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Effect of the type of dietary fat on cholesterolemia in rabbits fed brewer's yeast

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SUMMARY. To determine the effects of the kind of dietary fat on cholesterolemia in rabbits fed with brewer's yeast, diets based on soybean protein isolate or on a mixture of soybean protein isolate and brewer's yeast (1:1) were used. They were combined with corn oil and coconut oil in four balanced, cholesterol-free diets. Twenty four rabbits were fed with 79 grams of diet per day during 3 weeks. After 14 hours of fasting on the 22nd day, blood samples from the marginal ear vein were collected, animals were sacrified and their livers dissected. There were no significant differences in the final plasma concentrations of the total lipids, tryglycerides, total cholesterol, high density lipoprotein-cholesterol, and (low density lipoprotein + very low density lipoprotein)-cholesterol, even though groups fed yeast presented the highest values. Liver lipids were not affected by dietary treatment. However, when comparing the final plasma cholesterol with the initial cholesterol, a significant increase in the groups which consumed yeast with corn oil (48 mg/dL) and coconut oil (91 mg/dL) was observed in comparison with the groups which only consumed soybean protein with corn oil (21 mg/dL) and coconut oil (36 mg/dL). The two-way variance analysis of these data showed that there was no fat-protein interaction and the hypercholesterolemic effects observed were associated with brewer's yeast consumption.

RESUMEN. Efecto de la grasa dietaria sobre el colesterol del plasma en conejos alimentados con levadura de cerveza. Con el fin de determinar el efecto del tipo de grasa dietaria sobre la colesterolemia en conejos alimentados con levadura de cerveza, se usaron dietas basadas en aislado proteico de soya o en una mezcla de aislado proteico de soya y levadura de cerveza (1:1) en combinación con aceite de maíz y aceite de coco en cuatro dietas balanceadas libres de colesterol. Se alimentaron 24 conejos con 70 gramos de dieta al día durante tres semanas. Después de 24 horas de ayuno, se colectaron muestras de sangre de la vena marginal de la oreja, se sacrificaron los animales y se disecaron los hígados. No hubo diferencias en las concentraciones plasmáticas de los lípidos totales, triglicéridos, colesterol total, HDL-colesterol y LDL+VLDL-colesterol, si bien se observó que los grupos que consumieron la levadura presentaron los valores más altos. En el hígado, la concentración de los lípidos fue similar en todos los grupos. Sin embargo, al comparar las concentraciones final e inicial del colesterol plasmático, se observó un incremento significativo en los grupos que consumieron la levadura, bien con aceite de maíz (21 mg/dL) y con aceite de coco (36 mg/dL). El análisis de varianza de doble vía de los datos reveló que no hubo interacción de la grasa con la proteína y que los efectos hipercolesterolémicos observados estuvieron asociados al consumo de levadura de cerveza.

INTRODUCTION

Among the non-conventional protein sources, yeast have the highest potential for massive consume food. Yeasts have the advantage of using economic substrates such agroindustrial wastes and of duplicating their biomass in a short period of time in comparison with conventional protein sources (1). From the nutritional point of view, they are B vitamin sources and they contain enough essential amino acids, with the exception of the sulphur-amino acids. The yeast *Saccharomyces cerevisae* contains more methionine than other yeasts (2). Methionine super-producing strains have been developed (3) and a mutant *Saccharomyces cerevisae* was recently produced in agricultural extract substrate with three times more methionine than the control group wich increased the protein value of animal feed (4).

Nevertheless, yeast usage as a protein source in human food has been limited because it adds nucleic acids to the diet. When yeasts are consumed in high concentrations, uric acid blood concentration is raised (5). Yeast is mainly used in the food industry as an alcoholic beverage fermenter, a riser of dough in bakeries, a flavorant and to a lesser extent, as a B group vitamin supplement.

However, the yeast protein concentrate preparation constitutes an alternative protein source, because the concentration of the nucleic acids in it has been reduced. For instance, the use of a yeast protein concentrate has been proposed to enrich «tortillas», which are widely consumed in Mexico and Central America (6). Moreover, the commercialization of the nucleic acids as flavorants would make the process more profitable.

On the other hand, it has been established that the effects of diet on plasma cholesterol are associated not only with quantity and quality of fats, but also with the dietary quantity and quality of proteins. Many studies have demonstrated that animal proteins suchs as casein have a hypercholesterolemic effect on rats and rabbits in comparison with vegetal proteins such as soybean proteins (7). Hence, it is important to evaluate the effects of any source of protein on the lipid metabolism in order to contribute to establish consumption criteria.

We previously demonstrated that the substitution of 50% soybean protein isolate by brewer's yeast protein in diets for rabbits increased plasma cholesterol when compared with control group which only consumed soybean protein. Moreover, the substitution of casein with yeast in the same proportion, did not modify the hypercholesterolemic effect of casein (8).

These results greatly contradict those of other researchers who have demonstrated hypercholesterolemic activity using microorganism supplements with low protein value in the diet

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(9,10,11). It is important to remark that the objective of our studies has been to observe the effect of important concentrations of microbial proteins in the diet on the lipid metabolism. On the other hand, there was a possibility that the hypercholesterolemic effect observed by us in response to the yeast intake would be associated to extra cellular dietary factors such as the type of dietary fat or any unknown compound originating in the fermentation process. This possibility is based on the fact that in the above-mentioned study we used yeast coming from beer manufacturing, without bitter elimination treatment. As the lipid fraction of the diet was mainly based on coconut oil, we decided to find out if the effect of the yeast on cholesterolemia was associated only with yeast intake or with an interaction between yeast protein and saturated fatty acids contained in coconut out.

With this purpose, we compared the effect of replacing coconut oil with corn oil in cholesterol-free diets wich contained equal quantities of soybean protein isolate and brewer's yeast protein based on 21% protein in the diet.

MATERIALS AND METHODS

Animals and diets. Twenty-four New Zealand young rabbits (provided by Instituto Venezolano de Investigaciones Científicas) with approximately 2 kg each, were housed in single cages and conditioned to the animal room environment and to consume powder feed following the previously described protocol (8). They were distributed randomly in 4 groups, were fed with 70 grams of diet during 3 weeks and water was continously available. Body weight was registered weekly. At the begining and at the end of the experiment, blood from the marginal ear vein was taken after a 14-hour fasting. On the 22nd day, rabbits were sacrified. Their livers were dissected, washed in saline solution at 4 °C and were immediately frozen in liquid nitrogen. The livers were kept at -70 °C until their processing.

The protein sources were soybean protein isolate (89% protein, Protein Technologies, WI, USA) and brewer's yeast (35% protein, Polar Industries, Caracas). As we previously observed that washed yeast caused a decay in intake as well as in chicken growth (12), in our studies we use yeast without any treatment to eliminate bitterness.

The composition of the basal diet was identical to that described by West *et al* (13) Table 1 shows the protein and lipid composition of the experimental diets.

TABLE 1
Composition of the cholesterol-free experimental diets[1]

Protein Fat	Soy Coconut Oil	Soy Corn Oil	Soy:Yeast Coconut Oil	Soy:Yeast Corn Oil
g/Kg diet				
Soy Isolated	233	233	117	117
Brewer's Yeast	—	—	264	264
Corn Starch	333	333	185	185
Soy Oil 10	10	10	10	
Corn Oil	90	-	90	-
Coconut Oil	-	90	-	90
Constant Components	334	334	334	334

1 According to basal diet recommended by West et al (13).

Analitycal methods: Blood collected in plastic tubes with EDTA was centrifuged at room temperature in a clinic centrifuge to obtain plasma. To obtain the hepatic lipids, 10-12 g of liver were homogenized in distilled water and treated with a chloroform-methanol mixture (2:1) according to the method recommended by Folch (14). The chloroform phase was kept at -70 °C until its analysis. Total lipids, triglycerides, total cholesterol, total cholesterol and HDL-cholesterol were enzymatically determined by using commercial kits (Lab-Test, Sao Paulo, Brazil). The VLDL+LDL-cholesterol fraction was estimated as the difference between the total cholesterol and the HDL-cholesterol.

The level of significance of the interaction between fat and protein was analyzed by a two-way ANOVA (15). Data were presented as mean ± standard deviation and the differences were considered significant at $p \leq 0.05$.

RESULTS

On Table 2, data about final weight, food intake and the liver weight/body weigh ratio are detailed. In the three-week experiment, the body weight of the group which consumed diet based on brewer's yeast and coconut oil was lower than in any group, even though this difference was not significant. So, the food intake and the liver weight/body weight ratio was similar among groups, indicating that all experimental diets, regarding protein or fat sources, were nutritionally equivalent.

TABLE 2
Body weight, daily feed intake and liver weight to body weight ratio (x100) in rabbits

Protein Fat	Soy Coconut Oil	Soy Corn Oil	Soy:Yeast Coconut Oil	Soy:Yeast Corn Oil
Body Weight (kg)	2.39 ± 0.09^a	2.42 ± 0.09^a	2.36 ± 0.10^a	2.14 ± 0.11^a
Feed intake (g/day)	68.9 ± 0.7^a	68.3 ± 0.9^a	69.9 ± 0.4^a	66.5 ± 0.7^a
Liver wt x 100 Body wt	2.7 ± 0.2^a	3.0 ± 0.2^a	3.1 ± 0.2^a	3.1 ± 0.1^a

Values are mean ± SEM of six rabbits per group. Means with the same superscript letter in the row are not significantly different.

Plasma lipid concentration in rabbits fed semipurified diets is shown in Table 3. In the groups wich consumed brewer's yeast, not taking into account the kind of fat, a tendency to increase the total lipid concentrations, triglycerides, and total cholesterol was observed, even though differences were not significant at 5% level. The same pattern was observed when calculating the cholesterol values of VLDL+LDL. Concentration of HDL-C and HDL-C/total cholesterol ratio had a tendency to be lower in the group that consumed soybean protein and corn oil, though again the difference was not significant.

Mean total plasma cholesterol increases with respect to the rabbits initial cholesterolemia (70 mg/dL) are reported in Table 4. The highest increase of plasma cholesterol was observed in the group with brewer's yest and coconut oil diet. The two-way analysis of variance of such data showed that the protein-fat interaction was not significant (≤ 0.05) and that the observed increase was only associated with the dietary protein source. It was independent from fat effect.

TABLE 3
Plasma total lipids, triglycerides, total cholesterol, HDL-cholesterol, (LDL+VLDL)- cholesterol and HDL-cholesterol/ total cholesterol ratio in rabbits

Protein Fat	Soy Corn Oil	Soy Coconut Oil	Soy:Yeast Corn Oil	Soy:Yeast Coconut Oil
mg/100 ml				
Total lipids	426.3 ± 65.3[a]	423.7 ± 46.1[a]	547.4 ± 89.0[a]	393 ± 65.8[a]
Triglycerides	115.2 ± 27.4[a]	112.6 ± 10.4[a]	221.6 ± 45.2[a]	191.5 ± 21.8[a]
Total cholesterol	139.9 ± 20.9[a]	150.2 ± 18.4[a]	179.0 ± 21.4[a]	207.4 ± 33.9[a]
HDL-cholesterol	26.8 ± 2.9[a]	37.4 ± 4.7[a]	36.3 ± 2.9[a]	36.5 ± 3.1[a]
(LDL + VLDL)-cholesterol[1]	113.1 ± 16.5[a]	112.8 ± 12.5[a]	143.6 ± 18.1[a]	170.9 ± 31.0[a]
HDL-chol/ total cholesterol	0.20 ± 0.02[a]	0.22 ± 0.03[a]	0.26 ± 0.03[a]	0.19 ± 0.02[a]

Values are means ± SEM of six rabbits per group Means with the same superscript letter in the row are not significantly different.

1. Calculated from the total cholesterol and HDL-cholesterol data

TABLE 4
Plasma total cholesterol increase in rabbits[1]

Protein Fat	Soy Corn Oil	Soy Coconut Oil	Soy:Yeast Corn Oil	Soy:Yeast Coconut Oil	Fat	Protein	Interact.
Δ Cholesterol (mg/100 ml)	21.1±8.3	36.0±6.7	48.2±25.0	90.7±12.0	3.2 NS	8.78 S	1.24 NS

Values are mean ± SEM of six rabbits per group
1. Calculated from final and initial plasma total cholesterol levels.
NS: No significant
S: Significant ($p \leq 0.05$)

The data from the hepatic concentration of the total lipids, triglycerides and cholesterol shown in Table 5 indicate that there were no differences among the groups.

TABLE 5
Liver total lipids, triglycerides and cholesterol in rabbits

Protein Fat	Soy Coconut Oil	Soy Corn Oil	Soy:Yeast Coconut Oil	Soy:Yeast Corn Oil
mg/g Liver				
Total lipids	26.1 ± 0.38[a]	30.7 ± 9.5[a]	30.5 ± 4.9[a]	36.9 ± 7.1[a]
Triglycerides	51.1 ± 0.3[a]	3.7 ± 0.5[a]	4.4 ± 0.9[a]	4.8 ±± 0.4[a]
Cholesterol	2.4 ± 0.2[a]	2.0 ± 0.2[a]	2.0 ± 0.2[a]	2.4 ± 0.2[a]

Values are means ± SEM of six rabbits per group. Means with the same superscript letter in the row are not significantly different.

DISCUSSION

Nowadays it is accepted that the type of dietary fat can affect cholesterol levels in animals and human beings. Thus, saturated fatty acids increase plasma cholesterol and the degree of atherosclerosis compared with polyunsaturated fatty acids. Coconut and corn oils are the sources of saturated and unsaturated fats, respectively, that are more frequently included in semipurified experimental diets. Coconut oil contains 92% saturated fatty acids and is the dietary source of lauric and myristic acids wich are considered the main saturated fatty acids with hypercholesterolemic activity (16). In contrast, corn oil, with 12.7 % of satured fat, 24.2% of monounsaturated fatty acids and 58.7% of polyunsaturated fatty acids, has been recommended as a substitute for saturated fat in the diet (17).

We previously reported that brewer's yeast and coconut oil diet for rabbits showed a tendency to increase plasma cholesterol (8). The present study was carried out to find out whether the observed effect was due to the intake of yeast protein or saturated fat in the diet or to an interaction of these two factors.

Regarding the protein source, we found that rabbits fed brewer's yeast diets showed the highest concentration of blood lipids, while the group with soy protein with corn oil intake showed the lowest concentration of the HDL-C. We have previously reported decrease of HDL-C concentration associated to soy protein intake (8) and it has also demonstrated by other researchers (18,19). Loo et al (20), reported a decrease of HDL-cholesterol in rabbits fed corn oil (23 mg/dL) in comparison with those fed coconut oil (42 mg/dL). Our results showed a similar pattern. Thus, the lowest value of the HDL-C/total cholesterol ratio together with the highest cholesterolemia were observed in the group with brewer's yeast and corn oil intake. Although the data were not significantly different, the tendency of blood lipid increase as a response to brewer's yeast intake was observed. Therefore, it might be possible that this yeast intake for long periods of time might significantly increase cholesterolemia.

This suggestion is supported by the analysis of the significant ($p \leq 0,05$) increase of the final plasma cholesterol concentration in relation to initial values of the groups with brewer's yeast intake. This hypercholesterolemic activity of the diet was associated with the brewer's yeast intake and it was independent from the type of fat.

The results are in agreement with several studies which report in rat (21), African green monkeys (22) and guinea pigs (23) that the hypercholesterolemic effect of the saturated fat ocurred in the presence of dietary cholesterol. Kritchevsky, on the other hand, has reported that the hypercholesterolemic effect of saturated fat diminishes in the presence of soy protein (24). Our data suggest that when the diet contained only soy protein, cholesterolemia was not significantly different among the groups with either coconut or corn oil intake. The same pattern was observed in the brewer's yeast diets containing only 50% of soy protein; thus, it may be possible that even at this lower concentration level, soy protein modified the hypercholesterolemic action of the coconut oil.

Its very likely, therefore, that the present experimental conditions (intake of cholesterol-free, soy-protein based diets) may explain the absence of cholesterolemic reaction to the type of fat ingested.

In this study, a direct relationship between plasma and liver concentration of cholesterol accountable to the dietary protein or fat source was not observed. Beynen et al have proposed that the liver can absorb cholesterol up to a maximum level of 4.64 mg of cholesterol/g of liver (25). Above this level, the liver do not absorb additional cholesterol and it is accumulated in the plasma. In this study, concentrations of hepatic cholesterol varied from 2.0 to 2.4 mg of cholesterol/g of liver, far below the level proposed by Beynen. This would probably explain why the increase of cholesterolemia associated with yeast intake did not correspond with a proportional increase of hepatic cholesterol.

It has been suggested that a high value of the lysine/arginine ratio correlates positively with the hypercholesterolemic activity of the dietary protein (26). However, we demonstrated that the substitution of 50% of soybean isolated by brewer's yeast protein raised cholesterolemia levels in rabbits without a substantial increase of the lysine/arginine ratio (8). On the other had, Bergeron and Jackes (27) found a positive correlation between cholesterolemia in rats and tyrosine content of fish protein (3.8) and casein (5.8). In our study, the tyrosine content of soy protein (3.1) and brewer's yeast (3.8) do not seem to be the responsible factor of the cholesterolemia associated with brewer's yeast intake.

In summary, neither amino acids composition nor dietary fat-brewer's yeast interaction can explain hypercholesterolemia in rabbits fed brewer's yeast diets.

Recent data from our laboratory demonstrated that the brewer's yeast *Saccharomyces cerevisae* has hypocholesterolemic activity when it is produced in process other than beer production (28). This observation suggests that the brewer's yeast acquires hypercholesterolemic activity during the beer fermentation process. Due to the fact that in these studies we have used non-processed yeast, it may be possible that the hypercholesterolemic factor occurs from an extra-cellular source. To find out this possibility, further research is required.

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