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INTERACTION EFFECT OF IRON ENRICHMENT AND DEFATTED SOYBEAN SUPPLEMENTATION TO WHEAT FLOUR ON THE PROTEIN DIGESTIBILITY AND GROWTH IN GROWING 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 قابلية هضم البروتين مختبريا "وبايولوجيا". استخدم 60 من ذكور الجرذان البيضاء حديثة الفطام موديلا" بايولوجيا" للتجربة، قسمت الى مجموعتين رئيسيتين متساويتين، هما مجموعة جرذان السيطرة السليمة ومجموعة الجرذان المصابة بفقر دم نقص الحديد، قسمت كل من المجموعتين الى ستة مجاميع بالتساوي وفقا" لوزن الجسم، غذيت لمدة 10 أيام مدة التجربة.

تم تقدير وحساب وزن البروتين الغذائي المتناول والمهضوم ودليل قابلية هضم البروتين مختبريا "وبايولوجيا". كما قدر امتصاص الحديد الغذائي والهضم الظاهري والحالة التغذوية للجرذان المصابة بفقر الدم والسليمة، هذا بالإضافة لحساب كمية الطاقة المتأيضة البديلة لكل غذاء وحساب دليل نمو الجرذان.

بينت نتائج التحليل الإحصائي ان دليل هضم البروتين البايولوجي هو أدنى من نظيره المختبري، وان دليل هضم البروتين في الجرذان المصابة بفقر الدم هو أعلى مما هو عليه في الجرذان السليمة، وان دليل النمو في الجرذان السليمة هو أعلى مما هو عليه في الجرذان المصابة بفقر الدم، كماان دليل النمو يتناسب طرديا" مع مستوى الحديد الغذائي، وان تواجد فول الصويا منزوع الدهن في الغذاء يؤدي الى تحسين دليل النمو.ان طحين الحنطة استخلاص 85% المضاف إليه 50غم فول الصويا منزوع الدهن و50ملغم حديد/كغم غذاء أدى الى تحسين قابلية هضم البروتين والى ارتفاع دليل النمو في الجرذان.

ABSTRACT

Wheat flour (WF) of 85% meal extraction was blended with/without 50g defatted soybean flour (DSF)/kg and enriched with 50 or 100mg iron/kg diet used in vitro and In vivo study of protein digestibility. Sixty male weanling albino rats were used as a biological model for this study. Rats were divided into two major equal groups. First group as healthy control rats and second group as iron depleted rats. Each group was subdivided into six groups according to their body mass (BM), These subgroups fed experimental diets for 10 days.

Digestible protein (%) and its digestibility index, rat BM gain and its growth index were estimated using in vivo and in vitro methods. Dietary iron absorption, apparent digestibility, and rat nutritional status of iron depleted and healthy rats were determined. In addition the metabolic energy of the six diets and rat growth index were calculated.

Data analysis showed that, the in vivo protein digestibility index was lower than that index of in vitro. Protein digestibility index of the anemic rats was higher than that index of healthy rats. The growth index of the healthy rats was higher than that index of the anemic rats. The rat growth index positively proportional to the dietary iron level, The DSF presence in diet caused growth index improvement. The WF+50g DSF+50mg iron diet improves protein digestibility and rat growth index.

INTRODUCTION

It is well known that the people consuming cereal for long time may suffer from a shortage of protein, which is considered to be a major quality problem rather than quantity. In many lands in the world, cereal products provide most of the required calories and plant proteins in diet. Generally, in most of developing countries, as much as 64% of the daily protein intake is derived from cereal grains, (1). Soybeans are classified as oil seeds, not as dry beans. Whole dry soybeans contain about 40% protein (twice as much as most other pulses) and up to 20% fat. Whole soybeans are a good source of calcium, iron, zinc, phosphorus, magnesium, thiamin, riboflavin, niacin, and folacin, (2). It was recently recognized that the human diet contains, in addition to essential macro and micronutrients, a complex array of naturally occurring bioactive non-nutrients called phytochemicals (plant-derived compounds) that confer significant long-term health benefits, (3). Among these phytochemicals is the broad class of non-steroidal oestrogens called phytoestrogens that also behave as oestrogen mimics. The major classes of phytoestrogens that are of interest from a nutritional and health perspective are the lignin and isoflavones. Soybeans contain large amounts of the isoflavones diadzein, gesisten, and glycitein (1-3mg/g) and their acetyl and malonyl conjugates, (4).

The protein contents of soybean flour is approximately 50%. Wheat flour protein can be supplemented with soy flour protein, and improve the crust, colour and shelf life of baked goods when added to it. The nutritive value of the wheat products depends upon the protein level in flour and on the balance of various amino acids that make up protein, (5). Normal cereal grains including wheat are low or deficient in some essential amino acids such as lysine, threonine, methionine, tryptophan and isoleucine, they so-called limiting amino acids, (6). Raising the nutritional value of cereal products is done by blending wheat flour with different protein sources such as soybean meal or fish meal have been generally used in the world for improving protein utilization by the body. (7).

Food processing and the presence of anti-nutritional agents are the most important criteria that contribute the protein digestibility and consequently availability. Kent-Jones and Amos, (7) reported that digestibility of flours of different extraction falls as the extraction rate increases. They correlated the fall in digestibility with the increase in fiber (0.15% fiber increased in wheat flour caused 1.10% decreased in digestibility). This study was conducted to determine the protein digestibility of wheat flour, soybean meal alone, wheat flour with iron enrichment and wheat flour with iron and soybean supplementation, throughout the in vitro and In vivo of rat growth index methods.

MATERIALS AND METHODS

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 vein (8), with a heparinized capillary tube on the first and third days of starting the experiment. This group used as 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:

Group 1 considered to be control group(N= 5 rats).
Group 2 used for studying WF+50mg iron diet(N= 5 rats).
Group 3 used for studying WF+100mg iron diet(N= 5 rats).
Group 4 used for studying WF+50g DSF diet(N= 5 rats).
Group 5 used for studying WF+50g DSF+50mg iron diet(N= 5 rats).
Group 6 used for studying WF+50g DSF+100mg iron diet(N= 5 rats).

Those groups of rats were fed the below mentioned six diets separately for 10 days experimental period. The single rat lived separately in righted polyethylene cage covered with stainless steel net, fed approximately 10-16g diet daily, 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 aescivum* Gramineae) flour (85% extraction) was obtained from Nineveh Mill Company. While soybean (*Glycine max* Leguminosae) was obtained from local market, 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. Fat was removed by extracting soybean flakes oil with diethyl ether, then defatted flakes were completely dried, milled to get defatted DSF. Ferrous sulfate substance, which was used as a source for wheat flour iron fortification, was obtained from Flauka chemical Co., ferrous sulfate crystals was moistened with few drops of hydrochloric acid in order to dissolve it, before using it in the diets preparation. In order to meet the physiological status and nutritional requirements of the rats, various WF diets were prepared by blending certain amounts of wheat flour, defatted soybean flour and ferrous sulfate compound according to according to the suggestions and recommendation of American National Academy of Sciences (NAS)/Nutritional Research Council (NRC), (9) as in the following formula:

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

Therefore, six diets samples of WF and its blends were prepared separately by mixing it with suitable amounts of deionized water, formed in pellet shaped (12-16g weight), dried on stainless steel trays in electrical oven at 50- 55°C till completely dryness, then maintained in polyethylene bags in dry cool place. The various diets of WF, and its blends with DSF and iron supplementation and their chemical analysis were listed in Table (1).

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

Diets(kg)	Protein %	CHO %	Fiber %	Phytate-P g/kg	Ash %	Iron mg/kg	Metabolic energy Kcal/kg *
WF control	12.84	72.12	10.22	4.28	2.01	18.01	3398.4
WF + 50 mg iron	12.80	72.10	10.02	4.27	2.12	68.01	3396.0
WF + 100 mg iron	12.78	72.07	9.98	4.25	2.20	118.16	3394.0
DSF	42.71	38.05	11.91	3.17	3.20	43.11	3630.4
WF + 50g DSF	14.96	69.92	10.45	4.22	2.29	19.27	3410.8
WF +50g DSF+50mg iron	14.99	69.90	10.38	4.23	2.38	69.27	3409.2
WF +50g DSF+100mg iron	14.92	69.88	10.33	4.24	2.51	119.27	3407.2

Where, P: phosphate, CHO: carbohydrates.
*Calculated according to (9) where, each one g of carbohydrates and protein give 4 and one g of lipid gives 9 Kcal.

Chemical analysis: Crude protein, crude fiber, and ash were determined according to the Association of Official Analytical Chemists methods(10). Total hydrolysable carbohydrates were estimated according to the method described by Smith et al, (11). Calcium and phosphorus were determined according to the method fixed by Wheeler and Ferrel (12). In vitro protein digestibility was determined by pepsin at pH 1.5, followed by pancreatic enzymes at pH 7.6 according to the method stated by Akerton and Stahman, (13). The nitrogen contents of the sample and digestible protein was determined by the micro-kjeldahl method, (10) and calculated from the difference of total protein value before and after the action of proteolytic enzymes analysis. While protein digestibility% was calculated according to the following equation reported by McDonald (14):

$$\text{protein digestibility \%} = \frac{\text{digestible protein}}{\text{dietary total protein}} \times 100$$

Analytical and biochemical procedures: Diets, feces, liver and spleen were analyzed for iron contents according to the method described by Schricker et al(15),which modified by Miller et al, (16and17), using the disodiumbathophenanethroline reagent, light absorption was measured spectrophotometrically at 535nm. Hemoglobin(Hb) was determined by the cyanomethemoglobin method (18).In vivo determination:Experimentally using the albino rats as biological model for studying the protein digestibility. Apparent digestibility %, digestible protein (apparent iron absorption %) and protein digestibility index were calculated according to McDonald (14) and Sharaf (19), using the following equations:

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

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

$$\text{apparent protein absorption\% (digestible protein)} = \frac{\text{g dietary protein intake} - \text{g feces protein}}{\text{g dietary protein intake}} \times 100$$

$$\text{growth index} = \frac{\text{g body mass gain}}{\text{g final body mass}} \times 100$$

Where, final body mass = initial body mass + body mass gain.

Statistical analysis: Experimental data of the rats were statistically analyzed using the factorial experimental conducted completely randomized design (CRD), (20) using the variance analysis and Duncan multiple range test (spss computer program).

Results

Chemical analyses of WF and its blends with DSF and iron enrichment diets analyses represent protein, carbohydrates, fiber, phytate-p, ash, iron contents and metabolic energy content. All these components were identical in WF blends. Metabolic energy of these six diets gives a good values of energy content to meet the nutritional and physiological status of the rats, as shown in Table (1).

Table(2) showed the in vitro and In vivo digestible protein% and protein digestibility index of the tested six diets of WF and its blends only.

In vitro the level of digestible protein was lower for WF enriched with iron only and WF supplementated with iron and DSF with the increasing the iron level. The mean value of digestible protein for WF+50g DSF diet enriched with iron (9.42%) WF diet enriched with iron only (8.41%). Also in vitro the protein digestibility index % level for the WF diet enriched with iron only and WF+50g DSF diet supplemented with iron increasing iron level, was lower than WF control. The mean value of protein digestibility index of WF diet enriched with iron was higher (69.56) than that value of the WF+50g DSF diet enriched with iron (61.46).

In vivo ingested diets, digestible protein and protein digestibility index values of the healthy rat groups fed the six tested diets were higher than that values of the anemic rat groups fed the same diets. The mean value of the ingested diet of the rat groups fed the WF diet enriched with iron was higher (115.24g) than that value of the rat groups fed WF diet supplemented with iron and DSF (87.22g). The mean value of the ingested protein and digestible protein values of the rat groups fed WF diet enriched with iron was higher than that values of the rat groups fed WF diet supplemented with iron and DSF. The mean value of protein digestibility index of rat groups fed WF diet enriched with iron was higher (69.27) than that value of the rat groups fed WF diet supplemented with iron and DSF (59.68). The general mean of protein in vitro digestibility index was slightly higher than that value of the in vivo.

Table (2): In-vitro and in-vivo protein digestibility of wheat flour and it's blends with soybean and iron Enrichment

Diets (kg)	In-vitro		In-vivo*						
	Digestible Protein %	Protein digestibility index	Rat Physiological status	Ingested diet(g)	Ingested Protein %	Digestible protein %	Feces Protein %	Protein digestibility index	Mean of prot. digestibility
WF, control	9.22	71.81	Anemic	88.06±3.07 D	11.31±0.4 D	8.05±0.11 C	3.26±0.04 C	71.19±4.3 B	74.68
			Healthy	147.32±5.14 A	18.92±0.2 A	14.78±0.0 A	4.05±0.08 B	78.16±3.4 A	
WF+50mg iron	8.76	68.43	Anemic	103.53±7.27 B	13.25±0.5 B	8.65±0.09 C	4.60±0.09 B	65.28±2.2 D	66.60
			Healthy	149.72±4.11 A	19.16±0.6 A	13.02±0.1 A	6.14±0.10 B	67.92±2.6 C	
WF+100mg iron	8.75	68.44	Anemic	97.12±6.72 B	12.41±0.2 C	8.09±0.11 C	4.32±0.09 B	65.21±4.5 D	66.54
			Healthy	115.70±6.72 B	14.79±0.6 B	10.04±0.0 B	4.75±0.10 B	67.87±4.3 C	
General mean	8.91	69.56		115.24±5.51	14.97±0.8	10.44±0.0	4.52±0.09	69.27±2.4	69.27
WF+50g DSF	9.75	63.52	Anemic	88.31±5.17 D	13.21±0.6 B	8.00±0.11 C	5.21±0.02 A	60.56±2.6 E	61.07
			Healthy	95.99±7.25 C	14.36±0.6 B	8.84±0.06 C	5.52±0.10 A	61.58±3.9 E	
WF+50g DSF +50mg iron	9.29	60.62	Anemic	87.72±4.98 D	13.15±0.5 B	7.59±0.08 D	5.56±0.05 A	57.70±2.5 F	58.57
			Healthy	94.96±3.29 C	14.23±0.7 B	8.46±0.10 C	5.77±0.10 A	59.44±4.6 F	
WF+50g DSF +100mg iron	9.22	60.24	Anemic	70.10±4.62 E	10.46±0.2 D	6.14±0.09 E	4.32±0.11 B	58.66±2.2 F	59.39
			Healthy	86.21±4.75 D	12.86±0.4 C	7.73±0.09 D	5.13±0.09 A	60.11±2.5 E	
General mean	9.42	61.46		87.22±5.01	13.05±0.6	7.79±0.07	5.25±0.09	59.68±2.7	59.68
Total mean	9.17	65.49		101.23±5.26	14.01±0.8	9.12±0.09	4.89±0.10	64.47±2.2	64.47

*Mean of five rats.

There were a significant differences ($p<0.05$) in the BM gains, liver, spleen weights and their iron contents, and growth index of rats. There are no significant differences in the initial rat body mass, therefore, this weight considered to be identical and a good rat group used in the experiment. The highest BM gain was for the healthy rat groups fed the WF diet enriched with iron and with DSF comparing with the control group. The BM gains of the healthy rat groups were higher than those gains of the anemic rat groups fed the same diets. The mean value of the BM gain of the rat groups fed WF enriched with iron was lower (32.60g) than that gain of rat groups fed the WF diet supplemented with iron and DSF (39.07g).

Weights of liver and spleen give similar results to the distributing results of BM gains. The iron contents of those internal organs give similar results to each other. The highest weights of liver were found in the anemic rats fed WF+iron diet supplemented with/without DSF. While the highest weights of the spleen were found in the healthy rats fed WF diet supplemented with iron and DSF. On the other hand, the highest iron contents of liver and spleen were found in the healthy rats fed WF diet supplemented with iron and DSF. The mean values of the liver and spleen weights and their iron contents of the rats fed WF diet fortified by iron were lower than that mean values of the rats fed WF diet supplemented with iron and DSF.

There was a significant differences ($p<0.05$)in growth index of rat groups fed the six tested diets. The highest growth indices were found in the healthy rats fed WF diet enriched with iron and with/without DSF. The growth index value of the healthy rats was higher than that value of the anemic rats fed the same diet. The mean value of the growth index of rats fed WF diet enriched with iron was lower (34.16) than that mean value of rats fed WF diet supplemented with iron and DSF (39.00), (Table 3).

Table (3): Effect of iron enrichment and defatted soybean flour to the wheat flour alone and together on the nutritional status of growing male rats (mean ± S.E.) *

Diets(kg)	Rat status	Body mass (g)		Liver		Spleen		Growth index
		Initial	Gain	Weight (g)	Iron Cont. µg	Weight (g)	Iron cont. µg	
WF, control	Anemic	60.82±1.8 A	14.46±1.9 D	3.09±0.07 C	224.25±29.1 C	0.13±0.03 C	269.3±23.18 E	19.21±0.52 D
	Healthy	61.11±1.6 A	38.45±1.9 B	4.66±0.12 B	326.4±9.14 B	0.36±0.08 A	658.5±38.18 B	38.62±1.21 B
WF+50 mg iron	Anemic	59.32±2.0 A	28.11±2.5 C	4.21±0.22 B	304.8±32.19 B	0.21±0.05 B	338.6±47.25 D	32.15±1.13 C
	Healthy	60.17±1.9 A	39.92±2.2 A	4.85±0.13 B	339.7±11.29 B	0.38±0.07 A	662.8±26.31 B	39.88±1.76 A
WF+100mg iron	Anemic	62.04±1.8 A	34.42±2.1 B	5.16±0.27 A	313.8±18.25 B	0.25±0.14 B	371.2±28.62 D	35.68±1.80 C
	Healthy	61.84±1.7 A	40.25±2.3 A	4.88±0.08 B	342.5±12.64 B	0.38±0.05 A	673.9±47.63 B	39.43±1.12 A
mean		60.88±1.8	32.60±2.2	4.48±0.15	308.58±18.8	0.32±0.06	495.72±35.2	34.16±1.3
WF+50g DSF	Anemic	63.01±1.2 A	35.19±1.6 B	5.22±0.24 A	327.7±19.44 B	0.26±0.08 B	423.3±64.71 C	35.84±0.96 C
	Healthy	62.14±2.0 A	39.15±1.1 A	4.76±0.14 B	332.7±21.15 B	0.37±0.11 A	725.6±48.52 A	38.65±1.17 B
WF+50g DSF +50mg iron	Anemic	60.17±1.7 A	37.29±2.6 B	5.54±0.41 A	362.2±21.18 B	0.27±0.12 B	447.5±34.89 C	38.26±1.52 B
	Healthy	59.93±2.1 A	40.22±1.2 A	4.89±0.17 B	441.8±20.14 A	0.38±0.09 A	745.8±53.72 A	40.16±1.62 A
WF+50g DSF +100mg iron	Anemic	60.97±2.1 A	40.11±2.1 A	5.98±0.48 A	379.3±29.01 B	0.29±0.10 B	483.7±60.14 C	39.68±1.41 A
	Healthy	60.04±1.1 A	42.45±1.6 A	5.16±0.31 A	459.9±27.13 A	0.40±0.08 A	781.4±50.29 A	41.42±1.35 A
mean		61.04±1.8	39.07±1.8	5.26±0.29	383.93±23.0	0.33±0.10	601.22±52.1	39.00±1.3

*mean of five rats.

DISCUSSION

The reason of adding DSF to the WF in order to modify protein controls quantitatively and qualitatively because WF considered to be a poor nutrient materials with deficiency of essential amino acids especially lysine. In addition to soybean, iron was also added to the WF in the form of ferrous sulfate (the readily acidified water soluble and intestine absorbable ferrous iron),(21). Because soybean contains digestive enzymes inhibitors, divalent cations and mineral chelators (22). According to this fact, WF blends(WF+50 or 100mg iron, WF+50g DSF with 50 or 100mg iron) were prepared as experimental diets, in addition to the unblended control diets (WF and WF+50g DSF. The determination of carbohydrates, fiber, phytate-p, ash and the metabolic energy were identified and similar values between blends of diets, these results were agreed with the diets preparation postulated by Sharaf (19); Sharaf and Thannoun (23 and 24).

In vitro and in vivo conditions as the level of iron in diet increases, the level of digestible protein % and protein digestibility index decreases in other word there was an inverse relationship between the level of iron in diet and the two parameters mentioned above, which is due to the effect of iron ion, and considered to be an proteolytic enzymes inhibitor, and also due to the consideration of DSF as divalent cations and minerals chelators, which return to the presence of anti-nutrients (especially enzymes inhibitors) in the DSF. (21 and 22). The mean value of digestible protein of WF+50g DSF diet enriched with iron was higher than that value of WF diet enriched with iron only, this may be return to the high protein contents (42.71%) of soybean flour, this results agreed with the results reported by venter (25). Higher protein digestibility index value in vitro was higher than that in vivo value of rat groups may be due to anti-nutrients, enzyme inhibitors and mineral chelating agents content of soybeans, which may affect the dietary protein and other nutrients absorption (25).

In vivo digestible protein values of healthy rats were higher than that values of the anemic rats fed the same six tested diets, which might be due to the less appetite status of the anemic rats uptake diets than that status of the healthy control rats in agreements with report observed by Sharaf and Thannoun (26, 27 and 28 and 29). The higher value of protein digestibility index of healthy rats than the anemic rats fed the same six tested diets attributed to decreasing amounts of diet intake and digestible protein.

In vivo protein digestibility index was considered to be the real practical index, while the in vitro protein digestibility index was the theoretical index.

The highest BM gain was found in the healthy rats fed WF diet supplemented with iron and DSF, these results were agreed with results obtained by Sharaf (30, 31 and 32), who stated that the body mass gain was positively proportional to the dietary weights and content of iron level. However, the anemic rat group fed the WF+50g DSF+100mg iron diet was showed another highest BM gain (40.11g). Therefore, this diet may be considered the best diet according to the BM character. The highest weights of liver were found in the anemic rats, may be due to the enlargement of liver of iron-depleted (anemic) rats, while the spleen was not affected.

The highest iron contents of liver and spleen were found in the healthy rats fed WF diet supplemented with iron and DSF, these results were agreed with the results obtained by Sharaf and Thannoun (24), this may be due to the highly presence of ferritin (iron storage protein) shell in liver and spleen, these results were agreed with the conclusion reported by others (26).

The mean values of liver and spleen weights and their iron contents of rats fed WF diet enriched with iron were lower than that mean values of the rats fed WF diet supplemented with iron and DSF; it may be due to the affect of highly quantity and quality of protein contents of DSF.

The mean value of the growth index of rat groups fed WF diet enriched with iron was lower than that value of the rats fed WF diet fortified with iron and DSF, this was due to the effect of soybean flour contains highly qualitative and quantitative protein.

CONCLUSIONS

1.In vivo protein digestibility index had been found to be the lowest value, which lower than that value of in vitro analysis.
2.The protein digestibility index of the anemic rats was higher than that value of healthy rats.
3.The growth index of the healthy rats was higher than that value of anemic rats. The growth index of both rat trails was positively proportional to the dietary iron content with limitation to 100mg maximum iron level.
4.The addition of 50g defatted soybean flour and 50mg iron to the WF improves the protein digestibility and rat growth index.

REFERENCES

1.Unicef. The Prescriber, Anemia, pub. By the United Nations children's cooperation with the international course for health managers-ISTISAN (Italy); No. 11. P.1-16 (1994).
2.Langenhoven M., Kruger M., Gouws E. and Faber M. MRC food composition Tables. Prow Medical Research council (1991).
3.Setchell K.D.R., Am J Clin Nutr. 68(suppl): 1333s-1346s (1998).
4.Song T.; Barua K.; Buseman G. and Murphy A., Am J Clin Nutr 68(suppl): 14745-14795 (1998).
5.Howe E.E.; Jansen G.R. and Gilfillan E.W. Am J Clin Nutr. 16: 315-318 (1965).
6.Ibrahim N.A., Ph. D Thesis, Faculty of Agric., Biochem. Dept. Cairo univer. P.76 (1975).
7.Kent-Jones D.M. and Amos A.J. Modern cereal chemistry, 6th ed. Food Trade Press Ltd., WC. 2. London. P. 214-217 (1967).
8.Timm K. Orbital venous anatomy of the rat. Lab Animals Sci.29: 636-642 (1979).
9.American National Academy of Sciences/Nutritional Research Council, (NAS/NRC). Nutrient requirements of laboratory animals. 3rd ed., No.10, Washington DC. p. 2-27 (1978).
10.Association of official analytical chemist, AOAC., Chem.., Washington DC., Chap. 14, cereal food, p. 129, 211 and 227 (1980).

11.Smith F.; Dubois M.; Gilles M.A.; Hmifton J.K. and Rebers P.A., Ann.Chem.28:350-352 (1956).
12.Wheeler E.L. and Ferrel R.E., Cereal Chem. 58(5): 312-322 (1971).
13.Akerton WR and Stahman MA., J. Nutr. 83: 257-261 (1964).
14.McDonald P., Edwards R.A. and Greenhalgh J.F.D. Animal nutrition. Longman pub. Co., London and New York. P. 245 (1977).
15.Schriker B.R., Miller D.D. and Staffer J.R., J Food Sci. 47: 740-746 (1982).
16.Miller D.K., Gomez-basauri J.V.; Smith V.L.; Kanner J. and Miller D.D., Food Sci. 59(4): 747-750 (1994a).
17.Miller D.K.; Smith V.L.; Kanner J.; Miller D.D. and Lawless H.T., J Food Sci. 59(4): 751-755 (1994b).
18.Dacie J.V.and Lewis S.M. Practical Hematology. London; Churchill Livingstone, P. 51-60 (1986).
19.Sharaf Kh.H., Iraqi J Vet Sci. 14(1): 155-165 (2001).
20.Steel R.G.D. and Toorie J.H. Principles and procedures of statistics. New York, McGraw-Hill book Co. Inc (1980).
21.Sharaf Kh.H. and Mustaffa N.G., J Sci. 13 (4): 35-51 (2002).
22.Robbins J. What about soy? The food revolution. 1-19.(//A:\soyabean% 20review\ 2.HTM) (2004).
23.Sharaf Kh.H. and Thannoun A.M., Iraqi J Agric Sci. 2(1): 11-18 (2001a).
24.Sharaf Kh.H. and Thannoun A.M., Iraqi J Agric Sci. 2(2): 2-8 (2001b).
25.Venter C.S., J Family Ecology and Consumer Sci. 27(1): 0378-5254 (1999).
26.Sharaf Kh.H. and Thannoun. A.M., Mesopotamia J Agric.13(2): 9-19 (1999a).
27.Sharaf. Kh.H. and Thannoun A.M., Mesopotamia J Agric. 31(4): 17-28 (1999b).
28.Sharaf Kh.H. and Thannoun A.M., Mesopotamia J Agric. 31(4): 29-40 (1999c).
29.Thannoun A.M. and Sharaf Kh.H., Iraqi J Agric Sci. 2(1): 2-10 (2001).
30.Sharaf Kh.H., Iraqi J Vet Sci.16(2): 215-227 (2002a).
31.Sharaf Kh.H., Tikrit Agric Sci J. 2(1): 1-14 (2002b).
32.Sharaf Kh.H., Iraqi J Vet Sci. 16(2): 201-214 (2002).