THE EFFECT OF SUPPLEMENTING MICE BASAL DIET WITH SOLAR DRIED INDIGENOUS VEGETABLE FOOD FORMULATION ON GROWTH AND IMMUNE INDICATORS IN KIAMBU, KENYA

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Abstract

Kenya and other African countries are facing food and nutritional insecurity. Indigenous green leafy vegetables are of much importance among food crops as they reduce food insecurity, provide adequate amount of vitamins, immune boosting trace element and anti-oxidants. The nutritional value of the indigenous vegetable remains underutilized due to lack of awareness and appropriate technology for their effective use. The aim of the present work was to study effect of supplementing mice basal diet with solar dried indigenous vegetables rich in beta-carotene, iron and zinc food formulation on growth and immune indicators. The indigenous food supplement comprised of solar dried Gynandropsis gnandra (spider plant), Cucurbita maxima (pumpkin leaves) and Agaricus bisporus (button mushroom). Growth performance, feed efficiency and hematological parameters were determined using mice bioassay. The mice were fed with basal diet added either 25%, 50% or 75% prepared food supplement. The control was fed only basal diet. The results of bioassay showed the group fed with 25% of the prepared food supplement had the highest feed efficiency with a value of 6.96%, There was an increase in white blood cells, lymphocytes and monocytes on the group of animals fed with 25% of the indiginous vegetable supplement as compared to control, however the change was not statistically significant different ($p<0.05$). Inclusion of 25% of the solar dried indigenous vegetables mix in the basal diet gave the most promising results compared with 50% and 75%. The study findings promote the use of solar dried indiginous vegetables to provide low levels of zinc, iron and beta-carotene to fight malnutrition and increase immunity. The result show that taking a high percent of solar dried vegetables compared to other food rich in carbohydrate and protein may not improve immunity.

Key words: Indigenous vegetables, food supplement, feeding efficiency

1.0 Introduction

Indigenous vegetables are plants whose leaves, fruits or roots are used as vegetables (FAO, 1988; Chweya and Eyzaguirre, 1999). They are also those plants that have evolved within and spread throughout an area unassisted by humans. The indigenous vegetables have not received much attention although they grow very fast, can sustain harsh environmental conditions and have high nutritional value (Grivetti and Ogle, 2000).

They are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, mineral, vitamins, fiber and other nutrients which are usually in short supply in daily diets. They have a potential that has yet to be exploited in the effective management of the HIV/AIDS infected persons and healing stomach related ailments (Chweya and Eyzaguirre, 1999).

Lack of dietary diversity is particularly a severe problem among the poor populations in developing countries such as Kenya, Tanzania and Uganda. This has been attributed to the fact that the diets in such countries are mainly based on starchy staples with little or no fruits and vegetables (Chweya and Eyzaguirre, 1999). Low consumption of fruits and vegetables is the main contributor to micronutrient deficiencies (WHO**,** 2005).

Population increase and economic crisis has resulted to malnutrition in some places in Kenya such as Turkana, Marsabit and Samburu especially in children as high as 37.4 per cent due to shortage of food (WHO, 2005). The rich nutritive value of these indigenous vegetables need to be utilized to supplement the low levels of vitamin A, zinc and iron in the blood of HIV/AIDS patient (Tang and Lanzillotti, 2005) and others with similar complications. There is need for processing and formulating a food product from these vegetable to ensure that they are available all the year even in their low season Therefore, this study proposed to study effect of supplementing rats basal diet with analyzed indigenous vegetable *Gynandropsis gnandra* (spider plant), *Cucurbita maxima* (pumpkin leaves) and *Agaricus bisporus* (button mushroom) rich in beta-carotene, iron and zinc food formulation on growth and immune indicators.

2.0 Materials and Methods

2.1 Sampling and sample pretreatment

Fresh samples indigenous vegetables were obtained from two farms in Lari and Limuru in Kiambu County. Three types of indigenous vegetables were identified as *Gynandropsis gnandra* (spider plant), *Cucurbita maxima* (pumpkin leaves) and *Agaricus bisporus* (mushroom). Samples each 1kg was obtained from each indigenous vegetable indentified and transported to laboratory for analysis and preparation of food supplement.

2.2 Chemicals and reagents

The reagents and chemicals used to determine mineral elements of vegetable samples in this study were of analytical grade. Nitric acid, hydrochloric acid and beta-carotene used were purchased from Thomas Baker chemicals Ltd Mumbai India. Magnesium oxide Hyflosupercel, acetone, petroleum ether, Zinc, Manganese, Iron, sodium sulphate, EDTA, glacial acetic acid, gentian violet stain, methanol and polychromic staining solution were sourced from GmbH Chemical Company, inc. USA.

2.3 Sample analysis

Standard procedure was followed according to Horwitz, 2001 for determination of mineral elements where the digest was analyzed using Flame atomic absorption spectrophotometer for Zn and Fe (model AA-10). The extraction and analysis of beta-carotene was adapted from that given by Amaya (2001).

2.4 Formulation, preparation of experimental diet

A food supplement was formulated from the solar dried indigenous vegetables to meet the daily recommended allowance of vitamin A, Zn, and Fe (Institute of Medicine, 2001). The amount of each indigenous vegetable that was added to the food formula to provide the RDA of the three immune boosting nutrients was determined by linear programming in Excel by Toledo (1980). The ratio obtained from the mix of *Gynandropsis gnandra, Cucurbita maxima,*and *Agaricus bisporus* was 1:3:4 respectively. The indigenous vegetables were first solar dried at an average temperature of 45^{o}C using solar rooftop dryer and ground into powder form before preparing a vegetable mixture for formulation. The mixture was then blended together in the electrical mixer for full homogenization before coming up with a food formula which was later sieved through a silk sieve.

2.5 Experimental animals

2.5.1 Animal grouping and feeding

Male Mice of the inbred Bagg albino strain, reared in Kenyatta University zoology laboratory, were removed from their mothers when three weeks old and placed in separate, polypropylene cages on soft sawdust bedding at room temperature of 25 ± 2°C.They were fed with basal diet for four days to acclimatize to the new environment according to Reeves *et al,* (1993). The animals were distributed with the same weight into four (4) groups of five mice each.

The control group (5 animals) was fed throughout with the basal diet while the group 1, group 2 and group 3, which all consisted of five (5) animals each, received a composite blend of formulated vegetable mix at percentiles together with basal.

Control group (All weighing 20 g): Basal diet

Group 1: (All weighing 15g):25% indigenous vegetable mix blend + basal diet

Group 2: (All weighing 18g): 50% indigenous vegetable mix blend + basal diet

Group 3: (All weighing 22g): 75% indigenous vegetable mix blend+ basal diet

Figure 2.1 Pelleted solar dried indiginous vegetable mixture

The powdered food mixtures were made into pellets shown in figure 2.1 using a pelletor to allow easy feeding of the mice. Mice were given feed and water ad libitum for 42 days, measurements of food intakes and body weights being recorded at weekly intervals.

During the period of the experiment, the food intake and the mice were weighed twice weekly. Growth curves were drawn representing the relationship between the mice body weights and time. After the end of experimental period, biological changes; food intake, body weight gain, and food efficiency ratio (body weight gain/total food intake) were calculated and tabulated.

2.6 Collection of blood samples and measurement of hematological parameters

2.6.1 Determination of total leucocyte count by haemocytometry

The method of Gottfried and Gerard (1987) was adapted. Blood samples were taken at the beginning of feeding and at the end of feeding the 42nd day. Blood from mice were obtain by cardiac puncture into light-shielded centrifuge tubes and placed into sample bottles containing EDTA (1mg/ml) to be mixed with WBC diluting fluid that was taken in a watch glass. The glacial acetic acid was used to lyses the red cells while the gentian violet was used to slightly stain the nuclei of the leukocyte. Blood was drawn up to 0.5 mark of the WBC pipette and WBC diluting fluid was drawn up to 11 mark. The fluid and blood were mixed well and the first few drops of blood were discarded by holding the pipette vertically. The counting chamber was charged with a drop of blood mixed with diluting fluid. The chamber was left undisturbed for few minutes. The four corners of the chamber were visualized under a low power (10x) objective and the cells were counted in all the four marked corner squares.

$$\text{WBC per cubic mm} = \frac{\text{Number of cells x dilution factor}}{\text{Area counted x depth}}$$

Where:

(1) Dilution = 1:20

(2) Area counted = 4 sq mm

(3) Depth of fluid= 1/10mm

$$\text{WBC} = \frac{N \times 20}{0.4}$$

2.6.2 Determination of differential WBC count by Haemocytometry

The differential white blood cell count shows the various individual white blood cell types found in peripheral blood of which the predominant circulating leukocyte is the lymphocyte, followed by neutrophils, monocytes, eosinophils, and lastly basophils.

A thin smear was prepared by spreading a small drop blood evenly on a slide by another slide at an angle of 45°. The smear was stained by Leishman stain by placing the slide on a stain rock. It was allowed to react with the stain for 2 to 3 minutes. Then double distilled water was used to wash the excess stain on the slide. It was allowed to dry for 7 minutes and observed under the microscope. The film was examined by moving from one field to the next systematically. About 100 leucocytes were counted to give high degree of accuracy. To get the percent of each cell type, it was multiplied by the total white blood count to arrive at absolute differential counts for the various cell types, method by Gottfried and Gerard (1987). The WBCs were classified as Lymphocites, neutrophils and monocyte.

2.7 Analysis of data

The mean and standard deviation of means were calculated. A one way analysis of variance (ANOVA) and the least significance difference was carried out on the data obtained. Significance was accepted at $p \leq 0.05$.

3.0 Results and discussions

3.1 Chemical composition of indigenous vegetables

The chemical properties and beta-carotene composition of the vegetables were examined. The vegetables are good source of zinc, iron and beta-carotene as shown in table 3.1. *Gynandropsis gnandra* had the highest levels of iron and beta-carotene with a value of 90.24 mg/100 g and 6.32 mg/100 g respectively. Zinc levels was highest in *Cucurbita maxima* with a value of 1.23 mg/100 g.

Table 3.1 Levels of selected mineral content and beta-carotene in the analyzed indigenous vegetables (Mean ±SD, n=12) (mg/100 g)

Indigenous vegetables	Zinc	P-value	Iron	P-value	Beta-carotene	P-value
Gynandropsis gnandra	0.35±0.09[a]	0.000	90.24±23.14[a]	0.000	6.32±0.18[a]	0.000
Cucurbita maxima	1.23±0.21[b]		20.86±8.32[b]		2.86±0.32[b]	
Agaricus bisporus	0.80±0.43[c]		3.04±0.18[c]		4.23±0.43[c]	

Means in a column followed by same letter are not significantly different

3.2 Feed efficiency of animals fed with the formulated vegetable mix

Figure 3.1 shows the feed efficiency of mice fed with different proportion of indigenous vegetable mix when compared to a basal diet which served as a control. The group of mice fed with 25% of indigenous vegetable mix had the highest feed efficiency of 6.96. The lowest was the group fed with 75% of the indigenous vegetable mix with a value of 4.42. From the results, it can be stated that increasing the levels of indigenous food supplement in the diet of mice reduced the feeding efficiency while supplementation at the right proportion 25% increased the efficiency which suggests that the modified diet at this proportion was more accepted to the animals.

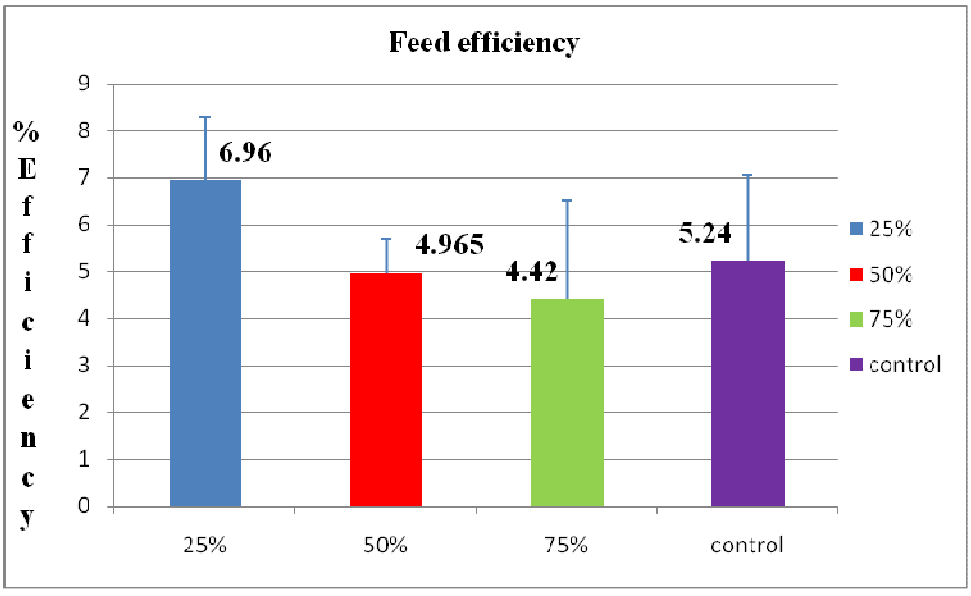

Figure 3.1 Feeding efficiency

3.3 Weight gain of animals fed with the formulated vegetable mix

The weight gain of mice fed with different proportion of indiginous vegetable mix blend is shown in figure 3.2. At the end of the experiment the highest mean weight gained was attained from the group of mice fed with control with a value of 37.5 g followed by that fed with 25% of indiginous vegetable mix with a mean value of 36.4 g. The lowest were those fed with 50% and 75% of the mix with a mean value of 32.5 g and 31.7g respectively. Supplimenting food with the right proportion of indiginous vegetable mix cotribute positively to growth and development as compared to the control group.

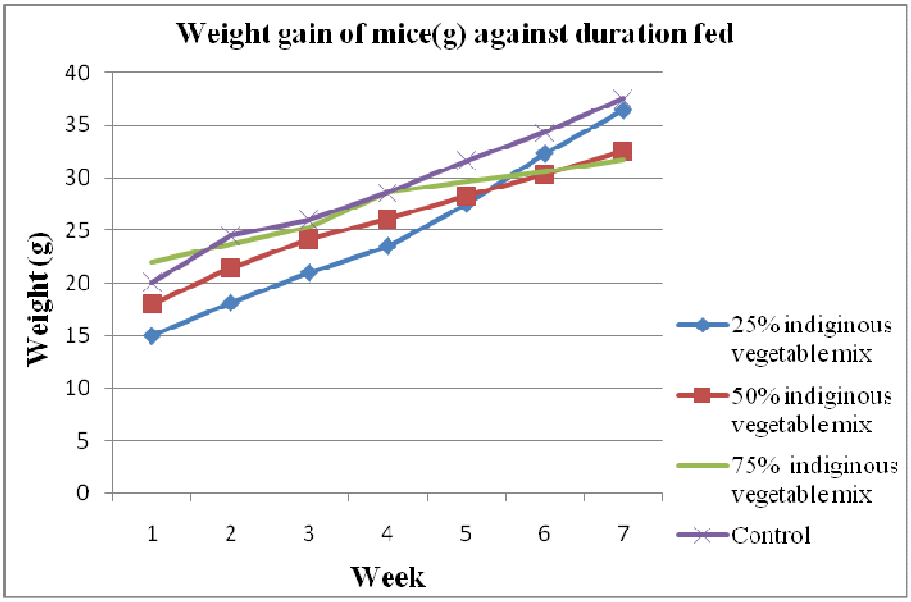

Figure 3.2 A curve of weight gain of mice against time fed

3.4 Hematological parameters

3.4.1 White blood cell count

Figure 3.3 gives a comparison of white blood cells in cubic millimeter of blood obtained at the beginning and at the end of the feeding period with 25%, 50% and 75% of the formulated indigenous vegetable mixture. There was a statistical significant different at $p<0.05$ in the number of white blood cells on the group fed with 25% and 75% of the feed mixture. The number of white blood increased at the 25% and decreased when the animals were fed with 50% and 75% of the prepared food mixture.

The results were comparable with that of Adepoju and Adebanjo, 2011 which showed an increase in white blood cell, which was statistically significant when compared with the control when they fed rats with a formulation of *Cucurbita pepo* seeds. Also they noted that when they increase the doses of the supplement the white blood cell decreased.

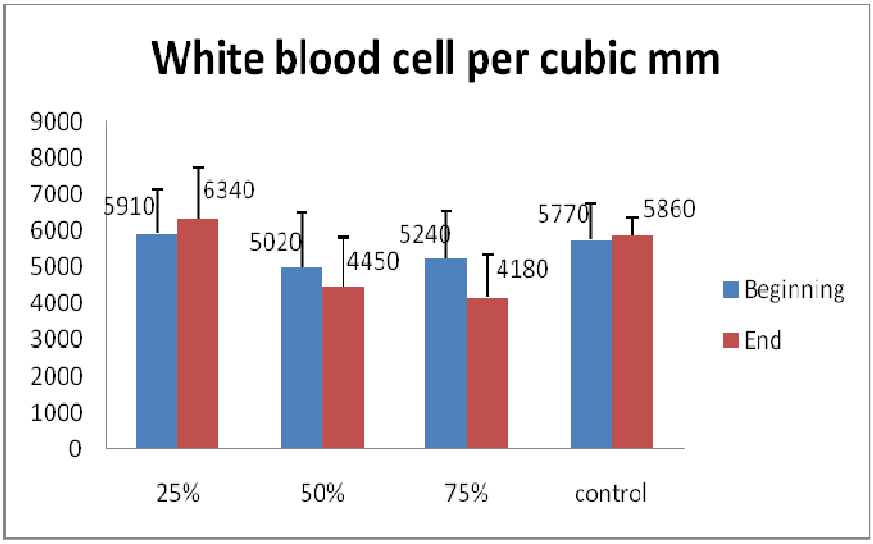

Figure 3.3 a graph of white blood cell in cubic mm

3.4.2 Lymphocytes

From figure 3.4 the study shows that there was an increase in the number lymphocytes on the group fed with with 25% and a decrease of the lymphocytes on the group fed with 50% and 75% however, the change was not statisticaly significant different ($p<0.05$).

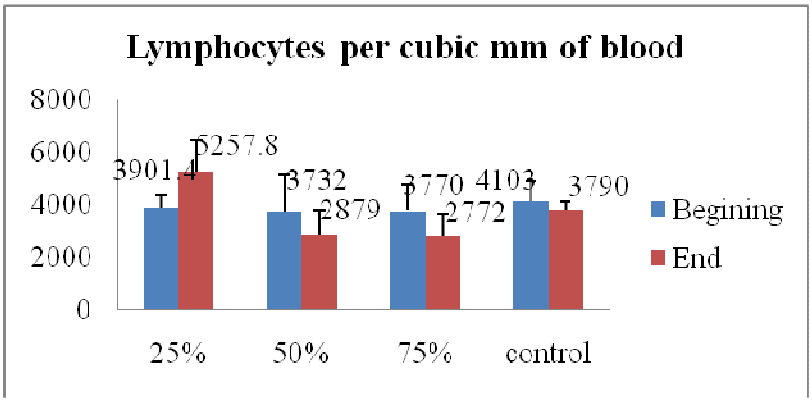

Figure 3.4 a graph of Lymphocytes in cubic mm

3.4.3 Neutrophlys

Figure 3.5 gives a comparison of Neutrophlys in cubic millimeter of blood obtained at the beginning and at the end of the feeding period with 25%, 50% and 75% of the formulated indigenous vegetable mixture. There was no significant change on the group fed with 25% and 75%, however there was a significant change to the group fed with 50% of the food mixture at ($p<0.05$).

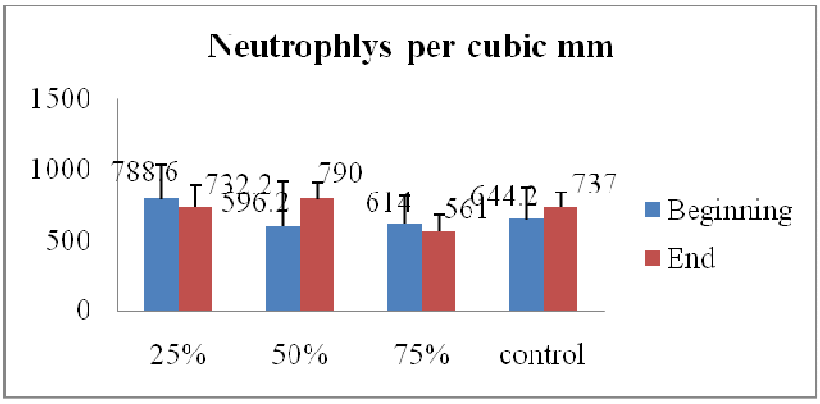

Figure 3.5 a graph of Neutrophlys in cubic mm

3.4.4 Monocytes

From the study, figure 3.6 shows the number of monocytes recorded from the beginning and at the end of the feeding period. The group fed with 25% showed an increase in number of monocytes while there was a decrease of monocytes to the group fed with 75% of the feed mixture, this change was statisticaly significant different at $p<0.05$.

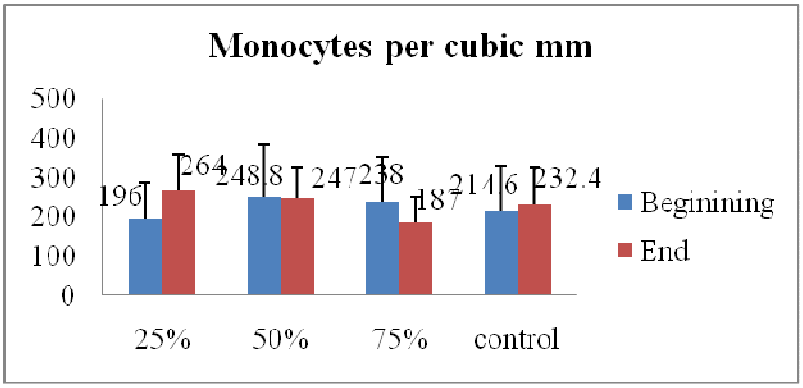

Figure 3.6 a graph of Monocytes in cubic mm

4.0 Conclusions

The aim of the present work was to study effect of supplementing mice basal diet with solar dried indigenous vegetable formulation on growth and immune indicators. Supplementing feeds at the right proportion resulted to an increase in weight gain and increase in feed efficiency. There was an increase in white blood cells, lymphocytes and monocytes on the group of animals fed with right poroprtion of suppliment in this case 25% of the indiginous vegetable supplement. The result show that taking a high percent of vegetables compared to other food rich in carbohydrate and protein may not improve immunity.
Locally available indigenous vegetables are more nutritious and can be solar dried and be used to formulate diet (blends) which can be used to support infants or malnourished in the society.

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