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Effect of addition of brewer's yeast to soy protein and casein on plasma cholesterol levels of rabbits

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SUMMARY. The purpose of this study was to determine whether the addition of high levels of yeast to casein and soy diets could modify the well known effects of any of these proteins on plasma cholesterol. Rabbits, were fed either a diet containing soybean protein-brewer's yeast or casein-brewer's yeast (each protein source providing 50 percent of the dietary nitrogen content) and casein and soybean protein basal diets. Brewer's yeast was obtained from a local beer factory in its non-debittered form. The diets contained 20 percent protein, 9 percent coconut oil and 1 percent corn oil, with no added cholesterol. After a 22 day experimental period, rabbits fed casein developed hypercholesterolemia whereas those fed the soybean protein diet did not. The replacement of 50 percent of the soy nitrogen by brewer's yeast nitrogen, increased the total cholesterol plasma level, but significant differences were only observed between rabbits fed casein and casein-yeast and those fed soybean protein. No differences in high density lipoprotein cholesterol could be detected among the groups. However, the HDL-cholesterol/total cholesterol ratio was significantly reduced in response to soy substitution by brewer's yeast. The (low density lipoprotein + very low density lipoprotein) - cholesterol was increased in all groups whith the exception of the animals fed purely soy protein. These data suggest a hypercholesterolemic activity of the dietary non-debittered brewer's yeast. Nevertheless, according to the amino acid composition, the factor responsible for the reported effects of dietary yeast was not associated with a high lysine to arginine ratio which could be due to extracellular components.

RESUMEN. Niveles de colesterol plasmático en conejos alimentados con dietas de soya y caseína enriquecidas con levadura de cerveza. El objetivo del presente trabajo fue determinar si altos niveles de levadura de cerveza modifican los efectos de la caseína y de la proteína de soya sobre el colesterol plasmático. Se alimentaron 4 grupos de conejos, 6 animales por grupo, con dietas que contenían mezclas de proteína de soya-levadura o caseína-levadura (cada fuente proteica aportando el 50 por ciento del nitrógeno dietario) y con las dietas control basadas en proteína de soya o caseína. La levadura se obtuvo de una planta local de fabricación de cerveza sin tratamiento previo de eliminación del amargor. Las dietas contenían 20 por ciento de proteína, 9 por ciento de aceite de coco y 1 por ciento de aceite de maíz y no contenían colesterol. Después de 22 días de tratamiento, los conejos alimentados con la dieta de caseína desarrollaron hipercolesterolemia comparados con los que consumieron la dieta basada en proteína de soya. La sustitución del 50 por ciento del nitrógeno en la dieta de soya por levadura de cerveza, incrementó el nivel plasmático del colesterol total aunque las diferencias no fueron significativas. Sólo se alcanzó significancia estadística entre las dietas que contenían caseína y caseína: levadura, comparadas con la dieta que contenía únicamente proteína de soya. No se observaron diferencias en el colesterol de las lipoproteínas de alta densidad entre los grupos. Sin embargo, la razón HDL-colesterol/colesterol total disminuyó significativamente en respuesta a la sustitución de la proteína de soya por levadura. El colesterol de las lipoproteínas de baja densidad + las lipoproteínas de muy baja densidad se incrementó en los grupos, excepto en el grupo que consumió únicamente proteína de soya. Estos datos sugieren un efecto hipercolesterolémico de la levadura dietaria no tratada. Sin embargo, de acuerdo a la composición de aminoácidos, el factor responsable de la actividad aquí observada en la levadura, no estuvo asociado a una razón lisina/arginina alta y posiblemente componentes extracelulares causaron estos efectos.

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INTRODUCTION

If rabbits or rats are fed animal proteins such as casein, they develop significantly higher levels of cholesterolemia than animals fed plant proteins, especially soybean protein (1).

The several theories proposed in an attempt to explain the possible mechanisms involved in the control of cholesterolemia by dietary protein have been recently reviewed (2). There is evidence that the role of dietary protein in the regulation of cholesterol metabolism is partially associated with their amino acid composition. Kritchevsky suggested that animal proteins such as casein with a high lysine to arginine ratio, increase the plasma cholesterol, whereas plant proteins, such as soybean with a low lys to arg ratio, reduced it (3). Van der Meer has proposed that the differential phosphorylation state of the casein and soybean protein can explain the inhibition of the intestinal steroid absorption in animals fed soybean protein when compared with those fed diets containing casein (4). This author proposed that casein phosphopeptides dissolves the calcium phosphate sediment in the intestine making the phosphate soluble. Consequently, bile acids and neutral steroids are re-absorbed and so, plasma cholesterol concentration is increased in animals fed casein.

On the other hand, single cell proteins have a potential use as an alternative protein source of good quality in poultry (5) and human (6,7) nutrition. For example, a protein concentrate from distilery yeast was used to supplement corn tortillas, a basic food in Central America and Mexico, showing a 60 percent increase of the protein content and a significant improvement in lysine content (8). Likewise, we demonstrated in chickens that whole cells of yeast *Saccharomyces carlsbergensis* from beer brewing, could be substituted by 50 percent of soy protein (9).

Several studies have demonstrated the hypocholesterolemic effect of microorganisms on man and animals. Phototrophic bacteria lowered the serum cholesterol in rats (10). Mycoprotein (11,12) and brewer's yeast (13,14) decreased serum cholesterol in humans. Recently a nicotinamide riboside, isolated from brewer's yeast extracts, has been identified as an inhibitory substance *in vitro* of 3-hidroxy-3 methyl glutaryl-coenzyme A reductase activity in rat liver preparations (15). Nevertheless, in these studies the microorganisms were added as dietary supplements and they were not an important source of protein in the diet. Little data is available on the effects of microbial proteins upon cholesterol metabolism where they significantly contribute to dietary protein contents. Since the concentrates or isolates, made principally from *Torula* or *Saccharomyces* yeasts, are potentially useful in the improvement of high consumption foods, it is necessary to evaluate the influence of high levels of microbial proteins up on lipid metabolism.

Therefore, the aim of the present study was to determine the effects on plasma cholesterol levels by substituting 50 percent of the dietary proteins, such as casein or soy protein, with brewer's yeast, an economical and abundant by-product of the Venezuelan beer industry.

MATERIALS AND METHODS

Animals and diets

Twenty-four young hybrid New Zealand-California rabbits (provided by Instituto Venezolano de Investigaciones Científicas) were housed individually in wire-bottom cages and maintained in a controlled environment at 20-22°C and with a 12 h light: dark cycle. During the acclimation period, the rabbits received a pelleted commercial rabbit chow (Protinal, Caracas) until they weighed nearly 2200 g. Then, they were fed ground chow diet for a seven-day period. At this point a first baseline blood sample was taken after which the animals were assigned by selective randomization to four groups, six animals per group with test diets mixed with ground chow, reducing progressibly the latter until, by day seven, they were fed only test. The amount of feed was 70g per day and water was freely provided for the 22-d feeding period. Animals were weighed weekly.

The protein sources were free vitamin casein (Teklad, Madison, USA), soy isolated protein (Ralston Purina, Missouri, USA) and flakes of brewer's yeast collected from the roll dryer of a brewery (Polar Industries, Caracas). The chemical composition of the brewer's yeast flakes was determined by us (9).

In a previous study wich we carried out with chickens, it was determined that the de-bittering process reduced the nutritive value of the yeast (16). Consequently, in this study, brewer's yeast without any treatment was used. The composition of the basal diets was identical to that described by West *et al* (17). The yeast diets were based on mixtures made with yeast and either soy protein or casein, providing a half nitrogen. All diets were isocaloric and they were formulated so as to contain 20 percent protein by weight and 10 percent fat, 9 percent from coconut oil and 1 percent from corn oil. Table 1 shows the composition of the experimental diets. The lysine to arginine ratio was calculated for each diet from the previously determined amino acid composition data of soy protein and yeast (9) and from data reported for casein (18).

TABLE 1
DIETS COMPOSITION

	Casein	Casein:Yeast	Soy	Soy:Yeast
	g/Kg diet			
Casein	240	121	—	—
Soy	—	—	233	117
Brewer's yeast	—	264	—	264
Corn starch	334	188	333	188
Methionine	2	2	2	2
Constant components[a]	426	426	426	426
Lys/Arg[b]	2.2	1.26	0.7	0.8

a. According to West et al (17)

b. Calculated from amino acid composition data as was indicated in Materials and Methods.

Analytical procedures

At the beginning of the trial and after 22-d of treatment, animals were fasted for 14 h and blood was drawn from the marginal ear vein into plastic tubes containing EDTA as anticoagulant. The blood was centrifuged at low speed for 10 minutes at room temperature to obtain plasma. Samples were analyzed by enzymatic colorimetric procedures for total lipids, triglycerides, total cholesterol and high density lipoprotein-cholesterol using commercial kits (Heiga, Caracas). Plasma values were determined using standard curves obtained by analyzing several concentrations of standards provided with the respective kits. The difference between total cholesterol and HDL-cholesterol was assumed to be cholesterol associated with VLDL+LDL.

Data were statistically analyzed using Duncan's multiple range test (19) and significant differences between initial and final plasma lipids were tested by student's «t» test (20). The level of statistical significance was pre-set at $p<0.05$.

RESULTS

Table 1 shows that the growth of the rabbits was not affected by inclusion of brewer's yeast into the diet. Lipid data are listed in Table 2. Total plasma lipids increased significantly in all group after 22 d of feeding as compared with initial values, although the rabbits fed purely soy protein had the lowest final value. No differences in plasma triglycerides could be detected among the groups during the trial as observed in Table 2. Rabbits fed the casein diet exhibited a much higher plasma cholesterol level than those on the soy diet. Addition of brewer's yeast to soy diet tended to increase plasma cholesterol level, though the differences were not statistically significant ($P<0.05$). HDL-cholesterol did not change in the groups but HDL-cholesterol/total cholesterol ratio decreased significantly in the casein or yeast diet groups, but not in the soy-fed group. (LDL + VLDL)-cholesterol showed a similar pattern to the one observed in total cholesterol content, indicating that the increase of plasma cholesterol was associated with these lipoprotein particles.

TABLE 2
EFFECT OF DIETARY CASEIN, CASEIN: YEAST, SOY PROTEIN AND SOY PROTEIN: YEAST ON BODY WEIGHT GAIN AND PLASMA LIPIDS IN RABBITS [1]

	DIET			
	Casein	Casein:Yeast	Soy	Soy:Yeast
Body weight gain (g/22 d)	154±92^{a}	271±53^{a}	206±63^{a}	271±58^{a}
Plasma (mg/100 ml)				
Total Lipids	961±166*	932±187^{a}	514±166^{b*}	668±57^{a*}
Triglycerides	136±32^{a}	89±20^{a}	160±37^{a}	89±21^{a}
Total cholesterol	405±89^{a}	394±83^{a*}	136±21^{b}	333±54^{ab*}
HDL cholesterol	52±6a	57±3^{a}	49±6^{a}	55±6^{a}
LDL + VLDL chol	353±92^{a*}	337±78^{a*}	87±24^{b*}	278±45^{ab*}
HDL-chol/Total chol	0.16±0.04^{a*}	0.18±0.04^{a*}	0.41±0.07^{b}	0.18±0.03^{a*}

1 Values are means ± SE of six rabbits per group

a,b means bearing different letters in the same row are significantly different ($p<0.05$) according to Duncan multiple range test.

* Different from initial value according to "t" test.

DISCUSSION

In developing countries it is necessary to have additional and economical protein sources to increase nutritional value of high-consumption food and feed. In this regard, microbial proteins, particularly from yeast, seem to have the greatest potential. Yeast has a relatively well-balanced amino acid composition, except for the sulfur-containing amino acids (21). However, for man, the protein supply from yeast is low because it is preferably used at low concentrations as a source of group B vitamins. Only some vegetarian groups commonly consume higher levels of yeast as a dietary protein supplement.

Since protein concentrates from yeast have potential usefulness in human nutrition and the role of the dietary protein upon the plasma cholesterol control is known (1), it is important to examine the long-term changes which could take place on plasma lipids in response to significant dietary levels of these proteins. For this reason, in this study we compared the effect of the replacement of 50 percent of the two most studied proteins, casein and soybean protein, by brewer's

yeast protein. Growth data of rabbits further confirm our previous findings that the substitution of 50 percent of dietary protein by brewer's yeast, maintains an adequate supply of amino acids without any side effects (15).

As would be expected, dietary casein increased plasma cholesterol when compared with dietary soy protein. The plasma lipid data, however, indicate that the inclusion of brewer's yeast in replacement of 50 percent of soy protein raised the total cholesterol and the (LDL+VLDL) cholesterol and lowered the HDL-cholesterol/total cholesterol ratio. On the other hand, the replacement of 50 percent of the casein by brewer's yeast did not cause any change in these parameters as compared with the group fed purely casein. Since the level of cholesterol in lipoprotein fractions has been shown to be a good indicator of the atherosclerosis risk in rabbits (22), the results of this study suggest that brewer's yeast tended to overcome the hypocholesterolemic effect of soy protein and did not change the hypercholesterolemic effect caused by casein consumption.

Nevertheless, the examination of the lysine to arginine ratio of the diets (Table 1), indicated that the addition of yeast to a soy protein diet did not significantly change the amino acid ratio despite the fact that it raised the plasma cholesterol. In contrast, the inclusion of yeast to casein diet, lowered the lysine to arginine ratio by 43 percent, without affecting the plasma cholesterol as compared to the casein diet. Therefore, the factor by which brewer's yeast developed its action was not associated with a high lysine to arginine ratio. Thus, these results are not in agreement with the atherogenic role of the lysine to arginine ratio as was suggested by Kritchesky (3).

On the other hand, we previously reported that the phosphorous content of the brewer's yeast in its non de-bittered and de-bittered form was 1.5 and 0.6 percent, respectively, and 0.7 percent in the soybean protein (16). So, it could be possible that the hypercholesterolemic effect shown here in response to non de-bittered yeast intake, was associated with the phosphate groups bound to insoluble hop resin. Such phosphate groups could have a similar role to that proposed by Van der Meer for casein-phosphopeptides and could explain the re-absorption of fecal steroids and bile acids in casein fed animals(4).

Taking into account that the brewer's yeast was not de-bittered, the effect observed here is not necessarily caused by yeast cells; it may also have resulted from extracellular components such as insoluble hop resins or from an interaction within the saturated dietary fat, based principally on coconut oil, and the brewer's yeast protein. Since the hypercholesterolemic effects presented here are hitherto unknown for yeast, both possibilities need further investigation in order to contribute to the establisment consumption criteria.

In summary, whatever the mechanism may be, the results presented in this work seem to suggest that the hypocholesterolemic effects reported by other authors for microbial proteins (10-14), could be modified by dietary factors.

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