Bull. Chem. Soc. Ethiop. 1998, 12(2), 95-103. ISSN 1011-3924
Printed in Ethiopia

BIOAVAILABILITY OF IRON AND ZINC FROM TEF IN RATS

Kelbessa Urga[1], H.V. Narasimha[2], B.V. Sasikala[2] and S. Vishwanatha[2]

[1]Ethiopian Health and Nutrition Research Institute, P.O. Box 5654, Addis Ababa, Ethiopia
[2]Central Food and Technological Research Institute, Mysore 570013, Mysore, India

(Received February 27, 1998; revised October 8, 1998)

ABSTRACT. Bioavailability of iron from tef (*Eragrostis tef*) was studied using anaemic rats by haemoglobin regeneration efficiency and apparent iron absorption. The bioavailability of zinc in tef prepared in the form of *kitta* and *injera* was evaluated with a rat bioassay, using the slope-ratio method and $\log_{10}$ total femur zinc or weight gain *versus* added zinc to the diets as response parameters. Diets were formulated by mixing *kitta* or *injera* prepared from tef with a semi-synthetic iron or zinc-free basal diet. Mean haemoglobin iron gain was lower in the groups fed *kitta* compared with *injera* fed groups. The respective mean haemoglobin regeneration efficiency and apparent iron absorption were 27.84% and 25.34% for anaemic rats when the *kitta* diet was fed and 76.85% and 79.40% when the *injera* diet was fed. For weight gain, slope-ratios relative to $ZnSO_4$ were 37.1% for *kitta* and 77.4% for *injera*. For $\log_{10}$ total femur zinc, slope-ratios relative to $ZnSO_4$ were 30.2% for *kitta* and 69.8% for *injera*. The phytic acid and phytate:zinc molar ratio of *injera* were 72% lower than that of *kitta*. The relative biological value (RBV) of iron and zinc in *kitta* was lowest compared to that of *injera*. The study suggested that natural lactic acid fermentation increased the RBV of iron and zinc.

INTRODUCTION

Tef (*Eragrostis tef*) is one of the most important cereal crops in Ethiopia and accounts for about two thirds of the daily protein content in the diet of the population [1]. Besides providing protein and calories, tef is a good source of minerals and phytic acid [2, 3]. On the basis of the high phytic acid, iron and zinc content of tef, it is important to learn whether such high level of phytic acid in tef will mean lower iron or zinc bioavailability. Thus, knowledge of the availability of iron and zinc in tef might aid in developing means of improving its bioutiliztion.

Several investigators have reported that fermentation improves the absorption of iron and zinc in humans and animals [4, 5]. Fermentation is also known to reduce phytic acid content of tef thereby, converting bound form of minerals to free form which is responsible for increased HCl-extractability and dialysability of the minerals of the fermented products [3, 6, 7]. Although the biological availability of iron and zinc from other diets have been measured by the rat bioassay [4, 5, 8], no such study has previously been carried out on Ethiopian diets prepared from tef. The purpose of this study was, therefore, to evaluate the bioavailability of iron and zinc from the two most popular food items, tef *injera* and tef *kitta*, from weight gain and total femur zinc using rat bioassay.

EXPERIMENTAL

Food preparation. The cereal tef, a mixture of white and brown tef variety (1:1 w/w), was purchased from the open market in Bishoftu, Shoa, Ethiopia. The seed sample was

transported to India and stored at 4 °C until used. Tef grains were cleaned of dust and other foreign material and ground into flour in an electric grinder (M/S Milone, Rajkot, India) using 0.5 mm sieve. *Injera* and *kitta* were prepared following the traditional methods as previously reported [7]. *Injera* and *kitta* samples were dried to constant weight in an air-draught oven pre-set at 60 °C before they were ground into fine powder as described earlier.

Animals. Weanling male albino rats of the Wistar strain obtained from a colony maintained at the Department of Biochemistry and Nutrition (Central Food Technological Research Institute) and weighing approximately 41 g were used. Animals were housed individually in suspended stainless steel cages with wire mesh bottoms and fronts. Housing was in a ventilated room with a 12 h light-12 h dark cycle.

Iron bioavailability. Bioavailability of iron from tef was tested by feeding rats diets in which equal amounts of iron was provided by *kitta* or *injera* in combination with AIN-77 vitamin mixes and iron-free mineral mixes [9]. A casein diet supplemented with $FeSO_4$ was the reference diet. Six rats in each group, a total of 18 rats, were used in the experiment. Body weight gain, haemoglobin-iron (Hb-Fe) gain and iron intake were measured and used for calculating HRE. Faecal iron and iron intake were measured and used to calculate apparent iron absorption, and measurements of liver weight and liver iron were used to determine changes in iron stores. The diets were formulated to provide 35 mg Fe/kg (ppm) diet. Total protein, fat and fibre were balanced across diets with casein, ground nut oil and cellulose. Calcium and phosphorus levels were also balanced in the diets, because these minerals can affect iron absorption. Composition of the $FeSO_4$ reference diet is listed in Table 1. Iron deficiency diet was formulated as the $FeSO_4$ reference diet without added iron.

Table 1. Formulation of diets containing varying proportions of iron from *kitta* and *injera* (g/kg diet).

Ingredients	Control diet[a]	*Kitta*[b] test diet	*Injera*[c] test diet
Casein	200	-	-
Kitta	-	380	-
Injera	-	-	435
Groundnut oil	100	100	100
Mineral mix* [18]	10	35	35
Vitamin mix* [18]	10	10	10
Cellulose	50	-	-
DL-Methionine	3	3	3
Choline chloride	2	2	2
Starch	600	220	220
Fe content, ppm	-	92.1±0.4	80.4±0.1
Zinc content, ppm	-	23.12	18.50
Phytic acid, mM	-	10.26±1.8	2.8±2.7
Phytate:zinc molar ratio	-	29	8

*The mineral mix is devoid of Fe. [a]Basal diet without iron. [b,c]35 ppm of Fe supplied by *kitta* or *injera* diet.

The diets were mixed using a stainless steel bowel and paddles and refrigerated (4 °C) in plastic bags until the rats were fed. Anaemia was induced in the animals by *ad libitum* feeding with the iron deficiency basal diet (10 mg/kg diet) for seven days (Table 1) and bleeding thirty drops of blood from the retroocular capillary bed (under light diethyl ether

anaesthesia) of the rats with heparinized glass capillary tubes on days 1 and 4 [10]. On day 8, haemoglobin and body weights were determined and six rats were allotted to each of the three diets (the two test diets and the reference diet), balancing for haemoglobin concentration and body weight (Table 2). The rats were assigned to groups to balance for haemoglobin so that variability among groups was similar at the start of iron repletion. Body weight was balanced by moving rats with similar haemoglobin levels and body weights across the groups as needed. Each rat was fed 10 g of its respective test and reference diets daily over a 10-day repletion period. Fresh double distilled water was given *ad libitum*.

Table 2. Formulation of diets containing varying proportions of zinc from $ZnSO_4$, *kitta* and *injera*[1].

Diet	Supplement level (%)	Basal diet[1] (%)	Zinc added[2] ppm	Phytate added[2] mM	PA/Zn[3]	Zinc content[4] ppm
$ZnSO_4$		100	3.17			3.26
		100	6.09			6.35
		100	9.23			9.39
Kitta	100		-	10.23	29	23.12
	8.94	91.04	3.27	0.92	17.60	3.37
	21.70	78.30	6.31	2.20	22.72	6.35
	34.50	65.50	9.19	3.53	24.70	9.29
Injera	100	-	-	2.86	8	18.50
	11.20	88.80	3.08	0.32	6.04	3.43
	27.10	72.90	6.14	0.78	7.81	6.45
	43.10	56.90	9.28	1.23	8.53	9.40

[1]Zinc deficient basal diet was substituted in the test diets at the expense of dextrose. [2]By calculation. [3]PA/Zn-phytic acid:zinc molar ratio. [4]Analysed zinc concentration in control and test diets fed to rats.

On day 18, haemoglobin and body weight was determined and the rats sacrificed by inhaling diethyl ether and their livers were excised. Livers were rinsed in cold isotonic saline (9 g NaCl/L), dried to constant weight at 65 °C in an air-draught oven and ground to a homogenous powder. Faeces were collected in glass test tubes, air-died for three days, and then weighed. All spilled and refused food was air-dried, weighed and subtracted from that offered to determine total dietary intake. Total iron intake was calculated by multiplying total feed consumed by dietary iron concentration. The blood samples taken from the orbital venous plexus of each animal were analysed in triplicate for haemoglobin using the cyanmethmoglobin method [11]. The calculation of haemoglobin-Fe (Hb-Fe, mg) was based on the assumption that 6.7% of body weight is blood, and haemoglobin contains 3.35 mg of iron per gram of blood [12-13]. The relative biological value was determined by dividing the individual HRE values of the test iron source by the mean HRE of $FeSO_4$.

Zinc bioavailability. Composition of the basal diet without zinc for the rats was used [9]. Casein (vitamin free, Hi Media, Bombay, India) was used instead of egg white as the protein source of all the control diets. As purchased, the vitamin free casein contained 40 ppm of zinc. Casein was washed with ethylenediamine tetracetate (EDTA) and double distilled water to remove inherent zinc [14]. The zinc content of the treated casein was 0.33 ppm. The low zinc diet contained the following ingredients on a percent basis: casein, 20; ground nut oil, 10; cellulose, 5; mineral mix [9], 3.5; vitamin mix [9], 1.5; DL-methionine, 0.3; choline chloride, 0.2; dextrose, 60. The standard source of zinc added as ($ZnSO_4$) to the

basal diet produced 3, 6 and 9 ppm. Test diets were made by supplementing the basal diet with three levels of zinc (3, 6, or 9 μg/g) using *injera* or *kitta* as zinc sources. The phytate concentration and phytate-to-zinc molar ratios for each level of supplementation of the basal diet with test diets are shown in Table 2. The protein content was not adjusted.

The rats were assigned by weight to 9 experimental groups of 6, so that the group mean weights were equal. Each diet was assigned to each group in a random manner. Diet and double distilled water were provided *ad libitum*. Food intake was recorded daily and the weight of each rat as well as food spillage was estimated triweekly. At the end of three weeks, the rats were killed under diethyl ether anaesthesia. The left femur bone removed from each animal was cleaned of soft tissue using stainless steel scissors and forceps and then dried to constant weight. Femurs were then ashed overnight in silica crucibles in a muffle furnace at 450 °C. The ashes were then dissolved in 1 mL concentrated nitric acid, taken to dryness on a hot plate and then fired for 1 min over a Bunsen burner. The resulting white ash was diluted with 10 mL of 1 M HCl. Samples of food and diets were digested with perchloric and nitric acid mixtures (5:1 v/v).

Iron and zinc in all digested samples and femur were determined by atomic absorption spectrophotometry (Model 3110, Perkin-Elmer, Norwalk, CT). The slope ratio assay was used to determine the relative biological value (RBV) of zinc in the test diets. Using weight gain and total femur zinc versus zinc intake as parameters, a partial slope was determined for each diet (control and test diets) [15]. Phytate in the test diets was estimated according to the method of Haug and Lantschz [16].

RESULTS AND DISCUSSION

The concentration of phytic acid, iron and zinc in *kitta* and *injera* is given in Table 1. The concentration of phytic acid in *kitta* was more than three-fold the concentration in *injera*. Heat treatment during the preparation of *kitta* exerted no significant effect on the content of phytic acid. Phytic acid content in *injera* was, however, reduced by 71%, which may be attributed to the natural fermentation process. This is in agreement with previous studies where natural lactic acid fermentation has significantly reduced the content of phytic acid in tef [3, 7]. The phytate:zinc molar ratio of *kitta* was three-fold higher than that of *injera* (Table 1). Phytate destruction by fermentation and lower phytate:zinc molar ratios has been reported previously for bread [4]. The iron and zinc content in both test diets, however, differed significantly following the food preparation schemes. *Injera* contained less iron and zinc compared with *kitta* which may be due to loss of soluble minerals when the top liquid layer of the fermenting dough is discarded prior to baking as reported previously [3].

The food consumption pattern as shown in Table 3 varied from the $FeSO_4$ diet group to the test diet group. Dietary iron in *kitta* and *injera* had no significant influence on actual food intake during the repletion period whereas, a significantly higher feed intake was noted for the $FeSO_4$ fed groups. Although no significant differences existed for food intake among the groups fed test diets, weight gain between *kitta* and *injera* fed groups were significantly different. Rats fed with the $FeSO_4$ diet gained more weight than any other group; those fed the *kitta* diet gained the least during the 10-day repletion period. Probably, the differences in weight gain observed are due to differences in dietary protein quality rather than to differences in amount of diet consumed by rats (Table 3). There were also significant differences in feed efficiency by the anaemic rats fed either the $FeSO_4$ diet or the test diets

Table 3. Body weight, haemoglobin concentration, HRE, liver and fecal iron, and apparent iron absorption for diets given to anaemic rats*

Parameter	$FeSO_4.7H_2O$	*Kitta*	*Injera*
Food consumed (g)	116.13±1.15	93.89±1.43	94.01±2.17
Body weight (g)			
Initial	38.73±3.27	41.83±4.13	39.19±2.63
Gain	83.45±2.13	16.17±0.31	57.30±1.31
Hb (g/dl)			
Initial	5.61±0.11	5.12±0.25	5.83±0.34
Gain	5.14±0.09	1.21±0.14	4.62±0.63
Hb-Fe (mg)			
Initial	0.55±0.01	0.56±0.03	0.57±0.01
Gain	3.30±0.13	0.92±0.09	2.53±0.13
Iron intake (mg)	4.06±0.09	3.29±0.06	3.29±0.25
HRE, %	81.10±0.48	27.84±0.68	76.85±0.56
RBV	100.01±0.08	34.33±0.83	94.76±0.16
Feed efficiency	0.72±0.01	0.17±0.02	0.61±0.06
Liver weight (mg)	482±22	693±93	676±43
Liver iron (μg/g)	560±56	329±69	402±3
Iron in faeces (μg)	679±28	2456±32	677±17
Apparent iron absorption, %	83.3±1.2	25.34±3.44	79.41±2.30
Available Fe, mg/100g	2.84±0.42	2.56±0.32	6.17±0.64

*Each value is the mean of 6 rats.

(Table 3). Feed efficiency (the ratio of body weight gain to food intake) was significantly lower in the *kitta* group compared with the *injera* or $FeSO_4$ group.

In Table 3, the iron gain efficiency was calculated by dividing haemoglobin-iron gain by iron intake. The iron gain efficiency was lower in the *kitta* group as compared with the $FeSO_4$ and *injera* groups due in part to the higher phytate intake. A decreased iron gain efficiency in the *kitta* group would be consistent with previous results found for wholemeal cereals where diets containing wholemeal cereals high in phytate content have a greater negative effect on iron uptake [17, 18]. The intake of phytic acid is thus a main determinant of iron nutrition especially in groups with a regular high phytate intake.

Analysis of blood from the rats immediately before repletion period demonstrated the degree of anaemia produced within 8 days by a combination of phlebotomy and low-Fe diet (Table 3). The efficiency of conversion of dietary iron into haemoglobin is shown in Table 3. Iron source affected haemoglobin gain. Anaemic rats gained most iron as haemoglobin when dietary iron was from $FeSO_4$ and *injera* and the results are consistent with those observed by Zhang *et al.* [19].

The HRE of $FeSO_4$ diet was about and 3 times the efficiency of the *kitta* diets whereas rats fed on the diet with iron from *injera* had significantly higher HRE compared with those fed with iron from the *kitta* diet (Table 3). In this study, 81% of the iron in the $FeSO_4$ diet was converted to haemoglobin. Similarly, Whittaker and Vander veen [20] showed in a recent study that the HRE ratios obtained with the ground Egyptian *balady* bread were lower than those with the casein-based meal for $FeSO_4$ groups, which demonstrates the inhibitory effect of phytate in the rat model. Of the iron in *injera* diet, 77% was converted to haemoglobin. The efficiency of converting the *injera* iron in to haemoglobin was 95% of the efficiency for $FeSO_4$. Thus, *injera* seems to be a very good source of food iron. In contrast, 28% of the iron in *kitta* was converted to haemoglobin iron by the rats in this experiment, which was 34% as effective as $FeSO_4$. This value agrees reasonably well with

the RBV values reported for corn meal, sorghum and spinach [21, 22]. The high utilisation of iron from *injera* diet may be attributed to the natural lactic acid fermentation process. A similar trend was found by the study of Derman *et al.* [23]. Moeljpawiro *et al.* [5] observed an increase in the RBV of iron in soybeans subjected to lactic acid fermentation. Stuart *et al.* [24] similarly observed that a significantly higher amount of iron was absorbed by rats fed with fermented *aceda* than those fed with maize gruel or other sorghum porridges.

The liver iron values are presented in Table 3. Liver weight and liver iron were affected by dietary iron source. Liver iron concentration was found to be significantly higher in rats fed $FeSO_4$ diet than those fed *kitta* or *injera* diets. However, there was a significant difference in liver iron concentration between anaemic rats fed the test diets. These results are in agreement with previous experiments using anaemic and non-anaemic rats [22]. In the present study, higher HRE was associated with higher apparent iron absorption values (Tables 2). Such association between HRE and apparent iron absorption indicates that HRE is a reliable measure of total dietary iron utilisation. Dietary iron source affected faecal iron excretion and apparent iron absorption (Table 3). Anaemic rats when fed $FeSO_4$ and *injera* diets excreted equivalent amount but less iron in the faeces and had a higher apparent iron absorption than those fed on diets with *kitta* food sources. However, the differences between apparent iron absorption in anaemic rats fed with $FeSO_4$ and *injera* diets were not significant.

The twenty-two-day weight gain, feed consumption and feed efficiency data are given in Table 4. Weight gains of the control and experimental animals increased as the added zinc in the diets increased but to a varying extent. The greatest mean weight gain, 118 g, was attained with the control diet containing 9 ppm of added zinc. Weight gain of animals fed diets with *kitta,* as the zinc source was less than the weight gain with either *injera* or $ZnSO_4$ diets. Thus, *injera* as zinc source supported weight gain as well as the control $ZnSO_4$ diets at equal added dietary zinc levels. These results are in full agreement with those of Morris and Ellis [25], who found that rats maintained on high phytate diets had reduced growth rates compared with control animals.

Table 4. Weight gain, feed intake, feed conversion and zinc intake in animals consuming, control and test diets[1].

Zn source	Weight gain g	Feed intake g	Feed efficiency[2]	Zn intake[3] µg
$ZnSO_4$	48±8	163±14	3.3±0.3	517±44
	93±6	187±13	2.0±0.2	1138±39
	118±10	207±16	1.8±0.9	
Kitta	38±5	159±15	4.2±0.3	519±49
	56±8	168±11	3.0±0.4	1060±69
	64±7	178±14	2.8±0.5	1636±28
Injera	42±5	160±18	3.8±0.3	493±37
	68±6	172±17	2.5±0.4	1056±38
	92±10	189±18	2.0±0.5	1754±67

[1]Group mean ± SD (n = 6). [2]Grams food consumed per gram body weight gain. [3]Zinc intake from added Zn.

Feed intake was lowest for rats consuming diets with 3 ppm zinc provided by the control and experimental diets probably a consequence of zinc deficiency. As the zinc content of the diets increased, feed intake increased. Feed efficiency, grams of food consumed per gram of weight gain, decreased as the amount of added zinc in the diets increased. Feed efficiency was higher in rats when the zinc source was attained with control

diets containing 9 ppm of added zinc. However, these results demonstrate clearly that the reduced weight gain and reduced food intakes and feed efficiency of the rats receiving *kitta* was due to a reduced zinc availability and suggest strongly that phytate in *kitta* was the agent responsible. The findings in this study are in agreement with those reported by Davies *et.al.* [26] and Franz *et.al.* [27] for wheat bran, boiled and raw white rice.

Bone zinc values, both concentration (μg/g) and total zinc (μg/femur) are given in Table 5. The mean dry weight of the femurs of control animals increased with zinc concentration in the diet and varied from 152 to 204 mg. Animals consuming the test diets also exhibited similar trends. Femur zinc also increased with increasing levels of zinc in test and control diets. There were also substantial differences between total zinc content of femurs of animals consuming experimental diets with comparable dietary added zinc. Total femur zinc content of animals fed *injera* diet varied from 10 to 16 μg whereas this parameter varied from 9 to 11 μg for those animals fed on *kitta* diet.

Table 5. Bone zinc values and relative biological value of zinc in control and experimental animals[1].

Zn source	Femur			Log_{10} total femur Zn	RBV[2]	
	dry weight mg	Total zinc μg/femur	Zn content μg/g		Weight gain %	log_{10} total femur Zn %
$ZnSO_4$	152±17	11.5±0.6	75.66±0.35	1.07±0.01	100	100
	184±14	15.4±0.1	83.70±0.07	1.19±0.02		
	204±17	21.4±0.3	104.90±0.17	1.33±0.01		
Kitta	154±7	8.7±0.2	56.49±0.29	0.94±0.01	37.1	30.2
	162±14	9.6±0.3	59.26±0.21	0.98±0.02		
	175±7	10.5±0.4	60.00±0.06	1.02±0.32		
Injera	155±16	10.4±0.4	67.10±0.25	1.01±0.01	77.4	69.8
	169±14	13.9±0.2	82.25±0.14	1.14±0.02		
	183±8	16.3±0.3	89.07±0.18	1.21±0.01		

[1]Group mean ± SD, (n = 6). [2]Relative biological value of zinc as determined by slope-ratio assays of weight gain and total femur zinc versus added zinc to the diets.

Zinc concentrations of the femurs of control animals varied from 76 to 105 μg/g dry weight. Femur zinc concentrations of rats fed *kitta* diet with zinc of 3 and 6 ppm (deficient for optimum growth) were similar but were lower than femur zinc of rats fed comparable $ZnSO_4$ and *injera* diets. The concentration of femur zinc of animals fed *kitta* diet was 43% lower than that of the control diet with equivalent levels of added zinc (9 ppm). When dietary zinc was 9 ppm, mean femur zinc value for *injera* was less than that from the reference group, but greater than that for the *kitta* group.

Relative biological value of zinc as determined by slope-ratio assays of weight gain and total femur zinc is presented in Table 5. The RBV was calculated using $ZnSO_4$ as 100%. Using the slope-ratio with log_{10} total femur zinc as response parameter versus added zinc, zinc from *kitta* and *injera* was utilised 30.2% and 69.8%, respectively, as efficiently as the zinc from $ZnSO_4$. However, zinc in the *kitta* and *injera* diets was 37.1 and 77.4% as efficiently utilised as when $ZnSO_4$ was added to the casein diet using weight gain *versus* added zinc as criterion. The ratios derived from total femur zinc were lower than those derived from weight gain and similar to previous reported studies [27]. The RBV of *kitta* was similar to the RBV of raw corn or brown rice while the RBV of *injera* was similar to leavened white bread with no phytic acid content [17]. Such improvement in the bioavailability of zinc from *injera* in contrast to *kitta* may be due to the marked reduction in

phytic acid during fermentation. Previous studies have demonstrated that leavening of wheat bread increased the bioavailability of zinc when compared with unleavened bread and the phytic acid concentration of cereals appeared to be inversely correlated to RBV of zinc [4, 25, 28].

In the present study growth responses of rats fed the *kitta* diets with phytate:zinc ratio of 29 was lower than the response to $ZnSO_4$ and *injera* as dietary zinc source. The combination of high phytate content and high phytate:zinc molar ratio severely depressed femur zinc values in *kitta* diet fed animals. The femur zinc values of the rats fed *injera* with ratio 8 as dietary zinc source supported greater accumulation of zinc in femur. These results are in agreement with previous reported studies [24, 25].

Tef is a major grain food for much of the Ethiopian population. The relatively high phytate content of the cereal grain tef contributes to the observed low mineral bioavailability from unfermented foods prepared from this flour [3, 7] and the prevalence of a high ratio of grain products to animal protein products in the diet may contribute to the poor nutritional status of the population. In conclusion, larger amounts of iron and zinc would be absorbed from tef food products prepared through fermentation process than from the same products prepared without fermentation.

ACKNOWLEDGEMENT

This study was financially supported by the United Nations University, Tokyo, Japan and Central Food Technological Research Institute, Mysore, India.

REFERENCES

1. Ethiopian Nutrition Survey, A Report; *Interdepartmental Committee of Nutrition on National Defence*, Washington, D.C., 1959.
2. Mengesha, M.H. *Econ. Bot.* **1966**, 20, 268.
3. Urga, K.; Fite, A.; Biratu, E. *Ethiop. J. Hlth. Dev.* **1997**, 11, 61.
4. Navert, B.; Sandstrom, B.; Cederblad, A. *Br. J. Nutr.* **1985**, 53, 47.
5. Moeljopaueiro, S.; Fields, M.L.; Gordon, D.T. *J. Food Sci.* **1988**, 53, 460.
6. Ramachandran, K.; Boldia, G. *Ethiop. Med. J.* **1984**, 22, 45.
7. Urga, K.; Narasimha, H.V. *Bull. Chem. Soc. Ethiop.* **1997**, 11, 10.
8. Moeljpawiro, S.; Gordon, D.T.; Fields, M.L. *J. Food Sci.* **1987**, 52, 102.
9. American Institute of Nutrition. *J. Nutr.* **1977**, 107, 1340.
10. Tim, K.I. *Lab. Anim. Sci.* **1979**, 29, 636.
11. Crosby, W.H.; Munn, S.E. *U.S. Armed Forces Med. J.* **1954**, 51, 693.
12. Forbes, A.L.; Adams C.E.; Arnaud. M.J.; Chichester, C.O.; Cook, J.D.; Harrison, B.H.; Hurrel, R.E.; Khan, S.G.; Morris, E.R.; Tanner, J.T.; Whittaker, P. *Am. J. Clin. Nutr.* **1989**, 49, 225.
13. Whittaker, P.; Mahoney, A.W.; Hendricks, D.G. *J. Nutr.* **1984**, 114, 1137.
14. O'Dell, B.L.; Burpo, C.E.; Savage, J.E. *J. Nutr.* **1972**, 102, 653.
15. Momcilovic, B.; Belonje, B.; Giroux, A.; Shah, B.G. *Nutr. Rep. Int.* **1975**, 12, 197.
16. Haug, W.; Lantsz, H.J. *J. Sci. Food Agric.* **1983**, 34, 1423.
17. McCance, R.; Widdowson, E. *J. Physiol.* **1942**, 101, 304.
18. Hallberg, L.; Rassander, L.; Skanberg, A.B. *Am J. Clin. Nutr.* **1987**, 45, 988.

19. Zhang, D.; Hendricks, D.G.; Mahoney, A.W. *Br. J. Nutr.* **1989**, 61, 331.
20. Whittaker, P.; Vander Veen, J.E. *Br. J. Nutr.* **1989**, 63, 587.
21. Ifon, E.T. *Nutr. Rep. Intl.* **1981**, 21, 25.
22. Zhang, D.; Hendricks, D.G.; Mahoney, A.W. *J. Food Sci.* **1985**, 50, 426.
23. Derman, D.P.; Bothwell, T.H.; Torrance, J.D.; Bezwoda, W.R.; MacPhail, A.P.; Kew, M.E.; Sayers, M.H.; Disler, P.B.; Charlton, RW. *Br. J. Nutr.* **1980**, 43, 271.
24. Stuart, M.A.; Johnson, P.E.; Hamaker, B.; Kirleis, A. *J. Cereal Sci.* **1987**, 6, 81.
25. Morris, E.R.; Ellis, R. *J. Nutr.* **1980**, 110, 1037.
26. Davies, N.T.; Hristic, V.; Flett, A.A. *Nutr. Rep. Int.* **1977**, 15, 207.
27. Franz, K.B.; Kennedy, B.M.; Fellers, D.A. *J. Nutr.* **1980**, 110, 2263.
28. Franz, K.B.; Kennedy, B.M.; Fellers, D.A. *J. Nutr.* **1980**, 110, 2272.