

EFFECT OF FEEDING WITH MIXTURE OF COLOURD RICE ON SERUM LIPID PROFILE AND ANTIOXIDANT STATUS IN EXPERIMENTAL RATS

Saleh, M. N.* ; M. A. Elbana* ; M. S. Goudaand S. M. Metwalli****

***Food Technology Research Institute. Agric. Res. Cent., Giza**

***Food Science and Technology Dept., Fac. of Agric., Kafrelsheikh Univ.**

ABSTRACT

The present investigation was carried out to study the influence effect of colourd rice mixture on serum lipid parameters and hepatic enzyme activities in rats and effect of protein, total lipids, crude fiber and ash of rice samples. The data pointed to the following results:

Milling processes led to decrease the percentage of protein, total lipids, crude fiber and ash of rice samples. Whereas it was increased the carbohydrate content in white rice samples.

The total phenolic compound content (TPC) of Brown rice varieties had the highest value (1.6 and 1.1 mg GAE/g) of Black and Giza 178, respectively, whilst milled rice varieties had the lowest ones (0.8 and 0.5 mg GAE/g).

Antioxidant activity was determined in brown black, white black, brown and white Giza 178 extracts. The present study cleared that brown black extract had the highest the antioxidant activity among the investigated extracts followed by brown Giza 178, white black and white Giza 178 extracts.

There are nine phenolic acids where identified. Ferulic acid was the major phenolic compound in all rice samples. Furthermore, the highest concentration of ferulic acid that found in brown black rice (4.82 mg/100g).

Results of the biological experiment revealed that, the final body weight and weight gain of rats fed on mixture of brown rice and black rice were significantly lower, but food intake was higher compared with the rats fed on basal diet at the end of the experimental period (8 weeks),

As for total Cholesterol (T.C), Low density lipoprotein cholesterol (LDL-C) and Plasma Triglyceride (T.G) of rats fed on white rice diet were higher than other groups. Furthermore, HDL-C significantly higher in rats fed on mixture of (white rice + black rice) and (brown rice + black rice) diets compared to those fed on white rice and mixture of white rice and brown rice ones.

The antioxidant levels in the rat livers ranged from highest in those fed on (brown rice + black rice) diet, followed by white rice + black rice then white rice + brown rice. Whilst, rice had the lowest level.

Finally, it can be concluded that, using brown and black rice have the pronounced effect for lowering cholesterol levels of the blood in experimental rats.

INTRODUCTION

Rice (*Oryza Sativa L.*) is among the oldest of cultivated crops and ranks as the most widely grown food grain crop, serving as the staple food for about half the world's population. World rice production increased from 520 million tonnes in 1990 to 605 million tonnes in 2004, while Iran's rice production increased from 1.3 million tonnes in 1980 to 3.4 million tonnes in 2004 (Faostat, 2005). For paddy rice, when the husk is removed, the grains

obtained are called brown rice due to the brown – color bran that covers the grain . White rice is obtained when the bran is removed in the regular milling process. Brown rice is nutritionally superior to white rice. Whereas, it has higher percentages of all nutrients except carbohydrate (Kennedy, 1980).

Black rice pigments are mainly located in the aleurone layer, which is characterized as dark purple to black in color and probably represents a mixture of anthocyanins. Most dietary intake of anthocyanins comes from fruits and vegetables (Clifford 2000).

Similar to cereal grains, rice is rich in many nutrient components including carbohydrates, proteins, certain fatty acids and micronutrients (vitamins and trace minerals). They are also sources of many bioactive non-nutrient compounds, known as antioxidant, including phenolic compounds (Frei and Becker, 2004).

Rice is a rich source of many bioactive compounds including phenolic antioxidants that have the potential to reduce the risk of disease, such as inhibiting platelet aggregation (Daniel *et al* 1999), reducing the risk of coronary heart disease and cancer (Martinez-Valverde *et al* 2000 and Newmark, 1996), and preventing oxidative damage of lipid and low-density lipoproteins (Morton *et al* , 2000).

A number of studies have shown the importance of antioxidants, including vitamin E and the antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), in protecting animals against the injuries due to oxidative stress, as well as the effect of an enhanced level of antioxidants in ameliorating the oxidant tissue injury (Stephens *et al*. 1996).

Genest (1986) and Kempner (1946) reported that consumption of white rice decreased blood pressure and lowered hypercholesterolemia in humans.

The present work done to study the influence effect of colour rice mixture on serum lipid parameters and hepatic enzyme activities in rats. Milling processes lowerd the percentage of protein, total lipids, crude fiber and ash of rice samples. Whereas it was increased the carbohydrate content in white rice samples.

MATERIALS AND METHODS

Materials:

Tow varieties of rice (*Oryza sativa* L.) namely black and Giza 178 were employed in this study. These samples were obtained from Rice Research and Training Center (RRTC) at Sakha KafrelSheikh Governorate, Egypt during the season of 2008,that under cultivated the recommended conditions for date of culture, fertilization, harvesting time and irrigation.

METHODS:

Preparation of rice samples:

Raw rice samples were dehulled to obtained the brown rice. The brown rice was divided into two parts, the first one was used as brown rice, where the second was milled to obtained the white rice. The brown and

white rice were kept in polyethylene bags and stored in freezer at -18°C until further analysis.

Gross chemical composition of rice samples:

Moisture, ash, crude protein, ether extract and crude fiber contents were determined according to the methods of A.O.A.C. (2005). Total carbohydrates content was calculated by subtracting protein, ash, total lipid and crud fiber from total mass of 100 as reported by A.O.A.C. (2005).

Total Polyphenols and antioxidant activity:

Extraction of total polyphenol content from rice samples:

Phenolic compounds were extracted using the method of Nara *et al.* (2006). Rice samples (2 g) was extracted twice with 80% methanol at a ratio of 1:20 (w/v). Each time, the mixture was kept on a mechanical shaker for 1 h at room temperature. After centrifuging at 4000 rpm for 5 min, the supernatants obtained from each time were combined and concentrated to dryness by using a rotary evaporator at 35°C. The dried methanol extract was redissolved in 5 mL of 50% methanol and used as crude extracts.

Determination of total phenolic content (TPC):

Total phenolic content presented in the sample extracts were determined spectrophotometrically using foline-Ciocalteu reagent according to the method described by Bonoli *et al* (2004).

Determination of DPPH radical scavenging activity:

This assay was based on the method of *Brand-Williams et al.* (1995) as modified by *Li et al.* (2007). Briefly, a 200 µL of appropriately diluted crude extract (or fractions) was added to 3.8 mL of freshly made DPPH radical solution (60 µM). After 60 min of incubation at room temperature, the absorbance at 515 nm was measured. DPPH free radical scavenging activities in crude methanol extracts and different fractions were expressed as µmol of trolox equivalents (TE) per 100 g of rice (dry weight basis).

Qualitative and quantitative determination of phenolic compounds by high performance liquid chromatography (HPLC):

A Hewlett- Packard Series 1,100 liquid chromatographic system (Waldbronn, Germany) loop 20 µl equipped with a diode array detector and a lichrosorb RP 18 Column (40 mm id × 250 mm ; partical size 5µm) (Merck, Darmstadt Germany) was used . Eluton was proformed at a flow rate of 1.0 ml/ min with mobil phase of water/ acetic acid (98; 2, v/v, solvent A) and A methanol / acetonitril (50: 50, v/v solvent B), starting with 5% B and increasing B to levels of 30 % at 25 % min, 40 % at 35 min., 52% at 40 min , 70 % at 50 min, 100 % at 55 min, and kept at this stage for 5 min. A re-equilibration time of 15 min was then required quantitation was achieved at 280 nm by internal standard method (*Ben-Hammouda et al., 1995*)

Biological evaluation:

Experimental animals and diets:

Thirteen rats of young male Albino rats, with an average weight of 99-101 gm were used. All animals were housed individually in cages with screen bottoms and fed on a basal diet for 7 days under laboratory conditions. Rats were given free access to food and water throughout the experimental period of 8 weeks.

The rats were weighted every week. Fed intake was closely monitored, an exact record of feed spillage was impossible to make due to the rats constant digging and scattering of the food. At the end of the experimental, weight gain and food efficiency ratio (calculated as gm of weight gain/gm of foods intake) were calculated for all rats. After acclimation, rats were randomly divided into 5 groups (each of 6 rats). The Experimental diets were prepared using a modified American institute of nutrition – 93 growth diet described by Reeves 1997.

Table (1): Composition of basal diet (control) and experimental diets (g/ kg).

Ingredient	Experimented diets				
	basal diet (control)	WH	WH:BR	WH:BL	BR: BL
Starch	500	-	-	-	-
Rice Powder	-	500	500	500	500
Casein	150	150	150	150	150
Sucrose	150	150	150	150	150
Oil	100	100	100	100	100
Cellulose	50	50	50	50	50
Mineral Mix	35	35	35	35	35
Vitamin Mix	10	10	10	10	10
DL-Methionin	3	3	3	3	3
Choline bitartrate	2	2	2	2	2

WH Diet: White Rice being 100 % Rice Powder.

WH: BR Diet: white rice being 333.35g and brown rice 166.65 g powder.

WH: BL Diet: white rice being 333.35g and black rice 166.65 g powder.

BR: BL Diet: brown rice being 333.35g and black rice 166.65 g powder.

Blood sampling:

In all the previously mentioned groups, blood samples were taken at the end of the experiment. The blood samples were collected after 12 hours fasting from Vein plexus eye into dry clean centrifuge tubes. The blood was centrifuged for 10 minutes at 3000 r.p.m. to separate the serum, it was carefully aspirated and transferred into clean quite plastic tubes. Then kept frozen at (-18 °C) until biochemical analysis (El-Khamissy, 2005).

Collection of organs:

All rats were scarified. The abdomen was opened, and the organs were separated by carefully dissection, cleaned from the adhesive matter. Then washed with running water, then weighted. The relative weight of the organs was calculated following the next equation:

$$\text{Relative weight} = \frac{\text{Organ weight}}{\text{Animal weight}} \times 100$$

Determination of serum lipids:

Triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C) concentrations were measured by enzymatic-colorimetc procedures using commercial available kits. Triglyceride was determined according to the method of Fossati and Prancipe (1982). Total cholesterol (TC) was carried out following the method of Richmond (1973). High-density lipoprotein cholesterol (HDL-C) was performed using precipitating reagent

according to the method described by Richmond(1973). Low-density lipoprotein cholesterol concentration was calculated as the difference between total and HDL-cholesterol according to the method of Freidwald *et al.* (1972). Phospholipids content was estimated by the method of Zilversmit and Davis (1950).

Preparation of liver tissue for measurement antioxidant activity

The liver tissues were rapidly removed, blotted dry, weighted, frozen in liquid nitrogen. Then stored at -80°C for antioxidant enzyme activity analyses. Before analyses, the liver tissue was homogenized in 10 volumes of a 50-mM phosphate buffer (pH 7.4) on ice for 30 s using a polytron homogenizer. The homogenate was transferred into centrifuge tubes and centrifuged at $9000 \times g$ at 4°C for 20 min. The supernatant was used for the measurement of antioxidant enzyme activities by the method of prased *et al.*1992.

Measurement of Hepatic Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was measured using the commercial Oxis kit. The method is based on the SOD-mediated increase in the rate of autoxidation of 5,6,6a,11b-tetrahydro-3,9,10-trihydroxy benzo [c] fluorine in an aqueous alkaline solution to yield a chromophore with maximum absorbance at 525 nm as outlined by Oyanatui (1984)

Measurement of Hepatic Catalase Activity:

Catalase (CAT) activity was measured using the commercial Oxis kit. The method is based on the disappearance rate of H_2O_2 with absorbance at 240 nm. (Oyanatui ,1984.)

Measurement of Hepatic GPx:

The GPx activity was measured using the commercial Oxis kit. Measurement of GPx activity assay is based on direct measurement of the activity of c-GPx. Oxidized GSH, produced upon reduction of organic peroxide by c-GPx, is recycled to its reduced state by the enzyme GSH reductase. The oxidation of NADPH to NADP^+ is accompanied by a decrease in absorbance at 340 nm, providing a spectrophotometric means for monitoring GPx enzyme activity as outlined by Oyanatui (1984).

Statistical analysis:

Most of the received data were analyzed statistically using the analysis of variance and the means were further tested using the least significant difference test (LSD) as outlined by Stell and Torrie (1980).

RESULTS AND DISCUSSION

Gross chemical composition of some rice varieties:

Data presented in Table (2) showed that, the moisture content of brown and white rice varieties ranged between 11.61 to 13.23 %. These values are in lien with those of Perez, (1993) and Dharmaputra, (1997). Apparent also moisture content of brown rice varieties were lower than those of white rice. Amorim *et al.* (2004) reported that, moisture content plays a great role during the storage of rice. From the same table, it could be observed that , brown rice of *Black rice* variety contains a relatively high

level of crude protein content (7.85%) while, white Giza 178 variety had the lowest level of crude protein content (6.9%).

These results revealed that, there were high significant differences in total lipids between brown and white rice of the same variety, also between the different varieties. The black rice variety had the highest total lipids content 2.31% for brown and 1.23% for white. Siebenmorgen and Sun (1994) and Pal *et al.* (1999) reported that surface fat content was inversely related to the degree of milling.

Table (2): Chemical composition (%) of some rice varieties.

Rice variety	Treatment	Moisture	Crude protein	Ether extract	Ash	Crude fiber	*Total carbohydrate
Black Rice	Brown	11.61d	7.85a	2.31a	1.65a	2.11a	86.08d
	White	12.3c	7.1b	1.23b	1.12bc	1.00c	89.55b
Giza 178	Brown	12.56b	7.71a	2.20a	1.39b	1.62b	87.08c
	White	13.23a	6.9c	0.87c	0.82c	0.64d	90.77a

Each value was an average of three determination

Values followed by the same letter in column are not significantly different at P < 0.05

*Total carbohydrate was calculated by difference.

High significant differences in ash content were recorded between the varieties as well as between brown and white rice in the same variety. The black variety contained the highest ash content (1.65 and 1.12%) for brown and white rice, respectively. Amorim *et al.* (2004) found that, the ash content in the rice was 0.4%.

In the same table showed that, the white rice variety of Giza 178 had the highest carbohydrates content in comparing with the other tested samples. In addition, carbohydrates content of rice samples were increased as a result of milling. These results may be due to the removal of the embryo and bran layer, to yield milled rice poor in fat, crude protein, fiber and ash. So, the level of available carbohydrates will be higher in milled rice than in brown rice (Singh *et al.*, 2000 and Suwansri and Meullenet, 2004)

Antioxidants from rice samples:

Total phenolic content

Phenolic compounds are common natural antioxidants in plant seeds and protect plant seeds from oxidative reaction. Table (3) shows the total phenolic content in the methanolic extracts of Brown black, white black compared to the content of brown and white of Giza 178 rice variety. The total phenolic compound content (TPC) of Brown rice varieties had the highest value (1.6 and 1.1 mg GAE/g) of Black and Giza 178, respectively, whilst milled rice varieties had the lowest ones (0.8 and 0.5 mg GAE/g).Whereas phenolic compounds stored in the out bran layer and hull, they removed during milling process. Similar results were obtained by Butsat and Siriamornpun (2010) and Qiu *et al* (2010).

Table (3): Total phenolic content of crude methanol extract from rice samples.

Variety	Treatment	TP (mg GAE/g)
Black	Brown	1.6a
	White	0.8c
Giza 178	Brown	1.1b
	white	0.5d

Each value was an average of three determinations

Values followed by the same letter in column are not significantly different $P < 0.05$

T.P = Total phenolic compounds

GAE= galic acid

Antioxidant activity (DPPH) of crude methanol extracts from rice samples.

The free radical scavenging of the extracts of rice samples were evaluated using the DPPH method and the results were presented in Table (4). The DPPH radical scavenging of Brown Black, White Black, Brown Giza 178 and White Giza 178 rice samples compared to BHA and TBHQ in concentration of 400 $\mu\text{g/ml}$ were 62.3, 50.3, 56.7, 44.6, 98.4 and 98.8 $\mu\text{g/ml}$. The varied radical scavenging activity of the extracts depended on the amount of total phenolic in each rice samples. This finding supports the data previously reported in a study where the antioxidant activity was dependent on the actual composition of milling fraction (Liyana-Pathirana and Shahidi 2007). In addition, all rice samples showed the ability to scavenge the DPPH radical at a rate lower than BHA and TBHQ at the all concentration. These results are in the same trend of those reported elsewhere (Butsat and Siriamornpun, 2010; Tananuwong and Tewaruth, 2010 and Qiu *et al*, 2010).

Table (4): Antioxidant activity by (DPPH) assays of crude methanol extract from rice samples.

samples	treatment	DPPH Scavenging			
		50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	200 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$
Black	Brown	32.08d	39.9c	50.4b	62.3a
	White	26.1d	29.9c	39.6b	50.3a
Giza 178	Brown	31.6d	38.3c	45.1b	56.7a
	white	22.0d	28.03c	35.2b	44.6a
BHA		50.3d	81.2c	90.4b	98.4a
TBHQ		75d	86.4c	93.1b	98.8a

Each value was an average of three determinations

Values followed by the same letter in row are not significantly different $P < 0.05$

BHA = Butylated hydroxyanisol

TBHQ=Tertiary butylhydroquinone

HPLC analysis of phenolic compounds extracted from rice samples:

The aforementioned set of experiments relevant to the antioxidant efficiency of phenolic compounds extracted from brown black, white black, brown Giza 178 and white Giza 178 rice varieties demonstrated that the phenolic compounds possessed remarkable antioxidant activity. Therefore, it

is quite necessary to characterize the phenolic compounds of total polyphenols.

High performance liquid chromatography (HPLC) was used for the qualitative and quantitative determination of total polyphenols. The results in table (5) indicated that, ferulic acid was the major phenolic compound identified in all rice samples. These results are consistent with finding of Tian *et al* (2004), who reported that in rice, ferulic acid and p-coumaric acid are the major phenolic compounds and exist in the free form or the insoluble bound form which is found in dietary fiber .The highest concentration of ferulic acid found in brown black rice was (4.82 mg/100g).Ferulic acid and syringic acid were the most dominant phenolic acids in white black rice, with concentration of 2.1 and 1.21 mg /100g, respectively. However, some phenolic acids as p-hydroxybenzoic acid and vanillic acids were not detected in brown and white rice (Giza178). Furthermore, the concentration of individual phenolic acid was higher in brown rice than milled rice. These results are in the same trend of those reported elsewhere (Zhou *et al.* 2004, Butsat and Siriamornpun 2010 and Vichapong *et al.* 2010).

Table (5): Phenolic compounds in rice samples:

Phenolic Compound	Amount of phenolic compounds in rice samples (mg / 100 g on dry weight)			
	Black		Giza 178	
	Brown	White	Brown	White
Galic acide	2.11	1.0	0.25	ND
Protocatethuic acide	2.27	1.01	0.07	ND
P.hydroxybenzoic acid	0.55	0.22	ND	ND
Vanillic acid	1,21	0.69	ND	ND
Caffeic acid	1.62	1.02	0.39	ND
Syringic acid	2.92	1.21	0.33	ND
P-coumaric acid	1.11	0.65	0.22	0.06
Ferulic acid	4.82	2.1ss	1.08	1.10
Guaiaicol	0.42	0.38	0.10	0.11

ND = Not Detected

Biological Evaluation of rice samples.

Body weight gain, food intake and feed efficiency ratio (FER) of rats fed on different rice diets:

Data in table (6) indicated that, the mean values of initial body weight of all groups were similar, it was ranged from 99.50 to 101.25 gm. Appeared also from the same table that, the rats fed on control diet had a higher final body weight. Comparing to with the rats fed on white rice diet, the final body weight and weight gain of rats fed on mixture of brown rice and black rice were significantly lower, but food intake was higher. Similar results were mentioned by kim *et al* (2006).They reported that transit time which means time to appearance of the first colored fecal pellet was shorter in black rice than brown and white rice. Furthermore, the reduction in transit time caused weight loss in rats and reduction in blood concentrations of total cholesterol (TC) and total triglycrides (TG) during the experiment. Accordingly , the highest feed efficiency ratio (FER) was seen in rats fed on control and white rice, but the lowest (FER) showed in rats fed on mixture of

(brown rice + black rice) in addition, (white rice + brown rice) and (white rice +black rice) had the same level of (FER). These results agree with those obtained by Read (1986), who reported that, absorption of food depends on the rate at which nutrients can be taken up from the lumen and the length of time nutrients are in contact with the absorptive epithelium. Furthermore, absorption of fat is said to be more influenced by changed in transit time than that of carbohydrates and proteins.

Table (6): Body weight gain, food intake and feed efficiency ratio (FER) of rats fed on different rice diets.

Test parameters Dietary Groups	Initial weight gain (gm)	Final weight (gm)	Weight gain		Food intake (8weeks)	Feed efficiency ratio (FER)
			(gm)	(%)		
Control	101.25a	164.45a	63.20a	38.43	720.30d	0.087a
WH	100.00a	159.20b	59.20ab	37.18	733.50cd	0.081ab
WH:BR	100.50a	156.80b	56.30b	35.90	765.00b	0.073c
WH:BL	100.25a	153.95c	53.70c	34.88	747.00c	0.072c
BR:BL	99.50a	151.80c	52.30c	34.45	805.90a	0.065d

Each value is an average of sex determination

Values followed by the same letter in column are not significantly different $p < 0.05$

Control=Basal diet content 100 starch

WH= Diet contain white rice 100% instead of starch in basal diet

WH: BR= Diet contain White rice: Brown rice at ratio 2:1 instead of starch in basal diet.

WH: BL= Diet contain White rice: Black rice at ratio 2:1 instead of starch in basal diet.

BR: BL= Diet contain Brown rice: Black rice at ratio 2:1 instead of starch in basal diet.

Effect of rats different diets on relative organ Weight (liver, kidney and spleem) in rats.

It is apparent from table (7) that, the relative liver weight of rats fed on diets containing different mixture of (brown rice + black rice) was relatively higher than that of other groups. The liver weight ranged between 3.31 to 2.94 gm at the end of the feeding priod (8 weeks) on diets contain different rice. Concerning liver, kideny and spleem weight, the percentage showed high significant changes in the relative weight by all different rice diets.

Surem lipid concentration in rats fed on different rice diets:

The lipid profiles in the serum of the 5 rat groups are presented in table (8). Total Cholesterol (T.C), Low density liboprotien cholesterol (LDL-C) and Plasma Triglyceride (T.G) in rats fed on the white rice were higher than other tested groups. The obtained results are on agreement with those obtained by Rubmini and Raghuram (1991), who reported that the beneficial influence of brown and black rice appeared to due to fiber and rice bran oil. Furthermore, rice bran oil contains oleic, linoleic and linolenic acid as unsaturated fatty acids and palmitic and stearic acids as saturated fatty acids. In addition, rice bran oil contains unsaponifiable materials including tocopherols, oryzanol, phytostrols, tocotrienols and squalene. A number of studies in humans and animals revealed that rice brain oil (RBO) consumption lower serum total T.G, T.C and LDL-C and increases plasma HDL-C concentrations (Nicolosi *et al* 1991, Purushothama *et al* 1995). In addition, pigmented rice varieties with high antioxidative activities provide a

source of antioxidants and are very efficient in reducing low density lipoprotein and total serum cholesterol (Zigoneanu *et al* 2008, Kim *et al* 2008).

HDL-C is considered to be good cholesterol in the circulation (stein and stein 1999). In this relation, apparent also from the same tables (8) that HDL-C was significantly higher in rats fed on mixture of (white rice + black rice) and (brown rice + black rice) diets compared to those fed on white rice and mixture of white rice and brown rice diets.

Table (7): Effect of different diets on relative organ Wight (liver, kidney and spleen) in rats.

Dietary Groups	Organs			
		Liver	kidney	spleen
Control	(gm)	3.0b	0.91a	0.39a
	(R.W.G)	1.82	0.55	0.24
WH	(gm)	3.19a	0.85ab	0.33bc
	(R.W.G)	2.0	0.53	0.21
WH+BR	(gm)	2.94c	0.81b	0.37b
	(R.W.G)	1.09	0.52	0.24
WH+BL	(gm)	3.03b	0.80b	0.30c
	(R.W.G)	1.97	0.52	0.19
BR+BL	(gm)	3.31a	0.78c	0.35b
	(R.W.G)	2.18	0.51	0.23

Each value is an average of sex determination

Value followed by the same latter in column are not significantly different P < 0.05

Relative weight gain (R.W.G) = organ weight / animal weight * 100

Control=Basal diet content 100 starch.

WH= Diet contain white rice 100% instead of starch in basal diet

WH: BR= Diet contain White rice: Brown rice at ratio 2:1 instead of starch in basal diet.

WH: BL= Diet contain White rice: Black rice at ratio 2:1 instead of starch in basal diet.

BR: BL= Diet contain Brown rice: Black rice at ratio 2:1 instead of starch in basal diet.

HDL-C carries the cholesterol or cholesterol esters from peripheral tissues or cells to the liver, where cholesterol is metabolized into bile acids. This pathway plays a very important role in reducing the reducing the cholesterol level in blood and peripheral tissues, and in preventing cardiovascular disease (Genest 1986 and kempner 1946). Therefore, the ratio of TC / HDL-C was higher in case of group fed on diet containing of white rice 3.06 and lower in group fed on diet containing of white rice + black rice 2.33. These results are in agreement with those of kim *et al* (2006) and Rubmini and ranghuram (1991), who reported that brown and black rice significantly increased plasma HDL-C compared with white rice.

Table (8): Serum lipid concentration in rats fed on different rice diets.

Test compounds Dietary Groups	Total cholesterol mg/dl	HDLc mg/dl	LDLc mg/dl	Total triglycerides mg/dl	TC/HDL-C
Control	105.35ab	33.12b	72.23b	110.67b	3.61
WH	109.12a	30.25c	78.87a	115.5a	3.06
WH:BR	99.10b	32.40b	66.70c	92.4c	2.57
WH:BL	92.26c	35.9a	56.36d	91.9c	2.33
BR: BL	88.20d	37.76a	50.44e	87.26d	3.18

Each value is an average of sex determination

Values followed by the same letter in column are not significantly different P <0.05

Control=Basal diet content 100 starch

WH= Diet contain white rice 100% instead of starch in basal diet

WH: BR= Diet contain White rice: Brown rice at ratio 2:1 instead of starch in basal diet.

WH: BL= Diet contain White rice: Black rice at ratio 2:1 instead of starch in basal diet.

BR: BL= Diet contain Brown rice: Black rice at ratio 2:1 instead of starch in basal diet.

Antioxidant status in the liver tissue of rats fed on different rice diets:

Antioxidant status in the liver tissue of the 5 tested groups are presented in table (9).The antioxidant levels in the rat livers ranged from highest in those fed on the (brown rice + black rice) diet, followed by the (white rice + black rice) then (white rice +brown rice) then white rice is the lowest diet . The aforementioned results coincide with those obtained by Neve (1995), Torrz *et al* (1997) and Chiang *et al* (2006), They found that, the consumption of brown and black rice leads to improve antioxidation and decreased peroxidation processes, damage from oxidative stress. Selenium, an antioxidant nutrient, was higher in the colored rice diets than in the white rice diet. This antioxidant nutrient might be responsible in part for the enhancement of antioxidant status in this study .Selenium is an integral part of the antioxidant enzyme GPX. In the present study,comparing the WH: BR groups and WH: BL group, GPX activity was higher in black rice than brown rice .

Table (9): Antioxidant status in the liver tissue of rats fed on different rice.

Antioxidant status Groups	SOD (U/mg Protein)	CAT (U/mg Protein)	GPX (U/mg Protein)
Control	12.5d	40.7c	27.1e
WH	16.3c	43.81b	32.51d
WH:BR	18.1b	44.11b	36.3c
WH:BL	22.9ab	46.22ab	39.8b
BR:BL	25.3a	50.12a	44.16a

Each value was an average of three determinations

Values followed by the same letter in column are not significantly different P <0.05

SOD = Superoxide Dismutase CAT = Catalase GPX = Glutathione Proxidase

REFERENCES

- A.O.A.C. (2005). Association of Official Agricultural Chemists. Official Methods of Analysis. 18th Ed., Washington D.C., USA.
- Amorim, J. A.;Eliziário, S. A .;Gouveia, D. S.; Simões, A. S. M.; Santos, J. C. O.;Marta M. C.; Souza, A. G. and Maria F. S. T. (2004). thermal analysis of the rice and by-products. *J. of Thermal Analysis and Calorimetry*, 75, 393–399.
- Ben-Hammouda,M.;Kremer,R.J.;Minor,H.c.andSarwar,,(1995). A chemical basis for differential allopathic potential of sorghum hybrids on wheat .*J. chem. Ecol.*, 21:775-786.
- Bonoli, M.; Marconi, E. and Caboni, M. F. (2004). Free and bound phenolic compounds in barley (*Hordeum vulgare* L.) flours. Evaluation of the extraction capability of different solvent mixtures and pressurized liquid methods by micellar electrokinetic chromatography and spectrophotometry. *J. of Chromatography A*, 1057: 1-12.
- Brand-Williams, W.; Cuvelier, M. E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Sci.and Tech.Research*, 28, 25–30.
- Butsat, S. and Siriamornpun, S. (2010). Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chem.*, 119: 606–613.
- Chiang, A.; Wu, H.L.; Yeh, H.; Chu, C.; Lin, H. and Lee, W.(2006). Antioxidant Effects of Black Rice Extract through the Induction of Superoxide Dismutase and Catalase Activities. *Lipids*, (41).
- Clifford, M.N. (2000). Anthocyanins: Nature, occurrence and dietary burden, *J. Sci. Food Agric.*, 80, 1063–1072.
- Daniel, O., Meier, M. S., Schlatter, J., and Frischknecht, P. (1999). Selected phenolic compounds in cultivated plants: Ecologic functions, health implications, and modulation by pesticides. *Environmental Health Perspectives*, 107, 109–114.
- Dhamaputra, O. (1997). The effects of milling degree and type of bag on fungal infection and some chemical contents of stored milled rice. *Biotropia.*, 10: 42-62.
- El-Khamissy, A. (2005). Studies on Biological Effects of Some Diabetes Foods. Ph.D. Thesis, Dept. Hom. Economics Faculty of Specific Education, Tanta Univ.
- Faostat (2005). Physical and Thermal Properties of the Rice. UFSM Didactic brochure, Santa Maria, Brazil, 68pp.
- Fossati, P. and Prancipe, L. (1982). Triglycerides determination after enzymatic hydrolysis. *Clin. Chem.*, 28: 2077.
- Frei, M. and Becker, K. (2004). Agro-biodiversity in subsistence-orientated farming systems in a Philippine upland region: nutritional considerations. *Biodiversity and Conservation* 13: 1591-1610.
- Freidwald, W.T.; Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein separate by three different methods. *Clin. Chem.*, 18: 499-502
- Genest, J. (1986). Nutritional management of hypertension: past, present, and future. *Can J Physiol Pharmacol*: 64: 836–839.

- Kempner, W. (1946) Some effects of the rice diet treatment of kidney disease and hypertension. *Bull. N.Y. Acad. Med.* 22: 350–370.
- Kennedy, B.M. (1980). Nutritional quality of rice and endosperm. In: *Rice Production and Utilization*, Luh, B.S. (ed.), AVI. Pub. Comp. Inc. Westport, Connecticut. pp. 439, USA.
- Kim, K. H.; Tsao, R.; Yang, R. and Cui, S. W. (2006). Phenolic acid profile and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chem.*, 95, 466 – 473.
- Kim, S. Y.; Kim, Y. S.; Kim, Y. S.; Kim, J. M. and Suh, H. J. (2008). The application of monascal rice in rice beverage preparation. *Food Scie. and Technology*, 41, 1204–1209.
- Li, W.; Pickard, M. and Beta, T. (2007). Effect of thermal processing on antioxidant properties of purple wheat bran. *Food Chem.*, 104, 1080–1086.
- Liyana-Pathirana, C. M. and Shahidi, F. (2007). Antioxidant and free radical scavenging activities of whole wheat and milling fractions. *Food Chemistry*, 101, 1151–1157.
- Martinez-Valverde, I.; Periago, M. and Ros, G. (2000). Nutritional importance of phenolic compounds in the diet. *Archives Latino American Nutri.*, 50, 5–18.
- Morton, L. W.; Abu-Amsha, C.; Puddey, I. B. and Croft, K. D. (2000). Chemistry and biological effects of dietary phenolic compounds: Relevance to cardiovascular diseases. *Critical and Experimental Pharmacology and Physiology*, 27, 152–159.
- Nara, K.; Miyoshi, T.; Honma, T. and Koga, H. (2006). Antioxidant activity of bound from phenolics in potato peel. *Bioscience Biotechnology and Biochemistry*, 70, 1489-1491.
- Neve, J. (1995). Human selenium supplementation as assessed by changes in blood selenium concentration and glutathione peroxidase activity. *J. Trace Elem. Med. Bio.* 9:65-73.
- Newmark, H. L. (1996). Plant phenolics are potential cancer prevention agents. *Advances in Experimental and Biology*, 401, 25–34.
- Nicolosi, R.J.; Ausman, L.M. and Hegsted, D. M. (1991). Rice bran oil lowers serum total and low density lipoprotein cholesterol and apo B levels in nonhuman primates. *Atherosclerosis*. 88:133-142.
- Oyanatui, Y. (1984). Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Anal. Bio.*, 142, 290-296.
- Pal, V.; J.P. Pandey and P.C. Sah (1999). Effect of degree of polish on proximate composition of milled rice. *J. of Food Sci. and Tech. Mysore*, 36, 160-162.
- perez, C.M. (1993). Gel consistency and viscosity of rice, International Rice Research Institute (IRRI). pp. 292.
- Prasad, K.; Lee, P.; Manthaa, S.V.; Kalra, J.; Prasad, M. and Gupta, J.B. (1992). Detection of ischemia-reperfusion cardiac injury by cardiac injury by cardiac muscle chemiluminescence. *Mol. Cell. Biochem.* 115:49-58.
- Purushothama, S.; Raina, P.L. and Hariharan, K. (1995). Effect of long term feeding of rice bran oil upon lipids and lipoproteins in rats. *Mol. Cell. Biochem.* 146:63-69.

- Qiu, Y.; Liu, Q and Beta, T . (2010) .Antioxidant properties of commercial wild rice and analysis of soluble and insoluble phenolic acids. *Food Chem.* 121,140-147.
- Read, N.W.(1986). Dietary fiber and bowel transit; in vahouny GV, kritchevsky D (eds): *Dietary Fiber Basic and Clinical Aspects*. New Yourk, Plenum Press, 81-100
- Reeves, P.G. (1997). Components of the AIN-93 diets are improvement in the AIN-76 diets . *J.Nutr* 127: 838s- 841S.
- Richmond, N. (1973). Calorimetric method of determination of total cholesterol and high density lipoprotein cholesterol (HDLC). *Clin. Chem.* 19: 1350-1356.
- Rubmini, C. and Ranghuram, T. C. (1991). Nutritional and biochemical aspects of the hypolipidemic zction of rice bran oil : areview . *J Am Coll Nutr .* 10 : 593-601.
- Stein, O. and Stein,Y.(1999). Atheroprotective mechanisms of HDL. *Atherosclerosis .*144:285-301.
- Stell, R.G. and Torrie, J.H. (1980). *Principles and Procedures of Statistics*, 2nd Ed. McGraw-Hill, New York, USA.
- Stephens, N. G., Parson, A., Schofield, P. M., Kelly, K., Cheeseman, M. J. and Mitchinson, M. J. (1996) .Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study. *Lancet.* 347: 781–786.
- Siebenmorgen, T.J. and Sun, H. (1994). Relationship between milled rice surface fat concentration and degree of milling as measured with commercial degree of milling as measured with commercial milling meter. *Cereal Chem.* 71(4): 327-329.
- Singh, D.; Chauhan, G.S.; Tyogi, S.M. and Suresh, I. (2000). Extruded snacks from composite of rice broken and wheat bran, *J. Food Sci. Technol.*, 37(1): 1-5.
- Suwansri, S. and Meullenet, J. (2004). Physicochemical characterization and consumer acceptance by Asian consumers of aromatic Jasmine rice. *J. of Food Sci.*, 69 (1): 30 - 37.
- Tananuwong , K. and Tewaruth, W. (2010). Extraction and application of antioxidants from black glutinous rice . *Food Sci. and Tech.* 43: 476-481.
- Tian, S.; Nakamura, K. and Kayahara, H. (2004). Analysis of phenolic compounds in white rice, brown rice and germinated brown rice. *J. of Agric. and Food Chem.*, 52, 4808-4813.
- Torrz , M.; Rodamilans ,M.; Montero, F. and Corbella , J. (1997) . Surum selenium concentration of a healthy northwest Spanish population. *Biol.Trace. Elem. Res.* 58:127-133.
- Vichapong , J.; Sookserm, M.; Srijesaruk, V.; Swatsitang , P. and Srijaranai, S.A. (2010). High performance liquid chromatographic analysis of phenolic compounds and their antioxidant activities in rice varieties *Food Science and Technology* 43 :1325-1330.
- Zhou, Z.K.; Robards, K.; Helliwell, S. and Blanchard, C. (2004). The distribution of phenolic acids in rice. *Food Chemistry* 87, 401–406.
- Zigoneanu, I.G.; Williams, L.; Xu, Z. and Sabliov. C.M. (2008). Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. *Bioresource Tech.* 99: 4910–4918.

Zilversmit, B.B. and Davis, A.K. (1950). Micro-determination of plasma phospholipids by TCA precipitation J.C. of Clin. Med. 35: 155-0160.

تأثير التغذية بخليط من الأرز الملون على صور مكونات الدم الليبيدية ووضع الاكسده في فئران التجارب

محمد نشأت صالح* , محمد احمد البنا* , محمود صابر جوده** و سمير محمود متولى**
* معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية
* قسم علوم وتكنولوجيا الأغذية - كلية الزراعة - جامعه كفر الشيخ

أجرى هذا البحث بهدف دراسة تأثير التغذية بخليط الأرز الملون على المكونات الليبيدية في السيرم وكذلك حالة الإنزيمات الكبدية في فئران التجارب وأظهرت النتائج ما يلي :

- اختلفت المكونات الكيماوية التالية: البروتين، الدهون ، الكربوهيدرات ، الألياف الخام، الرماد والمعادن في عينات الأرز المختلفة تبعاً للصنف و المعاملة فقد أدت عملية الضرب والتبييض للأصناف الأرز الى تقليل نسبة كل من البروتين ، والدهون ، الألياف الخام، الرماد والمعادن ، بينما أدت هذه المعاملات الى زيادة محتوى الكربوهيدرات في عينات الأرز الأبيض.
 - سجلت المركبات الفينولية الكلية في الأرز البني نسبة عالية للصنف الأسود وجزءه ١٧٨ وكانت ١,٦ و ١,١ مللجرام جالك أسيد / جرام على الترتيب، بينما كانت في الأرز الأبيض لكلا الصنفين كانت اقل وكانت نسبتهما ٠,٨ و ٠,٥ مللجرام جالك أسيد / جرام على الترتيب
 - تم تقدير نشاط مضادات الأكسدة في الأرز البني والأبيض للصنف الأسود وجزءه ١٧٨ ووجد أن نشاط المواد المضادة للأكسدة للأرز البني للصنف الأسود كانت عالية مقارنة بباقي العينات يليها الأرز البني لـصنف جزه ١٧٨ و الأبيض للـصنف الأسود ثم الأبيض للـصنف جزه ١٧٨ .
 - تم التعرف على تسعة مركبات فينولية في عينات الأرز ، و كان حامض الفيربولك هو المركب الفينولي الرئيسي في جميع العينات وكان تركيزه عاليا في الأرز البني للـصنف الأسود بالمقارنة بباقي العينات .
- أوضحت النتائج المتحصل عليها من التجربة البيولوجية لتغذية الفئران:**

أ- أن الوزن النهائي والزيادة في الوزن للفئران المغذاه على الأرز البني والأرز الأسود كانت اقل بينما الغذاء المتناول يكون عاليا مقارنة بالفئران المغذاه على وجبة الكنترول .

ب- سجل محتوى السيرم من الكلي والليبيروتين المنخفض الكثافة والجليسريدات الثلاثية في الفئران المغذاه على الأرز الأبيض نسبة عالية بالمقارنة بباقي المجاميع ، بينما سجل محتوى الليبيروتين عالى الكثافة مستوى عاليا في الفئران المغذاه على خليط من (الأرز الأبيض + الأرز الأسود) و (الأرز البني + الأرز الأسود) بالمقارنة بالفئران المغذاه على الأرز (الأبيض و خليط من الأرز الأبيض + الأرز البني

ت- اختلفت نسبه مضادات الأكسدة الموجودة في الكبد للفئران تبعاً لمحتوى الوجبات حيث سجلت نسبة عالية في الفئران المغذاه على وجبات تحتوي على خليط من (الأرز البني + الأرز الأسود) ثم (الأرز الأبيض + الأرز الأسود) يليها (الأرز الأبيض + الأرز البني) على التوالي وأخيرا الوجبات التى تحتوي على الأرز الأبيض والتي كانت اقلهم فى المحتوى .

مما سبق نستنتج أن :

استخدام الأرز البني والأرز الأسود يعمل على خفض الكوليسترول فى فئران التجارب وذلك لاحتوائه على نسبة عالية من مضادات الاكسده .

قام بتحكيم البحث

أ.د / مسعد عبد العزيز ابو ريه

أ.د / حنان السيد كساب

كلية الزراعة – جامعة المنصورة

كلية الزراعة – جامعة كفر الشيخ