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EFFECTS OF ANTINUTRTIONAL FACTORS AND TOXIC ELEMENTS ON BIOLOGICAL EVALUATION OF AL-BAN (*Moringa peregrina*) SEED PROTEIN

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ABSTRACT

The present study was carried out by using Al-Ban (AL-Yasser) *Moringa peregrina* seeds in which protein based products were prepared. The protein percentages in AL-Ban defatted flour (DF), protein concentrate (PC) and protein isolate (PI) were 59.7, 67.3 and 80.0%, respectively. Anti-nutritional factors (protease inhibitors, phytic acid and tannins) were calculated. Trypsin inhibitor activities of DF, PC, and PI were 11.72, 9.74 and 6.85 inhibitor unit/mg protein, recpectively. While phytic acid levels, in the same products were 1.90, 1.92 and 1.81%, respectively. Soaking of AL-Ban seeds in water followed by boiling effectively reduced trypsin inhibitor and phytic acid levels. Arsenic (<0.30µg/g), mercury (<89µg/g) and lead (<0.25µg/g) were found in AL-Ban seeds. Mice used for biological evaluation of AL-Ban seed protein were devided in ten groups. Five groups of mice received diets containing, A: heat treated PI; B: soaked boiled (40 min.) and defatted AL-Ban seeds; C: soaked, boiled (60 min.) and defatted seeds; D: soaked, boiled (60 min.) and defatted seeds 0.4% lysine; E: whole egg (reference group). The results showed that mice in these groups (A _ E) were still alive during the experiment period, except two mice in group A. Protein efficiency ratio (PER) values for mice in group A, B, C, D and E were : -0.1, 0.19, 0.57, 1.42 and 2.39, respectively. Protein

testability values for mice in the same groups were: 65.22, 68.22, 70.36, 72. 36 and 80.96%, respectively. Liver sections of mice in group A,B,C,and D showed the presence of inflammatory cells, edema, abscess and liver cirrhosis (group A). The damage to liver tissues, weight loss and decrease in PER and protein digestibility values in mice may be due to the presence of toxic elements and anti-nutritional factors in Al-Ban seeds.

Key work: *antinutrional factors, Moringa peregrina, seed protein, toxic elements.*

1. INTRODUCTION

Moringa peregrina is a perennial plant, of the family Moringaceae found in tropical and sub-tropical zones of the world. The plant is distributed in the North and South Hijaz areas of Saudi Arabia and locally called Al-Ban or Al-Yassar (Al-Yahya, *et al.*, 1990). Al-Ban seeds are characterized by having a high content of oil (52%) and protein (29.2%) and the defatted flour of the seed has a protein content of 53.8%. The protein of Al-Ban seeds contains a fair amount of all essential amino acids, but deficient in lysine (Al-Hussain and Abu-Tarboush, 1997). Because of its good contents of oil and protein; Al-Ban seed rendered itself to further investigations in purpose of using the seed products as food ingredients. Al-Khatani and Abu-Arab (1993) prepared a concentrate (64.4% protein) and an isolate (98.9% protein) from Al-Ban seed defatted flour, and these products showed good functionality. The presence of anti-nutritional factors (*e.g.* protease inhibitors, tannins and phytates) in Al-Ban seed was confirmed by Al-Khatani (1995). Al-Hussain and Abu-Tarboush (1997) stated that protease inhibitor activities in Al-Ban seed are lower *in vitro* protein digestibility and calculated protein efficiency ratio (C-PER) values. The presence of toxic elements, (*e.g.* heavy metals) at high doses in food and feed stuffs, affects nutrient utilization and biosynthesis due to the damage caused to body organs particularly the liver (Berman, 1980; Siewicki, 1981). No data on biological evaluation of Al-Ban seed protein were reported. The aim of the present study was to evaluate the presence of anti-nutritional factors (protease inhibitors, tannins

and phytate) and toxic factors (lead, mercury and arsenic) in Al-Ban seeds, and to determine protein digestibility and PER in mice. The effect of feeding Al-Ban seed products to mice was also elaborated histologically in sections of tissues of the liver.

2. MATERIALS AND METHODS

2.1- Materials

Al-Ban seeds were obtained from Al-Ola region of northwest Saudi Arabia. The seeds were cleaned, hand cracked, dehulled, and pulverized with a Waring commercial blender and sieved to pass through 425μm mesh. Trypsin (E.C.3.4.4.4.) from bovine pancrease, N. benzoyl.DL. argnine –p- nitronlilde- HCL (BAPA), α. chymotrypsin (E.C.3.3.3.5) from bovine pancrease, bovine serum albumin, vanillin, (+) catechin and sodium phytate were obtained from Sigma Chemical Co. St. Louis, Mo. Corn starch, corn oil, whole egg, cellulose, vitamin mix., minerals mix, choline chloride, butalyated hydroxy toluene (BHT), Lysine and bentonite were obtained from ICN Pharmaceuticals, Inc.

2.2- Preparation of defatted flours, protein concentrate and protein isolate

Soaked boiled seeds were prepared by soaking Al-Ban seeds in water at room temperature for 20 hours and in boiling water for one hour. Soaked boiled seeds were dried at room temperature and milled to pass 425μm mesh. The seed flours were defatted with n-hexane for 16 hours using a soxhelt apparatus. The defatted flours were dried for 24 hours at room temperature and then ground to 200 μm particles.

Protein concentrate was prepared according to the method of Mattil (1974). Defatted flour was dispersed in 70% ethanol at a ratio of 1:10 (W/V) for 30 minutes with continuous steering at room temperature before centrifugation at 3,000 r.p.m for 10 minutes. Supernatant was discarded. The precipitate was collected, dried at room temperature, and ground into flour (200-μm particles).

The protein isolate was prepared from Al-Ban seed defatted flour according to the method of El-Tinay, *et al.*, 1988,. Defatted

flour was dispersed in water (1:10 {W/V}) and the pH adjusted to 10.0 using NaOH (1.0 N) and kept with continuous steering at room temperature for two hours, this was followed by centrifugation at 3,000 rpm for 10 minutes. The extract was adjusted to pH 4.0 by using HCL (1.0 N) and centrifuged at 3,000 rpm for 10 minutes. The supernatant was discarded. The protein curd was spread as a thin layer on glass plates, air dried, and ground into flour (200-μm particles). The protein contents (Nx 6.25) of the defatted flours, protein concentrate and protein isolate were determined according to AOAC methods (1995).

2.3- Determination of trypsin and α - chymotrypsin inhibitors activities in AL-Ban seed products

Extracts for protease inhibitor assays were prepared by using 0.05M citrate buffer (PH 4.6). The protein content of the sample extract was determined by Lowry, *et al.*, (1951) method and bovine serum albumin was used as standard.

Trypsin inhibitor activity was assayed following the method of Kakade, *et al.*, (1969) using BAPA (N-benzol-DL-arginine-p-nitro-anihide hydrochloride as substrate and trypsin solution (40μg/ml). One trypsin unit (TU) was arbitrarly defined as an increase of 0.01 absorbance unit at 410 nm in 20 minutes for 10 ml of the reaction mixture, under the condition described in this method, and the trypsin inhibitor activity as the number of trypsin units inhibited (TUI).

The method of Kakade, *et al.*, (1970) was employed for determining α - chymotrypsin inhibitor activity using 1% casein as substrate and chymotrypsin soluyion (40μg/ml). One chymotrypsin unit (CU) was arbitrarly defined as an increase of 0.01 absorbance unit at 275 nm in 10 minutes for 10 ml of reaction mixture, under the conditions described in this method, and the chymotrypsin inhibitor activity as the number of chymotrypsin units inhibited (CUI).

2-4- Tannins determination

The modified vanillin-HCL method of Price,*et al.*, (1978) was followed. Tannin contents of the samples were obtained from (+) catechin standard curve and expressed as catechin equivalent (CE).

2-5- Determination of phytic acid in AL-Ban seed products

Dried samples were extracted with HCL (0.667 N), in 1:10 w/v ratio. The sample extract was passed through a column packed with ion exchange resin (50-100 mesh Dowex 1×8). The sample eluate was used to determine phytic acid content according to the coloremetric method of Lattn and Easkin (1980), sodium phytate was used as standard.

2.6- Determination of lead, mercury and arsenic

AOAC (1995) method for wet ashing was used to prepare AL-Ban seed samples for mineral analysis. An atomic absorbtion instrument (Perkin-Elmer 1100B) was used to determine the concentration of lead, mercury and arsenic in the samples following the instructions of the instrument.

2.7- Biological evaluation of AL-Ban seed protein

2.7.1- Preparation of diets

All the diets were prepared to contain about 8% protein. The following ingredients were used as protein sources in the diets: whole egg; AL-Ban seed defatted flour; heat treated (120 °c for 40 minuted), defatted flour; AL-Ban seed protein concentrate; AL-Ban seed protein isolate; heat treated (120c for 40 minutes) protein isolate; soaked, boiled (40 minutes) seeds defatted flour; soaked, boiled (60 minute) seeds defatted flour; soaked, boiled (60 minute) seeds defatted flour supplemented with 0.4% lysine. (see table (1) for diet composition).

2.7.2- Animal bioassays

Three-week old male weanling mice of SWR strain weighing 8-10 g were obtained from animals house, Faculty of Pharmacy, King Saud University, and used in bioassays to evaluate AL-Ban seed protein. Each assay was composed of 8 mice which were housed individually in stainless steel cage in a controlled-temperature and light-room. Each diet was given to one group (8 mice). Feed and water were provided *ad libitum* for 3 weeks. Feed consumption and body weight were recorded twice weekly. Feces were collected twice a week, screened to remove feed residues, and stored in a cold room. At the end of the experiment, they were dried

in an oven at 70° C and ground to pass through a 40-mesh screen. Proteins of diet and feceas within each experiment were used to determine protein content (Nx 6.25) following the standard micro-kjeldahl method (AOAC, 1995), these protein contents values were used to calculate the apparent digestibility of the protein. Protein efficiency ratio (PER) was determined according to AOAC (1995) method.

2.7.3- Histological examination of mice liver

Liver samples were obtained from each mouse, and kept in a deepfreezer (-40°c) until use.Before staining, liver specimens were

Taple (1): Composition of experimental diets.*(grams/100g) Diets .

Ingredients	Whole egg	Defatted flour (DF)	Protein concentrate	Protein isolate	Soaked boiled (40 min) DF	Soaked, boiled (60 min) DF
Whole egg	21.05	-	-	-	-	-
Defatted flour	-	13.4	-	-	-	-
Protein concentrate	-	-	11.89	-	-	-
Protein isolate	-	-	-	10.0	-	-
Soaked, boiled (40 min) seeds defatted flour	-	-	-	-	14.29	-
Soaked, boiled (60 min) defatted flour	-	-	-	-	-	13.49
Starch	68.248	71.04	73.825	79.298	70.148	70.548
Corn Oil	3.00	3.00	3.00	3.00	3.00	3.00
Cellulose	3.00	3.00	3.00	3.00	3.00	3.00
A/N Vitamin mix.	1.00	1.00	1.00	1.00	1.00	1.00
A/N Mineral mix.	3.50	3.50	3.50	3.50	3.50	3.50
Choline Chloride	0.20	0.20	0.20	0.20	0.20	0.20
BHT	0.002	0.002	0.002	0.002	0.002	0.002
Lysine	-	-	-	-	-	0.40
Bentonite	-	4.858	3.5893	-	4.86	4.86
Total	100	100	100	100	100	100

* According to Idouriane,1993.

fixed in 10% aqeous solution of formulation for 12-24 hours. The specimens were processed overnight by autotechion which was regulated by clockwork device, the specimens passed first through increasing concentration of ethanol for dehydration then through xylene for lipid extraction and finally through several changes of hot, melted paraffin. When the paraffin was allowed to harden, the specimens were cut on a rotary microtome into 5μm sections. The specimens were stained with hematoxylin-eosin stain according to the methods of U. S. Armed Forces Institute of Pathology (Luna, 1968).

2.8- Statistical analysis

Data were statistically analysed using the analysis of variance (Steel and Torrie, 1980). The differences among the means were determined for significance at 5% level using Duncan's new mutiple range test and SAS computer programs (SAS, 1982).

3- RESULTS AND DISCUSSION

Trypsin and chymotrypsin inhibitor activities, phytic acid and tannin contents of Al-Ban seed products are shown in Table 2. Untreated and heat treated defatted flours, protein concentrate and protein isolate samples contained appreciable amounts of protease inhibitors and phytic acid. Al-Ban seed products contained negligible amount of tannins. Although heat treatment is effective in reducing protease inhibitor levels in many food plant sources (Liener and Kakade, 1980; Al-Khatani, 1995; Abu-Tarboush and Ahmed, 1996; Vidal Dal verde *et al.*, 1997). In this study only slight reduction was observed in protease inhibttor levels following heat treatment (Table 2). Zivena *et al.*, 1991 stated that cooking faba beans for two hours at 125c reduced phytic acid content to a remarkable level. The same effect of phytic acid reduction by heat treatment was found in this study. Soaking Al-Ban seeds in water for 24 hours followed by boiling for 40 or 60 minutes appeared to be the most situable means of reducing trypsin and chymotrypsin inhibitors and phytic acid content of Al-Ban seed products (Table 2).

Soaking in water followed by boiling as means of reducing anti-nutritional factors were reported by Moneam (1990) for faba beans and Onyeike, *et al.*, (1991) for African yambean. The study

confirmed the presence of some heavy metals (*i.e* arsenic, mercury and lead) in Al-Ban seeds (Table 3). The important heavy metals pollutants of foods are arsenic, lead, cadmium and mercury (Mc Layghlin *et al.*, 1999). Burtis and Ashwood (1996) reported that arsenic, lead and mercury are considered to have acute toxicity when their levels in human blood exceed 10.100 and 15μg/ml, respectively.

Arsenic is a metalloid which has cumulative effect and at high doses affects all the body organs, *e.g* necrosis to liver cells

Table (2): Trypsin and chymotrypsin inhibitor activities, Tannins and phytic acid contents in Al-Ban seeds. *

Al-Ban seed products	Trypsin inhibitior (Units/mg) protein	Chymotrypsin inhibitior (Units/mg) protein	Tannins (CE) %	Phytic acid % (dry weight)
Defatted flour	11.73A ± 0.13	6.17A ± 0.00	0.0124B ±0.00	1.90A±0.001
Defatted flour (120° c, 40 min)	11.05B±0.11	5.96A+0.03	0.0129B±0.00	1.82B±0.01
Protein concentrate	9.74C±0.14	4.70B±47	0.013B+0.00	1.92A+0.00
Protein concentrate (120°c, 40min)	8.31D±0.38	2.79D±0.54	0.01C±0.00	1.84B±0.02
Protein isolate	6.85E±0.04	3.00 C±0.04	0.0176A±0.00	1.08C±0.01
Protein isolate (120° c, 40 min)	6.52E±0.15	2.82C±0.15	0.018A±0.00	0.72D±0.27
Soaked, boiled (40 min) seeds defatted flour	1.61F±0.04	ND	ND	0.26E±0.01
Soaked, boiled (60 min) seeds defatted flour	3.65G±0.01	ND	ND	0.23E±0.001
Soaked, boiled (60 min) seeds defatted flour + 0.4 lysine	2.52H±0.02	ND	ND	0.30G±0.00

* Mean ± standard error CE: Catechin equivalent ND: Not determined Different letters in the same column (A-H) mean significant difference (P≤ 0.05)

Table (3): Lead, mercury and arsenic concentration in Al-Ban seeds.

Metal	Concentration □g/g
Lead	< 0.25
Mercury	< 89
Arsenic	< 0.30

(Berman, 1980) and hypertrophy of liver and spleen in rats (Siewicki, 1981).

Table 4 shows some biological parameters in mice fed diets containing Al-Ban seed products. Five mice groups exhibited total mortality. These groups were fed on diets containing Al-Ban seed defatted flour (either untreated or heat treated), protein concentrate (untreated and heat treated) and untreated protein isolate. Idouraine(1993) stated that feeding untreated tepary beans to mice resulted in mice death within 10 days. Mortality rate was reduced in the group of mice which received diet containing heat treated protein isolate, six mice from this group remained alive to the end of the experiment, although these mice showed weight loss and weakness of the body. Heat treatment in this case could not prevent these adverse signs, but its significant effect was the reduction of mortality rate.

Mice fed on diets containing soaked and boiled Al-Ban seeds (boiling to 40 or 60 minues) and mice received whole egg diet no death occurred among the individuals of these groups. This could be attributed to the effects of soaking and boiling Al-Ban seeds in reducing the levels of anti-nutritional toxic element, the reduction of anti-nutrient levels by soaking and soaking was reported by Moneam (1990) for faba bean and Idouraire (1993) for teparybean.

There were remarkable significant differences in feed and protein intake values between the groups of mice fed on whole egg diet and other mice groups fed on diets containing Al-Ban seed products. Jenkins and Mitchell(1989)stated that the reduction in feed or protein intake in rats was due to the nature of the dietary protein, and to the imbalance or deficiency of amino acids in the protein, Palatability of the diet also affects feed and protein intake by affecting animal appetite. Weight gain values (Table 4) were significantly different among experimental diets, and this may be due to difference in protein intake. Supplementing the diet with lysine resulted in an increased weight gain value (Table 4), lysine is the limiting amino acid in Al-Ban seed protein (Al-Hussain and Abu-Tarboush, 1997). Leclrec, (1990) stated that supplementing a rat diet with methionine resulted in improving feed intake and weight gain values.

Table(4):Biological evaluation of AL-Ban seed protein. *

Source of protein	Feed intake (g)/21 days	Protein intake (g)/21 days	Weight change (g)/21 days	PER	Digestibility(%)	Mortality per 8 mice
Whole egg	84.84A±3.38	8.31A±0.33	19.86A±0.89	2.39A±0.08	80.96A±0.73	0
Defatted flour	ND**	ND	ND	ND	ND	8
Defatted flour (120°c, 40min.)	ND	ND	ND	ND	ND	8
Protein concentrate	ND	ND	ND	ND	ND	8
Protein concentrate (120° c, 40 min.)	ND	ND	ND	ND	ND	8
Protein isolate	ND	ND	ND	ND	ND	8
Protein isolate (120°c, 40 min.)	51.36C±1.00	3.39D±0.07	-3.46E±0.14	-1.00E±0.03	565.52D±0.68	2
Soaked, boiled (40 min.) seed defatted flour	53.40C±1.83	3.85C±0.18	-0.46D±0.24	-0.18D±0.07	68.96C±1.13	0
Soaked, boiled (60 min) seed defatted flour	55.58C±1.83	4.39C±0.15	2.50C ±0.14	0.57C±0.03	70.62CB±0.50	0
Soaked, boiled (60 min) seed defatted + 0.4 lysine	66.28B±2.34	5.50B+0.19	7.80B±0.34	1.42B±0.02	72.36B±1.27	0

* mean ± standard error (N=8)

** Not determined, different letters in the same column (A-E) mean significant differences ($P \leq 0.05$).

Values of PER (Table 4) showed significant differences between mice groups fed on whole egg diet and other groups fed on AL-Ban seed products. This may be due to the differences in protein intake or to the deficiency of AL-Ban seed protein in some essential amino acids compared to whole egg.

Apparent digestibility values in mice fed diets containing AL-Ban seed products (Table 4) were in the range 65.52 -73.36% . Cossack and Weber (1983) found that protein digestibility in mice fed diets containing varieties of common beans (*Phaseolus vulgaris*) had a mean value of 66.63%.

Soaking AL-Ban seed in water followed by boiling was found to be the most effective treatment in reducing anti-nutritional factor levels (Table 2) and improve biological parameters of AL-Ban seed protein. Idouraine, (1993) found that soaking tepary beans followed

by cooking, resulted in reducing anti-nutritional factor levels and improving PER and protein digestibility values in mice.

Table 5 and Figures 1,2,3 illustrate the diagnosis parameters and microscopic examinations of mice liver tissues. These groups of mice were fed diets containing whole egg and AL-Ban seed products and the mice of these groups remained alive up to the end of the experiment.

No negative signs were seen of liver tissues of mice receiving whole egg diet. Diagnostic parameters (Table 5) and microscopic examination. (Figure 1) revealed normal liver tissues and lobes distribution. Table 5 and Figure 2 showed some abnormal signs in mice liver tissues. These mice were fed diet containing heat-treated protein isolate. Appearance of liver cirrhosis was seen in liver tissues of this group. Infilteration of lymphocytes on portal tracts and liver abscess were major signs in liver tissues of mice fed diet containing AL-Ban seeds soaked boiled for 40 minutes (Table 2, Figure 3). Small numbers of negative signs were found in liver tissues of mice fed on diets containing AL-Ban seed soaked and boiled (60 min.) defatted flour.These signs (Table 5)were slight dilatation of central veins and scanty lymhocytes infiltration in portal tracts.

CONCLUSION

The presence of anti-nutritional factors (particularly protease inhibitors and phytic acid) and the presence of toxic elements may be the major cause of lowering the biological value of AL-Ban seed protein. Treatments such as heating, soaking in water then boiling and lysine supplementation, have positive effects on the nutritional parameters of the proteins. Although these treatments did not prevent completely the reduction in PER and protein digestibility values and also did not prevent the occurrence of negative signs in liver tissues. It could be said that the presence of heavy metals and other anti-nutritional factors limit the use of AL-Ban seeds as animal feed and in food applications. The study recommends the determination of arsenic and other heavy metals in other samples of AL-Ban seeds from different areas. Also the presence of other anti-nutritional factors (e.g. Saponins and lectins) in AL-Ban seeds may be investigated.

Table 5: Diagnosis of liver tissues.

Parameter	Mice groups (Diets)				
	Whole egg	**Protein Isolate**	**Soaked, boiled (40min) seed DF***	**Soaked, boiled (60min) seed DF**	**Soaked boiled (60min) seed DF+lysine**
Liver architecture	preserved	Some disturbance	preserved	preserved	preserved
Microsteatosis	-ve**	-ve	+ve**	-ve	-ve
Ballooning degeneration	-ve	+ve (focal)	+ve (focal)	-ve	+ve (focal)
Individual liver cell necrosis	-ve	+ve	-ve	+ve	+ve
Central veins	normal	dilated	Considerable dilatation	Considerable dilatation	Some dilatation
Sinusoids	normal	Slight dilatation	Slight dilatation	Slight dilatation	Slight dilatation
Portal tract	normal	Infiltration with small number of lymphocytes	Infiltration with small number of lymphocytes	Infiltration with small number of lymphocytes	Infiltration with small number of lymphocytes
Lobuler infiltration Lymphocytes and Neutrophils	-ve	-ve	Small number of lymphocytes and Neutrophils	-ve	-ve
Liver abscess	-ve	-ve	+ve (single)	-ve	-ve
Regenerated nodules (cirrhosis)	-ve	+ve (single)	-ve	-ve	-ve

* DF: Defatted flour.

** -ve and +ve not found and found, respectively.

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تأثيرات مضادات التغذية والعناصر السامة على التقييم الحيوى لبروتين بذرة البان

أمل عبدالله الحسين – عبدالله عبدالرحمن العثمان

قسم علوم الأغذية والتغذية – كليةالزراعة– جامعة الملك سعود– الرياض – المملكة العربية السعودية

ملخص

أجريت هذه الدراسة على بذور البان (اليسر). حيث تم تحضير عدد من منتجات البروتين من بذورها. كانت النسبة المئوية للبروتين فى دقيق بذور البان منزوع الدهن, مركز البروتين ومعزول البروتين ٥٩,٧, ٦٧,٣ و ٨٠,٠% على التوالى. كانت مضادات التغذية التى تمت دراستها هى مثبطات الانزيمات الهاضمة للبروتينات, حمض الفايتيك والتانين. كان نشاط مثبط إنزيم التربسين لدقيق البان منزوع الدهن ومركز البروتين ومعزول البروتين ١١,٧٢, ٩,٧٤ و ٦,٨٥ وحدة نشاط مثبط/ ملجرام بروتين. فى حين كانت أن مستويات حمض الفايتيك لنفس هذه المنتجات كانت ١,٩٠, ١,٩٢ و ١,٨١% على التوالى. وجد ان غمر بذور البان فى الماء ثم الغليان يخفض بكفاءة من مستويات إنزيم التربسين وحمض الفايتيك. وجدت فى بذور البان معادن الزرنيخ (<0.25µg/g)و الزئبق (<89µg/g) والرصاص (<0.25µg/g)و (<0.30).

التربسين وحمض الفايتيك. وجدت فى بذور البان معادن الزرنيخ (<0.30(<0.25µg/g)و الزئبق (<89µg/g) والرصاص (<0.25µg/g). قسمت الفئران التى أستخدمت فى التقييم الحيوى لبروتين بذرة البان إلى ١٠ مجموعات, خمسة مجموعات من هذه الفئران تناولت علائق إحتوت على مصدر بروتين عبارة عن دقيق منزوع الدهن, دقيق منزوع الدهن معامل بالحرارة (١٢٠م لمدة ٤٠ دقيقة), مركز البروتين, مركز البروتين المعامل بالحرارة ومعزول البروتين. ماتت كل الفئران فى المجموعات المذكورة خلال ٥-١٥ يوم من زمن التجربة (٢١ يوم). تناولت مجموعات الفئران الخمسة الأخرى علائق تشمل , (أ): معزول البروتين المعامل بالحرارة, (ب): بذور البان المنقوعة والمغلية لمدة ٤٠ دقيقة ومنزوعة الدهن, (ج): بذور البان المنقوعة والمغلية لمدة ٦٠ دقيقة ومنزوعة الدهن, (د): بذور البان المنقوعةوالمغلية لمدة ٦٠ دقيقة ومنزوعة الدهن ومدعمة باللايسين (٠,٤%), (هـ): البيض الكامل (كمجموعة مرجعية).

لم تمت الفئران فى المجموعات من (أ) إلى (هـ) أثناء فترة التجربة ماعدا فأرين من المجموعة (أ). كانت نسبة كفاءةالبروتين (PER) للفئران فى المجموعات أ, ب, ج, د, هـ على التوالى -٠,٠١, ٠,١٩, ٠,٧٥, ١,٤٢ و ٢,٣٩. وكانت قيم قابلية الهضم للبروتين فى الفئران لنفس هذه المجموعات ٦٥,٢٢, ٦٨,٢٢, ٧٠,٣٦, ٧٢,٣٦, ٨٠,٩٦% كانت على التوالى. أظهرت شرائح الكبد فى الفئران للمجموعات أ, ب, ج, د وجود خلايا التهابية وخراريج كما وجد تليف للكبد فى المجموعة (أ). يرجح التلف الذى حدث لأنسجة الكبد وفقدان الوزن والإنخفاض فى قيم PER وقابلية الهضم فى الفئران لوجود العناصر السامة ومضادات التغذية فى بذور البان.
