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Interaction of Dietary Wheat Bran and Dietary Calcium Levels on Calcium Utilization and Bone Mass in Post-breeding Female Rats*

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ABSTRACT

This study was conducted to determine the interaction of dietary wheat bran and dietary calcium levels on calcium utilization in post-breeding female rats. It was designed to compare the effects of four different levels (2.5, 5, 10 and 20%) of wheat bran and two different levels (0.5 and 1%) of calcium on bone and calcium balance in post-breeding female rats over a ten-week period. The effects of diet on animal weight gain, serum calcium, femur weight, femur calcium concentration, bone mass and calcium balance were determined and statistically analyzed. The addition of 20% wheat bran significantly ($p \leq 0.05$) decreased the weight gain of rats. Serum calcium and bone calcium contents were more affected by dietary calcium level than by dietary wheat bran level. There was no significant difference in fat-free solid, ash, percentage of ash to fat-free solid and percentage of calcium to ash among groups. Groups fed the 1% calcium diet had a higher percentage of calcium to fat-free solid. All rats were in positive calcium balance during the three-day experimental period. The average calcium balance of the rats fed 1% calcium diet ranged from 25.34 to 53.90mg and the average calcium balance of the rats fed the 0.5% calcium diet ranged from 26.71 to 32.90mg. In rats receiving 2.5% wheat bran, the difference in calcium balance between the group fed the 1% calcium diet and the group fed the 0.5% calcium diet was only 1.37mg, which was not significantly ($p \leq 0.05$) different. In rats receiving 20% wheat bran, the difference in calcium balance between the group fed the 1% calcium diet and the group fed the 0.5% calcium diet was 19.57mg, which was significantly ($p \leq 0.05$) different. The addition of wheat bran caused an increase in the calcium balance of the rats adminslesed the 1% calcium diet. On the other hand, the addition of wheat bran had no effect on the calcium balance of the rats adminislesed the 0.5% calcium diet. In conclusion, calcium utilization of rats were more positively affected by the interaction of both dietary wheat bran levels and dietary calcium levels than either dietary wheat bran levels or dietary calcium levels alone. Moderate wheat bran consumption did not interfere with the calcium metabolism of rats when calcium intake was high enough.

KEY WORDS : post-breeding · calcium balance · wheat bran · calcium.

INTRODUCTION

Calcium is the principal ingredient in bones of the human body. Osteoporosis is caused by calcium salt decrease in the bones. It is the most prevalent bone disease, and is especially prevalent among post-menopausal women.[1] The causes of osteoporosis are multiple in nature and are still the objects of intense investigation.[2-5] However, it is known that osteoporosis is aggravated by two factors : (1) lack of calcium in the diet and (2) poor absorption of dietary calcium by the intestine.[1)6-8] Unfortunately, both of these factors are common characteristics of the elderly population.

The age-related decline in capacity for intestinal absorption of calcium has been documented in many clinical studies. In both animals and humans, it has been observed that calcium absorption decreases with increasing age.[1)9)10] In the rat, there is a decrease in calcium active transport within a few months after weaning.[11)12] In man, there is a gradual decline in intestinal calcium absorption throughout life. In addition, the intestine loses its ability to adapt to a low-calcium diet with age.[6)13] The biochemical and physiological changes that result in this decreased intestinal capacity to absorb calcium with age are not well understood.

During recent years the beneficial effects of dietary fiber have received much attention. The lack of dietary fiber is the key factor in the development of a wide range of diseases,[14-17] and many physicians now advocate the use of dietary fiber in the treatment of constipation and other colonic disorders.[19-21] In view of the increase in consumption of dietary fiber, more attention should be given to some of the potentially deleterious effects of dietary fiber. One of the negative effects of unavailable carbohydrates is to lessen the intestinal absorption of calcium, zinc, and iron.[22-26] Calcium absorption from a high-fiber diet may depend largely on individual absorptive ability.[26] This ability may be adequate in some subjects, but the ability to adapt may decrease in the elderly.[23)27] Therefore, the in-

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crease in dietary fiber may be ditrimental to calcium metabolism in post-menopausal women when the reduced ability for calcium absorption is considered. Little is known about the metabolism of calcium in post-breeding female rats and even fewer studies have examined the interaction of dietary fiber and calcium levels. In view of the high incidence of osteoporosis among post-menopausal women, the effects of the addition of fiber, with or without supplementary calcium, was investigated in post-breeding female rats.

MATERIALS AND METHODS

1. Animals and diet

Ninety post-breeding female rats of the Holtzman Albino strain (Holtzman Co. Madison, WI) were used in this study. After a seven-day acclimation period, all rats were weighed and assigned to one of nine groups using the Cornell procedure.[28] Ten rats were assigned to each of eight diet groups and ten rats were killed to obtain data to represent the values on the first day of the study. All diets contained 1% American Institute of Nutrition (AIN) vitamin mixture 76 and 3.5% AIN mineral mixture 76. The dietary treatments were calculated to contain calcium and fiber at the levels shown in Table 1. Diets were calculated to contribute equal amounts of calorie, starch, fat and protein. Food and water were provided ad libitum for ten weeks. The rats were kepr in an air-conditioned (22± 1℃) room with a 12-hour light-dark cycle. All animals were weighed weekly and food intake was measured daily. All the equipment utilized during the study period was previously soaked in 10% nitric acid solution.

2. Calcium balance study

The calcium balance study was performed by housing the animals individually in stainless steel metabolic cages. Balances were calculated for a period consisting of 3 days during the last 9 days of the experiment. The calcium intake and calcium excretions (urine+feces) of each animal were analyzed by atomic absorption spectrophotometer[29] (Varian 475). The following formula was used to calculate the calcium balance of rats per day.

Calcium balance per day=

Calcium intake per day − Calcium excretion per day

Table 1. Calcium and wheat bran content of diets

	Group		Calcium[a](%)	Wheat bran[b](%)
1	2.5 WB		0.5	2.5
2	5 WB		0.5	5
3	10 WB		0.5	10
4	20 WB		0.5	20
5	2.5 WB	High Ca	1.0	2.5
6	5 WB	High Ca	1.0	5
7	10 WB	High Ca	1.0	10
8	20 WB	High Ca	1.0	20

a : Obtained from United States Biochemical Corp., Inc.
b : Whear bran from American Association of Cereal Chemists, St. Paul, Minn.

3. Serum analysis

At the end of the ten-week experimental period, blood samples were obtained by heart puncture from each rat. The blood samples were centrifuged at 5,000rpm for 30 minutes and stored in a freezer. The 0.1 ml serum samples were diluted to 5 ml with 0.5% KCl prior to Ca analysis with an atomic absorption spectrophotometer (Varian 475). Calcium concentration was measured against calcium standard solutions (Fisher Scientific Co.).

4. Bone analysis

Rat femurs were removed and cleaned of all soft tissue and weighed. Femurs was extracted in a soxhlet extractor with diethyl ether for 16 hours and with ethanol for an additional 16 hours. The bones were dried at 105℃ overnight and subsequently heated in a muffle furnace for 24 hours at 600−650℃. The ash weight was then measured. The results were expressed as ash percent on a fat-free dry basis. The ash bone was digested with HCl and made up to a final volume of 100ml with 0.5% KCl. Further dilution with 0.5% KCl was made up to 1 : 1000 dilution in duplicate from each sample for atomic absorption spectrophotometer analysis. Calcium concentration was measured against calcium standard solutions (Fisher Scientific Co.)

5. Statistical analysis

Statistical analysis was performed using a statistical package for social science (SPSS-X). The results were presented as mean±SEM and analyzed by one-way analysis of variance (ANOVA) in order to detect significant differences between the dietary treatments. Statistical evaluations were determined by Duncan's multiple range test at $\alpha=0.05$.

RESULTS AND DISCUSSION

1. Effect of wheat bran fiber and calcium on weight gain

There were no significant differences in the means of initial body weights of post-breeding female rats assigned to all the dietary treatments on the first day of the experiment because the rats were initially divided into nine

Table 2. Mean values of initial body weight, final body weight and weight gain for dietary treatment[1)]

	Group		Initial body Wt. (g)	Final body Wt. (g)	Wt. gain (g)
1	2.5 WB		355.10±36.22	404.20± 50.19	49.10±32.50[a2)]
2	5 WB		355.30±35.37	375.70± 21.94	20.40±46.40[a,b]
3	10 WB		355.70±35.12	367.20± 29.29	11.50±17.28[a,b]
4	20 WB		354.00±26.06	355.30± 31.20	1.30±13.84[b]
5	2.5 WB	High Ca	354.40±31.32	373.50± 29.39	19.10±27.39[a,b]
6	5 WB	High Ca	354.50±31.28	381.10±582.16	26.60±45.26[a,b]
7	10 WB	High Ca	351.56±30.01	375.00± 34.24	23.44±20.41[a,b]
8	20 WB	High Ca	353.80±28.47	354.30± 21.41	0.50±27.44[b]

1) Mean±S.D.
2) Values with different superscripts in the same column are significantly different at $\alpha=0.05$ level by Duncan's multiple range test

groups having equal average weight (Table 2). The average weight gain of rats (group 1) fed the 2.5% wheat bran-0.5% calcium diet was significantly higher than the rats (group 4, group 8) fed the 20% wheat bran-0.5% calcium diet or the 20% wheat bran-1.0% calcium diet over the 10 weeks of the experiment. The addition of 2.5, 5, or 10% wheat bran to the diet had no effect on the average weight gain of rats, but the addition of 20% wheat bran to the diet significantly decreased the weight gain of rats, irrespective of the amount of calcium intake. The results of this study disagree with a previous report[30] which showed that the addition of up to 10% wheat bran significantly increased the growth rate. The study by Bagheri and Gueguen[30] demonstrated that when all the diets had equal amounts of calcium, magnesium, phosphorus and zinc, the addition of wheat bran had no influence on the growth rate of the rats. Results of this study are similar in that average body weights of the groups receiving 20% cellulose or 20% glucomannan were decreased compared to that of the control group.[23]

2. Effect of wheat bran and calcium on serum calcium

The effects of dietary wheat bran and dietary calcium on serum calcium are presented in Table 3. The mean values of serum calcium of rats in groups 6 and 7 fed 5% wheat bran-high calcium and 10% wheat bran-high calcium diets were significantly higher than serum calcium of rats in group 4 fed a 20% wheat bran-0.5% calcium diet. There was no significant difference among groups 1, 2, 3 or 4 fed 2.5% wheat bran-0.5% calcium, 5% wheat bran-0.5% calcium, 10% wheat bran-0.5% calcium, or 20% wheat bran-0.5% calcium diets, respectively. Also, there was no significant difference among groups 5, 6, 7 or 8 fed 2.5% wheat bran-high calcium, 5% wheat bran-high calcium, 10% wheat bran-high calcium or 20% wheat bran-high calcium diets, respectively. It appears that serum calcium levels were less affected by dietary wheat bran fiber intake and more affected by dietary calcium intake. The results are in agreement with a number of studies related to high and low dietary wheat bran[25] and calcium supplementation,[31)32)] Serum calcium levels, however, were more affected by interaction of both dietary wheat bran fiber intake and dietary calcium intake than either dietary wheat bran fiber intake or calcium intake alone.

Table 3. Mean values of serum calcium of rats fed different levels of dietary fiber and calcium[1)]

	Group		Serum calcium (mg/100 ml)
1	2.5 WB		10.30±0.53[a,b2)]
2	5 WB		10.16±0.60[a,b]
3	10 WB		10.02±0.98[a,b]
4	20 WB		9.75±0.64[b]
5	2.5 WB	High Ca	10.51±0.68[a,b]
6	5 WB	High Ca	10.78±0.81[a]
7	10 WB	High Ca	10.78±0.74[a]
8	20 WB	High Ca	10.34±0.55[a,b]
9	Initial group		9.92±0.67[a,b]

1) Mean±S.D.
2) Values with different superscripts in the same column are significantly different at $\alpha=0.05$ level by Duncan's multiple range test

3. Effect of wheat bran and calcium on bone

The average femur weight, femur weight per 100 g body weight, and calcium content of the femurs are shown in Table 4. There was no significant difference due to dietary treatment in the average wet weight or in the average femur weight calculated as a perentage of the body weight. The calcium content of femurs of rats in group 6 fed the 5% wheat bran-high calcium diet was significantly higher than those of groups 1, 3 and 4 with 0.5% calcium diet. There was no significant difference among the calcium content of femurs of rats in groups 1, 2, 3 and 4 fed the 2.5% wheat bran, 5% wheat bran, 10% wheat bran and 20% wheat bran diets, respectively. Also, there was no significant difference among the calcium content of femurs of rats in groups 5, 6, 7 and 8 fed the 2.5% wheat bran-high calcium, 5% wheat bran-high calcium, 10% wheat bran-high calcium and 20% wheat bran-

Table 4. Mean weight and calcium contents of femur in rats fed different levels of dietary fiber and calcium[1]

	Group		Femur		
			Wt. (Wet) (g)	Wt.(g)/100g body Wt.	Ca contents (mg/g bone)
1	2.5 WB		1.049±0.092	2.627±0.298	181.68±16.94[b2]
2	5 WB		1.010±0.131	2.701±0.408	183.19±18.84[a,b]
3	10 WB		0.988±0.104	2.702±0.315	181.58±10.53[b]
4	20 WB		0.957±0.083	2.698±0.137	178.81±11.75[b]
5	2.5 WB	High Ca	0.978±0.117	2.623±0.291	190.71±12.51[a,b]
6	5 WB	High Ca	1.007±0.089	2.681±0.321	195.23±11.35[a]
7	10 WB	High Ca	1.004±0.166	2.692±0.454	190.26± 6.50[a,b]
8	20 WB	High Ca	0.974±0.097	2.750±0.249	189.47±16.50[a,b]
9	Initial group		0.980±0.114	2.848±0.226	175.42±18.23[b]

1) Mean±S.D.
2) Values with different superscripts in the same column are significantly different at $\alpha=0.05$ level by Duncan's multiple range test

high calcium diets, respectively. Calcium contents of femurs of the groups with high calcium intake were high compared to those of the groups with 0.5% calcium intake . This result is in agreement with a number of studies related to high and low dietary calcium intake.[31] Calcium intake was highly correlated to the mineral content of the bone of experimental animals.[33]

Table 5 shows the mean bone mass of the rats. The measurements of ash weight, fat-free solid and percentage of ash to fat-free solids must be viewed together to understand bone mineralization as well as bone development. There was no significant difference in fat-free solid, ash, percentage of ash to fat-free solid, and percentage of calcium to ash among the groups. Also, the bones of rats in group 6 fed the 5% wheat bran-high calcium diet had a significantly greater percentage of calcium to fat-free solid than the bones of rats in group 1 fed the 2.5% wheat bran-0.5% calcium diet, group 3 fed the 10% wheat bran-0.5% calcium diet and group 4 fed the 20% wheat bran-0.5% calcium diet. The bones of rats of the initial group had significantly less calcium content than the bones of rats of group 6 fed the 5% wheat bran-high calcium diet, but a significantly greater percentage of calcium to fat-free solid than the bones of rats in group 1, 3 and 4. This result suggests that bone mass decreased during the experimental period. Loss of bone mass with increasing age is a biological phenomenon ; bone becomes less dense and increasingly porous.[34]

Results of this study suggest that the calcium contents of femurs in post-breeding female rats were less affected by different dietary wheat bran levels and more affected by calcium supplements during the ten-week experimental period. This result tends to confirm the previous results[33] that calcium supplements decrease bone calcium loss and loss of bone mass.

4. Effects of wheat bran and calcium on calcium balance

1) Calcium intake

Calcium intake generally increased with increasing amounts of wheat bran in the diet (Table 6). This increase in calcium intake may be related to the increasing amounts of food eaten with increasing amounts of wheat bran to satisfy caloric requirements. Based on the three-day period, the average calcium intake of the rats fed the 1% calcium diet ranged from 120.65 to 208.93 mg, whereas the average calcium intake of the rats fed the 0.5% cal-

Table 5. Mean bone mass and mean calcium content in bones of rats fed different levels of dietary fiber and calcium[1]

	Group		FFS[3] (g)	Ash (g)	Ash/FFS (Percent)	Calcium/FFS (Percent)	Calcium/Ash (Percent)
1	2.5 WB		0.681±0.054	0.406±0.022	59.61±2.74	26.72±1.70[b2]	44.82±4.47
2	5 WB		0.662±0.087	0.395±0.048	59.67±1.80	27.64±1.39[a,b]	46.32±2.04
3	10 WB		0.672±0.050	0.391±0.034	58.18±1.16	26.91±1.06[b]	46.29±1.73
4	20 WB		0.665±0.066	0.397±0.038	59.70±0.99	26.92±1.13[b]	45.08±2.11
5	2.5 WB	High Ca	0.675±0.069	0.403±0.047	59.70±4.09	28.30±1.57[a,b]	47.39±4.16
6	5 WB	High Ca	0.661±0.040	0.395±0.024	59.75±1.52	29.50±1.41[a]	49.37±2.48
7	10 WB	High Ca	0.673±0.077	0.402±0.050	59.73±1.52	28.23±1.35[a,b]	47.26±3.14
8	20 WB	High Ca	0.668±0.055	0.400±0.035	59.88±1.19	28.29±2.34[a,b]	47.25±3.78
9	Initial group		0.594±0.059	0.366±0.040	61.62±1.61	29.41±2.40[a]	47.81±3.20

1) Mean±S.D.
2) Values with different superscripts in the same column are significantly different at $\alpha=0.05$ level by Duncan's multiple range test
3) FFS=Fat free solids

Table 6. Calcium balance of rats fed different levels of dietary fiber and calcium[1)]

	Group		Calcium intake (mg/day)	Calcium excretion (mg/day)	Calcium balance (mg/day)
1	2.5 WB		78.98± 9.06[f,2)]	52.26± 9.08[f,2)]	26.71± 6.78[b,2)]
2	5 WB		82.88± 6.64[f]	52.68± 6.70[f]	30.20± 7.88[b]
3	10 WB		95.26±13.03[e,f]	64.02± 6.67[e,f]	32.24±10.06[b]
4	20 WB		101.09± 9.18[e]	68.20±10.34[e]	32.90±11.39[b]
5	2.5 WB	High Ca	120.65± 8.79[d]	95.32±11.00[d]	25.34± 9.96[b]
6	5 WB	High Ca	156.54±10.35[c]	107.80±10.76[c]	48.75±13.31[a]
7	10 WB	High Ca	175.90±20.42[b]	122.00±16.24[b]	53.90±27.73[a]
8	20 WB	High Ca	208.93±24.54[a]	156.46±19.56[a]	52.47±14.56[a]

1) Mean±S.D.
2) Values with different superscripts in the same column are significantly different at $\alpha=0.05$ level by Duncan's multiple range test

cium diet ranged from 78.98 to 101.09 mg (Table 6). Of the eight groups, groups fed the 1% calcium diet (groups 5, 6, 7 and 8) had significantly ($p\leq0.05$) higher calcium intake than groups fed the 0.5% calcium diet (groups 1, 2, 3 and 4). When the animals were fed similar levels of wheat bran, the addition of calcium increased calcium intake significantly ($p\leq0.05$) in the eight groups. Among the groups fed the 1% calcium diet, the addition of up to 20% wheat bran increased calcium intake significantly ($p\leq0.05$). The addition of up to 20% wheat bran increased calcium intake significantly ($p\leq0.05$) in groups fed the 0.5% calcium diet. The interaction of calcium and wheat bran caused a significant ($p\leq0.05$) effect in calcium intake. The rats fed the high calcium diet with the addition of wheat bran had a sharp increase in calcium intake.

2) Calcium excretion

Table 6 shows the calcium excretion of groups fed different levels of wheat bran and different levels of calcium. Based on the three-day period, the average calcium excretion of the rats fed the 1% calcium diet ranged from 95.32 to 156.46 mg. On the other hand, the daily calcium excretion of the rats fed the 0.5% calcium diet ranged from 52.26 to 68.20 mg. Of the eight groups, groups fed the 1% calcium diet (groups 5, 6, 7 and 8) had significantly ($p\leq0.05$) higher calcium excretion levels than groups fed the 0.5% calcium diet (group 1, 2, 3 and 4). When the animals were fed similar levels of wheat bran, the addition of calcium increased calcium excretion significantly ($p\leq0.05$) in the eight groups. Among the groups fed the 1% calcium diet, the addition of up to 20% wheat bran to the diet increased calcium excretion significantly ($p\leq0.05$). The addition of up to 20% wheat bran to the diet increased calcium excretion significantly ($p\leq0.05$) in groups fed the 0.5% calcium diet. The interaction of dietary calcium and dietary wheat bran caused a significant effect in calcium excretion ($p\leq0.05$). The rats fed the high calcium and high wheat bran diet excreted more calcium in feces and urine.

3) Calcium balance

Table 6 summarizes the calcium balance of the rats fed different levels of wheat bran and different levels of calcium. All the rats were in positive balance during the three-day experiment period. The average calcium balance of the rats fed the 1% calcium diet ranged from 25.34 to 53.90 mg and the average calcium balance of the rats fed the 0.5% calcium diet ranged from 26.71 to 32.90 mg. Among the groups fed the 1% calcium diet, the addition of 5, 10 or 20% wheat bran to the diet increased the calcium balance significantly ($p\leq0.05$) more than the addition of 2.5% wheat bran to the diet. Among the groups fed the 0.5% calcium diet, there was no significant difference in the calcium balance. In the rats fed similar levels of wheat bran, the addition of calcium increased the calcium balance significantly except at the 2.5% wheat bran level ($p\leq0.05$).

The addition of wheat bran to the diet had a different effect on calcium balance depending on which level of calcium was given. The addition of up to 20% wheat bran to the diet caused an increase in the calcium balance of the rats when the 1% calcium diet was given. On the other hand, the addition of up to 20% wheat bran to the diet had no effect on the calcium balance of the rats when the 0.5% calcium diet was given. This study is in agreement with many studies[30)35)] that indicated that moderate dietary fiber consumption did not interfere with the calcium metabolism of rats when calcium intake was high enough. Regardless, numerous negative effects[23)26)] of dietary fiber on calcium balance have been reported.

CONCLUSION

Calcium metabolism of post-breeding female rats was not adversely affected by 20% wheat bran levels during the ten-week experimental period if a high-calcium diet

(1%) was administered. The rats fed the 1% calcium diet had a sharp increase in calcium balance with the addition of 5%, 10% and 20% wheat bran. This study suggests that the addition of high (1%) calcium levels to a high (20%) wheat bran diet may be a suitable method to improve the calcium balance in post-breeding female rats. Similar research should be conducted for longer periods to investingate the long-term nutritional effects of high-fiber diets.

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