as described by Passmore and Eastwood (1996), Rosewear et al. (1989).

Determination of lipid profile: Serum cholesterol level was measured using the method described by Allain et al. (1974). High density lipoprotein content was determined as described by Albers et al. (1978). Low density lipoprotein content was determined using the method of Assman et al. (1984). Triglyceride was determined using glycerol-phosphate oxidase method described by Albers et al. (1978).

$$\text{Low density lipoproteins } (\text{LDL}) = \text{Total cholesterol} = \text{HDL} - \frac{\text{TAG}}{5}$$

Very low density lipoproteins VLDL=HDL+LDL+Total Cholesterol.

Determination of liver function: The aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activity were determined by Reitman and Frankel (1957).

RESULTS AND DISCUSSION

Hematological Parameters

The Parked Cell Volume (PVC), White Blood Cells (WBC), Red Blood Cells (RBC) and Total Bilmbim significantly, p=0.5, increased from 38.00 ± 4.72-47.50 ± 1.87%, 6406.60 ± 15.00-8450.00 ± 920.33 10^6/mm^3, 286.0 ± 26.98-306.83 ± 48.45 10^9/L and 0.63 ± 0.16-0.81 ± 0.11 mg/dl, respectively, with increase in the percentage of added (0-25%) moringa paste as shown in Table 1. There are positive relationship(r) between the moringa seed flour and the respective assessed parameters (PVC=0.7, WBC=0.78, RBC=0.68). The increase could be due to inherent high protein (quantitative and qualitative) and bioactive components in moringa seed which could responsible for the improvement of the hematological parameters (Mishra et al., 2011; Olson and Fahey, 2001).

Lipid Profile

The addition of moringa seed flour affected the lipid profile of the rat as shown in Table 2. The total cholesterol (CHOL) and triglyceride (TAG) increased slightly from 135.11 ± 5.85-138.77 ± 5.46 mg/100 g, 134.78 ± 5.43-135.92 ± 3.35 mg/100 g, respectively, while there was a decrease in the High Density Lipoprotein (HDP), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) from 51.82 ± 3.95-50.46 ± 2.32 mg/100 g, 23.83 ± 2.95-23.08 ± 1.32 mg/100 g and 211.79 ± 5.13-209.81 ± 5.03 mg/100 g, respectively, with increase in added moringa seed flour. The high fat content of moringa seed (Mathur, 2006; Babayeju et al., 2014) could have contributed to the relative increase in the cholesterol and triglycerides. However, the maximum level of the cholesterol (138.77 mg/100 g) as observed in this work is relatively lower than upper limit (150 mg/100 g).

Liver Function

The effect of added moringa seed flour on liver function of rat fed with the acha-moringa seed flour blends are shown in Table 3. The aspartate aminotransferase (AST), alkaline phosphatase (ALP) and assay acid phosphatase (ACP) decrease slightly from 28.00 ± 4.15-23.17 ± 3.19 mg/100 g, 56.33 ± 11.60-42.67 ± 3.08 mg/100 g and 22.92 ± 1.39-22.52 ± 1.04 mg/100 g, while the lymphocyte increased from 29.50 ± 1.87-31.50 ± 3.73 mg/100 g, respectively, with increase in the added moringa seed flour (0-25%).The increase in the lymphocyte level could be due to the improvement in the amino acids and bioactive compounds resulting in the increase in the formation of

Table1: Effect of added moringa seed paste on the hematological quality of rat fed with acha and moringa composite flour

Moringa paste %	PVC (%)	WBC (x 10^6/mm^3)	RBC (x 10^9/L)	T Bilmbin (mg/dl)
0	38.00 ± 4.73^c	6466.66 ± 1500.22^b	286.00 ± 26.98^a	.63 ± .17^b
5	47.33 ± 6.12^b	8083.33 ± 899.81^a	300.83 ± 60.77^a	.66 ± .17^a
10	45.33 ± 5.47^b	8033.33 ±1335.16^a	306.00 ± 47.95^a	.65 ± .13^b
15	56.67± 4.46^a	8133.33 ±1440.37^a	290.50 ± 28.10^a	.81 ± .11^a
20	56.50 ± 6.57^a	8033.33 ± 880.15^{ab}	340.17 ± 42.08^a	.62 ± .13^{ab}
25	47.50± 1.87^b	8450.00 ± 920.33^a	306.83 ± 48.45^a	.45 ± .24^b

Note: Means average with the same alphabets(s) same column are not significantly different (p=0.05).

Table 2: Lipid Profile of Rat fed with moringa flour

Moringa: Acha flour	CHO (mg/100 g)	TAG (mg/100 g)	HDL (mg/100 g)	LDL (mg/100 g)	VLDL (mg/100 g)
0.0694444	135.11 ± 5.85^{a}	134.78 ± 5.43^{a}	51.82 ± 3.95^{a}	23.83 ± 2.95^{a}	211.79 ± 5.13^{a}
0.2743056	135.40 ± 11.69^{a}	132.16 ± 5.22^{a}	47.99 ± 2.09^{ab}	21.56 ± 2.02^{b}	204.95 ± 3.05^{ab}
0.4791667	130.28 ± 3.95^{a}	129.75 ± 4.69^{a}	47.16 ± 3.26^{ab}	21.21 ± 1.32^{b}	198.65 ± 3.65^{b}
0.6840278	134.34 ± 5.42^{a}	131.26 ± 3.94^{a}	48.92 ± 3.61^{ab}	22.67 ±2.31^{ab}	205.38 ± 5.23^{ab}
0.8888889	138.64 ± 6.34^{a}	133.81 ± 7.53^{a}	49.88 ± 3.61^{ab}	23.12 ± 1.72^{a}	211.64 ± 3.00^{a}
25:75	138.77 ± 5.46^{a}	135.92 ± 3.35^{a}	50.46 ± 2.32^{a}	23.08 ±1.32^{a}	209.31 ± 5.03^{a}

Note: Means average with the same alphabets(s) same column are not significantly different (p=0.05).

Table 3: Liver function test of Rat fed with moringa and acha blend flour

Moringa: Acha flour	AST (mg/100 g)	NLYM (mg/100 g)	ALP (mg/100 g)	ACP (mg/100 g)
0.0694444	28.00 ± 4.15^{a}	29.50 ± 1.87^{b}	56.33 ± 11.60^{a}	22.92 ± 1.39^{a}
0.2743056	27.33 ± 3.56^{ab}	32.67 ± 4.18^{ab}	53.50± 8.98a^{b}	23.23 ± 1.58^{a}
0.4791667	23.83 ± 3.06^{abc}	33.17 ± 3.06^{ab}	46.00 ± 6.36^{bc}	23.33 ± 1.85^{a}
0.6840278	21.83 ± 1.72^{abc}	33.00 ± 1.41^{ab}	58.67 ± 3.27^{a}	23.34 ± 2.16^{a}
0.8888889	24.00 ± 3.74^{c}	35.50 ± 2.89^{a}	61.67 ± 4.27^{a}	23.23 ± 1.55^{a}
25:75	23.17 ± 3.19^{c}	31.50 ± 3.73^{b}	42.67 ± 3.08^{c}	22.52 ± 1.04^{a}

Note: Means average with the same alphabets(s) same column are not significantly different (p=0.05).

lymphocytes. The increase in the level of lymphocytes could aid the boosting of the immune system of the blood with greater capacity to defend the system from external invaders (pathogenic microorganisms) and maintaining healthy individual(Mishra et al., 2011; Olson and Fahey 2001). The level of alkaline and acid phosphatase could be ideal for the adequate functioning of the aspartate aminotransferase needed in the synthesis of non-essential amino acids in the liver system. It could therefore be said that the addition of moringa seed flour would improve the health status of the consumer.

CONCLUSION

The studies have shown that the consumption of moringa seed flour could improve the heamatological components of the blood, while the effect on the liver function (improve production of lymphocytes) have showed a great potential for boasting the immune system of the blood with great capacity to defend the system against external invaders and also synthesis of protein consequence to production of relevant enzymes such as amino acid tranferase in the liver. However, the relative increase of the cholesterol on addition of moringa seed flour, though within the acceptable limit calls for caution with higher level of the flour. It can therefore be said the moringa seed flour can be used as nutritional tools to improve and sustain the health of the consumers. .

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