PHYSIOLOGICAL AVAILABILITY OF CALCIUM AND PHOSPHORUS IN SEVERAL MIDDLE EASTERN FOODSTUFFS

by

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Ca & P AVAILABILITY IN FOODSTUFFS

Rahman

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ABSTRACT

Over 70 percent of the ash content of the body consists of calcium and phosphorus, all of which is derived from the dietary sources. In the Middle East, human consumption of pulses, vegetables and cereals is more than that of the more expensive milk and dairy products. However, no information is available concerning the availability of calcium and phosphorus in the vegetables, pulses and cereals consumed. This study was carried out to determine the availability of calcium and phosphorus in six Middle Eastern foodstuffs, namely, cabbage, chickpea, lentil, okra, tahineh and jew's mellow leaf and in four "LAUBINA" infant food mixtures, developed at the division of Food Technology and Nutrition, American University of Beirut. In addition, the effect of oxalate content of okra on the availability of calcium was studied.

Proximate composition, calcium, phosphorus, phytate and oxalate content of the foodstuffs and mixtures were determined. For the availability study, slaughter technique was used in which the rat was used as the experimental animal.

The availability value for calcium carbonate was higher than that of the foodstuffs and laubina mixtures tested in these experiments. However, among the foodstuffs and laubina mixtures, cabbage showed the highest value for available calcium followed by chickpea, laubina mixtures, lentils and tahineh respectively. Calcium in okra and jew's mellow leaf was poorly utilized by rats.

No difference in the available phosphorus between potassium dihydrogen phosphate, tahineh, chickpea and lentil was observed. Phosphorus in okra, cabbage and jew's mellow leaf was of low availability.

No correlation was found between oxalate, phytate, fiber and fat intake and calcium availability. Nevertheless, the data obtained showed an indication of inverse relationship between oxalate, total intake of food and gain in weight of rats and calcium availability.

There was no correlation between fat, phytate and total intake of food and phosphorus availability. But there was a positive correlation between fiber intake, gain in weight and phosphorus availability.

The oxalate content of okra had no effect on the availability of calcium.

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INTRODUCTION

The primary function of calcium and phosphorus in the animal body is the formation of skeletal tissue and teeth. A hard soluble calcium phosphate salt, similar to that of hydroxyapetite ($3Ca_3(PO_4)$ $Ca(OH)_2$), is formed in both intramembranous and intracartilaginous ossification. The formation of bones and teeth is, therefore, dependent upon the presence of adequate amounts of calcium and phosphate ions within the milieu of calcification, and the levels of mineral elements in turn would be related to their nutritional supply.

Research workers in the field of animal and human nutrition have not been neglectful of mineral availability to the animal body and have given particular attention to calcium and phosphorus.

The results of the various investigations in which calcium in milk was used as a standard showed that availability of calcium in cereals and vegetables was low and that the availability values for any foodstuff varied to a great extent in different investigations.

The availability of calcium and phorphorus in the foodstuffs of the Middle East has not been investigated previously. Moreoever, the values found for the amount of calcium and phosphorus in the foodstuffs of the Middle East showed a considerable variation from the values found on analysis of the same foodstuffs in other countries. Therefore, it seems reasonable to think that the availability of these minerals in foods will vary from one country to the other.

Although milk and milk products are the richest sources of calcium and phosphorus, pulses, vegetables and particularly cereal grains often contribute the greatest portion of the daily calcium intake of the population of the Middle East. Cereals are frequently the chief contributors of calcium and phosphorus because of the large amounts consumed. In contrast, other foods that are very rich in calcium, but are eaten in such small amounts, that they contribute very little to the daily intake.

Because of the low socio-economic status of a large portion of the population of the Middle East, the consumption of the less expensive vegetables, pulses and cereals surpasses that of more expensive milk and dairy products. Due to the low consumption of milk and dairy products and lack of knowledge of the proper selection of the foodstuffs, calcium and phosphorus deficiency symptoms are commonly observed in the Middle East particularly among women and children.

The total consumption of calcium and phosphorus as calculated from the chemical analysis of foods consumed by human and animals does not give an accurate measure of the amounts of calcium and phosphorus utilized. Only a portion of the calcium and phosphorus in the foods consumed may be available to the animal body. The purpose of the present study is to determine the availability of calcium and phosphorus in the common foodstuffs of the Middle East to the rat. Six foodstuffs namely, cabbage, okra, jew's mellow leaf, tahineh, lentils and chickpeas were selected for the study because these are foodstuffs used in the diets of the Middle East. In addition, four "LAUBINA" food mixtures which have been developed in the division of the Food Technology and Nutrition, American University of Beirut, for infant feeding in the Middle East, were selected.

Information obtained from this study may be helpful in preparing nutritionally balanced diets for humans and rations for animals.

REVIEW OF LITERATURE

Calcium availability from different foodstuffs

Several studies by various workers have revealed that some commonly used vegetables are superior or equal to milk as an important source of calcium.

Sherman and Hawly (69), in their study on children, concluded that "milk is much superior to vegetables as a source of calcium for the growing child". The vegetables compared with milk were a mixture of carrots, spinach and celery. Blatherwick and Long (13), after doing a calcium balance experiment, reported that two women were able to maintain a positive balance when calcium was fed from different foodstuffs.

Sur and Subramanyan (74) have shown that the utilization factor for calcium derived solely from leucerne* on a dry basis is similar to that of milk. They found later that calcium in leucerne was equally utilized when an indian rice diet was supplemented with leucerne leaf powder or whole milk powder.

Devedatta and Appana (28) reported that calcium from some leafy vegetables of indian origin was utilized to the same extent as calcium of milk.

In a study of the retention of calcium in lettuce, Mallon *et al*. (56) found that more calcium was retained than that of pasteurized milk in two healthy young women.

Adolph and Chen (1) reported that cow's milk and soyabean

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* Leucerene is alfalfa.

curd are equally effective as sources of calcium in chinese diets. Kao et al. (46) have shown also that the calcium in chinese cabbage was almost as well utilized as that of milk.

In general, the availability of calcium from cereals and vegetables has been shown by various workers to be inferior to that of milk.

Shilds et al. (70) showed that the calcium in milk is definitely better utilized than the calcium in some vegetables. Under the condition of their experiment, calcium in fresh carrots, fresh lettuce and fresh stearing beans was respectively 85, 80 and 74 percent as available as calcium in milk.

Kelly (47) observed that calcium in milk was better utilized than calcium in some vegetables of New Zealand origin.

Finke and Sherman (33), in their study of availability of calcium from some typical foods, have shown that calcium in milk was the most available followed by calcium in kale and calcium in spinach was the least available.

Finke (34) observed the availability factor for calcium to be 87 percent, 79 percent and 69 percent for milk, broccoli and cauliflower respectively.

Speirs (72) found that the calcium in turnip greens to be as nearly as available as milk and that of tendergreen, collards and kale to be less.

Bendana-Brown and Lim (9) reported that calcium from fourteen vegetables of Phillipine origin was found to be less utilized than that of milk.

McClugage and Mendal (58) observed that calcium in spinach and carrots was poorly utilized by dogs, and suggested that these green vegetables should not be used as a dietary source of calcium in place of milk.

In a pairfeeding experiment, Fairbanks and Mitchell (28) reported that the calcium in fresh, cooked or canned spinach was poorly utilized by rats.

In a calcium balance study on rats, Alcaraz (3) found that the calcium in milk was better utilized than the calcium in seven vegetables tested in the experiment.

Bendana-Brown and Brown (8) have shown that calcium in dehydrated cabbage and carrots was more poorly utilized by albino mice than calcium in skim milk.

We can conclude from the above cited literatures that calcium in milk is either equally or more available than calcium in vegetables tested. But Kinsman et al. (48) in prolonged experiments on eleven preschool children, reported an average utilization of calcium in "dry milk solids" of 19%. In the same year, Steggerda and Mitchell (73) observed 20% utilization of calcium in "dry skim solids" by an adult subject. Whereas Ellis and Mitchell (31) showed that growing rats may deposit in their bodies 98% of the calcium consumed. This efficient utilization of calcium by the rats was confirmed by Fairbanks and Mitchell (32).

These results are in contrast with that reported by Kinsman et al. (48) in their studies with the eleven preschool children. They reported retention as low as 20% for school children. This shows that humans and rats do not retain calcium in their bodies to the same degree.

Few experiments have been conducted to find out the availability of calcium from different salts of calcium. If a high percentage of calcium availability is obtained when calcium salts are used then these salts could be used in place of milk to give a reference value for calcium availability.

In 1958, Causeret and Hugot (23) found that at 0.1% level of calcium in the diet, the availability of calcium from calcium carbonate, calcium sulfate and calcium lactate was 87, 88 and 89 percent respectively. However, when the calcium level was increased to 0.7% in the diet, the percent availability decreased to 40, 36 and 37 percent respectively.

Later, the same workers (24) compared the availability of calcium in $CaCO_3$ and in dry milk. They concluded that at levels of 0.1% to 0.7% of calcium in the diet, retention of calcium was similar when $CaCO_3$ or dry milk was fed to young rats.

Phosphorus availability from different foodstuffs

Phosphorus is present in all foodstuffs. In refined and processed foods, the phosphorus content may be greatly reduced. The best sources of phosphorus are those that also contain high amounts of calcium and protein. Due to this fact, Davidson and Passmore (27) proposed the following useful rule; "take care of the calcium and the phosphorus will look after itself". However, several experiments, later showed the contradictory results.

Bruce and Callow (21) established the fact that phosphorus in phytic acid is less available to rats than phosphorus in sodium phytate, and therefore claimed that the rachitogenic effect of cereals is due to the unavailability of phytin phosphorus. As a result of a long series of investigations, Mellanby (62) has demonstrated that certain cereals contain an anticalcifying factor that has the effect of producing or intensifying calcification of teeth in dogs.

Hoffjorgensen (41) showed that in puppies replacement of the calcium phosphate in a diet with pentacalcium phytate and the addition of sufficient amountsof sodium phytate to precipitate dietary calcium increased the retention of phosphorus in the body.

McCauce and Widdowson (57) pointed out that because approximately 40-70% of the phosphorus in cereal grains is in the form of phytic acid, cereals and cereal products are poor sources of available phosphorus for man. From the experiments, conducted on human subjects, they concluded that when cereals constitute the largest part of the diet, the low availability of phytin phosphorus can become a serious matter because about 20-60% of the phytin phosphorus injested by humans is excreted unchanged in the feces. Also in rats, Krieger _et al._ (49) found that the utilization of phosphorus from phytic acid was always less than that of inorganic phosphorus.

Burton (22) found that when boys and girls were given wheat and oat as a source of phosphorus, the retention of phosphorus was low, namely, 25-46% for the different boys and girls.

Blatherwick and Long (13) after performing a balance experiment on two women for 7 days, concluded that the phosphorus in celery, spinach, lettuce, asparagus and squash was able to maintain positive balance.

Sur and Subramanyan (74) reported that phosphorus was utilized much better when the poor rice diet was supplemented with leucerne than when supplemented with milk powder.

Boutwell _et al._ (18) in a study of the availability of wheatbran phosphorus to rats, showed that in the presence of adequate vitamin D, the utilization of wheatbran phosphorus was nearly equal to that of inorganic phosphorus, namely phosphorus of bone ash. They also found that presence or absence of phosphorus releasing enzymes in the diets did not alter the apparent availability of phytin phosphorus.

Williams _et al._ (80) observed that when the experimental diets contained phosphorus at a minimal level of adequacy and all other nutrients are at optimal levels, the phosphorus in a low phosphorus alfalfa hay (_Lespedeza serica_) was les available for growth and bone development of the rat than the phosphorus of high phosphorus hay of the same type.

Ashton _et al._ (6) reported that the rate of growth of 5-week-old chicks receiving about 50% of their phosphorus as phytate phosphorus was equal or greater than that of chicks receiving inorganic phosphorus, but there were indications that bone calcification was impaired. Balance studies showed that about 20% of the phytate phosphorus was used by chicks, and the use of radioactive phytin showed that some of this phosphorus was incorporated into the actively growing tissues.

Henser *et al.* (40) presented evidence indicating that the phosphorus of cereals and grains did not promote normal bone development in chicks unless the diet was supplemented by 2% of the steamed bone meal. In determining the availability of phosphorus in cereals and legumes McGinnis *et al.* (60) reported that phosphorus in cereals and legumes is not as available for bone development in chicks as phosphorus in inorganic form.

Effect of oxalates on calcium availability

It has long been known that the presence of oxalic acid in foodstuffs has a depressing effect on the availability of the calcium present, even to the extent that in certain cases calcium becomes totally insoluble. Spinach was always the classical example of such a foodstuff; Fairbanks and Mitchell (32) have shown that while the calcium in milk is utilized by rats to the extent of 89-100%, to that in Spinach is 0-11%. In a pair feeding experiment, they also found that calcium in calcium oxalate appeared to be entirely unutilizable.

Finke and Sherman (33) also found that calcium in spinach was poorly utilized by young growing rats and this was due to its high oxalate content.

Spiers (72) reported that the calcium in spinach not only was poorly utilized by rats, if at all, but the utilization of calcium in milk was diminished by the presence of New Zealand spinach which is due to its high content of oxalate, namely 4.8 percent.

Mclughlin (61) observed that calcium in vegetables is adequate in meeting the maintenance needs of man. His subjects were in calcium equilibrium when most of the milk of the diet was replaced by spinach. Bonner et al.(15) concluded that spinach is not harmful even when 100 grams are served daily to preadolescent children. These two studies showed contradictory results to the previous studies made by Fairbanks and Mitchell (32), Finke and Sherman (33) and Spiers (72) have shown that calcium from spinach was unavailable to rats due to its high oxalate content.

Majumder and De (55) reported that some indian green leafy vegetables and cereals contain unusual high amounts of oxalates. This observation was also made by Armstrong et al.(4) and Devedatta and Appana (28).

Armstrong et al.(5) found that there is no relationship between the oxalate content and calcium availability of the grasses tested in rats.

Oxalic acid also has a similar depressing effect on calcium retention in the ruminant, and this has been shown by Talapatra et al.(75).

Levelace et al.(53) have shown that, in rats, age is an important factor in determining the effect of soluble oxalates; and when present in equivalent amounts the soluble oxalates were almost fully effective in immobilizing calcium in rats at 50 days old. At 100 days, however, the effect was much less marked.

Imada et al.(42) using Ca^{45} oxalate mixed with calcium lactate, phosphate or carbonate in the diet of young albino rats, have shown that only 24 to 44% of the calcium in the diet was retained.

Jean and Hugot (43) showed that the retention of calcium when fed to rats as calcium oxalate was 40 to 50% of that of calcium when fed as calcium carbonate.

Effect of phytate

Phytic acid, a phosphorus containing compound found mainly in whole cereals and also present in few other foodstuffs, combine with calcium to form an insoluble compound and may interfere with the absorption of calcium.

Krieger and Stenbock (50) concluded from their studies that when calcium phytate was added to a cereal free low calcium ration, the calcium was found to be as readily available to the rats as the calcium of calcium carbonate.

Burton (22) found that whole cereal grains reduced calcium absorption. This may be due to its phytate content.

Hoffjorgensen (41) showed that in puppies, replacement of the calcium phosphate of the diet with pentacalcium phytate and addition of sodium phytate in sufficient amounts to precipitate dietary calcium of foodstuffs led to decreased retention of calcium.

Bronner *et al.* (20) observed that significantly less Ca^{45} was taken up by children in the presence of an equivalent quantity of phytic phosphorus supplied by oats. They concluded that "the effect of phytates in oatmeal containing moderate quantities of calcium is masked and of no practical importance".

Nicolaysen and Njaa (65) investigated the effect of phytic acid on the absorption of calcium in rats, pigs and men. They concluded that there is no effect of phytate content of cereals on the absorption of calcium.

Recently, Schreier and Ostheldr (68) reported that rats receiving phytin exhibited an inhibition of the incorporation of Ca^{45} into the bones by 25% and into the teeth by 13% as compared with the control. These experiments, however, have shown that the inhibitory action of the phytin in wheat grist and oat gruel on lime resorption and lime incorporation into the bone and teeth of rats was very small.

From a review of the subject. Brine and Johnston (19) concluded that the influence of phytic acid with the absorption of calcium apparently depends on the amount of both phytic acid and calcium in the diet. Probably if a diet for adults contains the recommended amount of calcium, a moderate amount of whole cereal will not have a harmful effect on calcium absorption.

Effect of crude fiber

It is generally accepted that there is an inverse relationship between the crude fiber content of a foodstuff and the digestibility of its major nutrients, and there are good indications to assume the existence of a similar relationship with calcium.

Bloom (14) found that the addition of 8% fiber to a diet of normal calcium content had no effect upon calcium and phosphorus retention by rats, and this was confirmed by Adolph _et al._ (2) where he used a low calcium diet.

Westerlund (77) concluded that an increase in cellulose consumption (the source of fiber being pulp) resulted in a significant increase in the amount of calcium in the feces of rats.

Armstrong *et al*. (4) found an inverse relationship between crude fiber content of three herbs of grassland and the utilization of their calcium by rats.

When studying adult human subjects consuming coarsely ground cellulose added to standard diet, Morgan (64) found that calcium balance tended to become negative during the period of high fiber content in the diet. Sjollema (71) observed that increased fiber consumption resulted in a negative calcium balance in rabbit where previously there had been a positive balance.

Finke and Sherman (33) concluded that the low utilization of calcium in spinach was not attributed to the presence of its fiber content but appears to be largely due to oxalate content of spinach.

Duckworth and Géodden (29), however, showed that the inclusion of upto 30% fiber in a diet fed to rats had no effect on calcium retention.

Effect of fat

The question of the influence of dietary fat on calcium utilization has received considerable attention. The results of many studies are conflicting. Many investigators used rats and have shown that the addition of fat to a fat-free diet often resulted in a favourable effect on calcium utilization.

Knudson and Floody (51) and Jones (44) presented evidence that moderate levels of fat in the diets favoured the utilization of calcium.

Jones (45) and French (35) have independently proven that in rats dietary fat increased the acidity of the intestinal contents and in particular in the jejunum and lower ilium portions of the intestine, and this change in acidity is responsible for the increased absorption of calcium and phosphorus.

Booth _et al_. (16) have found that on a high calcium, low phorphorus diet calcification of bones is definitely increased by the presence of fat in the diet. This is not evident, however, when a low calcium, high phosphorus diet is used.

McDongall (59) fed a diet deficient in calcium to rats and found that the rats were protected against rickets precipitated by low calcium diets when 11% lard was included in the diet. Olive oil and coconut oil were less effective than lard.

Telfer (76) outlined the mechanism of calcium absorption in presence of fat as follows: free fatty acids dissolve calcium phosphate with the formation of calcium soaps which are then absorbed. He also pointed out that if fats and phosphates are both low, calcium carbonate maybe excreted in an alkaline stool . The form in which calcium is eliminated from the intestinal tract is, therefore, associated with the pH of the intestinal contents as a consequence of the effect of acidity upon solubility.

It was, however, indicated by the work of Boyd _et al_. (17) that not all the calcium soaps are absorbed equally. The three soaps investigated were calcium oleate, calcium palmitate and calcium stearate. Calcium oleate showed the highest percentage of absorption and calcium stearate the least.

Basu and Nath (8) studied the effect of different fats on the utilization of calcium and phosphorus by man. They found that inclusion of mustard, sesame, groundnut or butterfat in the diet increased the utilization of these two elements more than in the absence of fat in the diet. With coconut oil the excretion of calcium in the urine and feces was greatly increased and thus in view of their studies they suggested that coconut oil cannot be recommended as the main dietary fat.

Effect of the ratio of calcium to phosphorus

An interrelationship in the metabolism of calcium and phosphorus has long been recognized and the proper ratios of the two elements have been established for many species. It is generally agreed now that the optimal ratio of Ca:P lies between 1:1 and 2:1.

Henry and Kon (39) found that when the ratio of Ca:P is unity and calcium and phosphorus content of the diet is adequate, growth and calcification of the bones are normal. The ratios from 1:1 to 2:1 are desirable for normal growth and calcification of bones in rats has been shown by Bethke *et al*. (10), Cox and Imboden (26) and Boutwell *et al*. (18).

In growing chicks it appears that satisfactory results are attained when Ca:P ratios from 1:1 upto 2.5:1 are used. This has been shown by Bethke *et al*. (12) and Wilgus (78).

Bethke *et al*. (11) found that growing swine exhibit optimal growth when fed diets containing ratios from 1:1 to 2:1 and Chapman *et al*. (25) found that the optimum ratio is about 1.4:1 for the 100 pound pig. Recently, using 0.2, 0.4, 0.5, 0.6, 0.7 and 0.8% phosphorus with 0.8% calcium from synthetic milk diet, Miller et al. (63) showed that pigs showed depressed growth, food intake and mineral retention when receiving 0.2% phosphorus in the diet. They also found that dietary phosphorus levels below 0.5% resulted in the reduced calcium retention whereas increasing phosphorus levels to 0.5% increased phosphorus retention.

Recently, Wise et al. (79) concluded that the optimal Ca:P ratio for the ruminant animals is higher than that of non-ruminant animals and that the ruminant will tolerate wide ratios of Ca:P (7:1) but ratios lower than unity are deletorius. However, The non-ruminant which tolerates a Ca:P ratio of less than 1:1 with ratios wider than 3:1 resulting in undesirable effects.

Gershoff et al. (36) had shown that more severe rickets was observed in the feline diets containing 1% calcium and 1% phosphorus (Ca:P ratio=1) than on diets containing 2% calcium and 0.65% phosphorus (Ca:P ratio = 3.1).

Haldi et al. (37), in their study, examined the effect of Ca:P ratio of the diet and dental carries and chemical composition of teeth of rats. The calcium content of the diet was maintained constant at 0.5% and the phosphorus content was varied from 0.24 to 1.48%, thereby altering the Ca:P ratio from 2.0 to 0.33. It was observed that as the phosphorus level of the diet increased with a concomitant reduction in the Ca:P ratio, the incidence of dental carries was reduced. However, Harris (38) was of the opinion that the Ca:P ratio becomes important only when the level of available phosphorus is low. In studies with hamster on a diet adequate in phosphorus, he concluded that the Ca:P ratio could be changed from 4.0 to 0.25% without affecting the incidence of dental carries.

Patton <u>et al</u>. (67) studied the relation of Ca:P ratio to the utilization of these minerals in 18 young college women. The balances of both minerals were more closely related to intakes than to the ratio of calcium and phosphorus.

MATERIALS AND METHODS

Six Middle Eastern foodstuffs were selected for the study of the availability of calcium and phosphorus. The scientific, english and arabic names of the foodstuffs are given in Table 1.

Four LAUBINA mixtures, supplemented and unsupplemented with boneash were chosen to study the availability of calcium. The LAUBINA mixtures are the mixtures developed at the division of Food Technology and Nutrition, American University of Beirut, for the feeding of the young children in the Middle East. The composition of the LAUBINA mixtures is given in Table 2.

Green samples of cabbage, okra and jew's mellow were obtained from Beirut market during the summer of 1963. Leaves of cabbage and jew's mellow (after removal from plants) were washed thoroughly with distilled water to avoid any calcium or phosphorus left on the leaves from the soil or irrigation water. Okra was also washed with distilled water. The washed samples were then dried in an air forced-draft oven at 50° C with the exception of okra which was dried at 60° C. After drying, the materials were ground to powder form in a Willey mill.

Dried samples of chickpeas and lentils were purchased and ground in the Willey mill.

"Tehineh" or crushed decorticated sesame, was defatted due to its high fat content. Tehineh was mixed with water in a

Table 1. Six Middle Eastern foodstuffs selected for calcium and phosphorus availability study

Scientific Name	English Name	Arabic Name[2]
Brasica oleracea	Cabbage	Malfuf
Hibiscus esculentus	Okra	Bamyah
Cicer arictenum	Chickpea	Hhimmas
Lens esculenta	Lentil	Adas
Corchorus olitorius	Jew's mellow	Malukhiyah
Sesame orientale	Tahineh[1]	Tahineh

1. Tahineh is the crushed decorticated sesame.

2. _Food Composition, Tables for Use in the Middle East_. Division of Food Technology and Nutrition, American University of Beirut, Lebanon, No. 20, 1963.

Table 2. Composition of laubina food mixtures

Mixtures	Ingredients								
	Parboiled Wheat	Chickpea	Tahineh	Corn Oil	Dry Skim Milk	Citric Acid	Vitamin A and D	Bone Ash	Sugar
	%	%	%	%	%	%	%	%	%
Laubina 102	60	20	10	—	10	—	—	—	—
Laubina 103	60	20	—	5	10	1	1	—	3
Laubina 102 (S)[2]	60	20	10	—	10	—	—	1	—
Laubina 103 (S)[2]	60	20	—	5	10	1	1	1	2

2. Means supplementation by bone ash.

Hobart mixer[3] until the fat seperated out, then the fat was decanted. The wet defatted tehineh was dried then in the oven at 60° C and ground to a powdery texture in a Willey mill.

Proximate analysis of foodstuffs and laubina food mixtures

Moisture, crude fat (ether extract), crude fiber, and ash were determined in duplicates using the methods described in A.O.A.C.[4] Handbook, 1960. Nitrogen was determined by the modified Kjeldahl method and percent crude protein was calculated by multiplying percent nitrogen by 6.25. Nitrogen free extract was calculated by the following formula:

$$\%\ \text{N.F.E.} = 100 - (\%\ \text{moisture} + \%\ \text{ether extract} + \%\ \text{crude fiber} + \%\ \text{crude protein} + \%\ \text{ash})$$

Determination of calcium and phosphorus

Calcium and phosphorus were determined in the foodstuffs and LAUBINA mixtures according to the methods described in A.O.A.C.[4] Two grams of ground samples were placed in a beaker and 10 ml. of conc. nitric acid were added and left overnight. The beaker was covered with a watch glass. Next day, 5 ml. of 70% perchloric acid were added and heated gently with the cover glass in place, until digestion was complete. The solution was cooled to room temperature and 25 ml. of hot water were added. The solution was then filtered and the

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3. Model H-600T, supplied by Hobart Manufacturing Company, Troy, Ohio, U.S.A.

4. Association of Official Agricultural Chemists, Bemjamin Franklin Station, Washington 4, D.C.

filtrate collected in a 100 ml. volumetric flask after repeated washing of the beaker with hot water. The solution was cooled, diluted to volume and mixed thoroughly and was labelled solution (A).

a) Calcium:

5 ml. aliquot of solution (A) was transfered to a 150 ml. erlenmeyer flask with a pipet and diluted with 25 ml. of distilled water. 5 drops of 0.5% methyl red indicator were added. Ammonium hydroxide (1:1) was added dropwise to a brownish orange colour, then 2 drops of hydrochloric acid (1:1) were added to produce pink colour instead of orange. After bringing the solution to boil, 25 ml. of hot concentrated solution of ammonium oxalate were added slowly with swirling to produce precipitates. The precipitates were allowed to settle by keeping the flask in the refrigerator overnight. Then the precipitates were filtered through Whatman filter paper number 42 or 44 and washed thoroughly with (1:50) ammonium hydroxide. The filter paper with the precipitates was placed in the original flask and 50 ml. of 5/125 sulfuric acid were added. The solution was then heated to 70° C. or above and titrated against standard potassium permanganate while hot until pink colour appeared. A blank was run with the sample, and correction for blank was made.

The calculation for calcium was made as follows:

$$\% \text{ of Calcium} = \frac{\text{N. of } KMnO_4 \times (\text{ml. of } KMnO_4 \text{ for sample} - \text{ml. of } KMnO_4 \text{ for blank}) \times \text{meq. of calcium} \times \text{Dilution} \times 100}{\text{Weight of sample} \times \text{Volume aliquot}}$$

b) Phosphorus:

5 ml. of solution (A) was transfered to 25 ml. volumetric flask, 2.2 ml. of molybdic acid solution were added, diluted to volume and mixed thoroughly. 5 drops of stannus chloride were added and mixed thoroughly. Spectrophotometer[5] readings were taken in 30 minutes after the addition of stannus chloride solution at 600 mμ in red tube (filter), setting it at zero with the blank.

The calculation for phosphorus was made according to the following formula:

$$\text{mg of phosphorus/100 gm. dried sample} = \frac{\text{ppm x dilution factor x 100}}{\text{Weight of sample}}$$

Determination of oxalate

The method of Eheart and Herst (30) was followed for the determination of oxalate in the foodstuffs and laubina mixtures. The determination of oxalates in plant materials consists of three steps; a) HCl extraction; b) liquid-liquid extraction of an aliquot of the HCl extract with subsequent precipitation of the oxalates as calcium oxalate; then filtering, washing and dissolving of the precipitates; and c) titration of oxalic acid with standard $KMnO_4$ solution.

The above mentioned steps in the determination of oxalates are performed in the following manner:

a) 1.0000 gram dried, ground, sample was transfered to an

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5. Spectrophotometer, Model sp 600. Unicam Instruments, Cambridge, London.

alundum thimble and extracted with 100 ml. 3.5 N HCl for six hours in soxhlet extractor. The HCl extract was filtered through 60 ml. medium porosity Buchner funnel into a 200 ml. volumetric flask with a 24/40 suction tube and an appropriate adapter. The residue was washed with water several times. The extract was allowed to cool and made to volume.

b) 50 ml. of the extract was transfered to a liquid-liquid extractor by means of a pipet and 10 ml. of water was added to make the volume 60 ml. 100 ml. of ethyl ether was placed in 250 ml. 24/40 ₸ erlenmeyer flask and extraction continued for 16 hours by applying gentle heat to the flask. This extraction stoichiometrically removes the dicarboxylic acids from the HCl aliquot.

The excess ether remaining in the extractor was transfered quantitatively to the erlenmeyer flask with a pipet. The inner tube of the extractor was washed with 10 ml. of distilled water, collecting the water in the erlenmeyer flask; the ether was evaporated by means of a gentle current of air. The sides of the flask were washed down with 25 ml. of distilled water. 2 drops of 0.1% bromocresol purple were added to the flask and contents were neutralized with 4 N ammonium hydroxide. 8 ml. of the buffer solution were added to the flask dropwise with swirling. This separated the calcium oxalate from the other dicarboxilic acids present.

The flask was allowed to stand overnight, then the calcium oxalate precipitates were filtered through a 60 ml. Buchner funnel (fine porosity), using slight suction with the 24/40 ₸ suction tube. The flask was washed several times with hot water and the water transfered to the funnel; then the precipitates were washed several times with hot water. The calcium oxalate precipitates were dissolved into the original flask with 12 ml. of hot 4 N sulfuric acid. The funnel was washed thoroughly with hot water by applying suction and collecting the washings in the flask.

c) The resulting oxalic acid solution was titrated, while hot, with the 0.2011 N potassium permanganate solution on a magnetic electric stirrer. The temperature was not allowed to exceed 80° C.

Determination of phytate

The method of Lopez (54) was followed for the determination of phytate in the foodstuffs and laubina mixtures. Eight grams of powdered sample were shaken frequently with 200 ml. of 2% hydrochloric acid for one hour. The HCl extract was filtered repeatedly until clear. 50 ml. of filtrate were added to 119 ml. of distilled water. After heating to 70-80° C., 2 ml. of a 10% solution of sodium salicylate were added and the solution was titrated against 0.05% solution of ferric chloride until the colour changes to violet. A blank was run with 50 ml. of water in place of extract.

Determination of body calcium and phosphorus in experimental rats

After the conclusion of experimental periods of 21 or 28 days, the rats were killed by the application of chloroform in a closed jar. For the determination of final body calcium and phosphorus, the rats, after killing, were dried in an air forced-draft oven at 100° C. for 48 hours and then preashed on a hot plate to get rid of the smoke developed. The final ashing was done in a muffle furnace at 600° C.

for a period of 36 hours. It was found necessary to increase the temperature of the furnace slowly in order to avoid loss by frothing. The ash was then dissolved in 50% hydrochloric acid and made to volume. This solution was labelled as solution (A).

The determination of calcium and phosphorus from this solution (A) was made according to the methods described earlier for the determination of calcium and phosphorus.

Calculation of calcium availability was made according to the following formula:

$$\% \text{ of calcium availability} = \frac{\text{Final body Ca of rats - initial body Ca of rats}}{\text{Calcium intake from diet}} \times 100$$

Phosphorus availability was calculated by the above formula only by replacing calcium with phosphorus.

Animal experiments

The experimental animals used were weanling, 21 to 23 days old, male, albino rats of the Sprague-Dawly[6] strain. The rats were placed on a stock diet[7] after receiving them for a recovery period of 2 days.

The animals were individually housed in mesh-bottom cages in an air-conditioned room held at 70 ± 2° F. and relative humidity of 60 percent. In all the experiments, animals were assigned to diets

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6. Obtained from Animal Suppliers (London) Ltd.

7. Obtained from Vitasni Feed Company, Beirut, Lebanon.

according to randomized block design. Animals received food and distilled water _ad libitum_; the spillage of individual rats were collected very carefully every three days. Food consumption was calculated by subtracting spillage from food intake by rats. Food consumption and weight gains were determined weekly. Three animal experiments were conducted in the following manner:

A. Experiment I.

For the study of the availability of calcium eight groups of rats, each consisting of seven rats were fed the different diets shown in Table 3. Another similar representative group of seven rats were killed at the beginning of the experiment to find the initial body calcium of the rats. At the end of the four weeks of the experimental period, the rats were sacrificed and final body calcium was determined. This method is referred to as "Slaughter Technique". The rats were starved 24 hours before sacrificing them to let the intestine empty their contents.

B. Experiment II.

Experiment II consisted of two parts; a) the availability study of calcium from LAUBINA mixtures and b) the availability study of phosphorus from six different foodstuffs.

These two studies were conducted in the same procedure of the Experiment I, except that phosphorus was determined in place of calcium for phosphorus availability and the experimental period was 3 weeks instead of 4 weeks for both experiments. The different diets fed to rats for both the experiments are shown in Tables 4 and 5.

Table 3. Composition of the diets fed to rats in experiment conducted for the study of calcium availability (Experiment I).

Diet No.	I (%)	II (%)	III (%)	IV (%)	V (%)	VI (%)	VII (%)	VIII (%)
$CaCO_3$	0.5	0.13	0.13	0.13	0.13	0.4	0.43	—
Cabbage	—	25.0	—	—	—	—	—	—
Okra	—	—	9.5	—	—	—	—	—
Jew's mellow	—	—	—	4.87	—	—	—	—
Tahineh	—	—	—	—	37.5	—	—	—
Chickpea	—	—	—	—	—	13.0	—	—
Lentil	—	—	—	—	—	—	63.0	—
No calcium	—	—	—	—	—	—	—	—
Casein[1]	20	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Corn starch	61.1	37.3	52.3	56.9	35.7	1.3	1.3	61.6
Corn oil	10	10.0	10.0	10.0	—	10.0	10.0	10
Non-nutritive cellulose[1]	5.0	5.0	5.0	5.0	5.0	—	—	5.0
Mineral mixture (Ca & P free)	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Vitamin mixture[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
KH_2PO_4	1.1	0.33	0.87	0.87	—	—	—	1.1

1. Obtained from General Biochemicals, Chagrin Falls, Ohio, U.S.A.

2. Obtained from Nutritional Biochemicals Cooperation, Cleveland, Ohio, U.S.A.

Table 4. Composition of the diets fed to rats in experiment conducted for the study of calcium availability of four LAUBINA mixtures (Experiment II a)

Diet No.	I (%)	II (%)	III (%)	IV (%)	V (%)
$CaCO_3$	0.5	—	—	—	—
Laubina 102	—	85.0	—	—	—
Laubina 102 (S)	—	—	33.0	—	—
Laubina 103	—	—	—	62.5	—
Laubina 103 (S)	—	—	—	—	28.2
Casein[1]	20.0	2.7	20.0	20.0	20.0
Corn starch	61.3	—	29.7	5.2	34.5
Corn oil	10.0	10.0	10.0	10.0	10.0
Non-nutritive cellulose[1]	5.0	—	5.0	—	5.0
Mineral mixture (Ca & P free)	1.3	1.3	1.3	1.3	1.3
Vitamin mixture[2]	1.0	1.0	1.0	1.0	1.0
KH_2PO_4	0.9	—	—	—	—

1. Obtained from General Biochemicals, Chagrin Falls, Ohio, U.S.A.

2. Obtained from Nutritional Biochemicals Cooperation, Cleveland, Ohio, U.S.A.

Table 5. Composition of the diets fed to rats in experiment conducted for the study of phosphorus availability (Experiment II b).

Diet No.	I (%)	II (%)	III (%)	IV (%)	V (%)	VI (%)	VII (%)	VIII (%)
KH_2PO_4	0.9	—	—	—	—	—	—	—
Cabbage	—	36.4	—	—	—	—	—	—
Okra	—	—	43.0	—	—	—	—	—
Lentil	—	—	—	71.65	—	—	—	—
Chickpea	—	—	—	—	56.1	—	—	—
Tahineh	—	—	—	—	—	21.0	—	—
Jew's mellow leaf	—	—	—	—	—	—	27.3	—
No phosphorus	—	—	—	—	—	—	—	—
Casein[1]	20.0	20.0	20.0	15.6	20.0	20.0	20.0	20.0
Corn starch	61.3	26.3	19.7	—	5.2	41.3	35.3	62.2
Non-nutritive cellulose[1]	5.0	5.0	5.0	—	5.0	5.0	5.0	5.0
Vitamin mixture[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mixture (Ca & P free)	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Corn oil	10.0	10.0	10.0	10.0	10.0	—	10.0	10.0
$CaCO_3$	0.5	—	—	0.45	0.4	0.3	—	0.5

1. Obtained from General Biochemicals, Chagrin Falls, Ohio, U.S.A.

2. Obtained from Nutritional Biochemicals Cooperation, Cleveland, Ohio, U.S.A.

C. Experiment III.

To find the effect of oxalate content of okra on the availability of calcium in okra four groups of rats, each consisting of seven rats, were selected. To find the initial body calcium a similar group of seven rats were sacrificed at the beginning of experiment. After three weeks of experimental periods, the rats were killed to determine the final body calcium after starving the rats for 24 hours to remove any calcium of the diet remaining in the intestine.

The composition of the diets used for experiments I, II a, II b, and III are represented in Tables 3, 4, 5 and 6 respectively.

The calcium and phosphorus free mineral mixture, used in all the experiments had the following composition:

Ingredients	Percentage
NaCl	48.1
$MgSO_4$, $7H_2O$	24.0
$MnSO_4$, H_2O	2.0
$ZnSO_4$, $7H_2O$	0.8
$CuSO_4$, $7H_2O$	0.8
$FeSO_4$, $7H_2O$	16.0
NaF	0.02
KI	0.01
KCl	8.27

For the calcium availability study, each of the diets was calculated to contain 0.2 to 0.3% of calcium. Small amounts were unavoidably contributed by other components. Analysis of diets I - VII

Table 6. Composition of the diets fed to rats in experiment conducted for the study of the effect of oxalic acid on the availability of calcium (Experiment III)

Diet No.	I (%)	II (%)	III (%)	IV (%)
$CaCO_3$	0.5	—	—	—
Okra	—	12.5	—	—
Okra and $CaCO_3$	—	—	6.25+0.25	—
$CaCO_3$ + oxalic acid	—	—	—	0.5+0.032
Casein[1]	20.0	20.0	20.0	20.0
Corn starch	61.3	49.5	55.4	61.25
Corn oil	10.0	10.0	10.0	10.0
Vitamin mixture[2]	1.0	1.0	1.0	1.0
Mineral mixture	1.3	1.3	1.3	1.3
Non-nutritive cellulose[1]	5.0	5.0	5.0	5.0
KH_2PO_4	0.9	0.65	0.8	0.9

1. Obtained from General Biochemicals, Chagrin Falls, Ohio, U.S.A.

2. Obtained from Nutritional Biochemicals Cooperation, Cleveland, Ohio, U.S.A.

in Experiment I showed the calcium content to be 0.225, 0.305, 0.310, 0.279, 0.272, 0.301, and 0.255 percent respectively; for Experiment II a., the calcium content of diets I - V was found to be 0.200, 0.220, 0.210, 0.205 and 0.201 percent respectively and for Experiment III, the calcium content of diets I - IV was 0.220, 0.266, 0.229 and 0.234 percent respectively.

In all these calcium availability experiments calcium carbonate diet was used as a reference standard diet. Because it was originally planned that the diets should contain approximately 0.2 to 0.3 percent of calcium, and as a calcium-phosphorus ratio of approximately 1:1, phosphorus supplementation of the diets was necessary. The supplementation of phosphorus was done by adding KH_2PO_4 in such a manner as to make the ratio of calcium-phosphorus as 1:1.

For the study of phosphorus availability, the reference standard diet was KH_2PO_4 which served as a sole source of phosphorus. To maintain the ratio of calcium-phosphorus 1:1, calcium carbonate was added to the diets. But in diets No. III and VII (Table 5), the ratio could not be maintained because of the great difference in calcium and phosphorus content of okra and jew's mellow leaf (Table 8). Analysis of the diets I - VII in Experiment II b., the phosphorus content found to be 0.200, 0.262, 0.201, 0.210, 0.205, 0.210 and 0.200 respectively.

The sub-optimal level of calcium and phosphorus was maintained in all the diets for complete utilization.

Statistical analysis applied to randomized block design and Dunken range test was made according to LeClerg _et al_. (52). The correlation for the factors affecting the availability of calcium and phosphorus was done according to Panse and Sukhtme (66).

RESULTS

Six different Middle Eastern foodstuffs and four "LAUBINA" food mixtures were analyzed for proximate composition and for calcium phosphorus, oxalate and phytate content.

Calcium availability in six Middle Eastern foodstuffs and in four laubina food mixtures was investigated using slaughter technique method whereas phosphorus availability study was made only in six foodstuffs using the same method. In addition, the study of the effect of oxalate on the availability of calcium in okra was made using the same procedure. The factors affecting the availability of calcium and phosphorus was also determined. The results obtained have been reduced to tabular form and are presented in Tables 7-30 inclusively.

Table 7. Proximate composition of six food ingredients and four mixtures (Percentage by dry matter)

Foodstuffs or Mixtures	Moisture	Ether Extract	Crude Fiber	Crude Protein	Ash	N.F.E.[1]
	%	%	%	%	%	%
Cabbage	11.3	2.8	11.9	17.8	8.0	48.2
Chickpeas	9.8	7.1	3.2	18.3	3.5	58.1
Jew's mellow leaf	9.0	5.3	9.8	25.5	18.3	32.1
Lentil	10.6	1.6	2.4	24.6	2.7	58.1
Okra	7.1	2.3	10.0	17.0	9.2	54.4
Tahineh	4.9	34.1	2.8	41.8	4.6	11.8
Laubina 102	6.9	8.3	2.8	19.0	2.9	60.1
Laubina 103	7.5	8.0	2.6	16.1	2.6	63.2

1. Nitrogen free extract.

Table 8. Calcium, phosphorus, oxalate and phytate content of the food ingredients and mixtures (mg/100 gm. dry matter)

Foodstuffs or Mixtures	Calcium	Phosphorus	Oxalate	Phytate
	mg.	mg.	mg.	mg.
Cabbage	600	548	100	194
Chickpea	70	350	50	108
Jew's mellow leaf	3121	731	2400	218
Lentil	44	280	60	110
Okra	1600	465	370	221
Tahineh	400	950	280	553
Laubina 102	210	396	41	21
Laubina 103	321	401	48	20
Laubina 102 (S)	536	482	49	18
Laubina 103 (S)	596	518	39	18

Table 9. Initial body calcium content of rats killed at the beginning of Experiment I.

Rat No.	Weight of Rats	Total Body Calcium	Calcium as % of Body Weight
	gm.	mg.	%
1	49.0	376.8	0.769
2	50.0	414.5	0.829
3	52.0	447.7	0.861
4	53.0	455.2	0.859
5	55.0	486.2	0.884
6	58.0	487.7	0.841
7	59.0	531.0	0.900
Mean	53.71	457.00	0.849

Table 10. Calcium and Phosphorus content of rats killed at the beginning of Experiment II a. and II b.

Rat No.	Weight of Rats	Total Body Calcium	Calcium as % of Body Weight	Total Body Phosphorus	Phosphorus as % of Body Weight
	gm.	mg.	%	mg.	%
1	48.0	381.6	0.795	238.5	0.496
2	51.0	401.8	0.788	250.0	0.490
3	52.0	416.5	0.801	255.5	0.491
4	53.0	447.8	0.845	241.0	0.454
5	54.0	464.4	0.860	259.0	0.479
6	58.0	442.5	0.763	264.0	0.455
7	59.0	519.7	0.881	270.0	0.457
Mean	53.57	439.18	0.819	254.0	0.474

Table 11. Initial body calcium content of rats killed at the beginning of Experiment III.

Rat No.	Weight of Rats	Total Body Calcium	Calcium as % of Body Weight
	gm.	mg.	%
1	49.0	355.2	0.725
2	50.0	418.0	0.836
3	52.0	360.8	0.694
4	53.0	426.1	0.804
5	55.0	480.7	0.874
6	56.0	496.1	0.886
7	57.0	493.6	0.866
Mean	53.1	431.5	0.812

Table 12. Calcium utilization by rats in Experiment I
(Average of 7 rates per group)

Diets Fed	Initial Wt. of Rats	Calcium Intake	% of Body Calcium (Assumed)[1]	Initial Body Calcium (Calculated)[2]	Final Body Calcium (Found)	Calcium Retained	Calcium Utilization[3]	Ca:P Ratio
	gm.	mg.	%	mg.	mg.	mg.	%	
$CaCO_3$	53.4	686.25	0.849	453.5	964.0	510.5	74.4	1:1
Cabbage	53.4	1000.1	0.849	453.5	1101.8	648.3	64.7	1:1
Chickpea	53.7	1040.9	0.849	455.9	1128.7	672.8	64.5	1:1
Lentil	53.4	901.6	0.849	453.5	896.8	442.3	49.0	1:1
Tahineh	53.3	1040.9	0.849	452.4	846.0	393.6	40.7	1:1.8
Jew's mellow leaf	53.6	676.7	0.849	454.7	678.4	223.7	32.9	1:1
Okra	53.6	901.6	0.849	454.7	695.0	240.3	28.7	1:1

* $CaCO_3$	Cabbage	Chickpea	Lentil	Tahineh	Jew's mellow leaf	Okra
74.4	64.7	64.5	49.0	40.7	32.9	28.7

* The average calcium utilization values underlined by the same line do not differ significantly at both 5% and 1% levels according to Duncan Range test.

1. Body calcium was assumed from a representative group of rats killed at the beginning of Experiment I (Table 7).

2. Calculated from initial body calcium of column 3.

3. % utilization of calcium calculated according to formula:

$$\frac{\text{Calcium retained}}{\text{Calcium intake}} \times 100$$

Table 13. Calcium utilization by rats in Experiment II a.
(Average of 7 rats per group)

Diets Fed	Initial Wt. of Rats	Calcium Intake	% of Body Calcium (Assumed)[1]	Initial Body Calcium (Calculated)[2]	Final Body Calcium (Found)	Calcium Retained	Calcium Utilization[3]	Ca:P Ratio
	gm.	mg.	%	mg.	mg.	mg.	%	
$CaCO_3$	53.4	504.0	0.819	437.4	805.5	368.1	73.0	1:1
Laubina 103 (S)	53.6	563.6	0.819	438.6	794.7	356.1	64.1	1:1.5
Laubina 102	53.4	554.8	0.819	437.4	786.0	348.6	62.6	1:1
Laubina 102 (S)	53.4	553.8	0.819	437.4	768.31	330.71	59.5	1:1.2
Laubina 103	53.7	492.7	0.819	439.8	731.3	291.4	58.5	1:1

* $CaCO_3$	Laubina 103 (S)	Laubina 102	Laubina 102 (S)	Laubina 103
73.0	64.1	62.6	59.5	58.5

* The average calcium utilization values underlined by the same line do not differ significantly at 5% level according to Duncan Range test.

1. Body calcium was assumed from a representative group of rats killed at the beginning of Experiment II a. (Table 8).

2. Calculated from assumed body calcium of column 3.

3. % calcium utilization was calculated according to the formula:

$$\frac{\text{Calcium retained}}{\text{Calcium intake}} \times 100$$

Table 14. Phosphorus utilization by the rats in Experiment II b.
(Average of 7 rats per group)

Diets Fed	Initial Wt. of Rats	Phosphorus Intake	% of Body Phosphorus (Assumed)[1]	Initial Body Phosphorus (Calculated)	Final Body Phosphorus (Found)	Phosphorus Retained	Phosphorus Utilization[2]	Ca:P Ratio
	gm.	mg.	%	mg.	mg.	mg.	%	
KH_2PO_4	53.42	504.0	254.0	136.14	427.71	291.0	58.0	1:1
Tahineh	53.85	523.28	254.0	137.28	439.28	302.0	58.0	1:1
Lentil	54.0	526.85	254.0	137.71	444.0	306.28	57.8	0.9:1
Chickpea	53.28	499.14	254.0	135.85	422.85	287.0	57.7	1.4:1
Cabbage	54.0	399.28	254.0	137.71	313.85	176.14	44.4	1:1
Okra	53.57	432.28	254.0	136.42	317.85	181.42	42.0	4:1
Jew's mellow leaf	53.71	238.85	254.0	137.3	236.71	99.42	41.7	4.3:1

* KH_2PO_4	Tahineh	Lentil	Chickpea	Cabbage	Okra	Jew's mellow leaf
58.0	58.0	57.8	57.7	44.4	42.0	41.7

* The average utilization values of phosphorus underlined by the same line do not differ significantly at both 5% and 1% levels according to Duncan Range test.

1. Body phosphorus was assumed from body phosphorus content of a representative group of rats killed at the beginning of the Experiment II b. (Table 8).

2. % phosphorus was calculated by the formula:

$$\frac{\text{Phosphorus retained}}{\text{Phosphorus intake}} \times 100$$

Table 15. Effect of oxalate content of foods on the availabilty of calcium in Experiment I (Averages of 7 rats per group)

Diets Fed	Oxalate Content of Diets	Total Intake of Oxalate	Availability of Calcium*
	gm./100 gm.	gm.	%
$CaCO_3$	—	—	74.4
Cabbage	0.015	0.040	64.7
Chickpea	0.031	0.107	64.5
Jéw's mellow leaf	0.035	0.085	32.9
Lentil	0.037	0.130	49.0
Okra	0.037	0.094	28.7
Tahineh	0.108	0.383	40.7

* There is no significant correlation between oxalate intake from the foodstuff and availability of calcium at both 5% and 1% levels ($r = -0.37$).

Table 16. Effect of oxalate content of foods on the calcium availability in Experiment II a. (Averages of 7 rats per group)

Diets Fed	Oxalate Content of Diets	Total Intake of Oxalate	Availability* of Calcium
	gm./100 gm.	gm.	%
$CaCO_3$	—	—	73.0
Laubina 103 (S)	0.009	0.023	64.1
Laubina 102	0.024	0.060	62.6
Laubina 102 (S)	0.010	0.024	59.5
Laubina 103	0.020	0.048	58.5

* There is no significant correlation between intake of oxalate from the mixtures and their availability at both 5% and 1% levels ($r = -0.31$).

Table 17. Effect of phytate content of foods on the availability of calcium in Experiment I (Averages of 7 rats per group)

Diets Fed	Phytate Content of Diets	Total Phytate Intake	Availability of Calcium*
	gm./100 gm.	gm.	%
$CaCO_3$	—	—	74.4
Cabbage	0.048	0.157	64.7
Chickpea	0.071	0.245	64.5
Lentil	0.069	0.244	49.0
Tahineh	0.207	0.735	40.7
Jew's mellow leaf	0.010	0.024	32.9
Okra	0.021	0.053	28.7

* There is no significant correlation between phytate intake from the foodstuffs and availability of calcium at both 5% and 1% levels ($r = 0.11$).

Table 18. Effect of phytate content of food on the availability of calcium in Experiment II a. (Averages of 7 rats per group)

Diets Fed	Phytate Content of Diets	Total Intake of Phytate	Availability of Calcium*
	gm./100 gm.	gm.	%
$CaCO_3$	—	—	73.0
Laubina 103 (S)	0.018	0.047	64.1
Laubina 102	0.021	0.053	62.6
Laubina 102 (S)	0.015	0.039	59.5
Laubina 103	0.020	0.048	58.5

* There is no significant correlation between the intake of phytate from the laubina mixtures and availability of calcium at both 5% and 1% levels ($r = 0.12$).

Table 19. Effect of phytate content of foods on the availability of phosphorus in Experiment II b. (Averages of 7 rats per group)

Diets Fed	Phytate Content of Diet	Total Intake of Phytate	Availability* of Phosphorus
	gm./100 gm.	gm.	%
KH_2PO_4	—	—	58.0
Jew's mellow leaf	0.059	0.07	41.7
Chickpea	0.061	0.15	57.7
Cabbage	0.067	0.13	44.4
Lentil	0.078	0.19	57.8
Okra	0.095	0.20	42.0
Tahineh	0.116	0.29	58.0

* There is no significant correlation between phytate intake of diets and phosphorus availability at both 5% and 1% levels ($r = 0.12$).

Table 20. Effect of fiber content of foods on the availability of calcium in Experiment I (Averages of 7 rats per group)

Diets Fed	Fiber Content of Diets	Total Intake of Fiber	Availability* of Calcium
	gm./100 gm.	gm.	%
$CaCO_3$	—	—	74.4
Cabbage	2.97	9.74	64.7
Chickpea	2.11	7.29	64.5
Lentil	1.59	5.62	49.0
Tahineh	1.05	3.72	40.7
Jew's mellow leaf	0.48	1.16	32.9
Okra	0.95	2.40	28.7

* There is no significant correlation between calcium utilization and fiber intake of the diets at both 5% and 1% levels ($r = 0.28$).

Table 21. Effect of fiber content of foods on the availability of calcium in Experiment II a. (Averages of 7 rats per group)

Diets Fed	Fiber Content of Diet	Total Intake of Fiber*	Calcium Utilization
	gm./100 gm.	gm.	%
$CaCO_3$	—	—	73.0
Laubina 103 (S)	0.55	1.34	64.1
Laubina 102	1.59	4.01	62.6
Laubina 102 (S)	0.51	1.45	59.5
Laubina 103	1.08	2.59	58.5

* Statistical analysis does not show any significant correlation between calcium utilization and total intake of fiber at both 5% and 1% levels ($r = -0.46$).

Table 22. Effect of fiber content of foods on the availability of phosphorus in Experiment II b. (Averages of 7 rats per group)

Diets Fed	Fiber Content of Diet	Total Intake of Fiber*	Availability of Phosphorus
	gm./100 gm.	gm.	%
KH_2PO_4	—	—	58.0
Tahineh	0.58	1.44	58.0
Lentil	1.7	4.26	57.8
Chickpea	1.8	4.38	57.7
Jew's mellow leaf	2.7	3.22	41.7
Okra	4.3	9.25	42.0
Cabbage	4.3	8.49	44.4

* Statistically there exists a significant positive correlation between phosphorus utilization and total intake of fiber at both 5% and 1% levels (r = 1.0).

Table 23. Effect of fat intake of diets on the availability of calcium in Experiment I (Averages of 7 rats per group)

Diets Fed	Fat Content of Diets	Total Intake of Fat	Availability of Calcium*
	gm./100 gm.	gm.	%
$CaCO_3$	10.00	30.50	74.4
Cabbage	10.70	35.07	64.7
Chickpea	14.69	50.79	64.5
Lentil	11.04	39.03	49.0
Tahineh	12.75	45.26	40.7
Jew's mellow leaf	10.25	24.48	32.9
Okra	10.21	25.84	28.7

* Statistical analysis does not show any significant correlation between total intake of fat and availability of calcium at both 5% and 1% levels ($r = 0.32$).

Table 24. Effect of fat intake of diets on the availability of calcium in Experiment II a. (Averages of 7 rats per group)

Diets Fed	Fat Content of Diet	Total Intake of Fat	Calcium Utilization*
	gm./100 gm.	gm.	%
$CaCO_3$	10.0	25.20	73.0
Laubina 103 (S)	11.54	30.12	64.1
Laubina 102	14.14	35.71	62.6
Laubina 102 (S)	11.42	30.58	59.5
Laubina 103	13.33	32.04	58.5

* Statistical analysis does not show any significant correlation between total intake of fat and availability of calcium at both 5% and 1% levels ($r = 0.56$).

Table 25. Effect of fat intake of diets on the availability of phosphorus in Experiment II b. (Averages of 7 rats per group)

Diets Fed	Fat Content of Diet	Total Intake of Fat	Phosphorus Utilization*
	gm./100 gm.	gm.	%
KH_2PO_4	10.68	25.20	58.0
Tahineh	14.77	36.79	58.0
Lentil	10.74	26.94	57.8
Chickpea	12.70	30.91	57.7
Jew's mellow leaf	10.96	13.08	41.7
Okra	10.65	22.90	42.0
Cabbage	10.68	21.10	44.4

* Statistical analysis does not show any significant correlation exist between phosphorus utilization and fat intake at both 5% and 1% levels ($r = 0.68$).

Table 26. Effect of total intake of food and gain in weight of rats on the availability of calcium in Experiment I (Averages of 7 rats per group)

Diets Fed	Total Food Intake[1]	Gain in Wt. of Rats[2]	Availability of Calcium
	gm.	gm.	%
$CaCO_3$	305	140.0	74.4
Cabbage	327.8	111.57	64.7
Chickpea	345.8	143.42	64.5
Lentil	353.6	138.85	49.0
Tahineh	355.0	137.28	40.7
Jew's mellow leaf	242.6	62.42	32.9
Okra	253.1	53.14	28.7

1. Statistically no significant correlation exists between intake of food and availability of calcium at both 5% and 1% levels ($r = 0.44$).

2. No significant correlation exists statistically between availability and gain in weight at both 5% and 1% levels ($r = 0.69$).

Table 27. Effect of total intake of food and gain in weight of rats on the availability of calcium in Experiment II a. (Averages of 7 rats per group)

Diets Fed	Total Intake of Foods[1]	Gain in Wt. of Rats[2]	Availability of Calcium
	gm.	gm.	%
$CaCO_3$	252.0	99.0	73.0
Laubina 103 (S)	265.0	98.6	64.1
Laubina 102	252.6	89.1	62.6
Laubina 102 (S)	263.8	83.7	59.5
Laubina 103	240.4	83.3	58.5

1. There is no significant correlation between intake of food and availability of calcium at both 5% and 1% levels ($r = 0.06$).

2. Statistically there is no significant correlation between gain in weight and availability of calcium at both 5% and 1% levels ($r = 0.67$).

Table 28. Effect of total intake of food and gain in weight of rats on the availability of phosphorus in Experiment II b. (Averages of 7 rats per group)

Diets Fed	Total Food Intake[1]	Gain in Wt. of Rats[2]	Availability of Phosphorus
	gm.	gm.	%
KH_2PO_4	252.0	94.0	58.0
Tahineh	249.1	89.3	58.0
Lentil	250.8	88.0	57.8
Chickpea	243.4	86.0	57.7
Cabbage	197.6	46.0	44.4
Okra	215.1	43.0	42.0
Jew's mellow leaf	119.4	4.7	41.7

1. There is no significant correlation between total intake of food and availability of phosphorus at both 5% and 1% levels ($r = 0.69$).

2. A significant correlation exists statistically between gain in weight of rats and phosphorus availability at both 5% and 1% levels ($r = 0.80$).

Table 29. Calcium intake of rats during 3 weeks of experimental periods (Averages of 7 rats per group) Experiment III

Diets Fed	Total Food Intake	Calcium in 100 g. Diet	Total Calcium Intake
	gm.	mg.	mg.
$CaCO_3$	185.0	220.0	407.0
Okra	208.8	266.0	555.0
Okra + $CaCO_3$	213.7	229.0	489.3
$CaCO_3$ + Oxalic Acid	200.8	234.0	468.2

Table 30. Effect of oxalate content of okra on calcium utilization (Average of 7 rats per group)
Experiment III

Diets Fed	Initial Wt. of Rats	Calcium Intake	% of Body Calcium (Assumed)[1]	Initial Body Calcium (Calculated)	Final Body Calcium (Found)	Calcium Retained	Calcium Utilization[2]	Ca:P Ratio
	gm.	mg.	%	mg.	mg.	mg.	%	
$CaCO_3$	54.28	407.0	0.812	440.77	741.45	300.68	73.9	1.1:1
Okra	54.2	555.5	0.812	440.77	668.5	227.7	41.3	1.2:1
Okra + $CaCO_3$	54.7	489.3	0.812	444.2	781.6	337.3	68.9	1:1
$CaCO_3$ + Oxalic Acid	54.1	468.2	0.812	439.6	641.3	201.7	42.5	1.1:1

* $CaCO_3$	Okra + $CaCO_3$	$CaCO_3$ + Oxalic Acid	Okra
<u>73.9</u>	<u>68.9</u>	<u>42.5</u>	<u>41.3</u>

* The average calcium utilization values underlined by the same line do not differ significantly at both 5% and 1% levels according to Duncen Range test.

1. The initial body calcium was assumed from a representative group of rats killed at the beginning of the experiment (Table 3).

2. % utilization of calcium was calculated by using the formula:

$$\frac{\text{Calcium retained}}{\text{Calcium intake}} \times 100$$

DISCUSSION

Proximate composition of the foodstuffs and food mixtures

Table 7 shows that the foodstuffs analyzed have different proximate composition except for the "LAUBINA" food mixtures. The more or less similar proximate composition of laubina mixtures is due to the fact that they contain the same amounts of burgol and chickpea. The composition of the laubina food mixtures is shown in Table 2. The difference in the crude protein content of the mixtures can be explained on the basis of the presence of "tahineh" or crushed decorticated sesame. Laubina 102 contains 10% tahineh while laubina 103 does not contain any tahineh. Tahineh is high in oil and protein content, and although it was defatted only 50% of the fat was removed and this doubled the protein content of tahineh. Cabbage, okra and jew's mellow leaf are high in fiber content.

Calcium and phosphorus content

The results presented in Table 8 show the calcium and phosphorus content of the foodstuffs and mixtures on a dry weight basis. It can be seen that the foodstuffs differ considerably in their calcium and phosphorus content. The calcium content is highest in jew's mellow leaf followed by okra and is lowest in lentils. The low content of calcium in lentils and tahineh is due to the fact that the seed coat of both lentils and sesame was removed during the preparation of lentils and processing the sesame seeds.

The phosphorus content also is highest in tahineh followed by jew's mellow leaf and is lowest in lentils.

It can be noticed from the results of calcium and phosphorus analysis that the difference in calcium and phosphorus content of the same foodstuffs may be great, such as, in lentil, chickpea, jew's mellow leaf and okra.

Oxalate and phytate content

Table 8 also shows the oxalate and phytate content of foodstuffs and laubina mixtures. The highest oxalate content was found in jew's mellow leaf followed by okra, tahineh, cabbage, lentil and lowest in chickpea. The oxalate content of four laubina mixtures were more or less similar and lower than that of the other foodstuffs. The highest phytate content was observed in tahineh followed by jew's mellow leaf. The laubina mixtures contained similar and low content of phytate.

Initial body calcium of rats

Data presented in Tables 9, 10 and 11 show that some differences were present in the body calcium and phosphorus contents of the representative group of rats killed at the beginning of experiments. The purpose of the representative group was to provide an assumed initial body calcium and phosphorus content of the experimental rats. However, these values were not considerably different and are close to values found by Kao et al. (46) and Armstrong et al. (4). The initial body phosphorus values were similar to that of Boutwell (18). The difference in initial body calcium and phosphorus of the representative group of rats may be due to the fact that the rats had different initial body weights and were not litter mates.

Calcium and phosphorus utilization

The values for percentage calcium utilization as determined by slaughter technique are presented in Table 12. There was a significant difference in the availability of calcium in calcium carbonate (the reference diet) and the other six foodstuffs used in Experiment I. The utilization of calcium was highest in calcium carbonate (74.4%) followed by cabbage (64.7%), chickpea (64.5%), lentil (49.0%), tahineh (40.7%), jew's mellow leaf (32.9%) and lowest in okra (28.7%). There was no significant difference between cabbage and chickpea and between jew's mellow leaf and okra in their availability of calcium. However, there was a significant difference in the availability of calcium in lentils and tahineh and when compared to foodstuffs tested in the experiment.

In this experiment, there was a calcium free diet, Table 3, fed to a group of seven rats. In the 3rd week of the experimental period four of the seven rats and in the 4th week five rats showed symptoms of rickets. After the experimental period of four weeks, the rats were analyzed for body calcium content and it was found that the rats lost an average of 37% of their initial body calcium. (Not shown in Table 12).

Results of determination of calcium availability in the four laubina mixtures are presented in Table 13. There was a significant difference in the utilization of calcium in calcium carbonate and in the four laubina mixtures. However, there was no significant difference among the four laubina mixtures tested in the experiment. Determination of the calcium availability in all the foodstuffs and mixtures tested in these experiments indicate that calcium in all the foodstuffs and mixtures was utilized to a lesser degree than calcium in calcium carbonate. These low values were not unexpected in view of the results obtained for calcium utilization in green vegetables. Bendana Brown and Lim (9), Alcarz (3) and Kelly (47) observed low values for calcium utilization from different vegetables.

Phosphorus utilization values for six different foodstuffs and potassium dihydrogen phosphate (KH_2PO_4, reference diet) are presented in Table 14. The utilization value was highest for KH_2PO_4 (58.0%) and tahineh (58.0%) followed by lentils (57.8%), chickpeas (57.7%), cabbage (44.4%), okra (42.0%) and lowest in jew's mellow leaf (41.7%). Statistical analysis of the results showed that there was no significant difference in the availability of phosphorus in KH_2PO_4, tahineh, lentil and chickpea. The utilization values of phosphorus in KH_2PO_4 and tahineh were the same. Moreover, there was no significant difference in the availability of phosphorus in cabbage, okra and jew's mellow leaf but there was significant difference in phosphorus utilization between these three and the other foodstuffs tested.

Effect of oxalate on the availability of calcium

Since it was observed that the foodstuffs and mixtures tested showed low calcium utilization values, it seemed pertinent to consider the factors that may influence the availability of calcium. The presence of oxalic acid has shown to have a depressive effect on the availability of the calcium and in certain cases, to the extent of rendering it totally insoluble.

Table 15 shows the relationship of oxalate intake to the availability of calcium. Statistical analysis of the results, however, showed that there is no significant correlation ($r = -0.37$). There was no significant correlation also between oxalate intake from the laubina mixtures and availability of calcium ($r = -0.31$, Table 16). These results, however, are not in agreement with that of Kelly (47), Fairbanks and Mitchell (32), Talapatra _et al_. (75) and Bendana-Brown and Lim (9). The absence of correlation between calcium availability and oxalate intake from the foodstuffs and mixtures appears to be due to the fact that the oxalate content of the diets were not sufficient enough to precipitate the dietary calcium. This result is in agreement with that of Armstrong _et al_. (5) and Bonner (15). Bonner who found that there was calcium equilibrium of human subjects when milk was replaced with spinach. Inspite of the high oxalate content the calcium equilibrium was not disturbed.

Effect of Phytate

Phytic acid, $[C_6H_6\ OPO(OH)_2]6$, a phosphorus containing compound may chemically combine with calcium and interfere in its absorption. Tables 17 and 18 show the phytate intake by rats from different foodstuffs and laubina mixtures and its effect on calcium availability. No correlation was found between phytate intake and calcium availability ($r = 0.11$ and $r = 0.12$). This result is in agreement with that of Nicolaysen and Njaa (65). They did not find any effect of phytate content of cereals on the absorption of calcium in man, pigs and rats. However, this result is not in agreement with the results obtained by Burton (22), Hoffjorgensen (41), Brooner <u>et al</u>. (20) and Schreier and Osthelder (68). This may be explained with the fact that in these experiments the phytate content of the diets, with the exception of tahineh diet, may not be sufficient enough to precipitate dietary calcium.

Table 19 shows that intake of phytate from the foodstuffs by the rats and its effect on the availability of phosphorus. Again there was no statistical correlation between phytate intake and phosphorus availability ($r = 0.12$). This result is not in agreement with that of Burton (22), McCause and Widdowson (57) and Krieger <u>et al</u>. (49) which may be due to the low phytate content of the foodstuffs. However, this result is in agreement with the result obtained by Blatherwick and Long (14). It can be concluded that the availability of calcium and phosphorus depends on the amounts of phytic acid, calcium or phosphorus content of diet.

<u>Effect of fiber</u>

Though it has generally been accepted that there is an inverse relationship between crude fiber content of foodstuffs and the digestibility of many of its nutrients, many workers have investigated the effect on calcium retention by increasing fiber content of diets and the results obtained have been conflicting. Table 20 and 21 show the total intake of fiber of the different foodstuffs and laubina mixtures. Statistical analysis showed that there is no correlation between fiber intake and availability of calcium. This result is in agreement with that of Bloom (15), Adolph et al. (2) and Duckworth and Godden (29). Bloom showed that there is no effect on calcium retention when 8% fiber was added to the diet, whereas Duckworth and Godden (29) found no effect on calcium retention upon adding upto 30% fiber to the diet.

However, statistical analysis showed a positive correlation between fiber intake and the phosphorus availability ($r = 1.0$, Table 23) which was not observed in case of calcium availability. From this result of the present experiment, it can be concluded that the fiber content of the foodstuffs affect only phosphorus and not calcium retention.

Effect of fat

The effect of dietary fat on the availability of calcium and phosphorus has also been investigated by many workers. The results of many studies are not in agreement with each other.

There was no correlation observed between the availability of calcium and intake of fat from the diets containing the foodstuffs and the laubina mixtures (Table 23, $r = 0.32$ and Table 24, $r = -0.56$). In each diet 10% fat was added except in the tahineh diet which is high in fat content. Small amounts of fat were contributed by the foodstuffs and food mixtures themselves. It can be noticed in Table 23, that the intake of fat in both jew's mellow leaf and okra was low. This low intake of fat may be a factor for the low availability of calcium in okra and jew's mellow leaf. From Table 25, it is seen that there is no correlation exists between total intake of fat and availability of phosphorus. Here also the intake of fat was low in the case of jew's mellow leaf, okra and cabbage and this may be a cause for low utilization of phosphorus in these foodstuffs.

Effect of ratio

It has been generally accepted that the maximum utilization of calcium and phosphorus is obtained when the ratio of calcium to phorphorus is 1:1 or 2:1.

In the present study on calcium and phosphorus availability, the ratio of calcium to phosphorus was maintained 1:1 except in two foodstuffs, namely, jew's mellow leaf and okra, because of the great difference in their calcium and phosphorus content (Table 8). The low availability of phosphorus in jew's mellow leaf and okra may be due to the high ratio of calcium to phosphorus, 1:4.3 and 1:4, respectively.

Effect of total intake of food and gain in weight of rats on the availability of calcium and phosphorus

Tables 26 and 27 show the total intake of food and gain in body weight of rats and relationship of both to the availability of calcium. Statistical analysis showed no correlation between the total intake of food and calcium availability or between gain in weight and availability of calcium ($r = 0.69$). Although there was no correlation between total intake of food and availability of calcium it can be noticed that in the case of jew's mellow leaf and okra, the intake is low; consequently the growth was slow and the availability low. Although there is no statistical correlation between gain in weight and calcium availability, the figures show that in most cases the rats with higher weights had higher calcium content. This was more pronounced in the study of laubina mixtures (Table 27).

No significant correlation again was found between total intake of food and phosphorus availability (Table 28, $r = 0.69$) whereas there was a significant correlation between gain in weight of rats and availability of phosphorus ($r = 0.80$).

Effect of oxalate content of okra on its calcium utilization

The result presented in Table 30, showed that there was no significant difference in calcium utilization between $CaCO_3$ and okra + $CaCO_3$. However, each of $CaCO_3$ and okra + $CaCO_3$ showed a significant difference from each of okra alone or $CaCO_3$ + oxalic acid. These results suggest that the oxalic acid in okra did not reduce significantly the retention of calcium in $CaCO_3$ whereas an equivalent amount of oxalic acid added separately reduce the retention of calcium in $CaCO_3$ to a significant level. Therefore, the low availability of calcium in okra does not seem to be due to its oxalate content.

These results are not in agreement with that of Speirs (72) who showed that calcium in spinach was very poorly utilized by rats, if at all and that the utilization of calcium in milk was diminished by the presence of a New Zealand spinach variety which has high oxalate content of 4.8 percent. Okra, on the other hand, has an oxalate content of only 0.37 percent. However, the results of

McLughein (61) and Bonner *et al*. (15) are in agreement with these results. They showed that when spinach was given to the diet for human subjects the normal calcium equilibrium was maintained.

SUMMARY AND CONCLUSIONS

Analyses for proximate composition and for calcium, phosphorus, oxalate and phytate content in six Middle Eastern foodstuffs and four LAUBINA food mixtures were done. The calcium availability in each of the foodstuffs and mixtures has been determined by a slaughter technique, using the rat as the experimental animal. Phosphorus availability has been studied in the six Middle Eastern foodstuffs only. In addition, the effect of okra on the availability of calcium has been determined, using the same procedure.

In all the availability studies, 20-23 days old, weanling albino rats were fed diets in which the dietary calcium or phosphorus was derived from the foodstuffs and food mixtures. The utilization values obtained were compared to those of similar diets in which all the calcium or phosphorus was derived from calcium carbonate or potassium dihydrogen phosphate.

In each case, the calcium in the six foodstuffs tested was found to be les utilized than the calcium in calcium carbonate. The calcium in cabbage and chickpeas had higher utilization values whereas calcium in lentil, tahineh and particularly jew's mellow leaf and okra was poorly utilized.

There was no significant difference in the utilization values of calcium in all the four food mixtures and was lower than calcium carbonate.

Three of the foodstuffs, namely tahineh, chickpeas and lentils had similar phosphorus utilization values (58.0, 57.8 and 57.7 percent respectively) and were similar to that of KH_2PO_4 which had an utilization value of 58.0 percent. In the other three foodstuffs, namely, cabbage, jew's mellow leaf and okra had relatively poor utilization value of phosphorus (44.4, 41.4 and 42.4 percent respectively).

No correlation was found between phytate intake of the foodstuffs and calcium availability between fat intake and calcium availability and between fiber intake and calcium availability. Nevertheless, there were some indications that an inverse relationship exists between oxalate intake of the foodstuffs and calcium availability, between total intake of food and calcium availability and between gain in weight of rats and availability of calcium.

The poor utilization of calcium in okra and jew's mellow leaf was probably due to the low intake of food and low gain in weight of rats during the experimental period.

There was no relationship observed between phytate intake, fat intake or food intake and phosphorus availability. However, there was a positive correlation between fiber intake and phosphorus availability and between gain in weight of rats and phosphorus availability.

The low availability of phosphorus in okra and jew's mellow leaf may be due to the low intake of food and low gain in weight of rats. The low utilization of phosphorus in cabbage may be due to its high intake of fiber.

The oxalate content of okra had no effect on the availability of its calcium because an equivalent amount of oxalic acid added separately reduced significantly the utilization of calcium in calcium carbonate, whereas the oxalate of okra showed no effect.

From these studies the following conclusions can be made:

1) Calcium in the six foodstuffs and four LAUBINA food mixtures was found to be utilized less than the calcium in calcium carbonate.

2) Calcium in cabbage, chickpea and four food mixtures is more available to the rat than calcium in tahineh, lentils, okra and jew's mellow leaf.

3) Phosphorus in tahineh, chickpea, and lentil is more available than phosphorus in the other foodstuffs tested.

4) Both okra and jew's mellow leaf are poor sources of calcium and phosphorus and are not adequate as the only source of calcium and phosphorus.

5) No correlation was found between fat, fiber, oxalate, phytate intake and calcium availability. Nevertheless, there was an indication of a relationship exists between total intake of food, gain in weight of rats, oxalate content of foodstuffs and calcium availability.

6) No correlation was also found between fat intake, fiber intake and phosphorus availability, but there was a positive correlation between fiber intake, gain in weight of rats and phosphorus availability.

7) The low utilization of calcium in okra was not due to its oxalate content.

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