Interaction effect of iron and defatted soybean (*Glycin max* L.) supplementation to wheat (*Triticum aestivum* G.) flour on hemoglobin regeneration efficiency of growing male rats

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الخلاصة

تم استخدام وخلط طحين الحنطة استخلاص85% من الحبوب مع طحين فول الصويا منزوع الدهن بنسبة 50غم/كغم أو بدونه والمدعم بنسبة 50 أو 100ملغم حديد/كغم غذاء في دراسة لكفاءة تخليق الهيموكلوبين في الجرذان النامية، بحيث غذيت على أغذية تجريبية(طحين حنطة كعينة سيطرة، وطحين حنطة+50ملغم حديد، وطحين حنطة+100ملغم حديد، وطحين حنطة+50غم فول الصويا منزوع الدهن فقط، وطحين حنطة+50غم فول الصويا منزوع الدهن+50ملغم حديد، وطحين حنطة +50غم فول الصويا منزوع الدهن+100ملغم حديد). استخدم60من ذكور الجرذان البيضاء حديثة الفطام موديلا" بايولوجيا" للتجربة، قسمت إلى مجموعتين رئيسيتين متساويتين، هما مجموعة جرذان السيطرة السليمة ومجموعة الجرذان المصابة بفقر دم نقص الحديد أستحدث بالنزف بأزالة 20-25قطرة دم من وريد منظمة العين الداخلية وذلك باستخدام أنبوبة شعرية محتوية على الهيبارين، قسمت كل من المجموعتين إلى ستة مجموعات بالتساوي وفقا" لوزن الجسم ومستوى الهيموكلوبين، غذيت لـ10 أيام مدة التجربة.

تم تقدير وحساب وزن الغذاء ووزن البروتين المتناولين والبروتين المهضوم ودليل قابلية هضم البروتين. كما قدر امتصاص الحديد الغذائي والهضم الظاهري والحالة التغذوية ومستوى الهيموكلوبين وزيادته وكمية حديد الهيموكلوبين وزيادته للجرذان المصابة بفقر الدم والسليمة، هذا فضلا" عن حساب كفاءة تخليق الهيموكلوبين ودليل نمو الجرذان، كما حسبت كمية الطاقة المتأيضة البديلة لكل غذاء.

بينت نتائج التحليل الإحصائي إن دليل هضم البروتين للجرذان السليمة هو أعلى مما هو عليه في الجرذان التي تعاني من فقر دم نقص الحديد وأن امتصاص الحديد الغذائي الظاهري يتناسب طرديا"مع كمية الحديد المدعمة للغذاء، كما أن طحين فول الصويا منزوع الدهن أدى إلى تحسين امتصاص الحديد الغذائي في الجرذان السليمة والتي تعاني من نقص الحديد، وأن

امتصاص الحديد الغذائي في الجرذان التي تعاني من فقر الدم هو أعلى مما هـو عليـه فـي الجرذان السليمة، كما أن الزيادة في مستوى هيموكلوبين الدم وحديده تزداد بزيـادة مـستوى الحديد الغذائي في كل من الجرذان السليمة والتي تعاني من فقـر الـدم، وأن كفـاءة تخليـق الهيموكلوبين في الجرذان التي تعاني من فقر الدم هي أعلى مما هـي عليـه فـي الجـرذان السليمة، وأن غذاء طحين الحنطة (استخلاص 85%) المدعم بـ50غم فول الصويا منـزوع الدهن و50ملغم حديد/كغم غذاء وجد بأنه يؤدي إلى تحسين هضم البروتين وتحسين امتصاص الحديد الغذائي وزيادة كفاءة تخليق الهيموكلوبين في الجرذان النامية.

ABSTRACT

Wheat flour (WF) of 85% meal extraction was blended with or without defatted soybean flour (DSF) meal and enriched with 50 or 100mg iron/kg diet. Experimental diets (WF control, WF+50mg iron, WF+100mg iron, WF+50g DSF, WF+50g DSF+50mg iron and WF+50g DSF+100mg iron) were prepared and used for protein digestibility and hemoglobin regeneration efficiency studies. Sixty male weanling albino rats were used as a biological and experimental model for this study, rats were divided into two equal major groups. The first group (healthy rats) divided into six subgroups according to the hemoglobin (Hb) value fed experimental diets separately for 10 days period. The second group was iron depleted by removing 20-25 drops of blood from ocular vein by using capillary tube and considered to be anemic rats, divided and fed diets as a above. Analysis of variance showed that, the protein digestibility index of the healthy rats was higher than that index of the anemic rats. Apparent iron absorption was positively proportional to the dietary iron enrichment. DSF protein was improved the dietary iron absorption of the rats. The dietary iron absorption of the anemic rats was higher than that absorption of the healthy rats. Hb value, Hb iron gain and HRE were increased with increase dietary iron level (18-121mg/kg diet) in both trails of rats. HRE% of the anemic rats was higher than that ratio of the healthy rats. WF+50gDSF+50gm iron diet was found to improve protein digestibility, dietary iron absorption and HRE in rats.

Introduction

Iron-deficiency causes the nutritional anemia, which is the major healthy problem in the affluent and developing countries, This problem is associated with inadequate or low intakes of iron and/or poor bioavailability of the iron from foods(1,2).

Some of the iron present in the diet may be in a chemical form that is poorly or not at all absorbable. The non-heme, due to the presence of dietary iron inhibitors is not readily absorbable from the intestine, However various enhancers affect its absorption(3). Diets in developing countries usually contain more non-heme iron, Which is mostly of plant sources, therefore considered to be the main source of dietary iron(4). In plant, iron is present in whole cereal and legume grains, but its availability is affected by several inhibitors such as fibers, phytate, tannins, and polyphenols,(5). Soybeans are high in phytic acid, (substance that can block the uptake of essential minerals especially calcium, magnesium, copper, iron and zinc in the intestinal tract). Phytate are found in soybeans, which interfere with zinc and other divalent cations absorption more completely than other minerals, there is absolutely no reliable evidence that vegetarians who consume soy foods for long period was severed from risk of mineral deficiencies therefore, vegetarian diets must be much higher in vitamin c, which is greatly enhances iron absorption(6).

The iron deficiency anemia is prevalent in populations of low socioeconomic status and of which food vehicles and iron compounds are most suitable for iron fortification(7). Supplementation of wheat flour with ferrous sulfate considered the best way for the treatment of iron-deficiency anemia(8). Ferrous sulfate, ferrous ammonium sulfate and ferric chloride are the available and good sources for the dietary iron fortification(9). Soy consumption reduced heart disease and cancer risk, that it lengthened lives and enhanced their quality and it provided ideal protein suitable for animal diet proteins. Soybeans are a food that can prevent and even cure disease. On the other hand, soybean contains large quantities of natural antinutrients including potent enzyme needed for protein digestion. They can produce serious distress, reduced protein digestion and chronic deficiencies in amino acid uptake and also cause divalent cations deficiencies. In addition, the protein of soybeans is slightly less digestible than that protein found in most animal foods, due to the presence of the enzyme inhibitors,(6).

The aim of this study is to evaluate the biochemical effects of iron enrichment (throughout the hemoglobin generation efficiency) and defatted soybean supplementation to the wheat flour throughout the dietary iron absorption, protein digestibility and nutrients especially amino acids vitamins and minerals, particularly iron absorption.

Materials and Methods

Pepsin and trypsin enzymes: Reliable enzymes produced by Merck Chemical Co. They were prepared in concentration 1% solution and used to digest the diet protein in test tubes according to (10).

Experimental laboratory animals. Sixty albino male weanling healthy rats (derived from Wister and Sprague-Dawley) having body mass ranging between 58-64 g, and aged 25-30 days were divided into two major equal groups. First group was the healthy rats, while the second group was iron depleted by removing 20-25 drops of blood from the ocular capillary bed eye vein(11), with a aheparinized capillary tube on the first and third days of starting the experiment. This group used as iron depletion-anemic rats. Handling and treatment of the anemic rats were identical in all other aspects to the healthy rats. Each of the healthy and anemic rats was divided into six subgroups as follows:

1st group considered to be control group fed WF diet.
2nd group fed WF+50mg iron diet.
3rd group fed WF+100mg iron diet.
4th group fed WF+50g DSF diet.
5th group fed WF+50g DSF+50mg iron diet.
6th group fed WF+50g DSF+100mg iron diet.

The single rat lived separately in polyethylene cage covered with stainless steel net, and fed approximately 10-16g diet daily for 10 days experimental period, food intake was determined by weighing the amount of offering diet, fed, spilled and refused diet. Deionized drinking water in polyethylene bottles was available *ad libitum*, animals room was maintained at 25°C and light/dark cycle was 12/12hrs.

Diets preparation. Commercial wheat (*Triticum aestivum* Gramineae) flour (85% extraction) was obtained from Nineveh Mill Company. While soybean (*Glycin max* Leguminosae) was obtained from Nineveh field croup's research station, grains were graded, cleaned with tap water, dried to about 14% moisture content and cracked to remove the hull. Dehulling soybeans were rolled into full fat flakes. Oil of soybean flakes was removed by extracting it using diethyl ether, then defatted flakes were completely dried, milled to get defatted soybean flour (DSF). Ferrous sulfate substance, which was used as a source for wheat flour iron fortification, was obtained from Flauka chemical Co., in order to dissolve ferrous sulfate crystals, it was moistened with few drops of concentrated hydrochloric acid. Various diets were prepared by blending certain amounts of wheat flour, defatted soybean flour and ferrous sulfate compound according to the following formula:

WF alone, control diet.
WF enriched with 50 or 100mg ferrous sulfate/kg diet.
WF supplemented with 50g DSF/kg diet.
WF supplemented with 50g DSF and 50 or 100mg ferrous sulfate/kg diet.

Six diets of wheat flour and its blends were prepared separately, mixing them with suitable amount of deionized water, formed in pellets shaped (12-16g weight), dried on stainless steel trays in electrical oven at 50-55°C till completely dryness, maintained in polyethylene bags in dry cool place.

Diet chemical analysis. Crude protein, fiber, and ash were determined according to the (13,14)methods. Total hydrolysable carbohydrates were estimated according to the method described by(15). Phytate-phosphate was determined according to the method fixed by(16).

Table (1): The chemical composition of wheat flour and its blends with soybean and iron enrichment, on dry weight basis.

Diets(kg)	Protein %	CHO %	Fiber %	Phytate-P % of total fiber	Ash %	Iron mg/kg diet	Iron mg/1000 Kcal	Metabolic Energy Kcal/kg*
WF control	11.97	73.14	10.80	83.11	2.29	18.64	5.48	3404.4
WF+50 mg iron	12.34	73.55	10.64	81.95	2.44	69.01	20.09	3435.6
WF+100 mg iron	12.16	73.21	10.19	81.78	2.59	119.54	35.01	3414.8
DSF	45.88	39.42	12.34	64.05	4.09	45.52	13.34	3412.0
WF+50g DSF	14.97	70.71	10.96	82.17	2.59	21.28	6.21	3427.2
WF+50gDSF +50mg iron	14.93	76.08	10.01	82.29	2.71	70.81	20.58	3440.4
WF+50gDSF +100mg iron	14.85	70.28	11.12	82.23	2.89	121.38	35.65	3405.2

Where, WF: wheat flour, DSF: defatted soybean flour, P: phosphate, CHO: carbohydrates.
*Calculated according to National Academy of Sciences/National Research Council, NAS/NRC (12), where one g of carbohydrates and protein gives 4Kcal, and one g of lipid gives 9Kcal.

Biological and biochemical analysis. Protein digestibility was determined experimentally using the albino rats as biological model for studying the protein digestibility. Diet, feces, liver and spleen were analyzed for iron contents according to the method described by (17), which modified by(18,19), using the disodiumbathophenanethroline reagent, light absorption was measured spectrophotometrically at 535nm. Hemoglobin (Hb) was determined by the cyanomethemoglobin method according to(20). Total dietary iron absorption was calculated as apparent iron absorption%, hemoglobin regeneration efficiency (HRE%), hemoglobin iron and apparent digestibility% were calculated according to (21,22,23), using the following equations:

$$\text{Apparent iron absorption\%} = \frac{\text{mg dietary iron intake} - \text{mg feces iron}}{\text{mg dietary iron intake}} \times 100$$

$$\text{HRE\%} = \frac{\text{mg final Hb iron} - \text{mg initial Hb iron}}{\text{mg consumed dietary iron}} \times 100$$

$$\text{mg Hb iron} = \text{g BM} \times \frac{\text{6.7ml blood}}{\text{100g BM}} \times \frac{\text{g Hb}}{\text{100ml blood}} \times \frac{\text{3.35mg iron}}{\text{1.0g Hb}}$$

Where, g BM: gram body mass, 6.7ml is the total blood volume per 100g rat body mass, and 3.35mg iron is the total iron per 1.0g Hb according to (24).

$$\text{Apparent diet digestibility \% (dry matter absorption, DMA)} = \frac{\text{g feces} - \text{g total diet intake}}{\text{g total diet intake}} \times 100$$

$$\text{protein digestibility index} = \frac{\text{g digestible protein}}{\text{g ingested total dietary protein}} \times 100$$

Statistical analysis. Experimental data were statistically analyzed using the statistical package for social-sciences (spss computer program), following the one way ANOVA variance analysis system and comparing the means in Duncan multiple range test to know the significant differences values at probability <0.05 according to(25).

Results

Table (1) showed the chemical composition of the WF (85% flour extraction from wheat grains). In order to enrich and modified the protein quality and quantity values of wheat flour, iron and DSF were added. Therefore, WF was blended with or without 50g DSF, and enriched with 50 or 100 mg iron/kg WF.

Table (2):Protein digestibility of wheat flour and it's blends with soybean and iron enrichment.

Diets(kg)	Rat Physiological status	Ingested diet (g)	Ingested protein(g)	Feces protein (g)	Digestible protein (g)	Protein digestibility index	Digest-ibility mean%
WF, control	Anemic	113.62±3.00B	13.60±1.04D	3.75±0.04C	9.85±0.11C	72.43±4.23B	75.01
	Healthy	106.92±5.11D	12.80±2.11E	2.87±0.08D	9.934±0.0C	77.58±3.14A	
WF+50mg iron	Anemic	117.12±7.21A	14.45±2.11C	4.47±0.09B	9.98±0.09C	69.07±2.52C	68.98
	Healthy	108.34±4.11A	13.37±1.11D	4.16±0.10B	9.21±0.10C	68.89±2.66C	
WF+100mg iron	Anemic	116.72±6.70A	14.19±1.21C	4.49±0.09B	9.70±0.11C	68.37±4.15C	69.56
	Healthy	116.41±6.71A	14.16±2.01C	4.14±0.10B	10.02±0.0B	70.75±4.13B	
mean		113.19±5.51	13.76±1.31	3.98±0.09	9.78±0.06	71.18±2.24	71.18
WF+50g DSF	Anemic	107.64±5.1D	16.11±1.44B	6.08±0.02A	10.03±0.1B	62.26±2.26E	64.23
	Healthy	117.18±7.21A	17.54±1.42A	5.93±0.10A	11.61±0.0A	66.19±3.09D	
WF+50g DSF +50g iron	Anemic	117.87±4.90A	17.60±1.32A	6.51±0.05A	11.09±0.0A	63.01±2.15E	62.76
	Healthy	112.37±3.29B	16.78±1.09B	6.29±0.10A	10.49±0.1B	62.51±4.06E	
WF+50g DSF +100mg iron	Anemic	111.84±4.62C	16.61±1.64B	6.37±0.11A	10.24±0.0B	61.65±2.12E	62.28
	Healthy	111.97±4.75C	16.63±1.83B	6.17±0.09A	10.46±0.0B	62.90±2.95E	
mean		113.15±5.01	16.88±1.82	6.23±0.09	10.65±0.07	63.09±2.17	63.09

* Mean of five rats. WF: wheat flour, DSF: defatted soybean flour.

Table (2) showed the digestible protein and protein digestibility index values for the anemic and healthy rats fed the experimental six diets separately. The healthy rats were ingested and digested higher protein, therefore caused higher digestible protein values and in turn caused higher protein digestibility indices than the iron-deficiency (anemic) rat groups. The mean value of the digestible protein values of the rat groups fed WF containing 50 and 100mg iron diets was lower (9.78g) than that mean value of the rat groups fed WF containing 50g DSF and 50 or 100mg iron diets (10.65g). While the protein digestibility index of the rat group fed WF containing iron was higher (69.10%) than that value of the rat groups fed the WF containing 50g DSF and 50 or 100mg iron diets (59.90%). These variations may be due to the feces protein value of the rat groups fed the WF containing iron was lower (3.98g) than that value (6.23g) of the rat groups fed the WF containing 50g DSF and 50 or 100mg iron diets. On the other hand, the healthy rat groups were ingested higher protein and digested more protein values than that values of the anemic rat groups fed the same diets. The mean values of the protein digestibility indices of the rat groups fed WF containing 50g DSF and 50 or 100mg iron diets were lower (62.76and 62.28respectively) than that values of the rat groups fed WF containing 50g DSF only (64.23) and in turn lower than that value of the rat groups fed WF containing 50 or 100mg iron diets (68.98 and 69.56 respectively). All the protein digestibility indices values of the rat group fed WF containing 50g DSF alone or with / without 50mg or 100mg iron diets were lower comparing with the rat group fed the WF control diet (75.01).

Table (3): Effect of iron enrichment and defatted soybean flour to the wheat flour alone and together in the dietary iron absorption for growing male rats (mean ± S.E.)*

Diets(kg)	Rat physiological status	Ingested diet		Eliminated feces		Apparent iron absorption %	Apparent diet digestibility (DMA) %
		weight (g)	iron content mg	weight (g)	iron content mg		
WF, control	Anemic	113.62±3.07B	2.12±0.05C	10.90±0.99A	1.18±0.09D	44.57±1.23C	90.41±0.8A
	Healthy	106.92±5.11C	1.99±0.09D	9.53±0.97B	1.30±0.12D	34.76±1.17D	91.09±0.7A
WF+50 mg iron	Anemic	117.12±7.27A	8.08±0.04B	11.18±0.92A	1.02±0.00D	87.42±2.11A	90.45±1.0A
	Healthy	108.34±4.11C	7.48±0.02B	9.71±1.05B	1.50±0.01D	80.00±1.82A	91.04±0.9A
WF+100 mg iron	Anemic	116.72±3.12A	13.95±0.03A	10.33±0.87A	7.74±0.01A	44.50±1.20C	91.15±0.7A
	Healthy	116.41±6.72A	13.92±0.03A	10.86±1.09A	8.72±0.02A	37.37±1.12D	90.67±1.1A
mean		113.19±5.51	7.92±0.04	10.42±0.98	3.58±0.04	54.77±1.44	90.80±0.89
WF+50gDF	Anemic	107.64±4.62C	2.29±0.02C	9.77±0.96B	1.11±0.12D	51.32±1.40C	90.92±1.1A
	Healthy	117.18±4.75A	2.49±0.04C	10.45±1.12A	1.37±0.21D	44.88±1.42C	91.08±0.8A
WF+50gDF +50mg iron	Anemic	117.87±5.17A	8.35±0.03B	10.86±0.86A	2.67±0.07C	68.07±1.73B	90.78±1.2A
	Healthy	112.37±7.25B	7.96±0.02B	10.56±1.13A	2.96±0.10C	62.81±1.68B	90.60±1.0A
WF+50gDF +100mg iron	Anemic	111.84±4.98B	13.58±0.02A	9.26±0.88B	5.70±0.04B	58.00±1.41C	91.72±0.9A
	Healthy	111.97±3.29B	13.59±0.03A	9.50±1.02B	6.83±0.08B	49.71±1.38C	91.52±0.8A
mean		113.15±5.01	8.04±0.03	10.07±1.00	3.44±0.10	55.80±1.50	91.25±1.10

* mean of five rats. WF: wheat flour, DSF: defatted soybean flour, DMA: dry matter absorption.

Table (3) showed the apparent iron absorption and apparent digestibility% (dry matter absorption %) for the anemic and healthy rat groups fed the WF alone and with/without DSF and iron diets. There were significant differences (p<0.05) In the apparent iron absorption and apparent digestibility% of the anemic and healthy rat groups fed various diets, which decreased with the increasing level of dietary iron from 50 to 100mg iron for WF and WF+DSGF alternatively comparing with control WF and WF+DSF diets, while it was improved and increased with the addition of 50g DSF to the three diets (WF, WF+50mg iron, and WF+100mg iron). There was no significant difference (p<0.05) in the apparent digestibility% (dry matter absorption%) between rat groups fed the six diets separately.

Table (4) showed no significant differences (p<0.05) in the initial hemoglobin (Hb) values and Hb iron contents of anemic or healthy rats, but there were significant differences (p<0.05) in the Hb gains, Hb iron gains and HRE % of the anemic and healthy rats fed six diets separately at the end of experiment. The highest Hb gains were found in the anemic rat groups fed WF+ 100mg iron and WF+ 50g DSF+ 100mg iron diets followed by the anemic rat groups fed WF+50mg iron and WF+ 50g DSF+ 50mg iron diets

Table (4): Effect of iron enrichment and defatted soybean flour to the wheat flour alone and together on the hemoglobin and hemoglobin iron content for growing male rats (mean ± S.E)*

Diets(kg)	Rat physiological status	Hb value (g/dl)		Hb iron content (mg)		HRE %
		initial	gain	initial	gain	
WF, control	Anemic	7.63±1.01B	1.31±0.04C	1.04±0.06B	0.20±0.01C	12.58±1.82D
	Healthy	12.20±0.31A	0.10±0.00D	1.67±0.03A	1.08±0.09A	40.75±2.63A
WF+50mg iron	Anemic	7.78±1.18B	2.02±0.03B	1.04±0.07B	0.88±0.04B	44.00±2.71A
	Healthy	11.98±0.42A	0.33±0.02D	1.62±0.08A	1.15±0.08A	39.79±1.92A
WF+100mg iron	Anemic	6.99±0.96B	3.00±0.08A	0.97±0.02C	1.19±0.10A	30.59±1.99B
	Healthy	12.11±0.29A	0.47±0.01D	1.68±0.10A	1.20±0.09A	23.30±1.42C
mean		9.78±0.70	1.21±0.03	1.34±0.06	0.95±0.07	31.84±2.08
WF+50g DSF	Anemic	7.27±1.05B	1.87±0.12C	1.03±0.07B	0.98±0.06B	14.22±0.92D
	Healthy	12.22±0.40A	0.27±0.03D	1.70±0.12A	1.14±0.07A	12.69±0.81D
WF+50g DSF +50g iron	Anemic	7.91±0.98B	2.64±0.12B	1.07±0.09B	1.24±0.12A	31.00±1.63B
	Healthy	11.89±0.29A	0.41±0.06D	1.60±0.11A	1.16±0.09A	22.35±1.35C
WF+50g DSF +100mg iron	Anemic	7.63±0.87B	3.42±0.13A	1.04±0.06B	1.47±0.12A	20.50±1.08C
	Healthy	12.37±0.33A	0.62±0.07D	1.67±0.08A	1.32±0.09A	14.52±0.95D
mean		9.88±0.65	1.54±0.09	1.35±0.09	1.22±0.09	19.21±1.12

- mean of five rats. Hb: hemoglobin, HRE: hemoglobin regeneration efficiency.

alternatively comparing with the group fed the WF control diet. The mean of Hb gains of the rat groups fed WF+ 50 or 100mg iron diets were lower than that mean gains of rat groups fed WF+ 50g DSF+ 50 or 100mg iron diets. The best Hb iron gains of healthy rat groups were found in the rat groups fed WF, WF+ 50mg or 100mg iron, WF+ 50g DSF and WF+ 50g DSF+ 50mg or 100mg iron diets. Also the best Hb iron gains of the anemic rat groups were found in the groups fed WF+100mg iron, WF+50g

DSF+50mg or 100mg iron diets alternatively. The mean of Hb iron gains of the anemic rat groups fed WF+ 50 or 100mg iron diets were lower than that mean gains of the healthy rat groups fed WF+ 50g DSF+ 50 or 100mg iron diets alternatively. There were significant differences ($p<0.05$) in the hemoglobin regeneration efficiency (HRE %) in the rat groups fed the six tested diets. The highest HRE ratios were found in the anemic and healthy rat groups fed WF+ 50mg iron diet, followed in decreasing order by WF+ 100mg iron, WF+ 50g DSF+ 50mg iron, WF+ 50g DSF+100mg iron, WF+ 50g DSF diets respectively. The HRE % of the anemic rat groups was higher than that ratio of the healthy rat groups. The HRE mean of the rat groups fed WF+ 50 or 100mg iron diets were higher than that HRE mean of rat groups fed WF+ 50g DSF+ 50 or 100mg iron diets.

Discussion

The form of ferrous sulfate, the easily soluble form in the stomach acid, and easily absorbable in intestine), the chemical form and concentration of the used iron showed the optimum values for rats nutrition(26,27), and in order to meet the rat physiological status and nutritional requirements, DSF protein supplementation and iron fortification done according to the recommendation of the American national academy of sciences/nutritional research council,(12). The analysis of fiber and phytates-P in the six WF diets and DSF were done due to their chelating roles of divalent cations absorption in the intestine, especially iron, which they naturally present in wheat and soybean,(6). In addition, the fiber have additional role, which was the arrangement of chymes (digested diets) movement in gastrointestinal track and also cleaning this track from the remain of any foods. In this case, fiber offered the practical maximum surfaces of diets to attack by digestive enzymes system. The tested diets showed identical metabolized energy, which was considered to be an important factor.

The diets content identical components (fiber, carbohydrates, phytates-p, ash, and metabolic energy) with exception of the DSF diet (it contains high protein, fiber and low carbohydrates). The limitation factors which used in this study were the protein and iron contents. These constituents were biologically used in the hemoglobin biosynthesis (heme iron group + globins protein) which used in the erythropoiesis process to generate red blood erythrocytes. The preparation of diets was identical and similar to the diets prepared by (28,29). The tested six diets showed identical metabolic energy, so was agreed with the results postulated by (28,29), Table (1).

The effect of iron enrichment, defatted soybean protein and the rat physiological status on the protein digestibility index. The digestible protein of the rat groups fed WF diet enriched with iron was lower (9.78g) than that value of the rat groups fed the WF diet enriched with iron and supplemented with defatted soybean (10.65g), this variation was due to the high protein contents of defatted soybean, and this result was agreed with the result reported by(30). The protein digestibility index of the rat groups fed WF enriched with iron diet was higher (71.18%) than that value of the rat groups fed WF enriched with iron and supplemented with defatted soybean diet (63.09%). This variation return to the feces protein content of the rat groups, which was higher (6.23g) in the rat groups fed WF+ iron+ DSF diets than that value of the rat groups fed only WF+iron diet (3.98g). The mean of protein digestibility index was decreased with the increasing levels of diet-iron and defatted soybean contents (68.98, 69.56, 64.23, 62.76 and 62.28%) comparing with the control value (75.01%). Those values depression return to the effect of dietary iron level, which considered an proteolytic enzymes inhibitor, while the defatted soybean considered to be chelater agent to the diet minerals (especially the divalent cations) and digestive proteolytic enzymes inhibitors, because soybeans contain large quantities of antinutrients, and this results agreed with the conclusion fixed by(6). The total mean value of the protein digestibility index (71.18+63.09/2=67.14%) was similar to the value of standard (ferrous sulfate) diet formulated by the NAS / NRC, (12) and used by many researches (21,22,25,26,27,28,29,30,31,32), who found that this value was equal to(64.47%),Table (2)

There were significant differences ($p<0.05$) between groups of anemic and healthy in the mean of ingested diet weights, ingested dietary iron contents, eliminated feces weights, feces iron contents, apparent iron absorption and dietary apparent digestibility in the same column. The highest ingested diet weight was for the anemic rat groups fed WF+50g DSF+50mg iron diet (117.87g) for 10days experimental period. The mean of the ingested diet weight of the rat groups fed WF enriched with iron was similar (113.19g) than that of diet weight ingested by rat groups fed WF enriched with iron and supplemented with DSF (113.15g). These results were consistent with the results obtained by(22,23,31,32). The highest iron content of the ingested diet was for the rat groups fed WF+50gDSF+100mg iron. The mean value of the dietary iron content of the rat groups fed WF enriched with iron was lower (7.92mg)than that dietary iron content of the rat groups fed WF enriched with iron and supplemented with DSF (8.04mg), this results consistent with the results obtained by(33). The eliminated feces weights were followed the distribution manner of ingested diets. They may be correlated to the diet amounts and its fiber contents. This conclusion was consistent with the results of many researches (22,23,24). The apparent iron absorptions of the anemic rat groups were higher than that absorption of the healthy rat groups. The mean value of apparent iron absorption of the rat groups fed

WF diet enriched with iron was lower (54.77%) than that absorption of rat groups fed WF diet supplemented with the iron and DSF (55.80%). These results consistent with the results obtained by(23,33). There were no significant differences ($p<0.05$) in the apparent digestibility% between the rat groups fed the six diets. While the apparent digestibility% of the rat groups fed WF diet enriched with iron was slightly lower (90.80%) than that value of the rat groups fed WF diet supplemented with iron and DSF (91.25%). These results consistent with the results obtained by (26,27,32), Table (3).

There were significant differences ($p<0.05$) in the Hb gains, Hb iron gains, and HRE of the rat groups fed the six tested diets. The highest Hb gain value was for the anemic rats fed WF diet supplemented with 100mg iron and 50g DSF (3.42mg) and WF diet enriched with 100mg iron comparing with the control group. The Hb gain values of the anemic rats were higher than those values of the healthy rats fed the same diet. The mean value of the Hb gain of the rat groups fed WF diet enriched with iron was lower (1.21g/dl) than that mean value of the rat groups fed the WF diet supplemented with iron and DSF (1.54 g/dl), these results were consistent with the results obtained by 21,22,23,30,33,35,36). The mean value of Hb iron gains of the rat groups fed WF diet enriched with iron was lower (0.95mg) than that mean value of the rat groups fed the WF diet supplemented with iron and DSF (1.22mg). The HRE % of the anemic rats fed the WF diet enriched with iron was higher (31.84%) than that value of the rat groups fed the WF diet fortified with iron and DSF (19.21%). The HRE percent of the anemic rats was higher than the HRE of the healthy rats fed the same diets. The highest HRE% (44.00 and39.79%)for anemic and healthy rats respectively was for rat groups fed WF diet fortified with 50mg iron. These results consistent with the results reported by (32,36,37), Table (4).

From the above results, it was found that, the protein digestibility index of the healthy rats was higher than that index value of the anemic rats fed the same diets. Apparent iron absorption was positively proportional to the dietary iron enrichment, and the dietary iron absorption of the anemic rats was higher than that absorption of the healthy rats. The DSF protein was improved the dietary iron absorption. Hb value, Hb iron gains and HRE% were increased with the increasing dietary iron level with limitation to 100mg maximum level. HRE% of the anemic rats was higher than that value of the healthy rats. Lastly the WF+50g DSF+50mg iron diet blend was found to improve protein digestibility, dietary iron absorption and HRE in rats.

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