Applied Biological Research 23(1): 37-44; (2021)
DOI: 10.5958/0974-4517.2021.00005.7

In vivo EVALUATION OF *Spirulina platensis* FOR NUTRIENT BIOAVAILABILITY IN MICE

Feven Tezera Damessa[1*], Musa Chacha[2], John-Mary Vianney[3]and Jofrey Raymond[1]

[1]Department of Food and Nutritional Sciences, [2]Department of Sustainable Agriculture and Biodiversity, [3]Department of Health and Biomedical Science, Nelson Mandela African Institution of Science and Technology, Arusha 23100 (Tanzania)
*e-mail: *damessaf@nm-aist.ac.tz*

(Received 24 August, 2020; accepted 1 December, 2020)

ABSTRACT

***Spirulina,* a photosynthetic blue-green alga (cyanobacterium), has drawn attention as a viable food supplement due to its suitable nutrient content. Despite its suitable nutrient composition, the bioavailability of nutrients Present in *Spirulina* is not well reported. In this study, the bioavailability of nutrients present in locally cultivated *Spirulina platensis* was evaluated by using *in vivo* method. A total of 54 mice, 5-8 weeks age were used. The mice were randomly divided into three groups. Group 1 (n = 18) served as a control and received a basal diet. Group 2 (n = 20) served as a test and received *Spirulina* blended with a basal diet. Group 3 (n = 16) serves as a standard and received a basal diet supplemented with nutritional supplements. The study revealed that test diet had apparent absorption of protein 67%, calcium 50.6%, iron 43.8%, zinc 42%, and vitamin A 56.5%, which was higher ($p < 0.01$) than control diet but similar ($p > 0.05$) with standard diet. Given the higher bioavailability of nutritional supplements mixed into the standard diet, the resemblance in nutrient absorption between test and standard diets illustrated that *Spirulina* mixed into the test diet also has higher nutrient absorption.**

Keywords: Nutrient absorption, mice, *Spirulina platensis*

INTRODUCTION

Micronutrient deficiency is an important global public health concern and is particularly high in developing countries (Rasolofoson *et al.*, 2018). Micronutrient deficiencies occur when intake and bioavailability of nutrients are inadequate to endure good health and development (Bouis and Saltzman, 2017). Bioavailability is a general term that refers to the proportion of an ingested nutrient that is absorbed from the diet and used for normal body functions (Nguyen *et al.*, 2012; Jafari and Mcclements, 2017). Several factors influence the bioavailability of ingested nutrients such as the presence of anti-nutritional factors in diet, nutrient interaction with each other, the release of nutrient from food matrix, digestive enzymes in intestine, adherence and uptake of nutrients by the intestine and transfer across the gut wall (Jafari and Mcclements, 2017).

Spirulina is a multicellular photosynthetic blue-green alga (cyanobacterium) that grows naturally in the marine and freshwater environment (Habib *et al.*, 2008). It is a naturally rich and most complete source of organic nutrition containing a substantial concentration of amino acids, polyunsaturated fatty acid, vitamin, protein, polyphenol and halogenated compounds (Dixit, 2018; Nouri and Abbasi, 2018). *Spirulina platensis* and *S. maxima* are the most common and widely available species of *Spirulina* (Saha and Murray, 2018). Although several studies have reported that

Spirulina is rich in essential nutrients, yet there is a dearth of information regarding how much of the nutrients in *Spirulina* can be absorbed and used by the body. Thus, the present study was aimed to evaluate the bioavailability of *Spirulina* nutrients by using the *in vivo* method. Moreover, the effect of phytic acid and nutrient interactions on *Spirulina* nutrient absorption was evaluated.

MATERIALS AND METHODS

This study was approved by Kibong'oto Infectious Diseases Hospital, Nelson Mandela African Institution of Science and Technology, Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) Health Research Ethics Committee-KNCHREC vide approval number KNCHREC00026.

Spirulina samples

Spirulina platensis was obtained from local producers from different areas of Kenya and was cultivated in a pond covered with a greenhouse. After harvest, the biomass of *S. platensis* was dried and grounded to obtain the powdered product.

Experimental animals

A total of 54 mice between the age group of 5-8 weeks and body weight of 21-38 g were used in the study. Animals were caged individually at room temperature with 12 h light and dark cycle. All mice had *ad libitum* access to feed and water. The mice were habituated to the experiment and housing condition (feeding and handling) for 3 days before the start of experiment.

Feeding and treatment

The mice were randomly divided into three experimental groups. Group 1 (n = 18) served as a control and received a basal diet once in a day for four weeks. Group 2 (n = 20) served as test group and received *S. platensis* powder (15%) blended with a basal diet. Group 3 (n = 16) served as a standard group and received a basal diet supplemented with appropriate quantities of casein protein, calcium carbonate, iron sulphate, zinc sulphate, phosphate, retinol, folic acid and cyanocobalamin to receive nutrient levels equivalent to the test diet. Daily intake of feed was recorded during the experiment and the mice were weighed before and after the feeding experiment.

Feces and liver samples collection

Apparent nutrient absorption and liver sample nutrient concentration after the mice been fed on the experimental diets were used to evaluate the bioavailability of nutrients from the diets. The feces samples were collected for 3 consecutive days after the mice had been fed the experimental diets for 27 days. At the end of experiment, the mice were anesthetized and dissected, and their livers excised. The collected feces and liver samples were dried overnight in a vacuum oven at 60°C and powdered for further analyses. The concentrations of vitamin, mineral and protein in the samples were measured using the standard methods proposed by Sami *et al.* (2014); Steponėnienė and Tautkus (2006); Mæhre *et al.* (2018), respectively.

Vitamin analysis

Analysis of vitamin A and B was performed as per the method proposed by Sami *et al.* (2014). Briefly, for vitamin A estimation 1 g pyrogallic acid, 70 mL ethanol and 30 mL (50%) KOH were added to 10 g powdered samples, stirred and refluxed for 40 min using a water bath at 50 ± 2°C. Extracts were obtained using different ether concentrations (50, 30 and 20 mL). The extracts were neutralized by double-distilled water, then dehydrated using anhydrous sodium sulfate, further concentrated to 5 mL in a water bath (50 ± 2°C), and then diluted to 10 mL by using methanol. The extracts of the samples prepared were filtered through 0.46 μm membrane and HPLC analysis performed on an Agilent 1200 series HPLC system (Agilent, USA). The Agilent Eclipse XDB-C18 column was used (5 μm, 4. 6 ×

150 mm), using methanol solvent, and UV detection was recorded at 325 nm. Vitamin separation was based on isocratic elution and the flow rate of the solvent was maintained at 1 mL min^{-1}. Preparation of the standard solution was done daily and followed a serial dilution to a concentration of 5 mg L^{-1} of vitamin A. Thereafter, 20 μL of standard solution and extract was directly injected into the HPLC column. The vitamin A was estimated by comparing its retention times with that of standard solutions. For vitamin B analysis, 25 mL H_2SO_4 (0.1 N) solution was added to 2 g powdered samples and incubated for 30 min at 121°*C*. The contents were cooled and adjusted to pH 4.5 with 2.5 M sodium acetate, then 50 mg Takadiastase enzyme was added and the contents stored at 35°C overnight. The mixture was filtered through a Whatman No.4 filter, the filtrate diluted with 50 mL pure water and filtered again through a micropore filter (0.45 mm). Then the extract (20 μL) was injected into HPLC system for the quantification of vitamin content which was accomplished by comparison with standards. Standard stock solutions for folic acid and cobalamin were prepared according to the standard methods (Sami *et al*., 2014). Chromatographic separation was attained on a reversed-phase (RP-HPLC) column through isocratic delivery mobile phase at 0.5 mL min^{-1} flow rate and ultraviolet (UV) absorbance was recorded at 270 nm at room temperature.

Mineral analysis

Minerals were analyzed by the method of Steponënienë and Tautkus (2006). For this, 10 mL of nitric acid was added to 10 g powdered samples. The mixture was heated for 10 min using a Kjeldahl block digester (Avishkar Int., Mumbai, India). After cooling, 5 mL nitric acid was added, heated again for 30 min, and the solution left as such for 10 min to cool. Then 2 mL distilled water, 3 mL hydrogen peroxide, and 2 mL hydrochloric acid were added and the mixture heated again for another 10 min. The solution was filtered using Whatman filter paper No. 1 and diluted to 100 mL using distilled water. Thereafter, the samples and standard solutions were injected into the atomic absorption spectrophotometer (Rayleigh, WFX-210). Readings on atomic absorption spectrophotometer were recorded at different wavelengths (248.3, 213.9, 422.7, and 213.6 nm) for iron, zinc, calcium and phosphorus estimation, respectively.

Protein analysis

The protein contents in samples were estimated as per Kjeldahl method (Mæhre *et al.*, 2018). Briefly, 1 g powder sample was hydrolyzed with 25 mL conc. sulphuric acid (H_2SO_4) containing two copper catalyst tablets in a heat block (Kjeldahl block digester, Avishkar Int., Mumbai, India) at 370°C for 2 h. After cooling, distilled water was added to the hydrolysates before neutralization and titration. Traditional conversation factor of 6.25 and microalgae specific conversion factor 4.08 (Templeton and Laurens, 2015) were used to convert the total nitrogen into protein for different samples.

Phytate analysis

Phytate was determined by using an anion-exchange method (Guansheng *et al*., 2005). For this, 1 g sample was transferred into 100 mL conical flask and 50 mL of Na_2SO_4 (100 g L^{-1})-HCl (1.2%) was added. The contents were shaken vigorously for 2 h on a rotator at ambient laboratory temperature and the supernatant filtered through qualitative filter paper No. 4. Then, 10 mL filtered extract was diluted to 30 mL with distilled water after mixing with 1 mL 0.75M NaOH and then passed through an anion resin column (resin AG1-X4, ~ 100-200 mesh, Biorad Laboratory Inc.), column 0.8 x 10 cm). The column was washed before use with 20 mL 0.5 mol L^{-1} NaCl solution and deionized water. After sample application, the column was washed with 15 mL distilled water and 20 mL 0.05M NaCl solution to remove the inorganic phosphates. The retained phytic acid was eluted with 0.7M NaCl. The post-column reagent was made up of 0.03% $FeCl_3$ solution containing 0.3% sulfosalicylic acid. Then 4 mL reagent was added to 5 mL collected eluate and centrifuged at 3000 rpm for 10 min. The absorbance of supernatant was measured at 500 nm using a spectrophotometer (LKB 4053, UK). A calibration curve for the colorimetric method was obtained by using phytate standards (P-8810 Sigma Co.). The phytate content of samples was calculated using the standard curve.

Statistical analysis

Data were analyzed using a statistical software IBM SPSS (23). Normal distribution was tested with the Kolmogorov–Smirnov test. One-way ANOVA (between-group variation) was used to evaluate statistically significant differences ($p < 0.05$) between the groups.

RESULTS AND DISCUSSION

Bodyweight

Ingestion of control, test and standard diet did not statistically affect the body weight and feed intake in the experimental mice. The mice weight at the start and end of the experiment did not significantly vary between different dietary treatments groups (Fig. 1). However, at the end of experiment, all the mice had significant weight increments ($p < 0.01$).

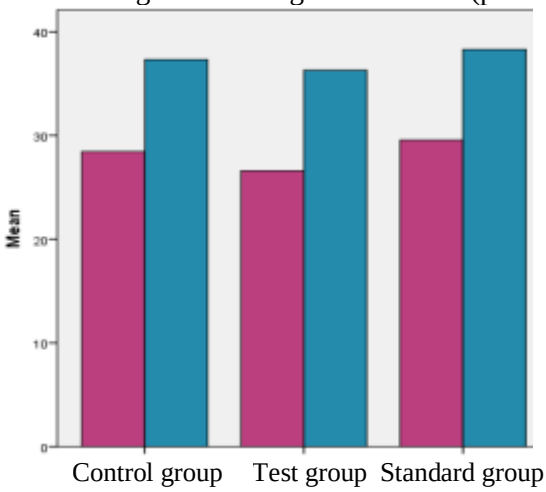

Fig. 1: The weight of control, test and standard group mice before (purple coloured) and after (blue coloured) the experiment

Feed intake

The daily feed intake of control, test and standard group mice was 4 ± 1.4, 4.3 ± 2.2 and 3.9 ± 1.5 g, respectively (Fig. 2), which did not significantly differ from one another ($p = 0.21$). Further, the total feed intake in control, test and standard group mice during the experiment was 99 ± 29, 104 ± 46 and 83 ± 32 g, respectively (Fig. 3), which was at par ($p = 0.24$).

Nutrient composition of experimental diets

The nutrient composition of control diet differed significantly ($p < 0.05$) from the test and standard diets (Table 1). The protein, calcium, vitamin A, and vitamin B_{12} contents in test and standard diets were statistically similar ($p > 0.05$); however, the phosphorus, iron, zinc and vitamin B_9 contents differed significantly ($p < 0.05$).

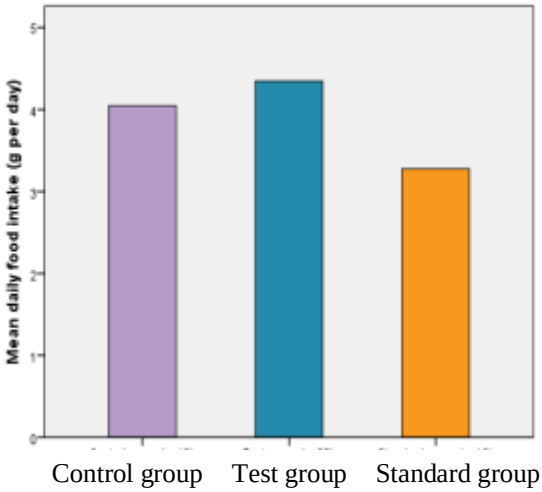

Fig. 2: Daily feed intake in control (light purple), test (blue) and standard (yellow) group mice

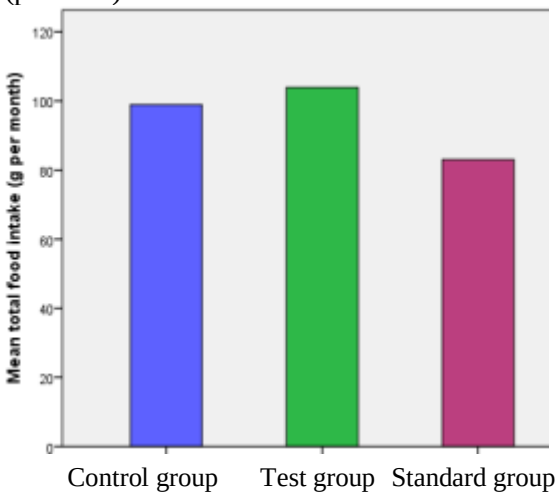

Fig. 3: The total feed intake of control (blue), test (green) and standard (purple) group mice during the experiment

Table 1: The nutrient composition (unit 100 g^{-1}) of control, test and standard diets

Nutrient	Control diet	Test diet	Standard diet
Protein (g)	$10.0^{b,c}$	14.5^{a}	15.0^{a}
Calcium (mg)	$199.4^{b,c}$	364.8^{a}	364.5^{a}
Phosphorus (mg)	$46.3^{b,c}$	$133.5^{a,c}$	$133.0^{a,b}$
Iron (mg)	$6.75^{b,c}$	$18.0^{a,c}$	$17.0^{a,b}$
Zinc (mg)	$0.9^{b,c}$	$13.4^{a,c}$	$12.3^{a,b}$
Vitamin A (μg)	$3.1^{b,c}$	6.65^{a}	6.6^{a}
Vitamin B_9 (μg)	$11.1^{b,c}$	$231.8^{a,c}$	$229.5^{a,b}$
Vitamin B_{12} (μg)	$0.2^{b,c}$	0.77^{a}	0.8^{a}

Mean values with letter 'a' mark a significant difference with control diet. Mean values with letter 'b' mark a significant difference with test diet. Mean values with letter 'c' mark a significant difference with standard diet (ANOVA, $p<0.05$)

Table 2: Nutrient intake in control, test and standard diet fed mice

Nutrient	Control diet	Test diet	Standard diet
Protein (g)	9.9 $(2.92)^{b}$	15 (6.7)	12.4 (4.89)
Calcium (mg)	204.7 $(62.9)^{b}$	379 (168.09)	302.5 (118.8)
Phosphorus (mg)	45.8 $(13.5)^{b,c}$	138.8 (61.5)	110 (43.4)
Iron (mg)	6.7 $(1.97)^{b,c}$	18.7 (8.29)	14 (5.54)
Zinc (mg)	0.89 $(0.26)^{b,c}$	13.9 $(6.17)^{a,c}$	10 $(4.01)^{a,b}$
Vitamin A (μg)	3.07 $(0.9)^{b,c}$	6.9 (3.06)	5.5 (2.15)
Vitamin B_9 (μg)	11 $(3.25)^{b,c}$	241 (106.8)	190.5 (74.9)
Vitamin B_{12} (μg)	0.19 $(0.06)^{b,c}$	0.8 (0.35)	0.7 (0.26)

Mean values with letter 'a' mark a significant difference with control diet. Mean values with letter 'b' mark a significant difference with test diet. Mean values with letter 'c' mark a significant difference with standard diet (ANOVA, $p<0.05$)

Table 3: Nutrient content (unit 100 g^{-1}) in the feces samples collected from control, test and standard diet fed mice

Nutrient	Control diet	Test diet	Standard diet
Protein (g)	7.3 $(0.37)^{b,c}$	4.9 $(0.54)^{a,c}$	4 $(0.37)^{a,b}$
Calcium (mg)	173.4 $(0.62)^{b,c}$	187 $(0.63)^{a,c}$	156 $(0.35)^{a,b}$
Phosphorus (mg)	39.4 $(1.02)^{b,c}$	94 $(0.85)^{a,c}$	72 $(0.68)^{a,b}$
Iron (mg)	5.2 $(1.21)^{b,c}$	10.5 $(0.39)^{a,c}$	8.5 $(0.59)^{a,b}$
Zinc (mg)	0.58 $(0.06)^{b,c}$	8 $(0.29)^{a,c}$	5.5 $(0.60)^{a,b}$
Vitamin A (µg)	1.7 $(0.10)^{b}$	3 $(0.37)^{a,c}$	1.5 $(0.46)^{b}$
Vitamin B_9 (µg)	7.9 $(0.54)^{b,c}$	150 $(0.71)^{a,c}$	115 $(0.55)^{a,b}$
Vitamin B_{12} (µg)	0.15 $(0.05)^{b,c}$	0.4 $(0.08)^{a,c}$	0.35 $(0.03)^{a,b}$

Mean values with letter 'a' mark a significant difference with control diet. Mean values with letter 'b' mark a significant difference with test diet. Mean values with letter 'c' mark a significant difference with standard diet (ANOVA, $p<0.05$)

Nutrient intake

The nutrient intake in mice was found dependent on the amount of feed ingested during the experiment. Vitamin A, vitamin B_9, vitamin B_{12}, iron and phosphorus intakes in control mice was significantly ($p < 0.01$) different from test and standard group mice (Table 2). The protein and calcium intake in control group mice differed significantly from test group mice but not from the standard group mice ($p > 0.05$). However, nutrient intake in test and standard group mice were statistically at par ($p > 0.05$), except for zinc intake. Zn intake significantly differed ($p < 0.05$) in all the experimental groups.

Feces nutrient content

The nutrient content of feces reflects how much of the ingested nutrient is absorbed (digested) by the body and how much is excreted. The nutrient content of feces samples collected from control, test, and standard group mice was significantly different ($p < 0.05$), however, the values were very similar for test and standard groups (Table 3).

Nutrient absorption

The amount of nutrients ingested during the experiment and the amount of nutrient excreted through feces was used to calculate the apparent nutrient absorption of experimental diets. Protein, calcium, phosphorus, iron, vitamin A and vitamin B_{12} absorptions from control diet differed significantly ($p < 0.01$) from the test and standard diets but not for zinc and vitamin B_9 absorptions. The apparent absorption of nutrients from test and standard diets was at par with each other, except for vitamin A (Table 4). Considering the similar nutrient composition between test and standard diets and nutrient intake between test and standard group mice, the results revealed that the absorption of nutrients from *S. platensis* used to prepare the test diet was comparable with that of nutritional supplements. The phytate content of *S. platensis* used to prepare the test diet was very little (1.86 mg 100 g^{-1}). Besides the computed iron to zinc (1.14), zinc to iron (0.88), calcium to phosphorus (1.6), phytate to iron (0.0019), phytate to zinc (0.0021), and phytate to

Table 4: Absorption of nutrients (%) in control, test and standard diet-fed mice

Nutrients	Control diet	Test diet	Standard diet
Protein	26 (8.1) [b,c]	67 (9.9)	67.7 (10.7)
Calcium	12 (4.4) [b,c]	50.6 (13.2)	48 (12.8)
Phosphorus	13.9 (4.6)[b,c]	32 (12.6)	34.5 (14.3)
Iron	22 (4.6) [b,c]	43.8 (13.9)	39 (12.3)
Zinc	34.8 (8.7)	42 (16.3)	45 (13.5)
Vitamin A	44.6 (7.6)[b,c]	56.5 (11.1)[a,c]	72.7 (5.2)[a,b]
Vitamin B_9	28 (9.9)	37.7 (19.3)	39.6 (9.6)
Vitamin B_{12}	21 (6.6) [b,c]	50 (13.1)	50 (12.3)

Mean values with letter 'a' mark a significant difference with control diet. Mean values with letter 'b' mark a significant difference with test diet. Mean values with letter 'c' mark a significant difference with standard diet (ANOVA, $p<0.05$)

Table 5: Nutrient content (unit 100 g^{-1}) in the liver samples of mice in control, test and standard diet groups

Nutrients	Control diet	Test diet	Standard diet
Calcium (mg)	22.5 (0.34)[b,c]	182 (1.24) [a,c]	148 (0.38)
Phosphorus (mg)	6.4 (0.6)[b,c]	43 (0.74) [a,c]	38 (0.64)
Iron (mg)	1.5 (0.07)[b,c]	8 (0.43) [a,c]	7.7 (0.53)
Zinc (mg)	0.3 (0.08)[b,c]	5.8 (0.38) [a,c]	5 (0.39)
Vitamin A (µg)	1.3 (0.54)[b,c]	3.5 (0.7) [a]	3.6 (0.85)
Vitamin B_9 (µg)	2.9 (0.43)[b,c]	87.3 (1.3) [a,c]	73.8 (1.48)
Vitamin B_{12} (µg)	0.03 (0.01)[b,c]	0.3 (0.05) [a,c]	0.31 (0.02)

Mean values with letter 'a' mark a significant difference with control diet. Mean values with letter 'b' mark a significant difference with test diet. Mean values with letter 'c' mark a significant difference with standard diet (ANOVA, $p<0.05$)

calcium (0.000086) molar rations of *Spirulina platensis* was below the critical values at which the absorption of minerals could be inhibited (Norhaizan *et al.*, 2009; Nguyen *et al.*, 2012). The absence of a cellulosic cell wall in *Spirulina* (Falquet, 2006), the oxalate free nature of *Spirulina* (Gutiérrez-Salmeán *et al.*, 2015), and the undetectable level of tannin (Mane *et al.*, 2019; Seghiri *et al.*, 2019) are reported reasons for the higher bioavailable nature of *Spirulina* nutrients.

Although the information is scarce, there are some human and animal studies reported on the bioavailable nature of *Spirulina* nutrients. For instance, after assessing the effect of *Spirulina*, calcium carbonate, and high calcium milk supplementations on the serum calcium and bone mass density of rats, Ekantari *et al.* (2017) reported that the calcium of *Spirulina* has higher bioavailability than those of calcium carbonate and high calcium milk. Madhubalaji *et al.* (2019) also reported that supplementation of *Spirulina* in the diet of vitamin B_{12} deficient rats leads to the normalization of vitamin B_{12} deficiency-induced circulatory and functional biomarkers, this explains that the vitamin B_{12} from *Spirulina* is absorbable and improves systemic vitamin B_{12} status. Moreover, a study administered 10 g *Spirulina* along with local diet to the intervention group children and only local diet to the control group children for 30 days reported that the weight-for-age Z scores and weight-for-height Z scores increased quickly and significantly in the intervention group than that of the control group ($p < 0.05$) (Matondo *et al.*, 2016). A blind randomized cohort study administered 1500 mg day^{-1} *Spirulina* supplementation to the intervention group of pregnant women and iron and folic acid supplementation to the control group of pregnant women reported that the hemoglobin level of intervention group pregnant women was significantly higher than that of iron and folic acid groups, thus the study concluded that *Spirulina* is a good option to control iron deficiency anemia and maternal mortality (Niang *et al.*, 2017). Furthermore, studies conducted to assess the impact of *Spirulina* supplementation on the nutritional status and health conditions of human and animal subjects proved that *Spirulina* contains highly absorbable nutrients which can improve the nutritional and health status of consumer (Marcel *et al.*, 2011; Dixit, 2018; Yu *et al.*, 2018).

Liver nutrient content

The nutrient composition of liver samples collected from the control group mice was significantly different from the test and standard group mice ($p < 0.01$). Liver samples collected from test and standard group mice had significantly ($p < 0.05$) different for calcium, phosphorous, zinc and vitamin

B_9 concentrations but not for iron, vitamin A and vitamin B_{12} concentrations ($p > 0.05$). Even though the nutrient composition of liver samples collected from test and standard group mice was almost similar, the concentrations were higher for test group mice (Table 5). In a similar study, Madhubalaji *et al.* (2019) reported concentrations of liver vitamin B_{12} 4.94 and 3.89 ng g^{-1} in rat fed with vitamin B_{12} deficient diet supplemented with standard vitamin B_{12} solution and *Spirulina* (32.5 g kg^{-1}), respectively, the values were higher (3.33 ng g^{-1}) than the deficient group fed with vitamin B_{12} devoid diet and lower (6.46 ng g^{-1}) than control group rats fed with the standard AIN-93 diet.

Conclusion: Given the higher bioavailability of nutritional supplements mixed into the standard diet, the resemblance in nutrient absorption between test and standard diets revealed that *Spirulina platensis* used to prepare the test diet also has higher nutrient bioavailability. The lower Phytate concentration and the suitable mineral to mineral molar ratios in *S. platensis* could be the reason for the higher nutrient absorption from the test diet.

Acknowledgment: The authors would like to thank the Nelson Mandela African Institution of Science and Technology (NMAIST), the Centre for Research, agricultural Advancement, Teaching Excellence, and Sustainability in Food and Nutrition Security (CREATES- FNS) and Arusha Technical College for their support.

Ethical statement: This study was approved by Kibong'oto Infectious Diseases Hospital, Nelson Mandela African Institution of Science & Technology, Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) Health Research Ethics Committee-KNCHREC vide their approval letter no. KNCHREC00026. All the methods involving animals were carried out in adherence with the relevant guidelines and regulations.

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

Bouis, H.E. and Saltzman, A. 2017. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003 through 2016. *Global Food Security*, **12:** 49-58.

Dixit, A. 2018. Effect of "*Spirulina*" on children aged 8-12 years. *BAOJ Nutrition*, **4:** 1-14.

Ekantari, N., Harmayani, E., Pranoto, Y. and Marsono, Y. 2016. Calcium of *Spirulina platensis* has higher bioavailability than those of calcium carbonate and high-calcium milk in Sprague Dawley rats fed with vitamin D-deficient diet. *Pakistan Journal of Nutrition*, **16:** 179-186.

Guansheng, M.A., Ying, J., Jianhua, P., Frans, K., Bonnema, G. and Evert, J. 2005. Phytate, calcium, iron, and zinc contents and their molar ratios in foods commonly consumed in China. *Journal of Agricultural and Food Chemistry,* **53:** 10285-10290.

Habib, M.A.B., Parvin, H., Huntington, T.C. and Hasan, M.R. 2008. FAO Fisheries and Aquaculture Department - A review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals. pp. 1-32. **In**: *FAO Fisheries and Aquaculture, Volume 1034 (Circular No. 1034, Issue 1034)*. Food & Agriculture Organization of United Nations, FAO Electronic Publishing Policy and Support Branch, Communication Division, Rome, Italy.

Jafari, S.M. and Mcclements, D.J. 2017. Nanotechnology approaches for increasing nutrient bioavailability. pp. 2-10. **In**: *Advances in Food and Nutrition Research, Volume 81* (1st edn.). Elsevier Inc, Amsterdam, the Netherlands.

Madhubalaji, C.K., Rashmi, V., Chauhan, V.S., Shylaja, M.D. and Sarada, R. 2019. Improvement of vitamin B_{12} status with *Spirulina* supplementation in Wistar rats validated through functional and circulatory markers. *Journal of Food Biochemistry,* **2019:** 1-10.

[*https://doi.org/10.1111/jfbc.13038*].

Mæhre, H.K., Dalheim, L., Edvinsen, G.K., Elvevoll, E.O. and Jensen, I. 2018. Protein determination - Method matters. *Foods*, **7:** 1-4.

Matondo, F.K., Takaisi, K., Nkuadiolandu, A.B., Lukusa, A.K. and Aloni, M.N. 2016. *Spirulina* supplements improved the nutritional status of undernourished children quickly and significantly: Experience from Kisantu, the Democratic Republic of the Congo. *International Journal of Pediatrics,* **2016:** 1-4. [*http://dx.doi.org/10.1155/2016/1296414*].

Norhaizan, M.E. and Faizadatul, N. 2009. Determination of phytate, iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in Malaysia. *Malaysia Journal of Nutrition,* **15:** 213-222.

Nguyen, P., Grajeda, R., Melgar, P., Marcinkevage, J., Flores, R., Ramakrishnan, U. and Martorell, R. 2012. Effect of zinc on efficacy of iron supplementation in improving iron and zinc status in women. *Journal of Nutrition and Metabolism,* **2012:** 1-8 [*https://doi.org/10.1155/2012/216179*].

Niang, K., Ndiaye, P., Faye, A., Augustin, J., Tine, D., Diongue, F.B., Camara, M.D., Leye, M.M. and Tal-Dia, A. 2017. *Spirulina* supplementation in pregnant women in the Dakar region (Senegal). *Open Journal of Obstetrics and Gynecology*, **7:** 147-154.

Nouri, E. and Abbasi, H. 2018. Effects of different processing methods on phytochemical compounds and antioxidant activity of *Spirulina platensis*. *Applied Food Biotechnology,* **5:** 221-232.

Rasolofoson, R.A., Hanauer, M.M. and Pappinen, A. 2018. Impacts of forests on children's diet in rural areas across 27 developing countries. *Science Advances,* **4:** 1-10.

Saha, S.K. and Murray, P. 2018. Exploitation of microalgae species for nutraceutical purposes: Cultivation aspects. *Fermentation,* 2018**:** 1-17 [*https://doi.org/10.3390/fermentation4020046*].

Sami, R., Li, Y., Qi, B., Wang, S., Zhang, Q., Han, F., Ma, Y., Jing, J. and Jiang, L. 2014. HPLC analysis of water-soluble vitamins (B_2, B_3, B_6, B_{12} and C) and fat-soluble vitamins (E, K, D, A, and β-carotene) of okra (*Abelmoschus esculentus*). *Journal of Chemistry*, 2014: 1-7. [*http://dx.doi.org/10.1155/2014/831357*].

Steponėnienė, L. and Tautkus, S. 2006. Determination of zinc in plants and grains by atomic absorption spectrometry. *Chemija (Vilnius)*, **3**: 3-6.

Templeton, D.W. and Laurens, L.M.L. 2015. Nitrogen-to-protein conversion factors revisited for applications of microalgal biomass conversion to food, feed and fuel. *Algal Research*, **11:** 359-367.

Yu, W., Wen, G., Lin, H., Yang, Y., Huang, X., Zhou, C., Zhang, Z., Duan, Y., Huang, Z., and Li, T. 2018. Fish and shell fish immunology: Effects of dietary *Spirulina platensis* on growth performance, hematological and serum biochemical parameters, hepatic antioxidant status, immune responses and disease resistance of Coral trout *Plectropomus leopardus*. *Fish and Shellfish Immunology*, **74:** 649-655.