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STUDIES ON THERAPEUTIC NUTRITION FOR IRON DEFICIENCY ANEMIA

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ABSTRACT

Iron deficiency anemia is a national problem most frequently occurring throughout the world. The objective of the present study was to evaluate the bioavailability of iron and protein from some biscuit samples provided to anemic rats and study the effect of these biscuit samples on the remedy of iron deficiency anemia. The biscuit samples were fortified by some natural sources rich in protein and iron. These samples were evaluated chemically, organoleptically, physically and biologically. Newly weaned male rats were divided into 9 groups of 5 animals each: 2 groups received standard diet supplemented with iron for 10 weeks; where the rest 7 groups received iron-free diet for 6 weeks until the hemoglobin levels dropped to 8-9 (g/dl) and then fed on the different biscuit samples for 4 weeks. It can be concluded from the biological results that the best samples that helped in the remedy of iron deficiency anemia and covered the daily requirements of protein, iron; parts of calcium, phosphorus and calories are samples No. (7), (6), (2) and (4), which contained: defatted soybean flour, roselle powder, chickpea flour and cinnamon powder, respectively.

INTRODUCTION

Adequate nutrition plays an important role in maintaining optimal health. Malnutrition increases health care costs by prolonging hospital length of stay due to the increased probability of medical complications which result from compromised immune function and impaired wound healing. Anemia is a condition in which there is deficiency in the size or number of erythrocytes or in the amount of hemoglobin they contain. Kent (1992) indicated that anemia, which affects approximately 30% of the world's population approximately half the children and women and 25% of the men in developing countries is Fe-deficient. Tolenen (1989) stated that, the last stage of iron deficiency occurs when stores are depleted and there is no longer sufficient iron to meet daily requirements which leads to significant compromise in cellular function in many organs. Freire (1997) mentioned that anemia in infants and children is associated with retardation in growth and cognitive development and with lower resistance to infection. Meanwhile, iron deficiency inhibits the ability of the body to regulate temperature when exposed to cold and alters hormonal production and metabolism, affecting neurotransmitters and thyroid hormones associated with neurological, muscular and temperature regulating functions. Fortification is generally considered the best long-term approach for combating iron deficiency. Wheat flour is the only food vehicle that has been extensively used for iron fortification at a national level (Darby, 1975). Recently, fortification of wheat flour and bakery products with iron provides about 20% of the Fe consumed in North America and nearly 40% of that in Sweeden. It is commonly assumed that fortification of wheat flour has contributed to the reduction in the prevalence of iron deficiency in countries where food iron fortification is mandatory (Hallberg *et al.*, 1979 and Cook *et al.*, 1986). In Egypt, biscuit is one of the major delicate and delicious foods. Its manufacturing in Egypt depends upon imported flour, whatever being soft or hard. In general, biscuits are easier to be produced than bread as they have much batter keeping quality. In fact biscuits are more popular and are cheaper products than many other prepared foods of equivalent caloric value and protein content. Biscuits represent an ideal means of supplementing the diet of any section of community suffering from deficiencies in vitamins or minerals. FAO/WHO/UNICEF (1969).

MATERIALS AND METHODS

Raw materials:

1- Wheat flour (72% extraction) was purchased from the " Eastern Delta Flour Mill Company ", Egypt.
2- Defatted soybean flour was obtained from Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.
3- Chickpea (*Cicer arietinum)* was obtained from Agricultural Research Center, Giza, Egypt. The grains were cleaned, washed, dried at 55°c for 12 hr, and then milled using an electric mill to pass through a 40 mesh sieve.
4- Green chicory (*Cichorium intybus)* was purchased from El-Mansoura local market. The raw green plants were cleaned, washed, dried at 75°c for 12 hr, milled using an electric mill, then sieved and packed in air tight kilner jars and kept until used.
5- Cinnamon park (*Cinnamomum zeylanicum)* was purchased from the local market of El-Mansoura, then milled using an electric mill, sieved and packed until used.
6- Cooked sugar cane syrup was obtained from local market at El-Mansoura.
7- Sun dried roselle leaves (*Hibiscus subdariffa*) were purchased from El-Mansoura local market, cleaned, milled using an electric mill, then sieved and packed until used.
8- Other materials used in the study such as yeast, sugar, fat (butter), eggs and salt were obtained from local market at El-Mansoura.
9- Animals [weanling albino rats (Sprague, Dawly strain)] of 23 days old were obtained from the Central Animal House of the National Research Centre.

Table (1): Wheat flour and its additives ratios.

Samples	The percentages of ingredients used						
	Wheat flour	Chicory powder	Roselle powder	Cinnamon powder	D. soybean flour	Cooked S. cane syrup	Chickpea flour
1	100	-	-	-	-	-	-
2	96	2.5	-	1.5	-	-	-
3	95	-	3	2	-	-	-
4	90	-	-	10	-	-	-
5	90	-	-	-	-	10	-
6	80	-	-	-	20	-	-
7	80	-	-	-	-	-	20

Preparation of biscuits:

The basic biscuit formula was prepared according to the following ratios: wheat flour, 100g; eggs, 25.3g; sugar, 50g; pure butter, 20.2g; salt, 0.5g and baking powder, 1.1g. Biscuits were baked in continuous band oven at 250°c for 10-15 min. Biscuit samples were packed in polyethylene bags after cooling.

Organoleptic evaluation of experimental biscuit samples:

Appearance, color, thickness, crispiness, shrinkage, taste and odor were evaluated by 10 panelists according to the method of Smith (1972).

$$\text{Ability value} = \frac{\text{overall acceptability of the sample}}{\text{overall acceptability of the control}}$$

Chemical analysis:

The moisture, protein, fat, fiber, ash, minerals (Fe-Ca-P) and tannic acid contents were determined as described in the methods of A.O.A.C. (1990).While total carbohydrates were calculated by the difference.

Determination of phytic acid.

Phytic acid was determined according to Mohamed *et al.*, (1986).

Feed Efficiency (FE).

Feed efficiency (FE) was calculated according to A.O.A.C (1990) using the following equation:-

$$FE = \frac{\text{Gain in body weight (g).}}{\text{Feed intake (g).}}$$

Protein Efficient Ratio (PER).

PER was calculated according to A.O.A.C (1990) using the following equation:-

$$PER = \frac{\text{Gain in body weight (g).}}{\text{Protein intake (g).}}$$

Digestibility coefficient (DC) of protein.

The digestibility coefficient (DC) was determined according to the method described by FAO/WHO (1989). It was calculated as follow:-

$$DC = \frac{I - (F - Fk)}{I} \times 100$$

Where:-

I = The nitrogen intake during the experimental period (g).

F = The fecal nitrogen value of the animal fed on the protein diet (g).

Fk = The fecal nitrogen value of the animal fed on the protein free diet (g).

Net Protein Utilization (NPU).

The NPU was determined according to the method described by Miller and Bender (1955) using the following equation:-

$$NPU = \frac{Bn - Bf}{N} \times 100$$

Where:-
Bn = Body nitrogen of the group fed on the tasted protein (g).
Bf = Body nitrogen of the group fed on free protein diet (g).
N = Nitrogen intake (g).

Biological Value (BV).

The BV was estimated indirectly using the relationship between NPU, DC and BV (FAO/WHO, 1989) using the following equation:

$$V = \frac{NPU}{DC} \times 100$$

Statistical analysis:

Data were analyzed according to SAS program SAS, (1990). The application of the least of means significance tested for the differences among the different treatments were done according to Duncan (1955).

RESULTS AND DISCUSSION

The chemical components of raw materials.

The results in Table (2) show that highest content was in cooked sugar cane syrup (23.09%) which approximately agrees with Pennington *et al.*, (1985) and USDA (2002). Meanwhile, the lowest content of moisture was in defatted soybean flour (6.55%) and this agree with Khalil (1998).

It could be noticed from the same results that the highest protein content was in defatted soybean flour (48.9%) followed by chickpea flour (26.03%), while the lowest value was in cooked sugar cane syrup (3.57%). These results are in agreement with those found by Salam *et al.*, (1992), Mansour (1996), Youssof (1999), Hussein (2000), Makhlouf (1991) and Romia (1998).

The crude fat content of raw materials was higher in chickpea flour (6.5%) and can not be detected in cooked sugar cane syrup. These results agree with those of Faheid and Hegazi (1991), Abo-zeid (1998) and Romia (1998).

The same results in Table (2) show that cinnamon powder had the highest fiber value (23.01%) followed by roselle powder (10.57%). While cooked sugar cane syrup did not contain any fibers, this result agrees with Makhlouf (1991) and USDA (2002).

Chicory powder had the highest content of ash (16.50%) followed by roselle powder (10.57%), while the lowest value of ash was in wheat flour (72% extraction). These results agree with those obtained by USDA (1998), Pennington *et al.*, (1985) and Khalil *et al.*, (2002a).

Table (2): Chemical components of raw materials (on dry weight basis).

Raw materials	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	*Carbo-hydrates (%)	Phytic acid (g /100g)	Tannic acid (mg	Minerals (mg/100g)		
									Fe	Ca	P
1	12.50	12.68	1.15	0.22	0.59	85.36	0.075	0.10	1.56	34.00	82.60
2	10.95	26.03	6.50	3.46	3.63	60.38	0.487	0.09	8.56	135.0	360.0
3	11.92	10.59	2.80	11.92	16.50	58.19	0.248	0.83	40.1	1248.0	453.8
4	6.76	5.55	5.30	23.01	3.60	62.54	0.265	1.99	42.01	1413.1	68.10
5	23.09	3.57	-	-	6.20	90.23	-	0.26	15.79	400.0	248.7
6	12.97	9.59	5.00	9.22	10.57	69.62	0.221	1.23	51.7	1536.0	278.2
7	6.55	48.90	6.00	4.20	6.76	34.14	0.662	0.59	47.08	577.7	670.0

* Carbohydrates were calculated by the difference.
(1) Wheat flour (72%). (2) Chickpea flour. (3) Chicory powder. (4) Cinnamon powder.
(5) Cooked sugar cane syrup. (6) Roselle powder. (7) Defatted soybean flour.

The results in Table (2) show that wheat flour (72% extraction) contained the highest value of carbohydrates (85.36%). These results are in the accordance to the results of Hussein (2001) who found that the percentage of carbohydrates in wheat flour (72% extraction) was 85.63%.

Concerning to Phytic acid values in Table (2), it could be noticed that defatted soybean flour contained the highest value (0.662g /100g), followed by chickpea flour (0.487g /100g), then cinnamon powder (0.265g /100g) and wheat flour (72% extraction) (0.075g /100g). While cooked sugar cane syrup did not contain any traces of phytic acid. These results are approximately agreed with Mahmoud (1996) and Hussein (2001).

Tannic acid contents were in the range between "0.096 to 1.99" (mg /100g). Cinnamon powder contained (1.99 mg /100g) followed by roselle powder (1.23 mg /100g).

Iron content of raw materials used in this study was higher in roselle powder (51.70 mg /100g) and defatted soybean flour (47.08 mg /100g) followed by cinnamon powder (42.01 mg /100g) and chicory powder (40.10 mg /100g), whereas the lowest content was in wheat flour (72% extraction) (1.56 mg /100g). These results approximately agree with Khalil *et al.*, (2002a); Pennington *et al.*, (1985); Iskander and Morad (1986); Khalil *et al.*, (2002c).

The highest content of calcium was in roselle powder (1536 mg /100g), cinnamon powder (1413.10 mg /100g). On the other hand, wheat flour (72% extraction) contained the lowest value (34 mg /100g). These results agree with those found by USDA (2002); Pennington *et al.*, (1985); USDA (1998); Khalil *et al.*, (2002a) and Khalil *et al.*, (2002c).

From the same table, it can be noticed that defatted soybean flour contained the highest content of phosphorus (670 mg /100g) followed by chicory powder (453.80 mg /100g) but cinnamon powder was the lowest material in phosphorus (68.10 mg /100g). This is approximately agreed with Abo-zeid (1998) and USDA (2002).

Organoleptic evaluation of experimental biscuit samples.

The average sensory panel scores of appearance, color, thickness, crispiness, shrinkage, taste, odor, overall acceptability and ability value for biscuit samples made from wheat flour and its mixtures with defatted soybean flour, chickpea flour, chicory powder, cinnamon powder, cooked sugar cane syrup and roselle powder are tabulated in Table (3).

The data show that sample No. (1) which contain 100% wheat flour recorded the best overall acceptability (90.53) and ability value (100%) followed by samples No. (5) [90% wheat flour + 10% cooked sugar cane syrup] and (4) [90% wheat flour + 10% cinnamon powder] which had (86.75 and 86.30, respectively) overall acceptability and (95.82% and 95.32%, respectively) ability value. The overall acceptability values for the rest samples varied between 79.43 to 85.17, while the ability value varied between 87.73 to 94.07 (%). These variations between biscuit samples revealed to its raw materials.

Table (3): Organoleptic evaluation of experimental biscuit samples.

Samples	Appearance (10)	Color (15)	Thickness (15)	Crispiness (15)	Shrinkage (15)	Taste (15)	Odor (15)	Overall acceptability (100)	Ability value (%)
1	9.15	14.00	13.52	13.60	13.25	13.46	13.55	90.53	100
2	8.14	12.85	12.87	12.38	13.00	12.10	13.30	84.64	93.49
3	7.80	10.50	12.88	11.26	12.75	11.14	13.10	79.43	87.73
4	8.79	12.80	12.90	11.90	12.83	13.58	13.50	86.30	95.32
5	9.14	13.35	13.21	12.60	12.75	12.20	13.50	86.75	95.82
6	8.42	10.70	12.70	12.14	12.50	11.80	13.40	81.66	90.20
7	8.71	12.42	12.64	12.50	13.50	12.15	13.25	85.17	94.07

Chemical components of experimental biscuit samples (on dry weight basis).

The results in Table (4) indicated that the moisture content of biscuits varied between 4.02% to 5.70%. The addition of 10% cooked sugar cane syrup to 90% wheat flour (72% extraction) in sample No. (5) raised the final moisture contents which was 5.70%. The addition of chickpea flour, chicory powder, cinnamon powder, roselle powder and defatted soybean flour to wheat flour in biscuit caused a decrement of biscuits moisture. These results are in the agreement with those of Khalil *et al.*, (2002b) and Mahmoud (1999).

Samples No. (7) [80% wheat flour + 20% defatted soybean flour] and (2) [80% wheat flour + 20% chickpea flour] had the highest protein content which were 17.65% and 14.80%, respectively; while other samples had protein content between 12.76% to 13.28%. These results agree with results of Lorimer *et al.*, (1991) who found that the replacement of wheat flour with legumes flours increased the protein content of products baked from the blended flours.

Table (4): Chemical components of biscuit samples (on dry weight basis).

SAMPLES	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	*Carbo-hydrates (%)	Phytic acid (g /100g)	Tannic acid (mg /100g)	Minerals (mg/100g)		
									Fe	Ca	P
1	4.75	13.28	14.74	0.26	1.14	70.58	0.056	0.060	2.83	54.41	189.02
2	4.15	14.80	15.58	0.66	1.37	67.61	0.170	0.053	3.77	66.79	223.82
3	4.02	13.15	14.90	0.75	1.51	69.69	0.088	0.183	3.97	92.35	194.20
4	4.14	13.14	14.98	1.58	1.62	68.68	0.094	0.266	5.30	138.18	180.94
5	5.70	12.76	13.67	0.23	1.47	71.87	0.045	0.089	3.64	75.73	198.63
6	4.04	13.19	14.85	0.67	1.41	69.88	0.061	0.234	4.17	97.71	192.11
7	4.55	17.65	15.02	0.69	1.96	64.68	0.257	0.122	8.17	117.95	258.27

* Carbohydrates were calculated by the difference.

Addition of chickpea flour or defatted soybean flour to wheat flour (72% extraction) in biscuit's formula increased its fat content.

The results also show that addition of cooked sugar cane syrup to wheat flour (72% extraction) increased the final carbohydrate content of biscuit, while the addition of chickpea flour, chicory powder, cinnamon powder, roselle powder, or defatted soybean flours to wheat flour (72% extraction) decreased the final carbohydrate content of biscuits.

The variation of ash content of samples depends on the kind and the ratio of additive material added to wheat flour, but all samples were higher in its ash content than the control sample [No. (1) {100%wheat flour}] especially sample No. (7) which had the highest value (1.96%).

It could be noticed from the same data in Table (4) that all additives added to wheat flour (72% extraction) raised the fiber contents of biscuits except cooked sugar cane syrup which caused the biscuit's fiber to be lower than the control sample. Sample No. (4) [90% wheat flour + 10% cinnamon powder] contained the highest value of fiber content due to the high ratio of fiber in cinnamon powder. All the previous obtained results agree with those obtained by Mahmoud (1999), Romia (1998), Khalil *et al.*, (2002b) and (2002c).

Concerning phytic acid content of biscuits, it was found that sample No. (7) scored the highest content followed by sample No. (2). That is due to the high content of phytic acid in defatted soybean flour and chickpea flour. These results are in the accordance of those of Lolas and Markakis (1975).

The data of Table (4) show that tannic acid contents were between 0.053 to 0.266 (mg /100g). The lowest value was for sample No. (2). On the other hand, sample No. (4) [90% wheat flour + 10% cinnamon powder] had the highest value followed by sample No. (6) [95% wheat flour + 3% roselle powder + 2% cinnamon powder] which were: 0.266 and 0.234 (mg /100g), respectively.

Regarding to minerals content in the same Table (4), sample No. (7) recorded the highest content of iron (8.17 mg /100g) followed by sample No. (4) and No. (6) which were 5.30 and 4.17 (mg / 100g), respectively. These data agree with those found by Romia (1998) and Khalil *et al.*, (2002b) who reported that fortification of wheat flour by defatted soybean flour, chickpea flour, cinnamon powder or cooked sugar cane syrup increased the total iron content of baked product.

Calcium contents of samples ranged from 54.41 (mg /100g) in sample No. (1) [100% wheat flour] to 138.18 (mg /100g) in sample No. (4) [90% wheat flour + 10% cinnamon powder].

Sample No. (7) [80% wheat flour + 20% defatted soybean flour] also had the highest value of phosphorus content (258.27 mg /100g). Generally, all fortified samples were higher than the control sample [No. (1)] in phosphorus content except sample No. (4) [90% wheat flour + 10% cinnamon powder] which had 180.94 (mg /100g).

Biological evaluation of experimental biscuit samples.

It can be noticed from the same Table (5) that sample No. (7) [80% wheat flour + 20% defatted soybean flour] had significantly the highest score of FE (Feed Efficiency) followed by casein diet and sample No. (2) [80% wheat flour + 20% defatted soybean flour] which were: 0.298, 0.270 and 0.254, respectively. On the other hand, the lowest FE value was for sample No. (1) [100% wheat flour] being 0.121, and there were no significant differences between samples No. (1), (4), (6) and (7) in FE values.

The obtained results of the same table show that biscuit fortified by defatted soybean flour had the high value of FE; this may be due to the high quality of soybean protein. These results approximately agree with those obtained by Saleh (1989), Mohamed (1998) and Hussein (2001).

The data in Table (5) show that basal diet (casein) gave significantly the highest value of PER which was (2.41). This is agreeing with Tsen (1980) and Hussein (2001) who reported that PER of casein was (2.45). The second value of PER was for samples No. (7) [80% wheat flour + 20% defatted soybean flour] and (2) [80% wheat flour + 20% chickpea flour] which scored PER of (1.99) and (1.794), respectively with no significant difference. That may be due to the high body weight of these samples rats. While sample No. (1) [100% wheat flour] had the lowest value of PER being 0.962 and this may be due to its low gain of body weight. In addition, there were no significant differences in PER values between samples No. (1), (3) and (6). These results are in accordance to those mentioned by El-Mahdy (1980), Magbool *et al.*, (1987), Abo-zeid (1998) and Hussein (2000).

Also, the results in the same table show that the DC values of samples No. (1) [100% wheat flour] and (5) [90% wheat flour + 10% cooked sugar syrup] were the lowest values with no significant difference, being 81.25% and 81.15% respectively. On the other hand, casein diet and samples No. (7)

and (2) were significantly higher than all experimental samples in the DC values, where they had 91.33%, 90.40% and 89.31%, respectively.

Table (5): Values of FE, PER, NPU, DC and BV for different biscuit samples.

Samples	FE	PER	DC (%)	NPU (%)	BV (%)
Casein	0.270 ab	2.41 a	91.33 a	77.88 a	85.27 a
1	0.121 d	0.962 e	81.25 c	56.89 e	70.02 d
2	0.254 b	1.794 b	89.31 a	70.36 b	78.78 b
3	0.127 d	1.012 e	84.91 b	59.90 cde	70.54 cd
4	0.177 c	1.44 c	84.70 b	63.55 c	75.02 bc
5	0.142 cd	1.180 d	81.15 c	58.66 de	72.28 cd
6	0.137 cd	1.084 de	82.75 bc	59.01 de	71.31 cd
7	0.298 a	1.99 b	90.40 a	74.26 a	82.14 ab
± SE	± 0.0142	± 0.073	± 1.013	± 1.274	± 2.015
$LSD_{0.05}$	0.0414	0.2128	2.9569	3.7179	5.8814
Sig.	**	**	*	**	*

The high score of DC for sample No. (7) [80% wheat flour + 20% defatted soybean flour] may be due to its high content of protein, this is agreed with those obtained by Zarkadas *et al.*, (1997) who found that soybean flour contained an excellent balance of essential amino acids. Also, the high DC value of Sample No. (2) which contain 20% chickpea flour may be due to that chickpea is a good source of protein with high content of lysine as reported by Bowes and Church (1983). While samples No. (3), (4) and (6), with no significant differences between them, had 84.91, 84.70 and 82.75 (%) of DC, respectively. These obtained results are in accordance of El-mahdy (1980) who reported that the digestibility coefficient (DC) of biscuits prepared from some cereals and legumes ranged from 85% to 97%. Also, these results are in agreement with those of Abo-Zeid (1998) and Hussein (2001).

The data in Table (5) indicate that the NPU of casein diet and sample No. (7) [80% wheat flour + 20% defatted soybean flour] were significantly higher than all experimental samples; since they had 77.88% and 74.26%, respectively. This result is accordant to results of Youssef (1999) and Hussein (2001) who found that NPU of casein diet were 74.3% and 78.04%, respectively.

Concerning to the experimental samples, it was clear that sample No. (2) [80% wheat flour + 20% chickpea flour] followed sample No. (7) in the NPU value (70.36%). These high values may be returned to the high quantity of soybean or chickpea which contain high level of protein. While sample No. (1) scored the lowest NPU value being 56.89%, and there were no significant differences between sample (1) and samples No. (3), (5) and (6). These results in agreement with results of Gruener *et al.*, (1986) who used soybean flour as partial substitution of ..heat bread and they found that NPU of the substituted bread was significantly higher than the control. Hallab *et al.*,

(1974) who found that, Arabic bread fortified by chickpea flour had NPU value higher than that of the control (un fortified). Also these results were supported by Magbool *et al*., (1987), Hussein (2001) and khalil *et al*., (2002b).

Regarding to the results of the biological values (BV) presented in Table (5), it could be noticed that the BV of casein diet was 85.27% which was significantly higher than those of the experimental samples, but it had no significant difference with sample No. (7) in the BV values. This result in accordance to those of Hassan (1976) who found that the BV of casein was 85%. Abo-zeid (1998) and Hussein (2001) found that the BV of casein was 87% and 86%, respectively.

Also, the results in the same Table (5) show that the biscuit sample with 20% defatted soybean flour (No. 7) had high BV value of (82.14%) followed by biscuit sample No. (2), with 20% chickpea flour, that had 78.79% (BV). This may be due to the high quality protein of these two samples that scored highest values of DC and NPU. On the other hand, samples No. (1), (3), (5) and (6) had no significant differences; since they recorded the lowest BV values being 70.02%, 70.54%, 72.28% and 71.31%, respectively. All previous obtained results agree with those of Faheid and Hegazi (1991), Abo-Zeid (1998), Hussein (2000) and Khalil *et al*., (2002b).

Hematological measurements of rats' blood after depletion and after repletion of iron periods.

Results in Table (6) show that all rats except basal diet (casein) group were anemic at zero time with no significant differences between values of Hb, Hct, RBCs and serum iron in all groups under investigations.

The results in the same table show that group fed on sample No. (7) [80% wheat flour + 20% defatted soybean flour] scored the highest hemoglobin level (14.35 g/dl) compared with the rest samples except the basal diet group. Such result may be due to its high ratio of iron and high quality protein with high values of DC, PER and BV. These obtained results are in accordance with those found by Khalil *et al*., (2002a).

The data of the same table show that there were no significant differences in both of Hb and Hb gain between samples No. (4), (6) and (7) in spite of the high content of iron in sample (7). Such result may be due to the high content of phosphorus and phytate in sample (7) which could reduce iron absorption as reported by Turnbull *et al*., (1962) and Garrow *et al*., (1993).

Rats fed on sample No. (7) [80% wheat flour + 20% defatted soybean flour] had the highest Hct value (57.33%) as compared with basal diet group (62.15%). This is may be due to the high level of hemoglobin in the blood of that group.

For the red blood cells count (RBCs) in the same table, it was clear that there were no significant differences between the basal diet (casein) and sample No. (6) [95% wheat flour + 3% roselle powder + 2% cinnamon powder] which had highest value of RBCs compared with the rest samples.

Table (6):Measurements of rats blood after depletion and after repletion of iron.

Measurements		SAMPLES								± SE	LSD 0.05	Sig.
		casein diet	(1)	(2)	(3)	(4)	(5)	(6)	(7)			
After depletion	Hb (g / dl)	14.92 ^{a}	8.28 ^{b}	8.26 ^{b}	8.23 ^{b}	8.27 ^{b}	8.28 ^{b}	8.20 ^{b}	8.25 ^{b}	± 0 096	0 2822	*
	Hct (%)	45.20 ^{a}	25.40 ^{b}	25.26 ^{b}	25.30 ^{b}	25.46 ^{b}	25.41 ^{b}	25.45 ^{b}	25.80 ^{b}	± 0 246	0.719	*
	RBCs(millions/ ml^{3})	6.314 ^{a}	4.514 ^{b}	4.515 ^{b}	4.510 ^{b}	4.499 ^{b}	4.502 ^{b}	4.513 ^{b}	4.515 ^{b}	± 0 015	0.0447	*
	Serum iron(µg/dl)	185.40 ^{a}	98.20 ^{b}	97.80 ^{b}	99.45 ^{b}	98.15 ^{b}	98.70 ^{b}	100.85 ^{b}	98.87 ^{b}	± 1 187	3.4663	*
After repletion	Hb (g / dl)	16.85 ^{a}	11.43 ^{e}	12.82 ^{cde}	12.57 ^{cde}	13.56 ^{bc}	12.02 ^{de}	13.43 ^{bcd}	14.35 ^{b}	± 0.477	1 3925	**
	Hb gain (g / dl)	1.93 ^{d}	3.15 ^{cd}	4.56 ^{bc}	4.34 ^{bc}	5.29 ^{ab}	3.74 ^{c}	5.23 ^{ab}	6 10 ^{a}	± 0.493	1 4414	**
	Hct. (%)	62.15 ^{a}	37.30 ^{d}	40.86 ^{cd}	42.28 ^{c}	51.44 ^{b}	42.31 ^{c}	53.89 ^{b}	57.33 ^{ab}	± 2.089	6 1289	**
	Hct gain (%)	16.95 ^{cd}	11.90 ^{d}	15.60 ^{cd}	16.98 ^{cd}	25.98 ^{ab}	16 90 ^{cd}	28.44 ^{a}	31 53 ^{a}	± 2.076	6 0615	**
	RBCs (millions/ml^{3})	8.475 ^{a}	5.000 ^{cd}	5.884 ^{c}	5.810 ^{c}	5.467 ^{c}	6.480 ^{bc}	7.447 ^{ab}	7.352 ^{ab}	± 0.385	1 1249	*
	RBCs gain (millions)	2.161 ^{ab}	0.486 ^{c}	1.369 ^{bc}	1 300 ^{bc}	0.968 ^{bc}	1.978 ^{abc}	2.934 ^{a}	2.837 ^{a}	± 0.385	1 1247	*
	Serum iron (µg /dl)	198.50 ^{d}	163.15 ^{e}	189.64 ^{d}	260.22 ^{b}	193.06 ^{d}	225.07 ^{c}	316.12 ^{a}	196.97 ^{d}	± 7 298	21 303	**
	Serum iron gain µg/dl	13.10 ^{f}	64.95 ^{e}	91.84 ^{d}	160.77 ^{b}	94.91 ^{d}	126.37 ^{c}	215.27 ^{a}	98.10 ^{d}	± 7 388	21 567	**

Hb : hemoglobin (normal value = 12 – 15 g /dl). Hct : hematocrit (normal value=40–45%).
RBCs : red blood cells count (normal = 6.30 – 8.23 millions/ml^{3}).
Normal serum iron = (185 – 200 µg /dl).
a,b,c: Means within the same row with different superscripts are significant.
LSD: Least Significant Difference. * : Significant at ($P<0.05$). ** : Significant at ($P<0.01$).
***** : Significant at ($P<0.001$).**

In addition, there were no significant differences between samples No. (6), (7) and (5) in value of RBCs and RBCs gain being (7.447, 7.352 and 6.480 millions / ml3) and (2.934, 2.837 and 1.978 millions / ml3), respectively; since they had the highest values compared with the rest samples.

Also, the same data in Table (6) indicate that group of rats fed on sample No. (6) [95% wheat flour + 3% roselle powder + 2% cinnamon powder] had significantly the highest values of serum iron and serum iron gain than all groups even the basal diet (casein) group, where it were 316.12 and 215.27(µg/dl) respectively followed by group of sample (3) [96% wheat flour + 2.5% chicory powder + 1.5% cinnamon powder] which scored 260.22 and 160.77(µg/dl), respectively.

Generally, it can be concluded that the best samples recorded high and best values of blood measurements after four weeks of repletion assay were samples No. (7) [80% wheat flour + 20% defatted soybean flour], (6) [95% wheat flour + 3% roselle powder + 2% cinnamon powder] and (4) [90% wheat flour + 10% cinnamon powder], since they had no significant differences between them. On the other hand, rats fed on the un-fortified sample [100% wheat flour] show the lowest gain of Hb, Hct, RBCs and serum iron. These obtained results approximately agree with Khalil *et al.*, (2002a).

Covering the daily requirements of protein, iron, calcium, phosphorus and calories according to recommendations of FAO/WHO (1988).

From tables (4) and (7), it was found that best samples covering the daily requirements of protein, iron, calcium, phosphorus and calories for different age periods can be arranged descendently as follow:

(1)- Sample No. (7) [80% wheat flour + 20% defatted soybean flour] considered the best sample covering the daily requirements under investigation, since consuming 130g from this sample covered the daily requirements of protein and 127% of iron, while the same amount covered 29% of calcium, 64% of phosphorus and 42% of calories daily requirements for 1-3 years old children. In addition, the biological value (BV) of this sample was 79.85%, while ability value was 94.07. Concerning to the other different age periods, it was found that the amount covering the daily requirements of the mentioned compounds ranged between 160 to 385 g.

(2)- Sample No. (2) [80% wheat flour + 20% chickpea flour] was the second sample that had 78.79 % BV (biological value) and 93.49 (ability value). It was found that 220 g from this sample covered 42.5% more than the daily requirements of protein; while covered 100% of iron requirements, 29% of calcium, 99% of phosphorus and 73% of calories daily requirements for 1-3 years old children. For the rest age periods, it was concluded that quantity from 250 to 460 g can cover the daily requirements of the mentioned compounds.

(3)- It was found that sample No. (6) [95% wheat flour + 3% roselle powder + 2% cinnamon powder] with 71.31% (BV) and 90.20 (ability value) was the third of best samples, from which 200 g covered 15% more than the daily requirements of protein; while covered 100% of iron requirements, 37% of calcium, 73.5% of phosphorus and 65% of calories daily requirements for 1-3 years old children. In addition, 225 to 513 g from the same sample covered the daily requirements of the mentioned compounds for the rest different age periods.

(4)- The fourth sample was No. (4) [90% wheat flour + 10% cinnamon powder] that gave 75.06% and 95.32 for the BV and ability value, respectively. Consuming 175 g from this sample covered the daily requirements of protein, 110% of iron, 46% of calcium, 59.5% of phosphorus and 56.5% of calories for 1- 3 years old children. While 215 to 516 (g) from this sample covered the daily requirements for the rest age periods from the mentioned components.

Taking into consideration that these samples were the only sources of these components.

Furthermore, all these samples were economically suitable for most families.

Table (7): The daily requirements of protein, iron, calcium, phosphorus and calories for different age periods as recommended by FAO/WHO (1988).

Age periods	Protein (g/day)	Iron (mg/day)	Calcium (mg/day)	Phospho -rus (mg/day)	Calories (mg/day)
1-3 years old children.	22	8	500	500	1400
4-8 years old children.	27	9	500	500	1800
7-9 years old children.	33	10	700	700	2200
10-12 years old male.	41	11	900	900	2500
10-12 years old female.	40	11	1000	1000	2300
13-15 years old male.	52	13	1200	1200	2800
13-15 years old female.	43	14	800	800	2200
16-18 years old male.	54	14	1000	1000	3200
16-18 years old female.	43	14	700	700	2100
19-60 years old male.	56	10	800	800	2800
19-60 years old female.	41	14	700	700	2200
Pregnant woman.	61	15	1200	1200	2500
Lactating woman.	65	15	1200	1200	2700

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دراسات على التغذية العلاجية لأنيميا نقص الحديد

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أنيميا نقص الحديد هى مشكلة قومية منتشرة فى أرجاء العالم، والهدف من هــذا البحث هو تقييم الإتاحة الحيوية للبروتين والحديد فى بعض عينات البســكويت التــى تــم إعطائها للفئران المصابة بالأنيميا ودراسة تأثير هذه العينات فى علاج أنيميا نقص الحديد. وهذه العينات مدعمة بمصادر طبيعية غنية فى البروتين والحديد. وقد تم إجــراء تقييمــاً كيميائياً وحسياً وطبيعياً لهذه العينات بالإضافة للتقييم البيولوجى. تم تقسيم فئران التجــارب إلى تسعة مجاميع رئيسية تحتوى كل مجموعة على خمسة فئران: مجموعتين تتناول وجبة قياسية مدعمة بالحديد لمده عشرة أسابيع فى حين أن السبعة مجاميع الباقية تتناول وجبــة خالية تماماً من عنصر الحديد لمدة ستة أسابيع حتى إنخفض مستوى الهيموجلوبين إلى ٨-٩ (جم/ ديسيلتر) ثم تم تغذيتها على عينات البسكويت المختلفة لمدة أربعة أســابيع. ومــن النتائج البيولوجية يمكن استنتاج أن أفضل العينات التى ساعدت فى علاج أنيميــا نقــص الحديد وغطت الإحتياجات اليومية من البروتين والحديد بالإضافة لأجزاء مــن الكالســيوم والفوسفور والسعرات الحرارية هى العينات رقم (٧) ، (٦) ، (٢) ، (٤) التى تحتوى على دقيق فول الصويا ومسحوق الكركديه ودقيق الحمص ومسحوق القرفه على التوالى.