Journal of Animal and Veterinary Advances 9 (15): 2085-2088, 2010
ISSN: 1680-5593


# Dietary Corn Oil Counteracts Casein-Induced Hypercholesterolemia in Rabbits

H.E. Mohamed, A. Alhaidary and A.C. Beynen
Department of Animal Production, College of Food and Agricultural Sciences,
King Saud University, Riyadh, Kingdom of Saudi Arabia

**Abstract:** In rabbits fed cholesterol-free, semipurified diets, an increase in the intake of casein raises serum cholesterol concentrations, whereas an increase in the dietary level of corn oil has a cholesterol-lowering effect. The question addressed in this study was whether the casein-induced hypercholesterolemia could be antagonized by a high intake of corn oil. Young growing rabbits were fed cholesterol-free, semipurified diets, containing either a relatively low (13.0 energy %) or high level of casein (21.6 energy %), to which extra corn oil (21.1 instead of 5.3 energy %) was added at the expense of an isoenergetic amount of corn starch and dextrose. An increase in casein level as only dietary variable elevated serum cholesterol, whereas an increase in corn oil caused a lowering. The addition of casein to the diet with low content of corn oil produced a high degree of hypercholesterolemia. However, the addition of casein to the diet with high content of corn oil only caused a relatively small increase in serum cholesterol. It is concluded that a high intake of corn oil negates the casein-induced hypercholesterolemia in rabbits.

**Key words:** Rabbit, serum cholesterol, casein, corn oil, semipurified diets, kingdom of Saudi Arbia

## INTRODUCTION

It has been well documented that the type of dietary protein affects serum cholesterol concentrations in rabbits. The replacement of soya protein in the diet by casein produces hypercholesterolemia (Kritchevsky, 2001; Carroll, 1992). The mechanism by which casein elevates serum cholesterol levels is not fully understood. One hypothesis as cribes the hypercholesterolemic effect of casein to its high degree of phosphorylation (Beynen *et al*., 1986a). The highly phosphorylated peptides derived from the digestion of casein bind calcium so that there is less calcium phosphate sediment in the small intestine. This implies that less bile acids are bound to the sediment and more bile acids are reabsorbed. The consequence is less conversion of cholesterol into bile acids through feed-back inhibition and thus less loss of cholesterol from the body in the form of bile acids, leading to hypercholesterolemia. The mechanism advanced would also explain the observation of Terpstra *et al*. (1981) that increasing amounts of casein in the diet lead to higher serum cholesterol concentrations in rabbits.

The type of dietary fat also influences serum cholesterol concentrations in rabbits. Substitution of corn oil for coconut fat in the diet reduces serum cholesterol concentrations in rabbits (Beynen *et al*., 1986b; Kritchevsky, 2001). The mechanism by which oils rich in polyunsaturated fatty acids such as corn oil, lower serum cholesterol when compared with fats rich in saturated fatty acids such as coconut fat is still a matter of debate (Connor *et al*., 1969; Beynen and Katan, 1985; Glatz and Katan, 1993).

There is some evidence for the idea that polyunsaturated fats may increase bile acid synthesis and thereby increase the loss of cholesterol from the body (Connor *et al*., 1969). It has recently shown in rabbits that the hypocholesterolemic effect of corn oil versus coconut fat was greater with higher concentrations of fat in the diet (Alhaidary *et al*., 2010).

It is clear that dietary casein and corn oil have opposite effects on serum cholesterol concentrations in rabbits. It is even possible that the mechanisms of action of casein and corn oil involve opposite effects on bile acid synthesis. It could thus be suggested that the hypocholesterolemic effect of dietary casein is antagonized by dietary corn oil. In this study with rabbits suggestions have been put to the test.

## MATERIALS AND METHODS

Random-bred, male rabbits of the New Zealand strain were used. The rabbits were housed individually as described earlier (Beynen *et al*., 1986b). Food and demineralised water were provided ad libitum. On arrival in the animal house, the rabbits which were aged about

**Corresponding Author:** H.E. Mohamed, Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Kingdom of Saudi Arabia

Table 1: Composition of the experimental diets

| | Diet code | | | |
|---|---|---|---|---|
| Composition | Low protein/Low fat | High protein/Low fat | Low protein/High fat | High protein/High fat |
| **Ingredient (g)** | | | | |
| Casein | 106.70 | 177.80 | 106.70 | 177.80 |
| Corn oil | 20.00 | 20.00 | 80.00 | 80.00 |
| Corn starch/dextrose (1/1) | 557.43 | 487.25 | 422.43 | 352.25 |
| Constant components[1] | 315.87 | 314.95 | 315.87 | 314.95 |
| Total | 1000.00 | 1000.00 | 925.00 | 925.00 |
| **Chemical analysis (g/100 g)** | | | | |
| Crude protein | 10.70 | 17.20 | 11.30 | 18.40 |
| Crude fat | 2.00 | 2.20 | 8.60 | 8.70 |

[1]The constant components consisted of (g): molasses, 100; cellulose, 150, monosodium phosphate, 9.1/9.9; calcium carbonate, 9.7/9.8; magnesium carbonate, 1.15/1.17; potassium carbonate, 18.0; sodium chloride, 5.0; vitamin premix, 12.0; mineral premix, 10.0. The composition of the vitamin and mineral premix has been described earlier (Beynen *et al*., 1986a ,b)

6 weeks were maintained on commercial rabbit pellets for 2 weeks. Subsequently, on the basis of their body weights, the rabbits were allocated to one of the four the experimental diets shown in Table 1. The diets contained two levels of casein as protein source and two levels or corn oil as fat source. Extra corn oil or extra protein was added at the expense of an isoenergetic amount of corn starch and dextrose in a 1:1 ratio. For diet formulation the following energy values for metabolisable energy were used (kJ $g^{-1}$): protein, 16.5; fat, 35.8; fiber, 4.1; carbohydrates, 15.9. The diets were in pelleted form. Each dietary group consisted of 12 animals. The experimental period lasted 56 days. Body weights and feed intake were measured.

At regular intervals, blood samples for the determination of total serum cholesterol were taken by incision from the marginal ear vein. At the end of the experiment, the rabbits were killed by cutting the carotid arteries and jugular veins. The livers were removed, weighed and then stored at -20°C until the analysis of total cholesterol.

The composition of the diets and serum and liver cholesterol concentrations were analysed as described earlier (Yuangklang *et al*., 2005). The growth performance and cholesterol data were subjected to two-way ANOVA to identify statistically significant effects of casein level, corn oil level and their interaction. The level of statistical significance was pre-set at $p<0.05$.

## RESULTS AND DISCUSSION

Final body weights were similar for the two low-fat diets and the low-protein, high-fat diet (Table 2). The rabbits fed the high-protein, high-fat diet showed a significant increase in body weight and also an increase in group mean feed intake. There was a significant interaction of the amounts of dietary casein and corn oil with regard to weight gain and feed intake. The addition of extra casein to the diet with low inclusion level of corn oil produced a marked increase in final serum cholesterol concentrations (Table 3). However, when extra casein was added to the diet with high amount of corn oil, there only was a relatively small increase in serum cholesterol. The diets with high content of corn oil systematically induced lower serum cholesterol concentrations than did the diets with low corn oil level. Figure 1 shows that the high intake of corn oil partly counteracted the hypercholesterolemic action of high casein throughout the entire course of the experiment. High versus low protein intake reduced relative liver weight and liver cholesterol concentration (Table 3). There was no significant effect of the amount of dietary fat on liver weight and liver cholesterol.

The present study confirms the cholesterolemic effects of extra intake of casein or corn oil that have been found earlier in rabbits (Terpstra *et al*., 1981; Alhaidary *et al*., 2010). The addition of extra casein to the low-fat diet caused a pronounced increase in serum cholesterol concentrations. The addition of extra corn oil to the either the low-casein or high-casein diet lowered serum cholesterol concentrations. The new finding is that a high intake of corn oil was able to negate a large fraction of the hypercholesterolemic effect of casein.

The low-protein, low-fat diet contained 13.0 energy percentage protein, 5.3 energy percentage of fat, 77.2 energy percentage of carbohydrates and 4.5 energy percentage of fiber. The addition of casein to the low-protein, low-fat diet raised the energy percentage of protein to 21.6 and lowered the energy percentage of carbohydrates to 68.6, thus leaving unchanged the energy percentage of both fat and fiber. Terpstra *et al*. (1981) added extra casein to the diet at the expense of corn starch. The low-protein diet used by Terpstra *et al*. (1981) contained 13.8 energy percentage of protein, 44.4 energy percentage of fat, 34.6 energy percentage of carbohydrates and 7.2 energy percentage of fiber. When the energy percentage of casein was raised to 27.5 percentage, the increase in serum cholesterol after 21 days was about 5.5 mmol $L^{-1}$. In this study, the increase in serum cholesterol after 21 days for the low-fat, high-casein diet, when compared with the low-fat, low-casein diet was about 3.7 mmol $L^{-1}$. The greater increase

Table 2: Body weights, feed intake and feed conversion in rabbits fed the experimental diets

| | Diet code | | | | |
|---|---|---|---|---|---|
| Parameters | Low protein/Low fat | High protein/Low fat | Low protein/High fat | High protein/High fat | Significance[1] |
| Body weight (kg) | | | | | |
| Day 0 | 1.53±0.18 | 1.52±0.10 | 1.53±0.16 | 1.53±0.16 | NS |
| Day 56 | 2.44±0.45 | 2.48±0.32 | 2.36±0.30 | 3.01±0.38 | P, F, P×F |
| Feed intake (g day$^{-1}$) | 85.30±14.2 | 81.60±9.30 | 76.30±9.10 | 95.40±11.7 | P, P×F |

Results are expressed as means±SD for 12 rabbits per dietary group, except for the rabbits fed the low protein, high fat diet which consisted of 11 animals. [1]Significance was calculated by analysis of variance. P = effect of amount of casein; F = effect of amount of corn oil; PxF = effect of interaction; NS = no significant effect

Table 3: Serum and liver cholesterol concentrations in rabbits fed the experimental diets

| | Diet code | | | | |
|---|---|---|---|---|---|
| Parameters | Low protein/Low fat | High protein/Low fat | Low protein/High fat | High protein/High fat | Significance[1] |
| Serum cholesterol (mmol L$^{-1}$) | | | | | |
| Day 0 | 2.08±0.39 | 2.04±0.43 | 1.99±0.35 | 1.53± 0.16 | NS |
| Day 56 | 3.92±2.40 | 12.56±4.39 | 2.64±1.26 | 3.36±0.30 | P, F, P×F |
| Liver weight (body weight, %) | 3.26±0.70 | 2.39±0.36 | 3.23±0.45 | 2.41±0.44 | P |
| Liver cholesterol (µmol g$^{-1}$) | 16.46±4.83 | 15.10±3.30 | 19.26±8.03 | 13.05±4.17 | P |

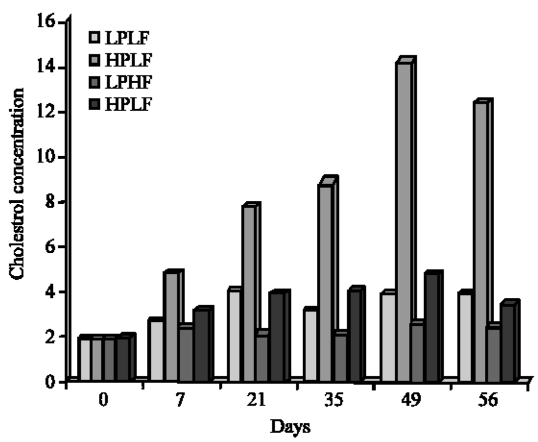


Fig. 1: Time course of serum cholesterol concentrations in rabbits fed the experimental diets. Diet codes: LPLF = Low Protein, Low Fat diet; HPLF = High Protein, Low Fat; LPHF = Low Protein, High Fat; HPHF = High Protein, High Fat

in the study of Terpstra *et al.* (1981) may be explained by the high content of coconut fat in the diets. The diets used by Terpstra *et al.* (1981) contained 1% corn oil and 13.8% coconut fat.

It is has been well documented that coconut fat versus corn oil in the diet of rabbits raises serum cholesterol concentrations (Alhaidary *et al.*, 2010; Beynen *et al.*, 1986b; Kritchevsky, 2001). Thus, it can be suggested that in the study of Terpstra *et al.* (1981) the hypercholesterolemic effect of an increase in casein intake was enhanced by the dietary background of high amount of coconut fat.

Replacement of dietary coconut fat by corn oil has been shown to decrease serum cholesterol concentrations in rabbits (Alhaidary *et al.*, 2010; Beynen *et al.*, 1986b). This study shows that an increase in the amount of corn oil in the diet at the expense of an isoenergetic amount of corn starch plus dextrose systematically lowered serum cholesterol concentrations. It has concluded earlier that the rabbit is more sensitive to the cholesterolemic effect of fat type than is man (Alhaidary *et al.*, 2010). The high sensitivity of the rabbit to the cholesterolemic effect of dietary fat type may be an advantage to study the underlying mechanism.

## CONCLUSION

In this study, the mechanism by which polyunsaturated versus saturated fatty acids lower serum cholesterol concentrations has not yet been unraveled. The hypercholesterolemic activity of dietary casein may be explained by an enhanced reabsorption of bile acids (Beynen *et al.*, 1986a). The antagonistic effect of high intake of corn oil on the casein-induced hypercholesterolemia could point at stimulation of the excretion of bile acids by the feeding of polyunsaturated fatty acids.

## REFERENCES

Alhaidary, A., H.E. Mohamed and A.C. Beynen, 2010. Impact of dietary fat type and amount on growth performance and serum cholesterol in rabbits. Am. J. Anim. Vet. Sci., 5: 60-64.

Beynen, A.C. and M.B. Katan, 1985. Why do polyunsaturated fatty acids lower serum cholesterol?. Am. J. Clin. Nutr., 42: 560-563.

Beynen, A.C., C.E. West, L.F.M. van Zutphen and M.B. Katan, 1986a. Relation between the responses of serum cholesterol to dietary cholesterol and to the type of fat in random-bred rabbits. Nutr. Rep. Int., 33: 71-78.

Beynen, A.C., R. van der Meer and C.E. West, 1986b. Mechanism of casein-induced hypercholesterolemia: Primary and secondary features. Atherosclerosis, 60: 291-293.

Carroll, K.K., 1992. Dietary protein, cholesterolemia and atherosclerosis. Can. Med. Assoc. J., 147: 900-900.

Connor, W.E., D.T. Witiak, D.B. Stone and M.L. Armstrong, 1969. Cholesterol balance and fecal neutral steroid and bile acid excretion in normal men fed dietary fats of different fatty acid composition. J. Clin. Invest., 48: 1363-1375.

Glatz, J.F. and M.B. Katan, 1993. Dietary saturated fatty acids increase cholesterol synthesis and fecal steroid excretion in healthy men and women. Eur. J. Clin. Invest., 23: 648-655.

Kritchevsky, D., 2001. Diet and atherosclerosis. J. Nutr. Health Aging, 5: 155-159.

Terpstra, A.H.M., L. Harkes and F.H. van der Veen, 1981. The effects of different proportions of casein in semipurified diets on the concentration of serum cholesterol and the lipoprotein composition in rabbits. Lipids, 16: 114-119.

Yuangklang, C., T. Wensing, A.G. Lemmens, S. Jittakhot and A.C. Beynen, 2005. Effect of sodium phytate supplementation on fat digestion and cholesterol metabolism in female rats. J. Anim. Physiol. Anim. Nutr., 89: 373-378.