Treatment of Dietary Casein with Formaldehyde Reduces Its Hypercholesterolemic Effect in Rabbits

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ABSTRACT Rabbits were fed cholesterol-free, semipurified diets containing 42% (wt/wt) casein or 21% casein plus one of the following nitrogen sources: soy isolate, amino acid mixture simulating casein, amino acid mixture simulating soy isolate, formaldehyde-treated casein or formaldehyde-treated soy isolate. Two additional groups of rabbits were fed the 42% casein diet and the diet containing casein plus soy isolate to which 0.4% (wt/wt) pure formaldehyde was added, this amount being identical to the amount of formaldehyde present in the diets with formaldehyde-treated proteins. Growth was somewhat reduced on the three diets containing 42% casein. The diet containing 42% casein to which no formaldehyde had been added induced severe hypercholesterolemia, the level of serum cholesterol after 8 weeks being about 10 mmol/L. The hypercholesterolemia was markedly reduced by the replacement of half of the casein by soy isolate, formaldehyde-treated soy isolate or formaldehyde-treated casein. No significant reduction of the concentration of serum cholesterol was seen when half of the 42% casein was replaced by an amino acid mixture imitating either casein or soy isolate. Formaldehyde per se did not significantly influence the level of serum cholesterol. We conclude that the differential tertiary structure of intact casein and soy isolate is an important factor in determining the cholesterolemic responses in rabbits to these proteins. J. Nutr. *114*: 17–25, 1984.

INDEXING KEY WORDS rabbits • serum cholesterol • dietary casein • dietary soybean protein • protein structure • formaldehyde

In young, growing rabbits the feeding of cholesterol-free, semipurified diets containing casein as a protein source produces hypercholesterolemia, whereas with soy protein low levels of serum cholesterol are maintained (1, 2). Huff and Carroll (3) have fed rabbits semipurifed diets containing an amino acid mixture resembling the composition of either casein or soy protein. It was found that the amino acid mixture equivalent in amino acid composition to casein produced concentrations of serum cholesterol similar to those obtained with casein, whereas the mixture imitating soy protein induced higher levels of serum cholesterol than the intact protein, but the levels were

 Received for publication 20 May 1983.

[1] KES was supported by the Netherlands Nutrition Foundation and by an International Agriculture Centre fellowship from the Dutch Ministry of Agriculture and Fisheries.

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significantly lower than those seen with casein. Thus, at least part of the differential effect of casein and soy protein on serum cholesterol appears to be related to differences in the amino acid composition of these proteins. It is possible, especially in the case of soy protein, that the structure of the proteins may also be involved in determining the level of serum cholesterol. In the present work this possibility was tested by modifying the structure of casein and soy protein by formaldehyde treatment and the feeding of these preparations to rabbits. Formaldehyde treatment of proteins effects cross-linking of the protein chains (4).

MATERIALS AND METHODS

Animals and diets. Male rabbits of the New Zealand White strain were obtained from the Broekman Institute, Helmond, The Netherlands. The rabbits were kept individually in cages with wire-mesh bases constructed of galvanized steel in a room with controlled lighting (14 hours/day), constant temperature (18°C) and humidity. On arrival in the animal house the animals, which were aged about 9 weeks, were maintained on commercial rabbit pellets (Trouw & Co. B.V., Putten, The Netherlands) for 2 weeks. They were then transferred to a semipurified diet containing soy isolate (Purina protein 500 E, Ralston Purina Co., St. Louis, MO) as a protein source for 4 weeks, when they were allocated for the experimental period to one of the 10 experimental diets on the basis of their serum cholesterol concentrations measured 2 days before the end of this preexperimental period. The diets contained either casein (acid casein, DMV B.V., Veghel, The Netherlands), soy isolate or amino acid mixtures imitating these proteins. In addition, some diets contained formaldehyde-treated casein or soy isolate (table 1). Two groups of animals were fed a diet containing 21% (wt/wt) casein or 21% soy isolate. The other groups received diets containing a basal proportion of 21% casein to which the following nitrogen sources were added: soy isolate, casein, amino acid mixtures simulating these proteins or formaldehyde-treated casein and soy isolate. The basal casein was added to the diets to ensure the availability of sufficient dietary protein. Two further groups of rabbits were fed the 42% casein diet or the diet containing casein plus soy isolate to which 0.4% pure formaldehyde was added. The amount of added formaldehyde was identical to the amount of formaldehyde present in the diets containing the formaldehyde-treated proteins. All diets were fed as pellets. The experimental period lasted 8 weeks.

In a separate experiment the digestibility of the formaldehyde-treated proteins was studied. The composition of the 21% casein and soy diets used in the digestibility trial (cf. table 3), was identical to diets 1 and 3 of table 1. The diets containing 10% of casein plus 20% of the formaldehyde-treated casein or soy protein consisted of 14% cornstarch, 17.8% (17.6% in the soy protein diet) sawdust and 15% dextrose; the contents of fats, minerals and vitamins were identical to those in the diets in table 1. Food was provided each day at 0900 hours on a restricted basis, the rabbits receiving 70 g/day, except for the animals on diets 5 and 6, which received 71 and 72 g, respectively, to correct for the lower protein and energy contents of these diets (see tables 1 and 2). Most rabbits consumed all their food within 4 hours. Water was provided ad libitum.

Analytical methods. Formaldehyde treatment of the proteins was performed as follows: 88 parts (by weight) of casein or soy isolate were added to 12 parts of a solution containing 18% (wt/wt) formaldehyde and 40% (wt/wt) of silica (Sipernat 22 S). After thorough mixing and incubating for 24 hours at 30°C the treated proteins were incorporated into the semipurified diets.

Formaldehyde in the protein preparations and the diets was determined according to Bremanis (5). Free formaldehyde in the formaldehyde-treated proteins was measured in the supernatant after centrifugation of a solution of 2 g of the protein in 100 ml of 0.287 M K_2HPO_4 (pH 8.9). The supernatants did not contain trichloroacetic acid–precipitable nitrogen. The amount of pepsin-digestible protein in the formaldehyde-treated proteins was estimated as described (6).

Nitrogen in protein preparations, feed, feces and urine of the rabbits was measured by the Kjeldahl method (7), and the protein

TABLE 1

Composition of the experimental diets

Ingredients	Amount for diet									
	1	2	3	4	5	6	7	8	9	10
						g				
Casein	21	42	—	21	21	21	21	21	42	21
Soy isolate	—	—	21	21	—	—	—	—	—	21
Methionine	—	—	0.2	0.2	—	0.2	—	0.2	—	0.2
Formaldehyde-casein	—	—	—	—	23	—	—	—	—	—
Formaldehyde–soy isolate	—	—	—	—	—	24.2	—	—	—	—
Casein amino acid mixture[1]	—	—	—	—	—	—	18.9	—	—	—
Soy amino acid mixture[1]	—	—	—	—	—	—	—	17.5	—	—
Cornstarch	17	7.5	17	7.5	7.5	7.5	8.5	9.0	7.5	7.5
Dextrose	21	9.5	21	9.5	9.5	9.5	10.6	11.3	9.5	9.5
Formaldehyde	—	—	—	—	—	—	—	—	0.4	0.4
Constant components[2]	41	41	40.8	40.8	41	40.8	41	41	41	40.8
Total	100	100	100	100	102	103.2	100	100	100.4	100.4

[1]The composition of the casein (and soy) amino acid mixture (grams/100 g of diet) was as follows: methionine, 0.548 (0.215); cystine, 0.079 (0.210); lysine, 1.402 (1.041); arginine, 0.643 (1.268); tryptophan, 0.284 (0.202); leucine, 1.706 (1.357); isoleucine, 0.957 (0.852); phenylalanine, 0.849 (0.892); tyrosine, 0.949 (0.644); threonine, 0.788 (0.641); valine, 1.227 (0.828); histidine, 0.616 (0.418); alanine, 0.547 (0.692); aspartic acid, 1.232 (2.003); glutamic acid, 3.897 (3.800); glycine, 0.335 (0.680); proline, 1.748 (0.833); serine, 1.139 (0.960). The amounts of added amino acids were 0.9 times those found in the intact proteins in order to correct for assumed differences in digestibility of amino acids (100% digestible) and proteins (90% digestible). [2]The constant components consisted of (grams): molasses, 4; coconut oil, 9; soybean oil, 1; dicalcium phosphate, 2.9; sodium chloride, 0.8 (in diets containing soy isolate, 0.6); potassium bicarbonate, 1.8; magnesium carbonate, 0.3; magnesium oxide, 0.2; vitamin premix, 1.2; mineral premix, 1.0. The vitamin premix contained (in milligrams, except as noted): thiamin, 6; riboflavin, 2.25; niacin, 15.2; Ca-pantothenate, 5.6; choline chloride, 200; inositol, 100; folic acid, 0.85; biotin, 0.06; pyridoxine · HCl, 2.25; *p*-aminobenzoic acid, 50; vitamin B-12, 1.5 μg; *dl*-α-tocopheryl acetate, 5; menadione, 0.4; vitamin A, 1500 IU; cholecalciferol, 300 IU; vitamin C, 40. The mineral premix contained (in milligrams): Na citrate · $2H_2O$, 153.5; $FeSO_4 \cdot 7H_2O$, 90; MnO_2, 14; $KAl(SO_4)_2 \cdot 12H_2O$, 10; $ZnSO_4 \cdot 7H_2O$, 20; KBr, 2; $NiSO_4 \cdot 6H_2O$, 0.85; $CuSO_4 \cdot 5H_2O$, 10; $CoSO_4 \cdot 7H_2O$, 0.5; $Na_2MoO_4 \cdot 2H_2O$, 0.5; KI, 0.5; As_2O_3, 0.02; NaF, 0.85; $Na_2B_4O_7 \cdot 10H_2O$, 0.5.

content of the protein preparations was calculated by multiplying the weight of nitrogen by 6.25. The apparent digestibility (in percent) of dry matter and nitrogen was calculated as the amount digested (ingested minus excreted in the feces) divided by the amount ingested times 100. Nitrogen retention (in percent) was calculated as the amount of nitrogen ingested minus nitrogen excreted in feces and urine divided by the amount ingested times 100.

Blood samples were taken from the rabbits between 0800 and 1000 hours after the removal of any remaining food at 1600 hours the previous day. The samples were taken from a marginal ear vein into tubes without anticoagulant, and after the tubes were standing at room temperature for at least 1 hour, serum was prepared by low speed centrifugation at room temperature. Cholesterol in serum was measured enzymatically (8) by using the kit (peroxidase method) supplied by Boehringer-Mannheim GmbH (Mannheim, West Germany). Three calibrated sera with low, medium and high cholesterol concentrations were used as cholesterol standards; the cholesterol concentration of these sera was determined by the method of Abell et al. (9).

RESULTS

Table 2 illustrates that formaldehyde-treated casein and soy isolate contained

TABLE 2

Characteristics of formaldehyde-treated casein and soy isolate preparations

	Chemical analysis of protein preparation[1]			
	Casein	F-casein	SI	F-SI
	wt %			
Total formaldehyde	—	1.7	—	1.9
Bound formaldehyde	—	1.0	—	1.1
Crude protein	89.2	79.5	85.0	75.8
Pepsin-digestible protein	87.4	36.2	83.0	61.7

[1]F, formaldehyde treated; SI, soy isolate.

similar amounts of free and bound formaldehyde. Formaldehyde-treatment of casein was more effective in reducing the amount of pepsin-digestible protein than the replacement of soy isolate. The formaldehyde content of the semipurified diets in table 1 was measured. Diets 1–4, 7 and 8, to which no formaldehyde was added, did not contain detectable amounts of formaldehyde. Diets 5 and 6, containing the formaldehyde-treated proteins, were found to contain 0.36 and 0.34% (by weight) of formaldehyde, respectively. In the control diets 9 and 10, to which pure formaldehyde was added, 0.34% of formaldehyde was found. Since 0.40% was added, it is likely that part of the formaldehyde evaporated during the pelleting of the diets.

Table 3 shows the digestibility coefficients of dry matter and nitrogen of rabbits on semipurified diets containing either casein or soy isolate. These values are somewhat less when the rabbits wore a collar to prevent coprophagy, but the difference did not reach statistical significance. Likewise, wearing a collar slightly reduced nitrogen retention. The digestibility of nitrogen appeared to decrease when formaldehyde-treated proteins were fed. In keeping with this observation nitrogen retention was lower in the animals fed the formaldehyde-treated proteins, the reduction being most pronounced in the rabbits fed formaldehyde-treated soy isolate. These effects induced by the formaldehyde-treated proteins should be interpreted with caution; the number of animals was limited and the content of total protein of the diets with formaldehyde-treated proteins was higher than in the other diets in the digestibility experiment.

As shown in table 4, the rabbits fed formaldehyde-treated casein (group 5) displayed the lowest body weight gain, which differed significantly from all the other groups, except for the animals that were fed 42% casein (groups 2 and 9). It would appear therefore, that the inclusion of 42% casein or 21% casein plus 23% formaldehyde-treated casein into the semipurified diet reduced

TABLE 3

Digestibility of dry matter and nitrogen, and nitrogen retention on semipurified diets containing either casein or soy protein[1]

Dietary protein,[2] *wt %*	Apparent digestibility: Dry matter	Apparent digestibility: Nitrogen	Nitrogen retention
		% of intake	
21% casein (5)	75.5 ± 0.8	91.3 ± 0.8	33.2 ± 1.9
21% soy (5)	76.3 ± 1.8	91.5 ± 1.4	34.3 ± 2.2
21% casein[3] (2)	72.1 (67.8, 76.4)	86.8 (85.0, 88.6)	28.0 (17.6, 38.4)
21% soy[3] (3)	69.5 ± 2.9	81.5 ± 2.9	23.7 ± 5.0
10% casein + 20% F-casein (2)		87.6 (86.4, 88.8)	25.8 (24.4, 27.1)
10% casein + 20% soy (2)		86.5 (87.8, 85.2)	18.3 (19.5, 17.0)

[1]The experimental period lasted 11 days; during the first 7 days the animals were allowed to become accustomed to the diet, followed by 4 days during which feces and urine were quantitatively collected. For calculations see analytical methods section. Results are expressed as means ± SE for three or five animals, or as the average (and range) for two animals, *n* in parentheses after protein descriptor. [2]F, formaldehyde treated. [3]The animals wore a collar to eliminate coprophagy.

TABLE 4

Body weights and growth of rabbits fed semipurified diets[1,2]

	Diet	Initial body wt[3]	Body wt gain
		g	*g/56 days*
1	21% casein	2209 ± 45	694 ± 48[a]
2	42% casein	2204 ± 44	615 ± 49[ab]
3	21% soy	2203 ± 64	760 ± 60[a]
4	21% casein + 21% soy	2205 ± 67	671 ± 56[a]
5	21% casein + 23% F-casein	2195 ± 69	508 ± 31[b]
6	21% casein + 24.2% F-soy	2200 ± 51	728 ± 57[a]
7	21% casein + 18.9% AA-casein	2198 ± 37	716 ± 50[a]
8	21% casein + 17.5% AA-soy	2217 ± 70	661 ± 64[a]
9	42% casein + 0.4% formaldehyde	2206 ± 68	556 ± 91[ab]
10	21% casein + 21% soy + 0.4% formaldehyde	2197 ± 66	665 ± 50[a]

[1]Results are expressed as means ± SE for 10 rabbits per group, except for group 8, which consisted of 9 animals. All animals were fed the 21%-soy diet until day 0 of the experiment, when the animals were allocated to diets 1–10. F, formaldehyde-treated; AA-casein, amino acid mixture imitating casein; AA-soy, amino acid mixture imitating soy isolate. [2]Means in the same column not sharing a common superscript are significantly different ($P < 0.05$; two-tailed Student's t-test). [3]The initial body weight refers to day −1 of the experiment.

growth of the rabbits. When the dietary groups with and without formaldehyde are compared, namely group 2 with groups 5 and 9 and group 4 with groups 6 and 10, it follows that the addition of formaldehyde does not affect growth of the rabbits.

The data in table 5 confirm that 21% casein in the diet, when compared with soy protein, induces elevated levels of serum cholesterol. The serum cholesterol levels of animals in groups 1 and 3 were already significantly different within 7 days, and this difference became gradually more pronounced during the course of the experiment. The twofold increase of casein in the diet at the expense of carbohydrates significantly elevated the level of serum cholesterol; the difference between groups 1 and 2 was statistically significant within 7 days. The concentration of serum cholesterol in the rabbits fed 42% casein continued to rise during the entire experimental period. When half of the 42% of dietary casein was replaced by soy isolate, the degree of hypercholesterolemia was markedly reduced (compare groups 4 and 2), but the levels of serum cholesterol were still significantly higher than those observed with the 21% soy isolate diet (compare groups 4 and 3). However, when half of the 42% of casein was replaced by an amino acid mixture imitating soy protein, the levels of serum cholesterol were not significantly different from those seen with the 42% casein diet (compare groups 8 and 2). Feeding the diet consisting of 21% intact casein plus an amino acid mixture equivalent in composition to casein (group 7) resulted in similar serum cholesterol concentrations as did feeding the diet containing the soy amino acid mixture (group 8).

The addition of 0.4% formaldehyde to the diet containing 42% casein did not significantly affect the level of serum cholesterol, except for day 28 of the experiment (compare groups 2 and 9). The mean cholesterol values found in rabbits fed the diet supplemented with formaldehyde were consistently higher, but this was due to two animals with extremely high values, the serum cholesterol concentrations of these rabbits being 46.5 and 26.6 mmol/L at day 56 of the experiment. Enrichment of the diet containing 21% casein plus soy isolate with formaldehyde did not influence the concentration of cholesterol in the serum (compare groups 4 and 10).

The incorporation of formaldehyde-treated soy isolate instead of native soy isolate into a semipurified diet containing 21% casein had no significant effect on the level of serum cholesterol (compare groups 4 and 6). In contrast, partial replacement of casein by formaldehyde-treated casein significantly reduced the degree of hypercholesterolemia, when compared with untreated casein, within 21 days (compare groups 2 and 5). The serum cholesterol–lowering effect of formaldehyde-treated casein was comparable to that of soy isolate (compare groups 4 and 5).

DISCUSSION

The hypercholesterolemic effect of dietary casein versus soy protein in rabbits has been

TABLE 5

Serum cholesterol concentrations in rabbits fed semipurified diets[1,2]

	Diet	Serum cholesterol for *day*						
		Initial (−2)	7	14	21	28	42	56
					mmol/L			
1	21% casein	2.18 ± 0.18	2.76 ± 0.28^{adefghj}	2.83 ± 0.21^{adefhj}	2.82 ± 0.16^{aefj}	3.15 ± 0.21^{adefj}	3.32 ± 0.30^{adefj}	3.73 ± 0.50^{adefgj}
2	42% casein	2.22 ± 0.16	3.99 ± 0.43^{bdefghi}	4.43 ± 0.50^{bdeghij}	5.96 ± 0.59^{bghi}	6.82 ± 0.75^{bgh}	8.84 ± 1.26^{bghij}	10.41 ± 1.75^{bghi}
3	21% soy	2.21 ± 0.21	2.02 ± 0.16^{c}	1.91 ± 0.16^{c}	1.94 ± 0.11^{c}	1.97 ± 0.13^{c}	1.91 ± 0.23^{c}	2.03 ± 0.18^{cj}
4	21% casein + 21% soy	2.21 ± 0.26	3.49 ± 0.38^{defghj}	3.32 ± 0.25^{defghj}	3.76 ± 0.37^{defghj}	3.88 ± 0.42^{defghj}	3.96 ± 0.45^{defghj}	3.90 ± 0.42^{defghj}
5	21% casein + 23% F-casein	2.21 ± 0.28	2.84 ± 0.34^{efghj}	3.25 ± 0.41^{efghj}	3.65 ± 0.54^{efghj}	3.96 ± 0.65^{efghj}	4.68 ± 0.97^{efghj}	5.58 ± 1.24^{efghj}
6	21% casein + 24.2% F-soy	2.16 ± 0.25	3.01 ± 0.24^{fghj}	2.82 ± 0.28^{fhj}	3.00 ± 0.39^{fhj}	3.13 ± 0.45^{fj}	3.06 ± 0.49^{fj}	3.33 ± 0.55^{fgj}
7	21% casein + 18.9% AA-casein	2.20 ± 0.17	3.64 ± 0.34^{ghj}	4.20 ± 0.49^{ghj}	4.73 ± 0.68^{ghj}	5.09 ± 0.79^{ghj}	6.45 ± 1.51^{ghj}	7.44 ± 2.02^{gh}
8	21% casein + 17.5% AA-soy	2.04 ± 0.16	3.17 ± 0.41^{hj}	3.93 ± 0.56^{hj}	4.93 ± 0.96^{hj}	5.42 ± 0.97^{hj}	6.10 ± 0.99^{h}	6.45 ± 0.90^{h}
9	42% casein + 0.4% formaldehyde	2.20 ± 0.19	5.52 ± 0.78^{i}	6.94 ± 1.12^{i}	8.52 ± 1.45^{i}	10.70 ± 1.67^{i}	14.96 ± 2.78^{i}	17.81 ± 3.83^{i}
10	21% casein + 21% soy + 0.4% formaldehyde	2.19 ± 0.23	2.80 ± 0.29^{j}	3.10 ± 0.39^{j}	3.38 ± 0.44^{j}	3.49 ± 0.59^{j}	3.34 ± 0.60^{j}	3.12 ± 0.52^{j}

[1]For experimental details, see table 4. The initial value refers to day −2 of the experiment. [2]Means of cholesterol values of two groups in the same column are significantly different ($P < 0.05$; two-tailed Student's *t*-test) if the first letter, which is characteristic of the group, does not occur in the superscript of a value above.

well established (1, 2, 10). It is not known, however, how in molecular terms dietary proteins interact with cholesterol metabolism. It is possible that both the amino acid composition and the structure of the protein are involved. This is indicated by several lines of evidence.

Huff and Carroll (3) have shown that the feeding to rabbits of an amino acid mixture corresponding to casein produced a hypercholesterolemic response similar to that seen with the intact protein. The amino acid mixture equivalent in composition to soy protein gave a significantly lower level of serum cholesterol, but not as low as that obtained with intact soy protein isolate. This suggests that at least part of the hypercholesterolemic effect of soy protein is associated with the structure of the protein. In the present study we were not able to demonstrate a difference in the cholesterolemic response to amino acid mixtures corresponding to casein and soy isolate (table 5, groups 7 and 8). The discrepancy between our data and those of Huff and Carroll (3) may lie in the fact that our diets also contained a background of 21% casein. The intact casein may have overridden the differential effect of the amino acid mixtures corresponding to casein and soy protein. In the experiments of Huff and Carroll (3) the diets contained either 25% (wt/wt) amino acids or intact protein.

In rats the differential effect of casein and soy protein on the level of serum cholesterol has also been demonstrated (11–14). In contrast to rabbits, rats display similar levels of serum cholesterol on diets containing intact casein or soy protein or amino acid mixtures simulating the amino acid pattern of these proteins (11, 13). This is difficult to explain in light of more recent observations. Rats fed intact casein as opposed to soy protein have an increased cholesterol absorption and decreased excretion of fecal steroids, but this difference was not observed in rats fed amino acid mixtures corresponding to casein and soy protein (15). Thus, intact proteins and free amino acids interact in different ways with cholesterol metabolism. This suggests that the structure of a protein is involved in determining its cholesterolemic effect.

Kirschenmann and Schneeman (16) have recently published their studies with mice fed either raw or heated egg albumin. It was found that plasma cholesterol levels were similar on both diets, but the mice fed unheated egg albumin had significantly lower cholesterol concentrations in the liver. This is suggestive evidence that the structure of dietary proteins affects cholesterol metabolism.

The present study shows that formaldehyde-treated casein had a similar hypocholesterolemic effect in rabbits as soy isolate (table 5, compare groups 4 and 5). This effect of formaldehyde-treated casein was unrelated to the formaldehyde present in the protein preparation, since formaldehyde per se did not have a hypocholesterolemic effect.

The hypocholesterolemic effect of intact soy protein (table 5, compare groups 2 and 4) disappeared when amino acids imitating the amino acid pattern of soy protein were added to a diet already containing 21% casein (table 5, compare groups 2 and 8). The differential effects of casein and formaldehyde-treated casein and soy protein and soy protein–like amino acids, respectively, indicate the importance of the structure of the intact protein in determining the level of serum cholesterol.

We have recently speculated that the digestibility of proteins is crucial with respect to their effect on the level of serum cholesterol (14). Proteins that are not completely digested interfere with the absorption of bile acids (17, 18) and may interrupt the enterohepatic circulation of bile acids, which in turn may result in an enhanced loss of steroids with the feces and consequently in lower levels of serum cholesterol. This idea would imply that soy protein is less digestible than casein, at least in the distal part of the small intestine where the absorption of bile acids takes place (19). Indeed, it has been shown that rabbits fed soy protein excrete more steroids than animals on casein diets (20, 21). Furthermore, Roy and Schneeman (22) observed that mice fed soy protein had increased amounts of material in their intestine compared with their counterparts fed casein, suggesting a slower rate of digestion of soy protein when compared with casein. Although rabbits fed casein or soy protein have the same mouth-to-anus digestibility of dry matter and nitrogen (table 3), it cannot be excluded that the maximum extent of digestion of soy protein occurs more distally in the gastrointestinal tract compared to that of casein. This is supported by the fact that the digestibility of soy isolate tended to be lower than that of casein when the rabbits wore a collar (table 3). Redigestion of soy isolate and casein after coprophagy not only increases the digestibility of the proteins but also results in an identical apparent digestibility of the proteins.

Formaldehyde treatment of proteins has been introduced in ruminant nutrition in order to protect the dietary protein against destruction by microorganisms in the rumen (23). The protein adducts formed with formaldehyde are stable at pH 6 to 7 in the rumen, but are broken down at pH 3 in the stomach thereby generating protein, which then becomes available to the ruminant. However, formaldehyde-treated proteins when they contain at least 0.2% of formaldehyde, are only poorly digested in cows (24) and also in nonruminants such as pigs (25) and rats (26). It is possible that also in rabbits formaldehyde-treated proteins are not as readily digested as the untreated proteins (table 3). Nitrogen retention is somewhat decreased after formaldehyde treatment of the dietary protein, which is compatible with the reduced growth of the rabbits fed formaldehyde-treated casein but not with the normal growth of the rabbits fed formaldehyde-treated soy protein. It could be suggested that the differential growth on formaldehyde-treated casein and soy protein is better predicted by the in vitro digestion of these preparations by pepsin. Formaldehyde-treated casein is not as well digested as treated soy isolate (table 2). This may also explain why the hypocholesterolemic effect of 21% soy isolate when present in the diet together with 21% casein (table 5, compare groups 2 and 4) was not increased by formaldehyde-treatment (compare groups 4 and 6), whereas formaldehyde-treated casein was clearly hypocholesterolemic when compared with untreated casein (compare groups 2 and 5). Thus the present work supports the idea put forward earlier (14) that differences in the digestion of proteins, at least at specific sites in the intestine and not necessarily in the overall digestion (i.e., mouth-to-anus digestion), affect the level of cholesterol in the serum. However, the observation that increasing amounts of casein in the diet further elevates the level of serum cholesterol (table 5, compare groups 1 and 2) may not fit in this concept, as it may be anticipated that the complete digestion of protein is reduced with increasing levels of protein in the diet. However, experiments with rats have shown that the apparent digestibility of soy protein and casein increased with increasing levels (up to 18% on the basis of dry matter) of these proteins in the diet (27).

To prove or disprove the hypothesis that the digestibility of proteins plays a role in their cholesterolemic effect, it will be necessary to measure intestinal contents of digesta and bile acids at different sites of the intestine in rabbits fed casein or soy protein as the structure of the protein may also interact in other ways with cholesterol metabolism. For instance, the order in which amino acids are released during digestion may also be important.

ACKNOWLEDGMENTS

We thank A. E. M. F. Soffers and K. J. Van Schalm for expert analytical assistance.

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