Nutritional Properties of Hard White and Hard Red Winter Wheats and Oatmeal. I. Effects on Cholesterol Levels and Fecal Fat, Neutral Sterols, and Bile Acids in Cholesterol-Fed Rats[1]

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ABSTRACT

The effects of hard white and hard red winter wheats (whole flour, bran, straight-grade flour) and oatmeal on rat weight gains, serum and liver cholesterol levels, fecal fat, neutral sterols, and bile acids were compared. No differences in weight gains or feed efficiencies were noted for animals fed red versus white whole flours, brans or straight-grade flours. Only animals fed the white wheat bran diet gained less weight than the control animals. The bran diets were the least efficient feeds. By the end of week 8, animals fed red wheat diets tended to have lower serum cholesterol levels than those fed respective white wheat diets, but the difference was significant only in animals fed whole wheat flour. The wheat brans, whole red flour, red straight-grade flour, and oatmeal diets were hypocholestrolemic compared to the control diet. No significant differences occurred in liver cholesterol levels between groups fed respective hard white versus hard red wheat diets. Animals fed bran diets had significantly lower liver cholesterol concentrations than did those fed whole flour or straight-grade flour, but concentrations were similar to those of animals fed the oatmeal diet. Correlation analysis showed significant inverse relationships between total serum cholesterol and dietary fiber (especially soluble fiber), amount of fecal fat and neutral sterols excreted daily, dietary phenolics and phytic acid, and diet viscosity. The relationships were stronger for liver cholesterol. All of these factors may contribute to the hypocholestrolemic properties of grain diets.

The effects of various sources of dietary fiber on serum lipids have been studied extensively and have been subjects of several comprehensive reviews (Judd and Truswell 1985, Kritchevsky et al 1990). It is well established that some water-soluble plant polysaccharides (Kritchevsky and Story 1986, Reiser 1987, Anderson et al 1990) and cereal 1-3,1-4-β-D-glucans (Klopfenstein and Hoseney 1987, Kashtan et al 1992) can lower serum and tissue cholesterol levels.

Most studies indicate that wheat bran, which contains about 5% soluble fiber, has little hypocholesterolemic effect. However, conflicting data continue to appear (Kies 1985, Pilch 1987, Anderson et al 1990, Kashtan et al 1992). For example, in 14 studies in which wheat bran was fed to humans at levels ranging from 9 to 38 g/day for 21-365 days, there was no change in serum cholesterol in nine of the studies; there was an increase of 7% in one study, and reductions of 7-22% in four studies (Pilch 1987). The wheat varieties fed are often not reported. Studies in which hypocholesterolemic effects were detected suggested that the variety of wheat and the coarseness of the bran might have modulated its effects (Munoz et al 1979).

A number of mechanisms have been suggested to explain the hypocholesterolemic effect of certain dietary fibers. Much atten-

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tion has been paid to the ability of dietary fiber to bind or trap bile acids, leading to their decreased absorption and increased excretion. That would increase the amount of cholesterol required for bile acid synthesis and lower the body cholesterol pool. Also, adsorption of bile salts by fiber in the small intestine could interfere with micelle formation, thereby reducing lipid absorption. Neutral steroid excretion would be increased and reentry of cholesterol into body pools would be reduced (Kies 1985). However, the changes noted in steroid excretion are not consistent and probably not large enough to fully explain the cholesterol-lowering effects of various dietary fiber components (Story 1985).

The objective of the experiment described here was to compare the effects of oatmeal and flours and brans of hard white and hard red winter wheats on rat weight gains; serum and liver cholesterol concentrations; and fecal fat, neutral sterols, and bile acids and to determine statistical relationships among the variables.

MATERIALS AND METHODS

Hard red winter wheat [Norkan (88-854)] and hard white winter wheat [KS84HW196 (88-850)] cultivars grown in 1988 at Fort Hays Agricultural Experiment Station, Hays, KS, were obtained. Each wheat cultivar was milled to whole wheat flour (100% of the grain) or straight-grade flour of 74% extraction and bran (26% of the grain). Whole wheat flour was milled using a stone mill (Europemill EM-25/251, Denmark). The straight-grade flour and bran were milled using the Miag Multomat S/100 (Braunchweig, Germany). After milling, fractions were stored in a cold room (2°C) until needed. Bran particle size was reduced using a Fitz mill model D-comminutor with a 1/16-in. (1.5-mm) screen (Fitzpatrick Co., Muncie, IN) before use. Diets were mixed in a Wenger ribbon mixer (Wenger Manufacturing Co., Sabetha, KS).

Eight groups of 10 male Wistar rats (SASCO Inc., Omaha, NE) were fed the following for eight weeks: 1) casein-based diet containing alphacel cellulose (control); 2) hard white wheat whole flour; 3) hard red wheat whole flour; 4) hard white wheat bran; 5) hard red wheat bran; 6) hard white straight-grade wheat flour; 7) hard red straight-grade wheat flour; or 8) oatmeal (Table I). Oatmeal was included as a comparison because it has been consistently shown to have a cholesterol-lowering effect when fed to animals or humans.

Diets were formulated to contain 20% protein and 6% fat and 45 or 50% by weight of the test product. Protein (%N × 6.25), fat, ash, calculated caloric value, insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and total dietary fiber (TDF) contents are presented in Table II. Standard AACC (1983) methods were used in analyzing diets: method 46-16 for protein; method 30-25 for fat; method 08-01 for ash; and method 32-07 for IDF, SDF, and TDF. Wheat bran diets (Diets 4 and 5) were high in IDF. The oatmeal diet (Diet 8) had about equal amounts of SDF and IDF. TDF was lowest for the straight-grade flour diets (Diets 6 and 7). Whole wheat flour diets had intermediate levels of TDF.

Animals, initially weighing 150 ± 5 g, were individually housed in stainless steel cages in an environmentally controlled room with a 12-hr light-dark cycle. Diets and water were provided ad libitum. Animals were weighed weekly, feed consumption records were kept, and feed efficiencies were calculated.

Total Serum Cholesterol

Blood samples were drawn by cardiac puncture from ether-anesthetized animals after weeks 4 and 8. Blood was allowed to clot at room temperature, then centrifuged at 12,000 × *g* for 15 min. Serum samples were analyzed in duplicate for total cholesterol using reagents from Sigma Chemical Co., St. Louis, Mo. (Procedure 352 and 352-3).

Liver Cholesterol

After eight weeks of feeding, the animals were sacrificed by placing them in an ether atmosphere. Their livers were removed, rinsed under cold tap water, blotted dry, weighed and frozen. Lipids were extracted with chloroform-methanol (Klopfenstein and Clegg 1980). In that method, lipids are extracted from tissue samples by homogenizing with 7.5 ml of 2:1 chloroform-methanol

TABLE I
Percent Composition of Rat Diets Containing Wheat, Oatmeal, or Cellulose[a]

Diet	Content	Cereal	Casein[b]	Corn Starch[b]	Fat[c]	Cellulose[d]
1	Control	0	23.5	47.0	5.0	6.0
2	White whole flour	50	14.9	12.4	4.2	...
3	Red whole flour	50	13.4	13.9	4.1	...
4	White bran	45	12.9	20.4	3.2	...
5	Red bran	45	12.0	21.2	3.3	...
6	White straight-grade flour	50	14.7	12.1	4.6	...
7	Red straight-grade flour	50	14.9	12.0	4.5	...
8	Oatmeal	50	13.0	17.1	1.4	...

[a]All diets contained 4% salt mixture XVII, 1% vitamin mix 2 (both obtained from ICN Nutritional Biochemicals, Cleveland, OH); 1% cholesterol, 0.30% DL-methionine, 0.2% choline bitartrate (all from Sigma Chemical Co., St. Louis, MO); and 12% sucrose.
[b]Vitamin-free casein and corn starch from Sigma Chemical Co., St. Louis, MO.
[c]Vegetable oil (soybean) from a local supermarket.
[d]Alphacel from ICN Nutritional Biochemicals, Cleveland, OH.

TABLE II
Protein, Fat, Ash, Dietary Fiber, Phytic Acid, Phenolic Acids, and Calculated Caloric Content and Viscosity of Rat Diets (as Fed)

Diet[a]	Protein (%)	Fat (%)	Ash (%)	IF[b] (%)	SF[b] (%)	TDF[b] (%)	Phytic Acid (%)	Total Phenolics (μg/g)	Diet Viscosity (cP)	Energy (kcal/100 g)
1	20.9	6.5	3.21	4.75	0.00	4.75	0.01	0.0	60	384
2	20.8	6.4	5.64	5.90	1.56	7.46	0.48	92.6	100	393
3	20.4	6.0	5.90	5.82	1.44	7.26	0.45	104.1	100	393
4	21.3	7.3	5.42	16.16	2.56	18.72	1.52	143.5	120	325
5	21.3	7.3	5.91	17.36	3.09	20.45	1.58	144.0	120	325
6	19.9	6.7	4.01	0.61	1.02	1.63	0.09	35.8	70	406
7	21.1	6.7	4.60	0.65	0.97	1.62	0.08	45.8	70	406
8	19.8	7.0	6.42	3.73	3.37	7.10	0.59	60.4	240	390

[a]Diet 1 = control, 2 = white wheat whole flour, 3 = red wheat whole flour, 4 = white wheat bran, 5 = red wheat bran, 6 = white wheat straight-grade flour, 7 = red wheat straight-grade flour, 8 = oatmeal.
[b]IF = insoluble dietary fiber, SF = soluble dietary fiber, TDF = total dietary fiber.

(v/v) as in the Folch et al (1957) method, then adding an additional 2.5 ml of chloroform (to facilitate separation of layers) along with 2.5 ml of wash water. After thorough remixing, the chloroform layer was recovered. An aliquot of the extract was evaporated to dryness under nitrogen; the lipid residue, redissolved in absolute ethanol, was used for determination of total liver cholesterol (Rosenthal et al 1957).

Fecal Fat

Feces were collected daily for three days during week 8. After each collection, feces were sealed in plastic freezer bags and frozen. At the end of the three-day collection period, feces from the same animal were pooled, weighed, and dried to constant weight in an air oven at 50°C (Klopfenstein 1990). Fat in feces was extracted from 1-g samples by the Soxhlet method using petroleum ether (AOAC 1984).

Neutral Sterols and Bile Acids in Feces

Total neutral sterols and bile acids were extracted with absolute ethanol from oven-dried and finely ground fecal samples by the method of Roscoe and Fahrenbach (1963). An aliquot of the extract was saponified, and neutral sterols were removed by extracting the saponified, dried extract with hexane. Bile acids were extracted from the residue with chloroform after removal of neutral sterols.

The chloroform was evaporated, and acidic pigments were removed by redissolving the residue in benzene-methanol (1:1 v/v) solution and adding activated charcoal. The mixture was then filtered through Whatman No. 1 paper. The filtrate was evaporated to dryness under nitrogen, and the residue was extracted with hexane to remove free fatty acids. The extracted residue was dissolved in 0.1 *N* sodium hydroxide solution (Collings et al 1979) and stored under nitrogen. Total bile acids were determined using the detection system of Kritchevsky et al (1963), modified for colorimetry by Collings et al (1979).

Phytic Acid

The method of Tangkongchitr et al (1981) was used to extract phytic acid from wheat whole flours, brans, and straight-grade flours and oatmeal. It was then measured by the method of Lindberg and Ernster (1956), as modified by Nahapetian and Bassiri (1975) (Table II).

Among the different wheat diets, the bran diets had the highest concentrations of phytic acid. The straight-grade flour diets had the lowest concentrations of phytic acid, whereas the whole flour diets had intermediate concentrations. No significant difference occurred in phytic acid concentration between respective red versus white wheat diets. Phytic acid was highly correlated with IDF ($r = 0.9579$, $P = 0.0001$) and TDF ($r = 0.9819$, $P = 0.0001$).

Total Phenolic Compounds

Total phenolic compounds were extracted from the grains by the method of Pussayanawin and Wetzel (1987) using dilute sulfuric acid. Clarase fungal amylase was added to the extract before centrifugation to clarify the extract. Determination of phenolic compounds was accomplished by the colorimetric method of Swain and Hillis (1959), and concentration in the diet was calculated (Table II).

The bran diets had the highest concentration of total phenolic compounds; the straight-grade flour diets had the lowest concentration; and the whole flour diets had intermediate concentrations.

Viscosity

Diet slurry viscosity (Table II) was measured with a Rapid Visco Analyzer (RVA-3C) interfaced to a computer (Newport Scientific Pty. Ltd., Sydney, Australia). The procedure for sample preparation as described by Deffenbaugh and Walker (1989) was used. Each slurry (28 g with 25% solids) was placed in an aluminum can 70 mm high and 38 mm in diameter. A paddle was inserted in the can, and then the can, sample, and paddle were placed in the RVA. The viscosity at 12 min was recorded. RVA units (stirring numbers) were converted to centipoise (cP) using the manufacturer equivalence of 1 stirring number = 10 cP.

Statistical Procedures

Data were analyzed with the Statistical Analysis System (SAS Guide 1989) using one-way analysis of variance with the Fisher's protected least significant difference (LSD) test for significant differences among means (Ott 1988). A complete randomized experimental design was used.

RESULTS AND DISCUSSION

Effects on Rat Weight Gains and Feed Efficiencies

By the end of week 8, animals fed the bran diets had lower overall weight gains than animals fed the other diets, but the difference was not significant in all cases (Table III). Weight gains and feed efficiencies were not significantly different for red versus white wheat diets. Diets containing brans were the least efficient feeds. Overall weight gains showed a significant inverse relationship with the amount of IDF fiber ($r = -0.4551$, $P = 0.0004$) and TDF ($r = -0.4419$, $P = 00001$). No significant correlation was found for SDF and weight gain.

Feed efficiencies tended to decrease as the amount of TDF in the diets increased ($r = -0.7486$, $P = 0.0001$). Weight gains were positively correlated with caloric contents of the diets ($r = 0.3627$, $P = 0.0060$).

Total Serum Cholesterol

At the end of four weeks, no significant differences were observed in total serum cholesterol levels in animals fed respective

TABLE III
Cumulative Weight Gains, Feed Efficiencies, and Feed Intake of Rats Fed Wheat and Oatmeal Diets for Eight Weeks[a,b]

Diet	Content	Weight Gain (g)	Feed Efficiency[c]	Feed Intake (g)
1	Control	286 ab	0.255 b	1,120 ab
2	White whole flour	312 a	0.265 a–c	1,179 a
3	Red whole flour	269 a–c	0.250 c	1,074 bc
4	White wheat bran	241 c	0.216 d	1,117 ab
5	Red wheat bran	254 bc	0.226 d	1,111 ab
6	White straight-grade flour	299 a	0.268 ab	1,110 ab
7	Red straight-grade flour	272 a–c	0.272 a	983 c
8	Oatmeal	296 ab	0.263 a–c	1,127 ab
	LSD[d]	43.4	0.0173	92

[a] Means in the same column not followed by the same letter are significantly different ($P < 0.05$).
[b] Diets contained 1% cholesterol.
[c] Grams gained per gram of feed consumed.
[d] Least significant difference.

TABLE IV
Effects of Wheat and Oatmeal Diets on Serum and Liver Cholesterol in Rats[a,b]

Diet	Content	Total Serum Cholesterol (mg/dL) Week 4	Total Serum Cholesterol (mg/dL) Week 8	Liver Cholesterol (mg/g of liver)
1	Control	98 a	93.1 a	32.2 a
2	White whole flour	108 a	84.5 ab	27.7 b
3	Red whole flour	104 a	60.5 d	26.3 b
4	White bran	64 b	69.8 cd	14.1 c
5	Red bran	73 b	56.7 d	12.1 c
6	White straight-grade flour	101 a	85.5 ab	29.9 ab
7	Red straight-grade flour	94 a	75.3 bc	27.9 b
8	Oatmeal	110 a	62.5 cd	17.1 c
	LSD[c]	16.6	13.9	5.9

[a] Means in the same column not followed by the same letter are significantly different at $P < 0.05$.
[b] Diets contained 1% cholesterol.
[c] Least significant difference.

hard white versus hard red wheat diets (Table IV). Animals fed the bran diets had lower total serum cholesterol than those fed any other diet. None of the other diets had any cholesterol lowering effect when compared to the control diet. Because the amount of cholesterol ingested by animals fed bran diets was not statistically lower than that ingested by animals fed any other diet, the data indicate that both white and red wheat bran fractions lowered serum cholesterol by the end of four weeks.

At the end of eight weeks, all animals except those fed white whole flour and white straight-grade flour had lower total serum cholesterol than did those fed the control diet, which contained no cereal product. Animals fed red wheat diets tended to have lower serum cholesterol levels than those fed respective white wheat diets, but the difference was only significant in animals fed whole flour diets. Among animals fed the different wheat fractions, those fed bran had the lowest serum cholesterol concentration and those fed straight-grade flour had the highest serum cholesterol concentration (Table IV). Animals fed red whole flour, white or red bran, or red straight-grade flour had serum cholesterol levels not significantly different than those of animals fed oatmeal. Ranhotra et al (1977) has reported that diets containing 50% wheat bran of different particle size lowered serum cholesterol when fed to rats for four weeks, with the fine bran having the greatest cholesterol-lowering effect. Munoz and colleagues (1979) demonstrated that soft white wheat bran had no effect on serum cholesterol, whereas hard red spring wheat bran significantly lowered plasma total cholesterol levels. In the present study, both hard red and hard white brans (ground to pass through a 1.5-mm screen) reduced serum cholesterol.

Liver Cholesterol

No significant differences in liver cholesterol levels occurred between groups fed respective hard white versus hard red wheat diets (Table IV). Among the different wheat fractions, bran gave significantly lower liver cholesterol concentrations than did whole flours or straight-grade flours. Liver cholesterol values were similar for animals fed the bran diets and the oatmeal diet. Animals fed all diets containing cereal fractions had lower liver cholesterol concentrations than did those fed the control diet containing no cereal.

Numerous studies have been conducted concerning liver cholesterol concentrations in relation to diet. One study by Chang et al (1979) showed liver cholesterol levels to be higher in rats fed whole wheat or low-grade flours than in those fed patent flour. Another study by Van Beresteyn et al (1979) showed that animals fed wheat bran diets had lower liver cholesterol concentrations than those fed cellulose-containing or fiber-free diets. In contrast, Klopfenstein (1990) reported no significant difference in liver cholesterol concentrations in animals fed diets containing 5% cellulose or 5% wheat bran. Oatmeal, which is rich in the water-soluble (1,3)(1,4)-β-glucan, has been shown to have significant hypocholesterolemic effects. However, in the present study, the bran diets had statistically the same cholesterol-lowering effect as the oatmeal diet.

Serum cholesterol concentrations after eight weeks were negatively correlated with TDF ($r = -0.3701$, $P = 0.0002$), IDF ($r = -0.3275$, $P = 0.0044$) and SDF ($r = -0.5186$, $P = 0.0001$). Correlations with liver cholesterol followed a similar pattern but were stronger (TDF: $r = -0.6994$, $P = 0.0001$; IDF: $r = -0.6483$, $P = 0.0001$; and SDF: $r = -0.8114$, $P = 0.0001$). Phytic acid and phenolic compounds in the diets were highly correlated with IDF ($r = 0.9579$, $P = 0.0001$ and $r = 0.8691$, $P = 0.0001$, respectively). That suggests that all dietary fiber fractions, as well as phytic acid and phenolic compounds, might play roles in lowering body cholesterol.

Diet Slurry Viscosity

The viscosity and gelling properties of soluble dietary fibers may have important effects on the hydrolysis and absorption of lipids (Anderson et al 1990). Also, SDF can increase the viscosity of the luminal contents in the small intestines. This process has been indicated as a possible means by which SDF lowers serum cholesterol (Gordon 1989). In this experiment, the oatmeal diet had the highest viscosity, whereas the straight-grade flour and control diets had the lowest (Table II). Correlation analyses showed a weak but significant inverse relationship between diet viscosity and total serum ($r = -0.3850$, $P = 0.0007$) and liver cholesterol ($r = -0.5321$, $P = 0.0001$).

Fecal Lipids (Ether Extract) and Total Neutral Sterols

Animals fed red wheat diets had significantly higher fat concentration in the feces than did animals fed white wheat diets (Table V), except for those fed the bran fractions (Diets 4 and 5). However, daily fecal fat loss was not significantly different for animals fed red versus white wheats. The oatmeal group (Diet 8) had the highest daily fecal fat loss. The control animals, whose diet contained no cereal product, had the lowest daily fecal fat loss.

Animals fed brans (Diets 4 and 5) had lower fecal lipid and total neutral sterol concentrations than did those fed straight-grade flours (Diets 6 and 7). Feces of animals fed whole wheat flours (Diets 2 and 3) had intermediate lipid and neutral sterol concentrations, and those of animals fed the straight-grade flours (Diets 6 and 7) had the highest concentrations. Feeding animals diets containing cereal products resulted in higher daily amounts of neutral sterols excreted than did feeding the no-cereal diet. Significant inverse relationships were present between fecal neutral sterol concentrations and dietary fiber, with the strongest correlations being with TDF ($r = -0.7868$, $P = 0.0001$) and IDF ($r = -0.8072$, $P = 0.0001$). Regression analyses showed a weak but significant relationship between amount of neutral sterols excreted and total serum ($r = -0.2782$, $P = 0.0314$) and liver ($r = -0.5167$, $P = 0.0002$) cholesterol levels.

TABLE V
Effects of Wheat Diets on Rat Fecal Fat, Total Neutral Sterols, and Bile Acids[a,b]

	Ether Extracted Lipid		Total Neutral Sterols		Total Bile Acids	
Diet[c]	mg/g	mg/day	mg/g	mg/day	mg/g	mg/day
1	5.8 d	14.2 c	9.1 d	22.8 e	19.1 c	49.9 ab
2	6.2 d	17.8 c	10.3 c	29.1 cd	18.6 c	52.9 ab
3	7.5 c	18.4 c	9.7 cd	24.6 de	20.9 bc	51.9 ab
4	4.5 e	25.3 b	6.8 e	34.4 ab	11.3 d	57.5 a
5	4.6 e	25.9 b	6.9 e	38.5 a	8.4 d	46.9 ab
6	13.9 b	23.4 b	17.9 a	29.4 cd	23.5 ab	40.5 b
7	15.9 a	26.9 ab	18.6 a	31.3 bc	27.3 a	45.2 ab
8	13.1 b	31.2 a	13.4 b	32.4 bc	18.7 c	45.3 ab
LSD[d]	1.05	4.96	0.94	4.80	3.84	13.38

[a] Means in the same column not followed by the same letter are significantly different ($P < 0.05$).
[b] Dry matter basis.
[c] Diet 1 = control, 2 = white whole wheat flour, 3 = red whole wheat flour, 4 = white wheat bran, 5 = red wheat bran, 6 = white wheat straight-grade flour, 7 = red wheat straight-grade flour, 8 = oatmeal. All diets contained 1% cholesterol.
[d] Least significant difference.

Total Bile Acid Concentration

Although no significant differences were observed in fecal bile acid concentrations in animals fed white versus red wheat, significant differences were observed for the various wheat fractions (Table V). In general, animals fed bran (Diets 4 and 5) had the lowest concentrations of fecal bile acids, whereas the straight-grade flour (Diets 6 and 7) produced the highest concentrations. The whole flour (Diets 2 and 3) and oatmeal (Diet 8) diets resulted in intermediate bile acid concentrations. Linear regression analysis showed significant negative relationships between total fecal bile acid concentration and IDF ($r = -0.8211$, $P = 0.0001$), SDF ($r = -0.6425$, $P = 0.0001$), TDF ($r = -0.8254$, $P = 0.0001$), and phytic acid ($r = -0.7986$, p=0.0001).

CONCLUSIONS

Weight gains in rats fed red versus white hard winter wheat diets were not significantly different. Both wheats were equally efficient feeds. Animals fed oatmeal gained the same amount of weight as those fed the whole wheat flours.

Both oatmeal and wheat diets were shown to be hypocholesterolemic, and the effect tended to be greater with the red wheat diets. Regression analysis of the data indicated that multiple factors are probably responsible. The data suggest that increased excretion of fat and neutral sterols, but not higher excretion of bile acids, may be one mechanism by which red and white wheat brans and oatmeal lowered serum and liver cholesterol. SDF was moderately correlated with serum cholesterol concentration and more strongly correlated with liver cholesterol levels. Therefore, it cannot be ruled out as a contributing agent to the cholesterol-lowering effect of wheat, especially bran, diets. Dietary phytic acid and phenolic compounds and diet viscosity were also correlated consistently with serum and liver cholesterol. These and possibly other factors associated with the bran may affect cholesterol status in animals.

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