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Effects of alkaline, hydrogen peroxide-treated fibres on nutrient digestibility, blood sugar and lipid profile in rats

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Abstract

Three diets containing different sources of dietary fibre, wheat bran, faba bean testas and rice hulls, were tested to determine the effects of alkaline, hydrogen peroxide (AHP)-treated fibre at 10% substitution level of dextrose corn flour on apparent digestibility, faecal characteristics, and levels of plasma glucose and lipid profile in rats. A fourth non-fibrous diet was used as a control. The results showed that, although AHP treatment of fibres resulted in reduction of their weight after washing, it caused an increase in their water absorption and in their swollen volume when mixed with an excess of water (up to three fold) as in the case of wheat bran. Rats consuming diets containing dietary fibres showed a significant loss of body weight but no significant effects on heart weight or liver weight were found. Faeces of rats fed on dietary fibre diets showed an increase in weight and volume, which resulted in reduction of faecal density. The consumption of the treated fibre significantly reduced total cholesterol, plasma triglycerides and blood sugar; wheat bran was the most effective followed by rice hulls and faba bean testas. On the other hand, it effected an increase in the plasma content of high-density lipoprotein HDL-C but this increase was only significant in rats fed with wheat bran. Alkaline hydrogen peroxide-treated fibres showed a great improvement in their absorbency, making them equal to or even better than untreated fibre in lowering blood lipids and sugar levels, in addition to their excellent effect on the physiological function of the intestinal tract. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

In the past, fibres were described simply as unavailable carbohydrates that occur naturally in plant foods (Eastwood & Passmore, 1984). Dietary fibres are defined as the endogenous components, or non-starch polysaccharides of plant materials in the diet, which are resistant to digestion by enzymes produced by man (Health and Welfare Canada, 1990). Many researchers have documented the beneficial physiological effects of dietary fibre in humans. A number of authors (Anderson, 1986; Fernandez, 1995; Galibois, Gueuin, & Jacques, 1994; Gillis & Le-Blanc, 1991; Overton, Beety, Chakraborty, Tredger, & Morgan, 1994; Sadek, 1993–1994; Schneeman, 1987), have proposed a relationship between increased fibre consumption and increase in faecal bulking. This prevents constipation, lowers incidence of colon cancer and other metabolic diseases such as diabetes,

hyperlipidaemia and ischemic heart disease (Fernandez, 1995; Galibois et al., 1994; Kahlon, Saunders, Chow, Chain, & Betschoit, 1990; Malkki, Torronen, Pelkunen, Myllymak, & Syrianen, 1993). Most recent nutritional studies recommend the inclusion of 20 to 35 g/day of dietary fibre in the diet (Beebe, Green, Powers, & Wylie, 1991). Gould (1984) and Kerley, Fahey, Berger, Gould, and Baker (1985) reported that the treatment of lignocellulosic materials at pH 11.5 with alkaline hydrogen peroxide (AHP) solution solubilizes a portion of the lignin present in the cell wall and that the ability of cell wall materials to absorb water and swell was significantly increased. This treatment improves the lignocellulosic matrix by increasing access to the sponge-like structure and thus increases the fibre action (Gould et al., 1989). Swain, Rose, Culey and Sacks (1990) have suggested the normalising action of dietary fibre on serum lipid levels. This effect has been associated with energy dilution of the diet with less tendency to overweight and (for soluble fibre) with lower blood lipids (Mongeau, Malcolm, & Shah, 1991).

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Reports by Jenkins, Reynolds, Leeds, Walter, and Cummings (1979), Kirby et al. (1981) and Kashtan et al. (1992) confirm the reports of Zhang et al. (1994b) and Shane and Walker (1995) on the hypocholesterolemic effect of the ingestion of dietary fibre in man. Madar (1983), Kahlon et al. (1990), Malkki et al. (1993), Sadek (1993–1994), Galibois et al. (1994), Overton et al. (1994) and Fernandez (1995) have studied the effect of dietary fibre in lowering serum lipids and sugar levels in animals. Mongeau et al. (1991) have shown that cereal brans have no long-term effect on body weight, although they have an influence on total serum lipid levels.

The present work studies the effects of using alkaline hydrogen peroxide treated fibres in rat diets on water absorbency, swollen volume, weight gain, faecal characteristics, levels of blood lipids and sugar in rats.

2. Materials and experimental procedures

2.1. Materials

2.1.1. Fibres tested

Three types of dietary fibres, wheat bran, faba bean testas and rice hulls were used. Wheat bran and faba bean testas were purchased from the local market in Riyadh, while rice hulls were brought from Al-Hofof, in the Al-Hassa region, in eastern Saudi Arabia. The fibres were treated with alkaline hydrogen peroxide (AHP) according to Gould et al. (1989), as follows.

The dry materials were suspended in water (40 g/litre) containing 10 g/litre of H₂O₂ and the slurry was adjusted to pH 11.5 with NaOH. The mixture was stirred gently for about 18 h. The resulting slurry was then neutralised with HCl to pH = 7. The insoluble fraction was collected by filtration through a fine muslin mesh cotton cloth. The treated materials were washed thoroughly with water and then dried in a forced air oven (Heraeus Proctorsch, wart WAG.) at 40°C for 24 h. The dried materials were kept in closed polyethylene packages until they were used as sources of dietary fibre in the animal feeding trial.

2.2. Animals

Thirty-two Wistar albino adult rat males weighing 100 g ± 5 g from the Animal Care House, University Centre for Women Students, King Saud University, Al-Malaz, were used.

2.3. Diets

The compositions of the control and test diets are presented in Table 1. The diets were prepared as in AIN93 as modified by Reeves, Nielson and Fahey (1993).

2.4. Determination of water absorbency and swollen volume

The relative ability of lignocellulosic materials to absorb water was estimated by mixing 5 g of dry AHP treated fibres, each with an excess of distilled deionized water (50 ml), and allowing the samples to hydrate for 12 h. The excess water was then removed by filtering the wet samples through a meshed cotton cloth until no more water separated (15 min). The wet samples were removed, weighed and then dried to constant weight in a forced air oven (110°C) (Heraeus, West Germany). The water absorbency (g water absorbed/g dry weight) and the swollen volume were estimated by mixing 1 g of dried AHP treated fibres, each with an excess of distilled deionized water (50 ml) in glass graduated cylinders. The suspensions were mixed to ensure complete hydration of the samples, and allowed to settle overnight. The volume in the cylinder occupied by the swollen particles was taken as the swollen volume in ml per g dry matter (ml/g dry wt).

2.5. Animal feeding studies

This experiment was conducted to determine the effect of AHP-treated dietary fibre (at 10% substitution level of dextrose corn flour (Table 1 in diets) on body and organ weights, faecal characteristics, lipid profile and sugar level in plasma. Animals were randomly divided into four groups, eight rats in each group. Animals were housed individually in stainless steel cages with wire mesh floors designed for the quantitative collection of faeces. The room temperature was maintained at 22 ± 2°C, humidity 50 ± 5% and the daily light–dark cycle was kept to 12 h each. The duration of the experiment was 6 weeks. Fixed amounts of daily diets were given and water was provided ad libitum. Rats were weighed weekly.

Table 1
Compositions of control and test diets containing 10% fibre

Ingredients	Control diet (GM%) ^a	Test diet (10% fibre) ^b
Dextrose corn flour 1:2	73.00	65.70
Source of fibre	–	07.30
Casein	15.10	15.10
DL-Methionine	00.20	00.20
Corn oil	06.00	06.00
Mineral mix	05.37	05.37
Vitamin mix	00.20	00.20
Choline bitartrate	00.10	00.10
MgSO ₄	00.03	00.03

^a As modified in (AIN 93) by Reeves et al. (1993) study.

^b Sources of fibre are rice hulls, faba bean testas and wheat bran in diets 1, 2 and 3, respectively.

2.6. Faecal measurements

Faeces were collected daily and air-dried at 55°C in a forced-air oven. Volumes of dry matter were determined according to Prosky et al. (1985). Faeces were ground so that they could pass through a 1 mm screen and pH was measured. Density was calculated by dividing the faecal weight by volume.

2.7. Biochemical measurements

At the end of the test period, fasted animals were sacrificed; plasma was collected and stored at -20°C until it was analysed. Analysis of total cholesterol was made according to the method described by Richmond (1973). High-density lipoprotein (HDL-C) was determined following the method of Arcol (1989). Low (LDL-C) and very low (VLDL-C) density lipoproteins were measured according to Van Horn et al. (1988). Triglycerides were determined as by Trinder (1969) and glucose by Teuscher and Richterich's (1971) method. All measurements were determined using an enzymatic colorimetric assay by using kits from Roche Diagnostic System Inc. Organs were removed immediately, washed in NaCl solution (0.85 g%), dehydrated on filter paper and weighed.

2.8. Statistical analysis

A Statgraphics software package (Rockville, MD) was used for statistical analysis of the data. Analysis of Variance (ANOVA) was used where statistical differences were noted for measurement. Differences between sample means were determined using the least significant difference test at a 5% level of significance.

3. Results and discussion

Table 2 compares the remaining weight of dry residues after AHP-treatment with the ability of treated fibres to swell when hydrated. From the results, it is clear that the treatment of lignocellulosic materials with

an alkaline (pH 11.5) hydrogen peroxide solution solubilizes a portion of the lignin present in the cell walls resulting in disruption of the substrates. Gould (1984) and Kerley et al. (1985) also found that AHP-treatment of lignocellulosic materials resulted in disruption of the substrate's morphological integrity and in a dramatic increase in susceptibility to hydrolysis. Our results support the findings of Gould et al. (1989) where AHP-treatment also significantly increased the ability of native cell wall materials to absorb water and swell when hydrated (Table 2). AHP-treatment increased the absorbency of AHP-treated wheat bran (by a ratio of 1:1, wheat bran to water). These higher absorbencies were consistent with the idea that AHP-treated cell walls have a more open, or sponge-like internal structure that provides greater access of water to the cell wall carbohydrates (Gould et al.). The effects of AHP-treatment on the aqueous swollen volume were particularly dramatic for wheat bran and faba bean testas, which were higher than the swollen volume of AHP-treated rice hulls. Gould also found a dramatic effect on a number of food-grade lignocellulosic materials such as wheat, corn and rice bran, where AHP-treatment increased the swollen volumes by 4 to 20-fold.

The effects of diets containing treated fibre (with AHP at pH 11.5) on faecal characteristics are shown in Table 3. The results show that adding different sources of fibre to the diet causes increased faecal weight and volume, and thus a reduction in both faecal density and pH. It is also clear that the increased faecal weight is due, in part at least, to increased excretion of endogenous faecal nitrogen, digestive gland secretions, some replacement mucosal cells and a small amount of plasma protein. The possibility that fibre might increase the sloughing of intestinal mucosal cells and may increase formation of short chain fatty acids, has been confirmed by Schneeman (1986) and Gillis and Le-Blanc (1991).

Rats which consumed diet containing faba bean gave the greatest faecal weight followed by rats, which consumed diets containing wheat bran testas and rice hulls. These results are in agreement with those reported by Ranhotra, Gelroth, and Bright (1988) and Sadek

Table 2
Effects of alkaline hydrogen peroxide (AHP) treatment on remaining fibre weight (g/100 g dry wt.) Water absorbency (g H₂O/g dry wt) and swollen volume (cm³/g dry wt)

Source of fibre	Remaining weight	Water absorbency	Swollen volume
Rice hulls	66.6 ± 2.1 ^a	3.6 ± 1.2 ^a	5.0 ± 1.5 ^a
Faba bean testas	48.6 ± 1.4 ^b	3.8 ± 1.3 ^{ab}	9.0 ± 2.4 ^b
Wheat bran	46.6 ± 1.3 ^b	5.3 ± 1.1 ^b	10.5 ± 2.3 ^b

Means in a vertical row with different superscripts are significantly different at $p < 0.05$.

Table 3
Effects of the AHP-treated fibre on faecal measurements

Fibre source	Weight (g)	Volume (cm ³)	Density (g/cm ³)	Faecal pH
Free group (control)	2.75 ± 0.7 ^a	3.87 ± 1.1 ^a	0.710 ± 0.102 ^a	6.64 ± 0.15 ^a
Rice hulls	8.80 ± 1.4 ^b	18.27 ± 2.1 ^b	0.458 ± 0.035 ^b	6.57 ± 0.18 ^a
Faba bean testas	8.99 ± 1.7 ^b	17.99 ± 1.9 ^b	0.512 ± 0.050 ^{ab}	6.45 ± 0.10 ^a
Wheat bran	8.82 ± 1.5 ^b	19.84 ± 1.6 ^b	0.468 ± 0.041 ^b	6.58 ± 0.19 ^a

Means in a vertical row with different superscripts are significantly different at $p < 0.05$.

(1993–1994) who attributed this increment to the bacterial mass, undegraded fibre, and to the excreted minerals and protein matter.

The present results confirm those of Alison et al. (1986) who studied the effect of dietary fibre on stool weight in healthy subjects (men and women). They found that increased stool weight was due to the increment of carbohydrate, non-starch polysaccharides and pentoses excretion. Nyman, Schweizer, Tyren, Reimann, and Asp (1990) have found that all vegetable fibres increased faecal weight and dry weight by 9–56%. They also found that faecal fat concentration decreased from 22% on basal diet to a mean of 11% with addition of vegetable fibre and faecal output of protein was higher with a diet containing fibre than with diet without fibre. Moreover, the excretion was low in all sugars except glucose, but had an increased degraded fibre content. Vegetable fibre resulted in higher faecal bulking and in higher faecal capacity than expected. Faecal volumes significantly increased with different fibre sources; wheat bran gave the highest faecal volume followed by rice hulls and faba bean testas.

Cummings (1985) reported that high faecal weight and high faecal volume, as maintained by dietary fibre, may protect against colon cancer, although low faecal parameters are not necessarily cancer-promoting factors.

Faecal density was significantly lowered by the presence of fibre in the diet. Density was affected by increased faecal weight associated with a great increase in faecal volume. The results are expressed as volume and density of faeces and show the useful effect of dietary fibre as a potential factor in diluting the metabolites

responsible for the incidence of colon cancer, diverticular disease, constipation, haemorrhoids, and appendicitis (Gillis & Le-Blanc, 1991; Lupton & Ferrel, 1986; Trowell & Burkitt, 1975).

Faecal pH values were negatively related to faecal weight. Faecal pH is affected by the metabolic reactions occurring in the gastrointestinal tract, including the degradation of fibre (Ranhotra, Gelroth, & Bright, 1987) and by the production of methane, which correlated with transit time. The associated slow transit time and lowered pH were due to methane production as a result of carbohydrate fermentation by the anaerobic flora of the large intestine. The change in pH values was due to the production of short-chain fatty acids in the caecum (Alison et al., 1986; Nyman et al., 1990; Sadek, 1993–1994).

The effects of fibre diets on body weight gain and weight of organs (liver and heart) are shown in Table 4. Weight gain in rats which were fed diets containing different fibre sources was less than the rats fed fibreless diets. Faba bean significantly reduced body weight compared to rice hulls and wheat bran. These results were in agreement with those reported by Kirby et al. (1981). Shah, Mahoney, and Pellet (1982) showed a significant increase in food intake with the presence of different dietary fibres such as pectin, guar gum and wheat bran in diets at a level of 20%, which produced a strong reduction in weight gain in rats. They suggested that such a reduction is associated with increase of nitrogen excretion in faeces. The results showed that the level of protein excretion was higher in rats fed wheat bran than in rats fed other fibres. Organ weights were affected by the body gain and the changes were significantly different compared to the control group. Malkki et al. (1993) recorded that oat bran concentrate in a hypocholesterolaemic diet decreased the weight by about 10%.

Table 5 shows levels of plasma lipids. In general the results confirm the hypocholesterolaemic action of the dietary fibres, and are similar to the results reported by Ranhotra et al. (1987), Hundemer, Nabar, Shriver, and Forman (1991), Malkki et al. (1993), Sadek (1993–1994), Jackson, Suter, and Topping (1994), Zhang, Hallmands, and Sterling (1994a), Overton et al. (1994), and Fernandez (1995) in different animals and to the

Table 4
Effects of AHP-fibre diets on weight gain (g) and organ weight (g) of rats

Fibre sources	Weight gain (g)	Liver weight (g)	Heart weight (g)
Control	105.25 ± 2.5 ^a	5.31 ± 0.7 ^a	0.58 ± 0.90 ^a
Rice hulls	90.50 ± 1.3 ^{ab}	4.90 ± 0.4 ^a	0.43 ± 0.05 ^b
Faba bean testas	83.00 ± 2.1 ^b	3.73 ± 0.2 ^b	0.44 ± 0.06 ^b
Wheat bran	91.38 ± 1.8 ^{ab}	4.15 ± 0.3 ^b	0.48 ± 0.4 ^b

Means in a vertical row with different superscripts are significantly different at $p < 0.05$.

Table 5
Effects of AHP-fibre diet on blood lipid profile and sugar (mg/dl) in rats

Fibre sources	Total cholesterol (TC)	High-density lipoprotein (HDL-C)	Low-density lipoprotein (LDL-C)	Very low-density lipoprotein (VLDL-C)	Triglycerides (TG)	Glucose (G)
Control	63.5 ± 7.2 ^a	31.4 ± 3.2 ^a	20.2 ± 4.2 ^a	7.1 ± 2.2 ^a	64.2 ± 3.6 ^a	88.9 ± 3.9 ^a
Rice hulls	58.4 ± 6.2 ^{ab}	35.3 ± 6.9 ^a	18.2 ± 3.2 ^a	5.1 ± 1.1 ^b	55.3 ± 1.7 ^{ab}	42.9 ± 1.6 ^b
Faba bean testas	60.5 ± 4.9 ^a	34.6 ± 4.7 ^a	16.1 ± 2.1 ^{ab}	4.2 ± 1.4 ^b	51.1 ± 1.1 ^{ab}	66.4 ± 3.7 ^{ab}
Wheat bran	50.7 ± 4.9 ^b	37.9 ± 4.4 ^b	8.7 ± 2.1 ^b	5.0 ± 1.5 ^b	39.5 ± 1.7 ^b	46.0 ± 3.8 ^b

Means in a vertical row with different superscripts are significantly different at $p < 0.05$.

results of Jenkins et al. (1979), Kashtan et al. (1992), and Zhang et al. (1994b) on humans. Total cholesterol and HDL-C content in plasma of the group fed fibreless diet were significantly higher than in the test groups. The changes were small in rats fed the diet containing rice hulls and faba bean testas. A strong difference in LDL-C was observed in diet containing wheat bran. No significant differences were found in VLDL-C levels among all groups except those fed faba bean testas, which showed a significant reduction effect. Reduction in blood cholesterol might be partly due to an increase in HDL-C or to lower LDL-C or associated with both of them. Davidson et al. (1991) reported that a water-soluble fraction of oat fibre, containing beta-glucans, might be responsible for the hypocholesterolaemic effects. The influence of dietary fibres on lipid absorption and excretion of cholesterol and the effect of short-chain fatty acids, especially propionate, on cholesterol synthesis are other possible mechanisms (Furda, 1990; Gillis & Le-Blanc, 1991).

Triglyceride levels showed a more profound drop in rats fed wheat bran diets than the other two fibre diets. Ranhotra, Gelroth, Astroth, and Rao (1990) found an unclear effect of oat bran on serum triglycerides whereas, in hypercholesterolemic rats fed oat bran concentrate at higher levels, triglyceride level was significantly lowered. Malkki et al. (1993) reported the effect of a concentrated fibre source (oat-bran) on serum lipids and reported that fatty changes in the liver led to an increase in serum triglycerides in all groups. The triglyceride increase means that the only marked abnormality was the change in fatty acids.

After 6 weeks of feeding rats with treated fibre diets, negative effects were observed in blood glucose levels of fasted animals compared to the control group which was fed a non-fibrous diet. The results, in Table 5, show that both rice hulls and wheat bran in the diet caused a significant reduction in blood glucose levels. The results indicate that rice hulls diet are the most effective in slowing down intestinal absorption of glucose, while rats fed with the faba bean testas diet showed the least effect. The present results confirm the view that dietary fibre plays the most important role in modulating glucose absorption as shown by Madar (1983), who studied the effect of brown rice and soybean dietary fibre on the control of glucose metabolism. The results of Anderson (1986), Beebe et al. (1991) and Galibois et al. (1994) also confirm the effect of dietary fibres in controlling blood glucose levels.

In summary, this study indicates that AHP-treated fibre induces weight gain, and lowers both blood lipid and sugar levels in tested groups of rats compared to the control group. Therefore, AHP improves the effects of lignocellulose materials of different fibres, producing a great improvement in their absorbency and thus making them equal to, or even better than, other forms.

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