

## **Original Research Article**

**Body weight, hematological and biochemical parameters of winstar rats fed with chia seed concentrate**

**Comment [A1]:** What is this, Winstar?

### **Abstract**

**Comment [A2]:** The research objectives and research methods are unclear.

Chia seeds are among commonly used natural remedies to control obesity, diabetes mellitus and cardiovascular diseases. In the current study, rats were fed with chia seed powder mixed with broiler chicken feed mash at 0, 5, 10, 15 and 20%. Body weight gain, hematological parameters, blood glucose, and lipid profiles were assessed. Results showed no significant effect on weight gain in chia treated groups compared to control group. Hematological parameters also indicated that there are no significant ( $p > 0.05$ ) changes in most of hematological parameters but there was significant increase ( $R^2 = 0.87$ ;  $p = 0.032$ ) in white blood cell (WBC) count and lymphocytes in a dose dependent manner. Plasma glucose and lipid profile were observed to decrease significantly in a dose dependent manner whereby for glucose, group 4 rats decreased from  $138.5 \pm 0.8$  to  $80.3 \pm 12.9$  mg/dL from day 0 to day 28 of treatment respectively. In conclusion, findings in this study illustrated that chia seeds can be the good candidate for controlling body weight, blood glucose, lipid profile and improving hematological parameters in animals.

**Keywords:** Chia seeds, blood glucose, lipid profile, rats

**Introduction :**

Recent reports from the [1] have indicated increased mortalities of up 17.9 million deaths per year attributable to metabolic disease conditions such as obesity, diabetes mellitus and cardiovascular diseases [1]. About 1.5 million of the deaths being caused by diabetes mellitus [1]. Treatment of metabolic disorders often requires changes of lifestyle habits such as consumption of healthy diets and engaging in routine physical exercises [2][3]. However, it can be challenging for patients to adopt this lifestyle and faithfully adhere to it.

There are several industrial medications available to treat some metabolic disorders. However, there are many challenges in treatment and management of these conditions [4][2]. For instance, finding the right medication and dosage for each patient can be a challenge. Additionally, some patients have adverse reactions to certain medications while others have limited access to healthcare resources, [5][6]. These challenges necessitate for search of alternative therapeutic approaches including functional foods supplementation towards preventing and controlling these conditions and other associated health risks. Several herbs, spices and natural remedies have been used as alternative therapeutics to these medications [7][8][9]. Chia seeds are among the commonly used natural remedies for controlling obesity, diabetes mellitus and cardiovascular diseases [10][11][12]. The seeds have been in use for instance for medicinal purposes by Mexican communities, among others since 3500 BC [13]. Nowadays, Chia seeds have extended use beyond medicine as used by ancient communities and have many industrial uses including; Oil industry to produce edible oil and mayonnaise, beverage industry where the seeds and products are used to enrich beverages, milk industry where it is used in butter, cheeses, and fresh dairy products such as yoghurt, packaging industry where the seeds are used to produce biodegradable films, meat, snacks and extrusion industries among others [14][15][16][17].

Chia is an annual flowering plant that belongs to a family Lamiaceae. The seeds are known to contain mainly; protein, fat, carbohydrate, dietary fibre, ash and dry matter contents [18][19]. They also contain phenolic compounds, minerals and omega-3 fatty acids, and  $\alpha$ -linolenic

acid[20]. Omega-3 fatty acid are essential fatty acids known to have a large number of physiological functions in human body[21][22],for instance, they function as antioxidants attributable to chlorogenic acid, caffeic acid, myricetin, quercetin, and kaempferol [23]. These agents are believed to have renal, cardiac, hepatic protective effects, anti dyslipidemia, anti hypertension, anti-ageing and anti-carcinogenic characteristics [24][10].

Chia seed are also known to improve insulin sensitivity, maintenance of bone mass, decrease inflammation of the tissue and decrease vascular reactivity[25][26][7]. They also contain high fiber content important in the control body weight gain and obesity[27][28]. Chia seeds however have a hardy seed coat which makes the hydrolysis of the coat lipids and proteins difficult hence difficult to release the bioactive components of the seed matrix. This makes an overall difficulty in bioavailability of nutrients from the seeds [29].In addition, they are small in size and lowly cohesive, making them difficult to incorporate into food and feed products [30].Processing of the seeds could simplify the release of the valuable chia seeds nutrients making them bioavailable. Soaking, sprouting or fermenting, the seeds could make digestion easier in the gut for easy release of nutrients [30].Sprouting is one of the cheapest methods for chia seeds processing that can improve bioavailability of the nutrients from the seeds. Grinding of the seeds is another simpler way to expose the seeds matrix from the hard seed coat to make the nutrients bioavailable [30] while preserving the nutrients. In this research we used chia seed powder after sun drying and mixed with commercial broiler chicken mash to compose experimental animal feeds.

This study evaluated the effect of ground dried chia seed on body weight, selected hematological and biochemical parameters using Wistar rats as model animals.

## Materials and methods

### Study area

The study was conducted at College of Veterinary Medicine and Biomedical Science (CVMBS), Sokoine University of Agriculture (SUA), Morogoro.

**Comment [A3]:** You don't need to examine the germination of chia seeds in the background. Are you researching germination?

Just focus on chia seeds and their benefits on weight, hematological parameters, and biochemical parameters that you are investigating.

### **Research design**

An experimental study was done where mature healthy male and female **winstar** rats were fed on different percentages of chia seed meal and broiler chicken mash as basal diet to study the effects of the seeds in growth rate and selected hematological parameters.

**Comment [A4]:** What is this, Winstar?

### **Chia seed meal preparation**

Five chia seed feed formulations were prepared for the experiments at 0, 5, 10, 15 and 20% commercial broiler chicken feeds mash. The above formulations were prepared by addition of 0, 50, 100, 150, and 200g of chia seed powder to make 1,000g of the commercial broiler chicken mash for different feed formulations making up five formulations referred to as formulation 0 (F0) to formulations 5 (F4). The feeds were compounded three times (once a week) and stored in cool dry place without direct sun light through the experiment period.

### **Preparation of experimental animals**

A total of 100 mature male and female wistar rats weighing between 180 to 200g were used for the experiments. The rats were divided into five subgroups each with 20 Wistar rats designated group 0 (G0) to group 4 (G4). G0 was used as the control group while G1-G4 were the treatment groups. All rats were housed in zoo-sanitary cages and had full access to potable tap drinking water and the formulated broiler chicken mash feeds with chia seed powder. Feeding the rats were such that G0 was fed. The groups were fed on the different feed formulations containing the chia percentages such that: G0 (Control group) was the control group and was fed on F0. The rest of the groups were fed on F1 for G1 to F4 for G4 respectively daily for 28 days. The room temperature and humidity conditions in the experimental rooms were adopted to the general room environmental weather conditions at average of 26°C temperature and 75% humidity of the the Sokoine University of Agriculture environmental conditions. Dark and light conditions were also adopted the environmental cycles of dark and light which approximated to 12 hours light and 12 hours of darkness where light was allowed through open windows.

### **Data and samples collection**

Data on feed intake and body weights were recorded daily for all the rats in all cages during the entire period of the experiment. Intracardial blood samples were collected where approximately 4ml of blood samples were collected following procedures explained by Parasuraman *et al.*, [31]. for approach to collection of blood samples in small laboratory animals where blood was collected on days 0, 7, 14, 21 and 28. Briefly, the blood samples were collected through cardiac puncture after humane sacrifice of three rats from each group on every sampling day. About 1ml of the blood was then transferred into blood sample bottles containing ethyl diamine tetraacetate (EDTA) for haematological parameters analyses. 2ml of the blood samples for the respective rats and groups were immediately centrifuged at 3,000 revolution per minute (rpm) for 10 minutes to obtain fresh plasma. The plasma samples were used for analyses of hematological biochemical parameters using spectrophotometer (Cole Patner 1,100 rs). All samplings were done out in the mornings between 7 and 9 A.M in order to reduce on diurnal variability. The collected blood samples in EDTA vacutainer tubes were immediately analysed using the MS<sup>TM</sup>4-s, MS laboratories, France, an automated hematological analyzer. For glucose assays, blood samples were collected in sodium fluorinated vacutainer tubes to preserve glucose while for the other biochemical parameters, the blood samples were collected in heparinized vacutainer tubes. The blood were centrifuged to obtain plasma which were stored at 4°C prior to assays.

**Comment [A5]:** Is euthanasia performed on the mouse samples?  
If yes, please describe the method used

### **Determination of plasma glucose and lipid profiles**

Determination of the blood glucose, total protein and lipid profile levels was done by the glucose-oxidase principle as explained by Trinders [32].

**Comment [A6]:** Explain the methods and tools you use in collecting weight data.

### **Data analysis**

Data were statistically analyzed to check for differences of the means among the groups using one way analysis of variance (ANOVA). Differences were considered significant when *P values* were equal to or less than 0.05. Differences between the means of the groups were also analyzed for significance using the student's *t*-test and a Tukey's Honest Significance Difference (Tukey HSD) where differences were considered significant at *P* equal or less than 0.05. The means were

expressed with their corresponding standard error of the means (mean  $\pm$  SE). The data were analyzed in the statistical package for social sciences (SPSS) version 29, 2022 [33].

## Results

### Body weight

Feed consumption and body weight measurements were carefully monitored in all groups of rats throughout the experimental period. Feed consumption and body weight gain results for the control and experimental groups are indicated in Table 1. From the results, mean body weights of the rats in the control group were slightly lower than, but not significantly different ( $p=1.069$ ) from the treatment groups.

Table 1: Means  $\pm$  SE of body weights (g) for control of the rats which were fed on basal diet and treatment groups which were fed on chia seed feed formulations for 28 days.

Groups	Treatment time in days				
	0	7	14	21	28
G0	155.9 $\pm$ 16.5	193.8 $\pm$ 14.5	177.9 $\pm$ 12.3	179.7 $\pm$ 12.6	185.3 $\pm$ 12.3
G1	190.6 $\pm$ 15.2	182.5 $\pm$ 17.7	180.5 $\pm$ 12.1	173.1 $\pm$ 10.5	175.1 $\pm$ 14.2
G2	178.7 $\pm$ 14.3	183.4 $\pm$ 12.8	170.3 $\pm$ 17.5	175.0 $\pm$ 14.4	167.5 $\pm$ 21.7
G3	189.9 $\pm$ 12.9	183.7 $\pm$ 17.8	184.0 $\pm$ 12.3	178.6 $\pm$ 21.2	165.9 $\pm$ 13.4
G4	178.7 $\pm$ 12.7	173.4 $\pm$ 22.0	165.3 $\pm$ 13.5	177.0 $\pm$ 14.4	177.9 $\pm$ 11.7*

\* = the mean of body weight differed significantly from the rest of the group means

### Hematological results

Generally there were no significant differences ( $P>0.05$ ) in hematological parameters among the rats groups as indicated in Table 2. For red blood cells (RBC) count, blood hemoglobin concentration (Hb) and hematocrit (Hct). But there was a significant dose dependent increase ( $p<0.05$ ) in the white blood cell count (WBC), lymphocytes (LYM) and monocytes (MON) concentrations. The mean platelet volume (MPV) and platelets (PLT) were observed to be significantly lower than ( $p = 0.008$ ) the control group G0.

**Table 2.** Mean hematological parameters before and 28 days after administration chia seed in rats

Parameters	G0		G1		G2		G3		G4	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
WBC(M/mm <sup>3</sup> )	5.9±0.3	5.8 ± 3.7	5.6 ± 1.0	6.2 ± 2.9	5.8 ± 0.5	8.5 ± 3.1	5.3 ± 3.4	10.1 ± 2.4	5.9 ± 0.3	7.7 ± 0.3
Lym(%)	60.8 ± 1.8	57.1 ± 10.5	66.0 ± 18.2	64.7 ± 12.0	59.9 ± 6.	68.8 ± 9.3	61.8 ± 3.1	68.6 ± 14.2	60.8 ± 1.8	68.9 ± 2.3
Mon(%)	12.5 ± 0.5	16.2 ± 4.5	14.4 ± 6.2	17.5 ± 6.1	18.1 ± 1.1	14.6 ± 4.2	18.4 ± 0.1	13.0 ± 3.9	12.5 ± 0.5	14.5 ± 1.2
Neu(%)	6.7 ± 1.3	8.7 ± 4.6	9.6 ± 12.90	7.6 ± 4.3	14.3 ± 2.4	6.5 ± 5.0	6.55 ± 0.2	13.6 ± 5.5	6.7 ± 1.3	10.5 ± 0.9
RBC(M/mm <sup>3</sup> )	5.8 ± 0.1	6.3 ± 0.0	5.1 ± 0.2	6.1 ± 1.1*	6.5 ± 0.1	5.7 ± 0.6	4.2 ± 1.52	6.12 ± 0.2	5.8 ± 0.1	7.5 ± 0.4
MCV(ft)	54.9 ± 2.5	65.4 ± 1.5	55.7 ± 0.2	55.1 ± 1.2	61.1 ± 0.4	55.5 ± 1.3	45.5 ± 15.5	60.0 ± 2.7	54.9 ± 2.5	62.4 ± 0.8
Hct(%)	31.9 ± 2.0	41.6 ± 1.5	28.7 ± 1.2	33.5 ± 5.3*	39.6 ± 1.0	31.9 ± 4.2	25.1 ± 9.5	36.6 ± 0.4	31.9 ± 2.0	46.0 ± 2.2
MCH(pg)	24.2 ± 0.8	24.0 ± 0.1	25.7 ± 0.3	24.5 ± 1.7	24.2 ± 0.9	25.7 ± 1.5	20.2 ± 7.0	24.8 ± 1.8	24.2 ± 0.8	22.2 ± 0.5
MCHC(g/dL)	44.3 ± 0.5	36.7 ± 0.6	46.5 ± 0.3	34.5 ± 7.7	39.7 ± 1.8	46.5 ± 3.7	33.45 ± 11.3	41.3 ± 1.1	44.3 ± 0.5	35.7 ± 0.3
RDW	11.9 ± 1.3	14.3 ± 0.7	11.4 ± 0.3	11.2 ± 0.5	10.9 ± 0.7	12.1 ± 0.2	10.0 ± 0.6	10.4 ± 0.1	11.9 ± 1.3	10.8 ± 1.0
Hb(g/dL)	14.1 ± 0.7	15.2 ± 0.2	13.2 ± 0.4	14.9 ± 1.4	15.7 ± 0.3	14.7 ± 0.7	11.2 ± 4.2	15.1 ± 0.5	14.1 ± 0.7	16.5 ± 0.6
THR(M/mm <sup>3</sup> )	1034.5 ± 51.5	2310.5 ± 46.0	1337.5 ± 16.5	1221.0 ± 61.0	1265.5 ± 36.4	1961.0 ± 87.0	1427.0 ± 36.0	997.7 ± 107.2	1034.5 ± 51.5	882.2 ± 12.2
MPV(fl)	9.0 ± 0	8.8 ± 0.0	8.6 ± 0.15	8.7 ± 0.1	8.9 ± 0.2	8.25 ± 0.1	7.6 ± 0.1	6.7 ± 0.2	9.0 ± 0	6.4 ± 0.0
PDW	7.6 ± 0.1	7.7 ± 0.0	8.4 ± 0.25	8.2 ± 0.5	6.85 ± 0.0	8.1 ± 0.0	6.4 ± 0.1	0.7 ± 0.1	7.6 ± 0.1	0.6 ± 0.0

*Plasma glucose concentration*

At baseline, the plasma glucose levels were not significantly different between the groups. The level of plasma glucose was observed to decrease significantly ( $p < 0.05$ ) in all treated groups in a dose dependent manner. The decrease was more prominent on 7<sup>th</sup> day of G3 and G4 ( $R^2 = 0.78$   $p = 0.032$ ).

Table 3: The table indicates the mean plasma glucose levels (Mean  $\pm$  S.E) for the control group of rats fed on basal diet treatment group of rats fed on different chia seed feed formulations()

Groups	Treatment days				
	0	7	14	21	28
G0	105.7 $\pm$ 4.2	111.5 $\pm$ 3.1	118.7 $\pm$ 20.5	117.6 $\pm$ 2.7	119.9 $\pm$ 9.9
G1	113.5 $\pm$ 3.5	122.8 $\pm$ 2.6	123.8 $\pm$ 7.5	73.5 $\pm$ 6.3	61.3 $\pm$ 10.9
G2	116.5 $\pm$ 2.0	118.7 $\pm$ 4.7	122.1 $\pm$ 14.9	102.9 $\pm$ 5.6	80.3 $\pm$ 12.9
G3	131.1 $\pm$ 1.8	158.1 $\pm$ 9.6	131.3 $\pm$ 1.1	86.2 $\pm$ 7.5	83.1 $\pm$ 14.1
G4	138.5 $\pm$ 0.8	110.7 $\pm$ 4.7	107.1 $\pm$ 4.9	89.9 $\pm$ 5.9	80.3 $\pm$ 12.9

Note: Normal value 76- 175 mg/dL

*Plasma cholesterol concentration*

Results in Fig 1 illustrates that there is significant in all lipid profile in a dose dependent manner ( $R^2 = 99.1$ ;  $p = 0.000335$ ) compared to the control group G0. The decrease was much in the LDL and VLDL

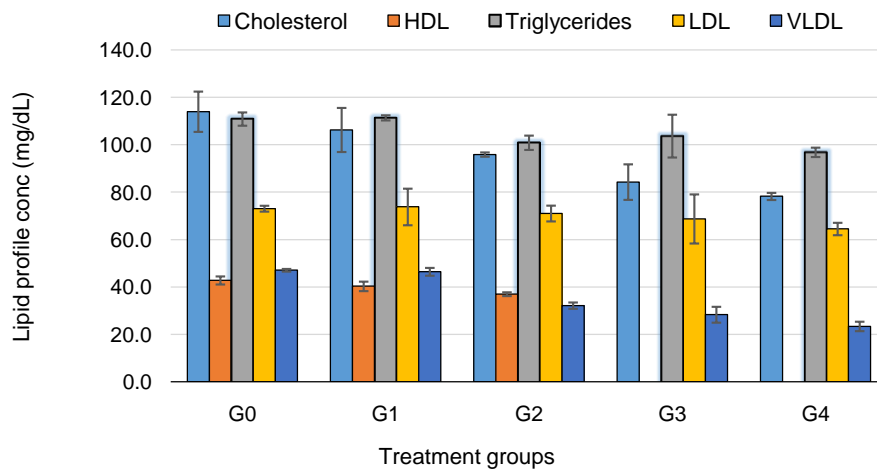


Fig 1: Plasma lipid profile of rats fed with chia concentrate (Mean±S.E)

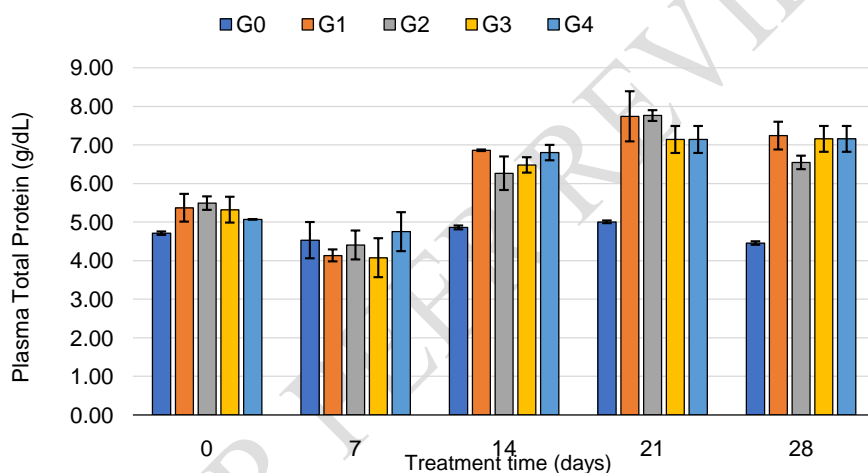


Fig 2: Plasma total protein of rats fed with chia concentrate (Mean±S.E)

For plasma total protein, results indicated there was significant increase ( $p=0.048$ ) in the levels compared to the control group G0. The level ranged from 4.71 to 7.16 g/dL for G0 to G4 that is, the rats fed with high concentration of chia seed concentrate. For the control groups, the value does not change insignificantly.

## Discussion

The current study evaluated the effect of chia seed on body weight, some selected hematological and biochemical parameter using rats as model experimental animals. From the study results revealed that the chia seed concentrate had no significant change in body weight of rats fed with chia seed concentrate as compared to the control group. The results concurred with the findings of the study done by Marineli *et al.*, [34], Da Silva *et al.*, [35] and Alamri, [36] who reported insignificant variation of body weight following inclusion of chia seed in feeds. The insignificant weight changes could be due to high fiber contents (more than 80%) in chia seed [37]. Dietary supplementation of chia seeds resulted into increased value of red blood cell indices such as RBC count, Hb, Hct and RDW compared to the control group (G0). This could be explained as the results of chia in boosting micronutrients such as iron and calcium in the body [38] which are among factors promoting blood formation. The increased level of white blood cell indices such as WBC, lymphocytes and monocytes was the indication that chia seeds are capable of boosting the immune system [39][7][40].

All these results are in agreement with the study done by Alarcon *et al.*, [41] Alagawany *et al.*, [42] Mihafu *et al.*, [43]. who reported improvement of hematological markers following dietary supplementation with chia seed in different animals. The decreased levels of platelets in blood could be due to effect of omega 3 fatty acids which is the important in reducing the content of arachidonic acid in membrane phospholipids of platelets, endothelial cells, and inflammatory cells. This reduces the amount of pro-inflammatory factors that are derived from arachidonic acid. Also Alpha-linoleic acid is found to down-regulate the activity of a nuclear factor NF- $\kappa$ B which plays an important role in generating inflammatory responses which is known to cause cardiovascular diseases [10][44][43]. Alpha-linolenic acid decreases the risk of thrombosis by inhibiting platelet aggregation. They act upon the mRNA expression of the growth factors of platelets, thus stop abnormal growth and proliferation of platelets and play a key role in preventing formation of clots and plaques in the arteries thus help to prevent cardiovascular diseases [10][44][43]. The plasma glucose and lipid lowering effect of chia seeds have been reported in different animal models as reported from a study by Gian *et al.*, [45].

**Comment [A7]:** Does high fiber consumption not aid in weight loss?

**Comment [A8]:** Explain the mechanisms that occur leading to a decrease in blood glucose levels and lipid profile after the administration of chia seeds

**Conclusion:** findings in this study illustrated that chia seeds can be the good candidate for controlling body weight, blood glucose, lipid profile and improving hematological parameters in animals. This which are marked as good indicators for controlling metabolic diseases such as as type 2 diabetes mellitus, obesity and cardiovascular diseases are increasing and causes death of both livestock and humans. Further researches are necessary for more confirmation of chia seed on their mode of action and toxicity in animal body.

#### **Ethical Clearance**

The study and all procedures involving the animals were conducted with strict adherence to guidelines and procedures reviewed and approved by the Institutional Ethical Clearance Committee from the Directorate of the Post graduate, Research and Consultancy Committee of Sokoine University of Agriculture.

**Comment [A9]:** move to the methods section

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