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STUDIES ON INTESTINAL ENZYME ACTIVITY AND NUTRITIVE VALUES OF DIETARY FIBRES IN RATS

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ABSTRACT

Components of dried and milled spiny cactus (*Opuntia sp.*) fruit peel (SCFP) and sugar beet leaves (SBL) were determined. The major component was total non–starch polysaccharides, NSP (64.27 % and 60.16 %, respectively). Results indicated the presence of the highest level of soluble non–starch polysaccharides, NSP in SFP (74.53 % of total NSP), was nearly double that in SBL (47.51 % of total NSP) and the reverse was true for insoluble NSP (25.48 % and 52.29 % of total NSP, respectively). The nutritive values of SFP and SBL dietary fibres (calculated as NSP) and the effect on intestinal enzyme activities were studied by feeding rats for 8 weeks on three different diets (control and two experimental diets supplemented with SFP and SBL). Results showed that the body weight gain, food consumption and feed efficiency ratio (FER) of rats fed on the two experimental diets were significantly lower than those fed control diet. The results indicated that sucrase activity was highly stimulated in rats given diet with SFP and stimulated for diet with SBL but less than diet with SFP compared with those given control diet. The diet with SBL-fed rats had the lowest activities of maltase and lactase, compared with the other two diets fed groups, particularly with those given diet with SFP. For alkaline phosphatase, the activity was similar in rats received both experimental diets, significantly lower than those given control diet. Thus, both by-products could be

considered as good and inexpensive sources of natural dietary fibres having high nutritive values and effects on intestinal enzyme activities. These dietary fibres ,when used as supplements to the diets at the level of 100 g /kg, (10%) diet may be a factor protecting against metabolic and heart diseases.

Key words *: dietary fibres , NSP - SFP - SBL , intestinal enzyme, nutritive values.*

1. INTRODUCTION

A great deal of interest has recently, been focused on the potential of converting agricultural and industrial byproducts into useful products and also for diminishing the problems of pollution. Several workers have however, converted waste plant materials into commercially valuable products, specially protein, and polysaccharides (Rossi *et al.*,1988 and Jwanny *et al.*, 1990,1996). The high incidence of many metabolic diseases, including diabetes and ischemic heart disease, could be associated with low intake of fibre. Since many experimental studies have confirmed the beneficial effects of dietary fibre and particularly soluble fibre, upon carbohydrate and lipid metabolism (Pedrose *et al.*,1990 and Cameron –Smith *et al.*,1994). Most of nutrition recommendations (Health and Welfare Canada 1990 and Beebe *et al.*, 1991) suggested to include generous amounts of fibre in one ' s diet, quantitative (40 g/day or more)' as well as qualitative (insist soluble fibre) (Beebe *et al.*, 1991).Different effects on body weight gain, food consumption, feed efficiency ratio, apparent digestibility (Dapp.) and food conversion efficiency (FCE) , were observed in the rats when different types of dietary fibres were studied (Stanley and Newsholme 1985, Stanley *et al.*, 1986,Johnson and Gee 1986, Kritschevsky *et al.*, 1988 and Jwanny *et al.*, 1996). Other workers reported lowering effects on plasma lipid, glycaemic response and activity of liver and intestinal enzymes in rats fed different types of dietary fibres (Pedrose *et al.*, 1989, 1990, Morgan *et al.*, 1990 and El – Beih *et al.*, 1996). However, it has been found that the long term consumption of various types of dietary fibres (guar gum, pectin, wheat bran, oat bran and

pectin) leads to increase caecal size, mucosal growth and delayed intestinal absorption and transit time in rats (Sakata 1987; Walter *et al.*, 1988 and Cherbut *et al.*,1991). The main constituents of dietary fibres or un–available carbohydrate are non–starch polysaccharides (NSP) (British Nutrition Foundation,1990), which disappear within the human gut by large bowel microflora as a result of fermentation (Cummings and Englyst 1987), and the same way applies to the rat (Goodlad and Mathers1990). The activity of the intestinal enzymes could be influenced by dietary fibre supplemented diets as well as the other nutrients. Thus, feeding rats with a diet containing soluble fibre increases their sucrase and maltase activities compared with fibre free – diet fed rats (Chun *et al.*,1989 and Schneeman and Gallaher 1993). Other workers reported that the prolonged consumption of various types of dietary fibres was associated with the adaptive changes in mucosa including a reduction in the activity of intestinal enzymes (Thomsen and Tasman – Jones 1982; Johnson and Gee 1986 and Onning and Asp 1995). The present study was carried out to investigate the effects of spiked fig peels (SFP) and sugar beet leaves (SBL) on intestinal response and biological values in the rats. Therefore, both by-products with different constituents were fed to rats and the influences on the intestinal enzymes activity was measured.

2. MATERIALS AND METHODS

2.1. By-products and analysis

Sugar beet leaf (SBL) and spiny cactus (*Opuntia sp.*) fruit peel (SCFP) were oven-dried at 60–80°C and milled to very fine powder ,then stored till use for analysis and biological assay.

Total nitrogen was determined by the Kjeldahl procedure.The protein content was caculated as N × 6.25. Lipids were determined according to the method of Folch *et al.*, (1957). Starch and non-starch polysaccharides (either soluble or insoluble) were estimated according to the method described by Englyst and Cummings (1988).

2.2. Animals and diets

Ninty six male adult white rats (*Rattus norvegicus*), weighing about 198-203g, (obtained from the Egyptian Organization for

Biological Products and Vaccines), were divided into three groups, each comprised of 32 rats and individually housed in wire screen bottomed cages. Each group received commercial diet for 5 days before receiving an experimental diet. The composition of the diets are given in Table (1). The first diet was used as fibre free - control diet (diet I). The second and third diets were based on the first (diet I) but differed in that they contained 100g dietary fibre of SFP or SBL /kg diet (experimental diets, II and III, respectively), substituted for a proportion of the starch as shown in Table (1) as described by Johnson and Gee (1986).

Table (1) : Composition of the diets used in the biological assay (g/kg diet).

Ingredients[a]	Diet I	Diet II	Diet III
Maize starch	360.00	219.68	212.87
Sucrose	300.00	300.00	300.00
Casein	200.00	194.86	190.73
Maize oil	80.00	69.87	70.19
Mineral mixture[b]	40.00	40.00	40.00
Vitamin mixture[b]	20.00	20.00	20.00
SFP*	-	155.59	-
SBL**	-	-	166.21

a Johnson and Gee (1986).

b Motzok *et al.*, (1975).

100 g dietary fibres from SFP and SBL replaced 100 g starch.

SFP* Dry powder of cactus fruit peels, 155.59g/kg containing 100g dietary fibres (approximately soluble NSP, 74.53g, insoluble NSP,25.48 g), 40.32 g starch, 5.14g protein and 10.13 g lipid.

SBL** Dry powder of sugar beet leaves , 166.21g/kg containing 100g dietary fibres (approximately soluble NSP,47.71g, insoluble NSP,52.29 g), 47.13 starch, 9.27g protein, 9.81g lipid.

All animals were given food and water *ad libitum* for 8 weeks. The faeces were collected daily and dried at 105°C, weighed and tested for nitrogen content (Jwanny *et al.*, 1996). Food consumption, body weight gain, feed efficiency ratio (FER), apparent digestibility (Dapp) and food conversion efficiency (FCE) were calculated according to Silva and Nicoli (1985) and Johnson and Gee (1986). After the end of 4 and 8 weeks of feeding, rats were killed by

stunning and cervical dislocation as rapidly as possible. The small intestine of 8 rats separately from each group was removed and extended on the laboratory bench without stretching. The length of the intestine was immediately measured. The small intestine was slit open, laid mucosa was scraped, then frozen and stored at – 20°C for subsequent enzyme assay.

Maltase (EC3.2.1.20), lactase (EC3.2.1.23) and sucrase (EC 3.2.1.48) activities of intestinal enzymes in the scraped mucosa were assayed by the method of Dahlqvist (1964 and 1968). Mucosal alkaline phosphatase (EC3.1.3.1.) activity was assayed by the method of Belfeld and Goldberge (1971) using Bio-Merieux kits. Mucosal protein content was determined by the method of Lowry *et al.*, (1951). DNA content of the mucosa was estimated according to the method of Fiszar–Szarfarz *et al.*,(1981). The data of the present study were statistically analyzed using student t - test according to Fisher (1970).

3. RESULTS AND DISCUSSION

An increased dietary intake of plant fibre is currently being recommended for a variety of reasons. These include the lowering of intestinal absorption of nutrients and regulating the digestive transit time (Cherbut *et al.*, 1991). The quantity and the quality of dietary fibre (NSP) are responsible for specific physiologic effects on biological values and enzyme activities (Johnson and Gee 1986 and Jwanny *et al.*, 1996). Thus, the chemical constituents of SFP and SBL have been determined in the present study. The chemical composition of SFP and SBL are shown in Table (2). It can be observed that the major components were total NSP (64.27% and 60.16%) and starch (25.91% and 28.36%), respectively for SFP and SBL. Results also indicated that SFP contain a high amount of soluble NSP (74.53 % of total NSP), low amount of insoluble NSP (25.48 % of total NSP), while SBL contains nearly equal amounts of both (47.71% and 52.29 %, respectively of total NSP), which revealed that both by-products are rich sources of dietary fibre compared with orange peel which had 21% NSP (Menezes de Barreto *et al.*,1989).These results are within the range reported by other workers (Goodlad and Mathers 1990 and Galibois *et al.*,1994) but

higher than those obtained by Englyst and Cummings (1988) and El–Beih *et al.*,(1996), who reported that the levels of dietary fibre (as NSP) in plant and agricultural by-products ranged from 3 to 38 % . Similarly high estimates were obtained by Bach - Knudsen and Hessove (1995).

Table (2) :Compsoition of SFP and SBL by-products (g/100g dry weight).

Ingredients	SFP	SBL
Protein	3.30±2.20	5.58±3.72
Lipid	6.51±4.35	5.90±3.94
Starch	25.91±17.19	28.36±18.81
Soluble non-starch polysaccharides (NSP)	47.98±31.97	28.70±19.15
Insoluble non-starch polysaccharides (NSP)	16.37±10.92	31.46±20.99
Total non-starch polysaccharides (NSP)	64.27±42.90	60.16±40.14

Mean of three batches (mean ± SE).

3.1.Nutritional evaluation

The diets were readily accepted by the rats and the three rat groups continued without any signs of ill - health over the feeding period (8weeks). The body weight gain increased significantly in all groups after 4 and 8 weeks of feeding. Significantly lower increases were observed in rats receiving diets II or III as compared with those given diet I. Results also showed significant increase in body weight gain of rats fed diet III compared with those fed diet II after 4 and 8 weeks of feeding (Table 3). Food consumption for rats fed diets II or III were significantly lower than those given diet I. Significant decreases in feed efficiency ratio (FER) were observed in rats fed diet II compared with the other two diets (I and III). The final body weights of rats fed diets II and III were nearly similar after 4 weeks of feeding and significantly decreased than those fed diet I, while after 8 weeks of feeding, highly significant increase in final body weight was observed in rats fed diet III compared to those fed diet II (Table 3). However, body weight gain, food consumption and final body weight, were significantly increased in all groups after 8 weeks of feeding than that after 4 weeks of feeding. The lowering of these biological values in rats fed on both experimental diets (II and III) might be related to the delaying effect of 100 g dietary fibres of SFP

Table (3) : The body weight gain, food consumption, feed efficiency ratio (FER), apparent digestibility (Dapp), food conversion efficiency (FCE) and final body weight of white rats fed on three different diets for 4 and 8 weeks. (Mean values of 8 rats/group).

Parameters		4 weeks			8 weeks		
		Control	Experimental diets		Control	Experimental diets	
		I	II	III	I	II	III
Initial body weight (g)	Mean	203	202	198	203	202	198
	±SE	3.50	3.48	3.42	3.50	3.48	3.42
Body weight gain (g)	Mean	162	122	136	314	234	262
	SE	7.84	4.52**	4.57	9.42	7.32	8.16
	T1		4.42**	2.86*	—	6.71**	4.17**
	T_2	—	—	2.18	—	—	2.55*
	T_3	—	—	—	12.39**	13.02**	13.48**
Food consumption (g)	Mean	560	452	485	1080	890	940
	SE	13.4	14.82	16.20	16.32	18.94	19.80
	T1		5.41**	3.57**	—	7.60**	5.46**
	T_2	—	—	1.50	—	—	1.83
	T_3	—	—	—	24.82**	18.21**	17.79**
Feed efficiency	Mean	0.29	0.27	0.28	0.29	0.26	0.28
ratio (FER)	SE	0.01	0.01	0.01	0.01	0.01	0.01
	T1		1.42	0.71	—	2.13	0.71
	T_2	—	—	0.71	—	—	1.42
	T_3	—	—	—	0.00	0.71	0.00
Apparent digestibility	Mean	92.54	90.04	89.05	92.47	89.99	89.03
(Dapp)	SE	1.40	1.52	1.64	1.72	0.94	1.24
	T1		1.21	1.62	—	1.27	1.62
	T_2	—	—	0.44	—	—	0.62
	T_3	—	—	—	0.03	0.03	0.01
Food conversion	Mean	28.93	26.99	28.04	29.07	26.29	27.87
Efficiency (FER)	SE	1.53	1.98	2.01	1.42	1.81	1.62
	T1	—	0.78	0.35	—	1.21	0.56
	T_2	—	—	0.37	—	—	0.65
	T_3	—	—	—	0.07	0.26	0.07
Final body weight (g)	Mean	365	324	334	517	436	460
	SE	6.42	5.40	7.22	8.70	9.82	6.80
	T1		4.89**	3.21*	—	6.17**	5.16**
	T_2	—	—	1.11	—	—	2.01
	T_3	—	—	—	14.06**	9.99**	12.70**

T_1:Significance test for diets II or III comparing to diet I.

T_2:Significance test for diet III comparing to diet II.

T3 Significance test for 8 weeks to 4 weeks.

* Significant ($P < 0.05$)

* Highly significant ($P < 0.01$).

and SBL on digestion and absorption of various nutrients (Cherbut *et al.*, 1991 and Bach - Knudsen *et al.*, 1994). The present results (Table 3) are in agreement with observations of other workers (Stanley *et al.*, 1986, Kritchevsky *et al.*, 1988, Morgan *et al.*, 1990 and Jwanny *et al.*, 1996), in which the use of 100 g dietary fibre / kg diet of various types such as guar gum, cellulose, wheat bran and date waste NSP, caused lowering effects on biological values over the 5-8 weeks of feeding. Walter *et al.*, (1988) reported that a period of 7-8 weeks was reasonable to study adaptive response of long term feeding of dietary fibres added to fibre free -diet.

3.2.Intestinal adaptaton

Results in Table (4) show the experimental diets fed groups (II and III) had similar intestinal length,significantly greater than those given fibre free-control diet (1). Results also show significant increases in intestinal length of all rats after 8 weeks of feeding than that after 4 weeks. For sucrase, the activity was highly stimulated in rats given diet II and lower increase for diet III fed group compared with those given diet I. The diet III fed group had the lowest activities of maltase and lactase, significantly lower than diets I and II fed groups, particularly after 8 weeks of feeding. Alkaline phosphatase activity for rats given diets II and III was significantly lower than those of diet I fed rats. No variations in alkaline phosphatase activity was observed between diets II and III fed rats. The highest content of mucosal protein was observed in rats given diet II than that of the other two diets groups (I and III), particularly after 4 weeks of feeding ,but significantly lower after 8 weeks of feeding was illustrated. The mucosal DNA content was similar in rats fed on the three different diets after 4 and 8 weeks.. Intestinal enzyme activities were unaffected with increasing the feeding period from 4 to 8 weeks of feeding. The present results show an increase in intestinal length and mucosal DNA content in rats fed experimental diets (II and III). These changes may be related to the presence of soluble and insoluble NSP in SFP and SBL.(at ratio of 3:1 and 1:1 respectively), that cause adaptive response of the intestine to dietary fibre. Similar results were obtained by other workers (Johnson *et al.*, 1984; Johnson and Gee 1986; Bach-Knudsen *et al.*,1994), they

Table (4): Intestinal length ,mucosal enzyme activites and protein and DNA contents in rats received experimental diets (II and III) and fibre free-control diet (I). (Mean values of 8 rats/group).

Diets and feeding period.		4 weeks			8 weeks		
		Control	Experimental diets		Control	Experimental diets	
		I	II	III	I	II	III
((Length of small intestine(cm) (cm)	Mean	109	122	126	125	144	149
	SE	1.52	1.86	2.08	2.16	2.36	2.24
	T_1	--	5.41**	6.60**	--	5.94**	7.71**
	T_2	--	--	1.43	--	--	1.54
	T_3	--	--	--	6.06**	7.32**	7.52**
Sucrase	Mean	1.82	2.98	2.02	1.78	3.20	2.26
(EC 3.2.1.48)	SE	0.10	0.40	0.20	0.09	0.44	0.26
	T_1	--	2.81*	0.89	--	3.16*	1.74
	T_2	--	--	2.15	--	--	1.84
	T_3	--	--	--	0.30	0.37	0.73
Maltase	Mean	17.8	18.60	9.82	17.60	19.10	9.60
(EC 3.2.1.20)	SE	2.10	2.30	1.50	1.90	2.50	1.40
	T_1	--	0.26	3.09*	--	0.48	3.39**
	T_2	--	--	3.20*	--	--	3.31**
	T_3	--	--	--	0.07	0.15	0.11
Lactase	Mean	0.34	0.20	0.05	0.36	0.16	0.04
(EC 3.2.1.23)	SE	0.05	0.02	0.01	0.04	0.03	0.01
	T_1	--	2.60*	5.69**	--	4.00**	7.77**
	T_2	--	--	6.70**	--	--	3.80**
	T_3	--	--	--	0.31	1.11	0.71
Alkaline phosphatase (ALP)	Mean	8.30	5.06	5.20	8.12	4.86	4.98
(EC 3.1.3.1)	SE	0.84	0.32	0.54	0.80	0.36	0.40
	T_1	--	3.60**	3.10*	--	3.72**	3.51**
	T_2	--	--	0.22	--	--	0.22
	T_3	--	--	--	0.16	0.42	0.33
Mucosal protein (mg/g dry	Mean	405.00	458.10	376.30	452.00	398.00	386.00
Weight)	SE	9.50	10.10	8.60	8.80	6.10	5.60
	T_1	--	3.83**	2.24	--	5.04**	6.33**
	T_2	--	--	6.17**	--	--	1.45
	T_3	--	----	--	3.63**	5.09**	0.95
Mucosal DNA (mg/gdry	Mean	15.60	16.10	16.20	15.40	16.60	16.80
Weight)	SE	1.10	0.82	0.90	0.94	1.02	1.12
	T_1	--	0.36	0.42	--	0.87	0.96
	T_2	--	--	0.08	--	--	0.13
	T_3	--	--	--	0.14	0.38	0.42

T_1:Significance test for diets II or III comparing to diet I. * Significant ($P < 0.05$)

T_2:Significance test for diet III comparing to diet II. ** Highly significant ($P < 0.01$).

T3 Significance test for 8 weeks to 4 weeks

used guar gum and other non-available polysacchrides with rats. Contradictory result was obtained by Onning *et al.*, (1995), who reported that no changes in intestinal length in oat fed rats. The differences in intestinal enzyme activities could be attributed to the presence of different levels of soluble NSP in diets II and III (74.53 % and 47.71 % of total NSP, respectively). Different changes were observed in the activities of the intestinal enzyme as to the effect of various components of the diets. Thus feeding rats with a diet containing higher amount of soluble fibre as diet II (74.53 % of total NSP) in the present study stimulated their sucrase activity (Table 4) compared with rats fed diet I (Chun *et al.*, 1989 and Schneeman and Gallaher 1993). Other workers obtained contradictory results with rats fed pectin and galactomannan (Thomsen and Tasman – Jones 1982 and Tomson *et al.*, 1983). Other reports are consistent with the present results of diet III (Johnson *et al.*, 1984 and Onning *et al.*, 1995). The present results also are consistent with those obtained by Johnson and Gee (1986) to the extent that the experimental diets (II and III) fed groups had reduced lactase and alkaline phosphatase activities, particularly the lowest decrease of lactase activity of diet III fed group. Maltase activity was inhibited significantly in the intestinal mucosa of diet III fed rats (Containing 52.29 % insoluble NSP and 47.71 % soluble NSP). Similar results were obtained by other workers (Johnson and Gee 1986, Schneeman and Gallaher 1993 and Onning *et al.*, 1995), they gave rats diet containing cellulose and guar gum (100g/kg diet). However, the induction of colonic carcinogenesis in the rat is enhanced by increased mucosal growth brought about by wheat bran (Stasse 1981). The use of glucosidase inhibitors such as acarbose, to reduce the rate of disaccharidase hydrolysis and hence delay carbohydrate absorption in metabolic diseases is now being actively pursued (Johnson and Gee 1986). If such reduction could be achieved in man by the consumption of particular soluble fibre supplements, this might provide a useful alternative to use of drugs (Stasse 1981 and Johnson and Gee 1986). However ,the addition of SFP or SBL to the diet (100 g/kg), may lead to mucosal growth but tended to reduce the activities of intestinal enzymes. It can be concluded that the present study suggests that the prolonged consumption of soluble and insoluble dietary fibre may

reduce the rate of digestion and absorption of carbohydrates. It is clear too that future work is necessary to detect the physiological effects of individual componenets of these materials on functions and enzymes of the intestine.

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دراسات على نشاط انزيمات الأمعاء والقيمة الغذائية للألياف في الجرذان

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ملخص

تم تحليل وتقدير مكونات قشور التين الشوكي واوراق بنجر السكر المجففة والمطحونة جيدا فتبين احتواءهما على كمية كبيرة من عديدى السكريات غير النشوى (64.27 % و 60.16 % على التوالي) .وتشير النتائج أيضا إلي وجود نسبه مرتفعه من عديدى السكريات غير النشوي الذائب (74.53 %) في قشور التين الشوكى عن تلك الكميه الموجودة في أوراق بنجر السكر (47.51) والعكس صحيح بالنسبة لعديدى السكريات غير الذائب (25.48 % و52.29 % علي التوالي). كما تم دراسة القيمة الغذائية لتلك الألياف وتأثيرها على نشاط إنزيمات الأمعاء وذلك بتغذية الجرذان لمدة 8 أسابيع على ثلاث وجبات مختلفة (وجبة الكنترول ووجبتي التجربة الأولى على مطحون قشور التين الشوكى الجاف والثانية على مطحون أوراق بنجر السكر الجاف المضافة إلي العليقة الأساسية). وتبين النتائج انخفاض واضح في زيادة وزن الجسم وكمية الغذاء المستهلك وكفاءة التغذية بالنسبة للجرذان التي تم تغذيتها على وجبتي التجربه مقارنة بمثيلاتها التي تغذت على وجبة الكنترول كما تبين النتائج ارتفاع نشاط انزيم السكريز في الجرذان التي تغذت على وجبتي التجربة مقارنة بتلك التي تغذت على وجبة الكنترول وأظهرت النتائج أن الجرذان التي تغذت على الوجبة المحتوية على مطحون أوراق بنجر السكر الجاف تعطى أعلى انخفاض في نشاط المالتوز واللاكتوز مقارنة بالوجبتين الأخرتين اما عن نشاط إنزيم الفوسفاتيز القلوي فقد وجد أنه متشابه في الجرذان التي تغذت على وجبتي التجربة بينما ينخفض عن تلك التي تغذت على وجبه الكنترول.

ومما سبق يتضح أن تلك المواد تعتبر مصادر جيده ورخيصة للألياف الغذائية الطبيعية لما لها من قيمه غذائية عاليه وتأثيرات متباينة على نشاط إنزيمات الأمعاء و لذلك فان تلك الألياف عندما تضاف إلي الوجبات الغذائية بمقدار 100 جم/كيلو جرام (10%) قد تستخدم كعامل وقائي لامراض القلب وسوء التغذية .

المجلة العلمية لكلية الزراعة – جامعة القاهرة المجلد (51) العدد الرابع (أكتوبر 2000): **431-446.**