Cholesterol-Lowering by Rice Bran
and Rice Bran Oil Unsaponifiable Matter in Hamsters

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ABSTRACT

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Unsaponfiable matter (U) was prepared from both raw and extrusion stabilized (130°C) rice bran and tested for cholesterol-lowering activity in hamsters by addition to diets containing cellulose, raw rice bran, or stabilized rice bran at either the level found in the rice bran diet (0.4%, 1X) or twice that level (2X). All diets contained 0.3% cholesterol, 10% total dietary fiber, 10.1% fat, and 3% N (same plant-to-animal N ratio). After 21 days, plasma cholesterol was significantly reduced by rice bran diets containing added U compared to the cellulose control diet, while the high density lipoprotein cholesterol-to-low density lipoprotein cholesterol ratio remained unchanged in all treatment groups. Liver cholesterol was significantly reduced by all rice bran-containing diets and with cellulose diets containing 2X added U when compared to the control diet. Rice bran diets plus added U resulted in cholesterol values lower

than cellulose diets containing the same level of U. Stabilization of rice bran did not appear to affect the plasma and liver cholesterol responses to the unsaponifiable matter prepared from the extracted oil. There appears to be a dose response to rice bran unsaponifiable matter in plasma and liver cholesterol reductions. After 2 weeks, fecal fat and neutral sterol excretion were significantly greater with all treatment diets compared to the control diet. Fecal fat was negatively correlated with liver as well as plasma cholesterol ($r = -0.97$, $P \leq 0.0001$ and -0.91, $P \leq 0.0006$, respectively). Under the conditions of this study, cholesterol-lowering activity of rice bran is present in its unsaponifiable matter in addition to other components. Increased fecal excretion of fat and neutral sterols appears to be a possible mechanism for cholesterol-lowering by rice bran.

Hypocholesterolemic effects of rice bran and some of its fractions (neutral detergent fiber, hemicellulose, rice bran oil, and unsaponifiable matter) have been observed (Suzuki and Oshima 1970; Ayano et al 1980; Ishibashi and Yamamoto 1980; Suzuki 1982; Sugano et al 1984; Sharma and Rukmini 1986, 1987; Seetharamaiah and Chandrasekhara 1988, 1989; Raghuram et al 1989; Hegsted et al 1992; Nicolosi et al 1991). We have previously reported plasma and liver cholesterol-lowering with stabilized full-fat rice bran, and liver cholesterol-lowering with defatted rice bran when combined with rice bran oil or degummed-dewaxed rice bran oil (Kahlon et al 1990; 1992a,b) in cholesterol-fed hamsters.

In the study reported here, male hamsters were fed 0.3% cholesterol diets to evaluate the cholesterol-lowering activity of raw vs. extrusion-stabilized (130°C) rice bran and of unsaponifiable matter (U) prepared from raw or stabilized rice bran and added to diets at two concentrations. Raw rice bran and its U were investigated to determine the effects of the stabilization process on cholesterol-lowering properties.

MATERIALS AND METHODS

Male, 25-day-old weanling Syrian golden hamsters (Sasco, Inc., Omaha, NE) were housed individually in wire bottom cages in a controlled environment (20–22°C, 60% rh, 12-hr light and dark cycle) and fed ad libitum for the 21-day study. On arrival, each animal was weighed and assigned to one of nine treatments by selective randomization (blocked by weight, one animal per treatment from each block), 10 animals per treatment. Total feed consumption was measured, fresh feed was provided twice weekly, and animals were weighed once a week. All the proce-

dures described were approved by the Animal Care and Use Committee of the Western Regional Research Center, USDA, Albany, CA, and conformed to the principles in *Guide for the Care and Use of Laboratory Animals* (Committee on Care and Use of Laboratory Animals 1985).

Rice bran was obtained from a local mill, and unsaponifiable matter (U) was prepared from either raw or extrusion stabilized (130°C) rice bran. Oil was extracted from the rice bran by slurrying with four volumes of hexane at room temperature for 1 hr, a total of four times. Extracts were combined and hexane was evaporated in a rotary evaporator (40°C under 200 torr vacuum). Unsaponifiable matter was obtained from the oil by alcoholic potassium hydroxide saponification (AOCS Ca 6a-40 1988). Peanut oil (No. 403440), casein (No. 400625), and soy protein (No. 400050) obtained from Dyets, Inc., Bethlehem, PA were additional sources of fat and protein.

Unsaponifiable matter (U) was 0.4% (1X) in the diets containing rice bran. Diets were: cellulose control; cellulose diet plus U prepared from either raw or stabilized rice bran added at 1X or 2X levels; raw rice bran; stabilized rice bran; and stabilized rice bran plus 1X U (total 0.8%) prepared from either raw or stabilized rice bran. All diets contained 10% total dietary fiber, 0.3% cholesterol, 10.1% fat, 11.8% casein, and 3.0% nitrogen. Soy protein was added to the cellulose diets to keep the ratio of animal to plant nitrogen constant (56:44). Total neutral sterol content of each diet was calculated based on the neutral sterol analysis of all dietary ingredients, using the same procedure described for liver cholesterol. Composition of the diets is given in Table I.

After two weeks of feeding the treatment diets, total feces were collected for 72 hr (days 13–15) and samples were analyzed for dry matter at 70°C under vacuum for 16 hr (AOAC method 934.01, 1990), crude fat (AOAC method 20.39C, 1990), and total neutral sterols (same procedure described later for liver cholesterol). At the end of the 21-day feeding period, all animals were fasted for 16 hr and anesthetized with CO_2 for tissue sample collection. Blood was drawn by cardiac puncture into plastic tubes containing anticoagulant (ethylenediamine tetraacetic acid, dipotassium salt, 0.8 mg/ml of blood) and centrifuged at $1,500 \times g$ for 30 min at 4°C to obtain plasma. Livers were excised, rinsed, blotted, weighed, and kept on dry ice. Liver and plasma aliquots

were stored at –70°C until analysis. Plasma samples were analyzed by an enzymatic colorimetric procedure for cholesterol (diagnostic kit 352, Sigma Chemicals, St. Louis, MO) and triglycerides (diagnostic kit 23422, Gilford Systems, Oberlin, OH). Plasma cholesterol and triglycerides values were determined using standard curves obtained by running several concentrations of standards provided with the respective kits.

Fresh plasma pooled samples were prepared (two animals per pool) using an equal volume of plasma from each animal. Protease inhibitor (epsilon-amino caproic acid, ICN Biomedicals, Inc., Costa Mesa, CA, 1.3 mg/ml of plasma) and antimicrobial agent (garamycin 50 mg/ml, Schering Corp. Kenilworth, NJ, 10 μl/ml of plasma) were added to stabilize the plasma. Lipoproteins were fractionated using density gradient ultracentrifugation (Havel et al 1955). After adjusting the background density of 1 ml of plasma to 1.019 g/ml with 5 ml of NaCl solution (1.0214 g/ml), plasma was centrifuged in an L8 Beckman ultracentrifuge (Beckman Inc., Palo Alto, CA) at 40 K for 18 hr at 17°C in a Beckman 50.3 rotor. The top 1 ml was removed as the very low density lipoprotein (VLDL) fraction, and another 1 ml was removed as background. The subnatant density was adjusted to 1.067 g/ml and centrifuged similarly for 24 hr. The top 1 ml was removed as the low density lipoprotein (LDL) fraction, and another 1 ml was removed as background. The subnatant contained the high density lipoprotein (HDL) fraction. With each ultracentrifugation two salt solution tubes with similar density were run, and the densities of their fractions were monitored with a DMA-48 Density Meter (Anton Paar, Inc., Richmond, VA). Lipoprotein fractions were analyzed for cholesterol and triglycerides by the procedures described for plasma.

Each liver was individually thawed, minced, and thoroughly mixed to obtain a homogeneous 1-g sample for extraction of total lipids by the procedure of Folch et al (1957). Liver total lipid was determined by drying an aliquot of the extract that appeared to be free of protein contamination. Liver cholesterol was determined in aliquots (30 μl) of extract after evaporation under nitrogen and solubilizing with Triton X-100 (Carlson and Goldfarb 1977), using the same enzymatic kit as with plasma. Liver cholesterol values were determined from standard curves obtained by running National Bureau of Standards reference material for cholesterol (SRM 911b) through the procedure as described for the samples.

All analyses were conducted in triplicate. Data were statistically analyzed using analysis of variance and Duncan's new multiple range test (Steel and Torrie 1960). A value of $P \leq 0.05$ was considered the criterion of significance.

RESULTS AND DISCUSSION

Initial body weight (64 ± 1 g, mean ± standard error mean), final full-fed body weight (107 ± 1 g), fasting body weight (101 ± 2 g), total weight gain (43 ± 2 g/21 days) and feed intake (8.7 ± 0.3 g/day) in hamsters fed treatment diets were similar to those of

TABLE I
Composition of Diets (% dry matter)[a–d]

Diets	Cellulose	Stabilized Rice Bran	Raw Rice Bran	Unsaponifiable Matter (U) [Total U]	Peanut Oil	Soy Protein
Cellulose	10	· · ·	· · ·	· · ·	10.1	8.1
Cellulose + raw U (1X)	10	· · ·	· · ·	0.4 [0.4]	9.7	8.1
Cellulose + stabilized U (1X)	10	· · ·	· · ·	0.4 [0.4]	9.7	8.1
Cellulose + raw U (2X)	10	· · ·	· · ·	0.8 [0.8]	9.3	8.1
Cellulose + stabilized U (2X)	10	· · ·	· · ·	0.8 [0.8]	9.3	8.1
Raw rice bran	· · ·	· · ·	45	· · · [0.4]	0.9	0.5
Stabilized rice bran	· · ·	48	· · ·	· · · [0.4]	0.4	· · ·
Stabilized rice bran + raw U (1X)	· · ·	48	· · ·	0.4 [0.8]	· · ·	· · ·
Stabilized rice bran + stabilized U (1X)	· · ·	48	· · ·	0.4 [0.8]	· · ·	· · ·

[a] All diets contained: casein, 11.8%; mineral mix, 3.5%; vitamin mix, 1%; DL-methionine, 0.3%; choline bitartrate, 0.2%; cholesterol, 0.3%; corn starch for balance of matter as needed.

[b] Composition of rice bran oil (Kahlon et al 1992a): unsaponifiables, 4.1%; saturates, 19.4% (palmitic, 17.0%; stearic, 1.7%; arachidic, 0.6%); monounsaturates, 40.9% (oleic, 39.4%; vaccenic, 0.9%; gadoleic, 0.6%); polyunsaturates, 35.6% (linoleic, 34.3%; linolenic, 1.3%).

[c] Composition of rice bran oil unsaponifiables: sterols, 42%; higher alcohols, 24%; ferulic acid esters, 20%; hydrocarbons, 10%; unidentified compounds, 2% (Akiya 1962).

[d] Composition of peanut oil (data provided by Dyets, Inc.): unsaponifiables, 0.4%; saturates, 17% (palmitic, 10.9%; stearic, 2.3%; arachidic, 0.7%; behenic, 2.3%; lignoceric, 0.8%); monounsaturates, 51.8% (palmitoleic, 1.0%; oleic, 50.8%); polyunsaturates, 30.8% (linoleic, 30.8%).

TABLE II
Effect of Stabilized Rice Bran, Raw Rice Bran, and Rice Bran Oil Unsaponifiable Matter (U) Diets Containing 0.3% Cholesterol on Feed Intake, Weight Gain, Body Weight, and Feed-Gain in Hamsters[a–c]

Diets	Feed Intake (g/day)	Weight Gain (g/21 days)	Final Weight Fasting (g)	Apparent Dry Matter Digestibility (%)[d]	Feed/Gain
Cellulose	8.6 ± 0.3ab	43 ± 3a–c	102 ± 3ab	85.1 ± 1a	4.3 ± 0.1b
Cellulose + raw U (1X)	9.2 ± 0.4a	44 ± 2ab	102 ± 3ab	84.7 ± 1ab	4.4 ± 0.2ab
Cellulose + stabilized U (1X)	8.5 ± 0.2ab	42 ± 2a–c	100 ± 2ab	84.2 ± 1a–c	4.3 ± 0.1b
Cellulose + raw U (2X)	8.2 ± 0.2b	37 ± 2c	95 ± 2b	83.2 ± 1c–e	4.8 ± 0.2a
Cellulose + stabilized U (2X)	8.5 ± 0.2ab	38 ± 2bc	97 ± 2ab	83.1 ± 1c–e	4.7 ± 0.2a
Raw rice bran	9.0 ± 0.2ab	45 ± 2ab	104 ± 2a	83.4 ± 1b–d	4.3 ± 0.1b
Stabilized rice bran	8.8 ± 0.2ab	45 ± 1ab	103 ± 1a	83.8 ± 1a–d	4.1 ± 0.1b
Stabilized rice bran + raw U (1X)	8.3 ± 0.4ab	42 ± 3a–c	99 ± 3ab	81.8 ± 1e	4.2 ± 0.2b
Stabilized rice bran +stabilized U (1X)	8.9 ± 0.3ab	47 ± 2a	104 ± 3a	82.5 ± 1de	4.0 ± 0.1b

[a] All diets contained 10% total dietary fiber, 10.1% crude fat, and 3% N with the same animal-to-plant nitrogen ratio (56:44).

[b] Mean ± standard error mean. Values with different superscript letters differ significantly ($P \leq 0.05$).

[c] Initial and final full-fed body weights were similar among all treatments (64 ± 1 and 107 ± 1 g, respectively).

[d] Based on feed intake and fecal excretion data for 72 hr on days 13–15.

animals fed the control diet (Table II). However, animals fed cellulose with 2X raw U diet had significantly lower weight gain than did four other treatment groups. Apparent dry matter digestibility (digestibility = [intake – excretion]/intake) after two weeks was significantly ($P \leq 0.05$) lower in all treatments containing 2X U (stabilized or raw) compared with those fed the control diet, and significantly more feed was required per unit gain in hamsters fed cellulose diets with 2X added U compared to those fed the control diet. All diets containing rice bran or cellulose with 1X U were similar in feed efficiency to the control. Feed intake, weight gain, feed efficiency, and apparent feed digestibility were similar in animals fed diets containing either stabilized or raw rice bran or equivalent levels of their unsaponifiable matter.

Total Plasma Cholesterol

Total plasma cholesterol (TC) was significantly lower in hamsters fed the two stabilized rice bran diets with added U compared to those fed the control diet (Table III). Unsaponifiable matter from either stabilized or raw rice bran oil produced similar cholesterol-lowering effects. All diets containing cellulose and unsaponifiable matter from rice bran oil, as well as the stabilized or raw rice bran diets, tended to produce lower TC values than the control, but differences were not significant at $P \leq 0.05$. In previous studies we have reported significant TC reductions in hamsters fed stabilized rice bran diets compared with those fed cellulose control diet (Kahlon et al 1990; 1992a,b). In those studies, the control diet had casein as the sole source of nitrogen, whereas the rice bran diets provided nitrogen from both rice bran and casein. In the present study, the plant to animal (casein) nitrogen ratio was kept constant by using soy protein in diets containing cellulose to provide an amount of nitrogen equivalent to that supplied by rice bran protein in rice bran diets. This addition of soy protein to the control diet apparently contributed to a 15% lower level of hypercholesterolemia (281 mg/dl) in the control animals, compared with 322 or 327 mg/dl in control groups in the previous studies. Cholesterol was fed at 0.3% of the diet in each case. This lower plasma cholesterol level in animals fed the control diet might have resulted in the lack of significant TC reductions with rice bran diets in the present study. Plant proteins have been shown to be hypocholesterolemic relative to animal proteins (Sugano et al 1984, Terpstra et al 1991). Others have also observed a similar relationship between the cholesterol-lowering effect of a test substance and the degree of hypercholesterolemia in test animals (Shinnick et al 1988, Newman et al 1992). The nonsignificant TC-lowering effect with diets containing cellulose and rice bran oil U suggests that intact rice bran contains additional cholesterol-lowering components that act together with the unsaponifiable matter to produce significant TC reductions.

Previously, we observed that intact full-fat rice bran was more effective in lowering cholesterol than selected isolated rice bran fractions or their combinations (Kahlon et al 1992a,b). Other researchers have reported mixed results. Cholesterol lowering by rice bran and some of its fractions has been reported (Suzuki and Oshima 1970; Ayano et al 1980; Ishibashi and Yamamoto 1980; Suzuki 1982; Sugano et al 1984; Sharma and Rukmini 1986, 1987; Seetharamaiah and Chandrasekhara 1988, 1989; Raghuram et al 1989; Hegsted et al 1992; Nicolosi et al 1991), whereas no cholesterol-lowering effects by rice bran or its fractions were observed (Malinow et al 1976, Miyoshi et al 1986, Ranhotra 1989, Kestin et al 1990). The different findings reported by other investigators may be due to the level of rice bran or its fractions fed, the level of hypercholesterolemia, and/or the species tested. Results from the present study suggest that the cholesterol-lowering activity of rice bran may be influenced by the level of unsaponifiable material in the lipid fraction in addition to other rice bran components.

VLDLc, LDLc, and HDLc

Very low density lipoprotein cholesterol (VLDLc) was significantly lower only in hamsters fed stabilized rice bran with 1X added U from raw rice bran oil compared to those fed the control diet, and low density lipoprotein cholesterol (LDLc) values were not affected by any of the treatment groups compared with the control. When VLDLc and LDLc levels were calculated as percent of pooled lipoprotein cholesterol (PC = VLDLc+LDLc +HDLc), they were not significantly affected by any of the treatments compared with the control group (mean values: 17.6 and 23.6%, respectively), suggesting that VLDLc and LDLc production and clearance rates were not affected by any of the treatment diets. High density lipoprotein cholesterol (HDLc) values were similar to the control in all treatment groups except those fed stabilized rice bran with 1X added U from stabilized rice bran oil, where values were significantly lower. HDLc levels as percent of PC ranged from 57 to 62% and were similar to the control in all treatments except for significantly higher values in animals fed stabilized rice bran with added U from raw rice bran oil. The HDLc to LDLc ratio was similar in all treatment groups (mean = 2.5 ± 0.2). Newman et al (1992) reported significant lowering of LDLc while HDLc increased in chicks fed full-fat rice bran diet, and others reported that the ratio of HDLc to PC was significantly raised in subjects consuming rice bran (Kestin et al 1990). Differences in HDLc response may be related to the species studied or the methodology used for HDLc quantitation (precipitation vs. ultracentrifugation).

TABLE III
Effect of Stabilized Rice Bran, Raw Rice Bran, and Rice Bran Oil Unsaponifiable Matter (U) Diets Containing 0.3% Cholesterol on Plasma Total Cholesterol (TC), Very Low Density Lipoprotein Cholesterol (VLDLc), Low Density Lipoprotein Cholesterol (LDLc), High Density Lipoprotein Cholesterol (HDLc), and Triglycerides in Hamsters[a–c]

Diets	TC (mg/dl)	VLDLc (mg/dl)	LDLc (mg/dl)	HDLc (mg/dl)	HDLc/PC[d] (%)	Triglycerides (mg/dl)
Cellulose	281 ± 8a	43 ± 1a	58 ± 10a	134 ± 2a	58 ± 2b	315 ± 39a
Cellulose + raw U (1X)	275 ± 9ab	39 ± 2ab	53 ± 4a	124 ± 7ab	57 ± 1b	257 ± 28a
Cellulose + stabilized U (1X)	276 ± 12ab	37 ± 5ab	49 ± 9a	131 ± 7ab	61 ± 1ab	299 ± 38a
Cellulose + raw U (2X)	261 ± 10a–c	36 ± 5ab	51 ± 3a	124 ± 6ab	58 ± 2b	235 ± 32a
Cellulose + stabilized U (2X)	261 ± 10a–c	44 ± 3a	47 ± 3a	122 ± 4ab	57 ± 1b	329 ± 46a
Raw rice bran	267 ± 6a–c	34 ± 2ab	52 ± 1a	132 ± 4ab	60 ± 1ab	238 ± 26a
Stabilized rice bran	266 ± 10a–c	42 ± 5ab	51 ± 4a	128 ± 5ab	58 ± 1b	303 ± 47a
Stabilized rice bran + raw U (1X)	242 ± 12c	30 ± 3b	46 ± 3a	124 ± 8ab	62 ± 1a	214 ± 25a
Stabilized rice bran +stabilized U (1X)	246 ± 13bc	34 ± 3ab	50 ± 4a	112 ± 7b	57 ± 1b	263 ± 43a

[a] All diets contained 10% total dietary fiber, 10.1% crude fat, and 3% N with the same animal-to-plant nitrogen ratio (56:44).
[b] Values with different superscript letters differ significantly ($P \leq 0.05$).
[c] Mean ± standard error mean.
[d] PC = (VLDLc+LDLc+HDLc).

Plasma Triglycerides

There were no significant differences in plasma triglycerides in animals fed any of the treatment diets, compared with those fed the control diet, in agreement with findings by Chochi et al (1984), Fadel et al (1987), and Kahlon et al (1990, 1992a, 1993). However, rice bran consumption has been reported to result in significant plasma triglycerides reduction in animals (Suzuki 1982, Newman et al 1992, Kahlon et al 1992b) and in humans (Kestin et al 1990, Hegsted et al 1992). The distribution of triglycerides in the VLDL, LDL, and HDL fractions was not significantly influenced by any of the treatments compared with the control group (76, 11, and 12%, respectively).

Liver Weight and Liver Lipid

Although liver weights per 100 g of fasting body weight were within a narrow range (4.37–4.86 g) among all groups, animals fed stabilized rice bran with 1X added U or raw rice bran diets had significantly lower liver weights compared to those fed the control diet (Table IV). Lower liver weights were not attributable to lower feed intake or feed efficiency. Liver lipid per 100 g of liver was significantly lower in animals fed cellulose diets containing 2X U and in animals fed any of the rice bran diets com-

pared with those fed the control or cellulose with 1X U diets. Liver lipids in animals fed rice bran with added U were significantly lower than those in all other treatment groups. The data suggest that rice bran unsaponifiable matter and other components of rice bran may prevent fatty infiltration of the liver in cholesterol-fed animals, in agreement with previous observations (Kahlon et al 1992a).

Liver Cholesterol

As with total liver lipids, significantly lower cholesterol/g of liver was found in hamsters fed cellulose diets with 2X added U or any rice bran diet, compared with control or cellulose with 1X U diets. Significantly greater liver cholesterol reductions were observed in animals fed rice bran with 1X added U diets compared with those fed all other diets. This agrees with our previous observations of significant reductions in liver cholesterol with rice bran diets in hamsters (Kahlon et al 1990; 1992a,b; 1993). No significant liver cholesterol reductions were observed with 0.4% U cellulose diets compared with cellulose control diet, in contrast to the observations by Sharma and Rukmini (1987), who reported significant liver cholesterol reductions in rats fed 0.4% rice bran oil unsaponifiable matter and 1.0% cholesterol in 2% cellulose diets.

Liver cholesterol reductions with rice bran diets were 1.5 to 3-fold greater than reductions with cellulose diets containing equivalent levels of unsaponifiable matter. Cellulose diets with 0.4% unsaponifiable matter resulted in 11% liver cholesterol reductions compared to 30% reductions with stabilized or raw rice bran, while cellulose diets with 0.8% added U resulted in 30% reductions compared to 45% reductions for rice bran with added U. Liver cholesterol and liver lipid were highly positively correlated ($r = 0.98$, $P \leq 0.0001$).

Fat and Neutral Sterol Digestibilities

Fat intake during the fecal collection period (days 13–15) was similar in all treatments except for the group fed cellulose with 1X raw U (Table V). Significantly higher fat intake (1,006 mg/day) in that group appears to be the result of one animal that excessively spilled the diet and made it difficult to measure actual feed intake. Excluding intake data from this animal would bring mean fat intake for this group to 941 mg/day, thus eliminating the difference. Fecal fat excretion was significantly greater, and fat digestibility significantly lower for animals fed all treatment diets compared with those fed the control diet. In addition, animals fed rice bran diet with added U had significantly higher fat excretion and significantly lower apparent fat digestibility than all other treatments. Some of the increased fat excretion observed with the rice bran diets may be related to differences in apparent fat absorption of endogenous rice bran oil versus supplemented pea-

TABLE IV
Effect of Stabilized Rice Bran, Raw Rice Bran, and Rice Bran Oil Unsaponifiable Matter (U) Diets Containing 0.3% Cholesterol on Liver Weight, Lipid, and Cholesterol in Hamsters[a–c]

Diets	Liver wt/100 g of Fasting Body wt (g)	Lipid (g)/100g of Liver	Cholesterol (mg/g)
		Liver	
Cellulose	4.86 ± 0.12a	12.7 ± 0.3a	46 ± 1a
Cellulose + Raw U (1X)	4.64 ± 0.11a–c	12.1 ± 0.3a	40 ± 2a
Cellulose +Stabilized U (1X)	4.76 ± 0.12ab	12.6 ± 0.3a	42 ± 1a
Cellulose +Raw U (2X)	4.52 ± 0.08a–c	10.9 ± 0.3b	32 ± 2b
Cellulose +Stabilized U (2X)	4.66 ± 0.11a–c	10.9 ± 0.4b	32 ± 2b
Raw Rice Bran	4.51 ± 0.09bc	10.9 ± 0.3b	32 ± 2b
Stabilized Rice Bran	4.57 ± 0.07a–c	10.9 ± 0.4b	32 ± 2b
Stabilized Rice Bran +Raw U (1X)	4.37 ± 0.12c	9.4 ± 0.5c	26 ± 3c
Stabilized Rice Bran +Stabilized U (1X)	4.43 ± 0.13bc	9.4 ± 0.4c	25 ± 2c

[a] All diets contained 10% total dietary fiber, 10.1% crude fat, and 3% N with the same animal to plant nitrogen ratio (56:44).
[b] Values with different superscript letters differ significantly ($P \leq 0.05$).
[c] Mean ± standard error mean.

TABLE V
Effect of Stabilized Rice Bran, Raw Rice Bran, and Rice Bran Oil Unsaponifiable Matter (U) Diets Containing 0.3% Cholesterol on Fat and Neutral Sterol Intake, Excretion, and Apparent Digestibilities in Hamsters[a–d]

Diets	Fat			Neutral Sterols	
	Intake (mg/d)	Excreted (mg/d)	Digestibility (%)	Intake (mg/d)	Excreted (mg/d)
Cellulose	866 ± 30bc	37 ± 3f	96 ± 1a	43 ± 2d	8 ± 1d
Cellulose + raw U (1X)	1,006 ± 68a	79 ± 3d	92 ± 1b	60 ± 4a	21 ± 2b
Cellulose + stabilized U (1X)	856 ± 27bc	64 ± 3e	93 ± 1b	51 ± 2bc	18 ± 1bc
Cellulose + raw U (2X)	853 ± 15bc	107 ± 2c	87 ± 1cd	59 ± 1a	33 ± 1a
Cellulose + stabilized U (2X)	846 ± 26c	99 ± 6c	88 ± 1c	59 ± 2a	34 ± 2a
Raw rice bran	960 ± 32ab	124 ± 6b	87 ± 1d	NA	NA
Stabilized rice bran	911 ± 17a–c	106 ± 4c	88 ± 1c	47 ± 1cd	15 ± 1c
Stabilized rice bran + raw U (1X)	944 ± 36a–c	150 ± 7a	84 ± 1e	57 ± 2ab	34 ± 3a
Stabilized rice bran + stabilized U (1X)	947 ± 30a–c	147 ± 5a	84 ± 1e	58 ± 2a	31 ± 2

[a] All diets contained 10% total dietary fiber, 10.1% crude fat, and 3% N with the same animal-to-plant nitrogen ratio (56:44).
[b] Values with different superscript letters differ significantly ($P \leq 0.05$).
[c] Based on feed intake and fecal excretion data for 72 hr on days 13–15. Values are means of three days. NA = not available.
[d] Mean ± standard error mean.

nut oil. The data suggest that rice bran diets with endogenous U or supplemented with isolated U result in greater interference with fat absorption than cellulose diets supplemented with equivalent levels of isolated U. The significantly greater fecal fat excretion in animals fed rice bran with added U diets was reflected in significantly greater plasma and liver cholesterol reductions in these groups.

Neutral sterol intake with all treatments except the stabilized rice bran group was significantly greater than that of the control group during the fecal collection period. The lack of significant difference with stabilized rice bran compared to the control diet may be attributable to the similar total neutral sterol content of the two diets (0.5%), whereas all other diets contained from 0.6–0.7%. Neutral sterol excretion was significantly greater in hamsters consuming any of the treatment diets when compared with the control diet. Diets containing 0.8% U (cellulose + 2X U and rice bran + 1X U) resulted in significantly greater neutral sterol excretion compared with all other treatments. The increased fecal excretion of neutral sterols probably included nondietary as well as dietary sources of neutral sterols. Other investigators have reported increased fecal fat and cholesterol excretion with dietary plant sterols, due to interference by plant sterols with absorption of dietary fat and cholesterol as well as increased endogenous cholesterol excretion (Best et al 1954, Kudchodkar et al 1976, Lees et al 1977). Fecal neutral sterols and fat were significantly negatively correlated with liver cholesterol ($r = –0.89$, $P \leq 0.0014$ and $–0.97$, $P \leq 0.0001$, respectively); but only fecal fat was significantly negatively correlated with plasma cholesterol ($r = –0.91$, $P \leq 0.0006$), while fecal neutral sterols and plasma cholesterol had a nonsignificant negative correlation ($r = –0.83$, $P \leq 0.0052$). The lipid and neutral sterol excretion data suggest that possible mechanisms for cholesterol-lowering by rice bran and rice bran unsaponifiable matter in hamsters may be through diminished absorption-reabsorption of cholesterol and lipid from the digestive tract and interference with cholesterol metabolism by sterols from rice bran oil U.

In summary, liver cholesterol was significantly lowered by stabilized or raw rice bran diets with or without added unsaponifiable matter from rice bran oil (U = 0.8 or 0.4%), and by cellulose diets with added rice bran oil U (0.8%). Plasma cholesterol was significantly lowered in animals fed rice bran diets with added unsaponifiable matter while maintaining the ratio of HDLc to LDLc. Rice bran diets appeared to lower both plasma and liver cholesterol to a greater extent than cellulose based diets with equivalent levels of unsaponifiable matter, but differences were significant only for liver cholesterol. Fecal fat excretion was significantly negatively correlated with liver and plasma cholesterol. The data suggest that unsaponifiable matter and other components of rice bran have cholesterol-lowering activity, possibly through increasing the excretion of fat and neutral sterols.

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