J. Food Sci. Nutr.
Vol. 4, No. 1, p. 70~74 (1999)

Screening of 3-Hydroxy-3-Methylglutaryl-Coenzyme a Reductase Inhibitors *In vitro* and Its Application to Pullets

Keum-Hee Yeom, Suk-Yeun Hwang*, Kyung-Woo Lee**, Moon-sook Woo, Sun Park, Dae-Gyu Min, Bong-Duk Lee** and Chang-Keun Sung†

Dept. of Food Science and Technology, ChungNam National University, Taejon 305-764, Korea
**Dept. of Microbiology, HanNam University, Taejon 306-791, Korea*
***Dept. of Animal Science, ChungNam National University, Taejon 305-704, Korea*

Abstract

The primary objective of these studies was to screen the materials showing inhibitions of HMG-CoA reductase *in vitro*. The secondary objective was to determine the effect of garlic, lovastatin and copper on cholesterol concentrations in plasma, liver and breast muscle of pullets. In experiment 1, the degree of inhibition of the selective samples on HMG-CoA reductase activity was determined in *vitro*. The inhibition rate of water soluble garlic extracts, lovastatin and copper to HMG-CoA reductase activity were 51.3%, 87.5%, and 82.0%, respectively. In experiment 2, control diet (basal diet), garlic powder (3% in diet), lovastatin (300 mg/kg of diet) and copper (200 mg/kg of diet) were fed to pullets in order to investigate the changes of cholesterol concentration in plasma and tissues. Plasma total cholesterol, and LDL-cholesterol were significantly reduced in pullets fed a diet containing 3% garlic powder. However, copper significantly increased total cholesterol compared to controls and lovastatin did not affect plasma cholesterol concentration. Total cholesterol in liver and breast muscle in pullets were not affected by adding cholesterol-lowering materials to the diets. The data suggests that it is not easy for HMG-CoA reductase inhibitors to reduce cholesterol levels in the body due to complication in cholesterol metabolism. However, garlic administration can lower the levels of plasma cholesterol in pullets.

Key words: HMG-CoA reductase, cholesterol, inhibitor, pullets

INTRODUCTION

The evidence of correlating plasma cholesterol levels with coronary heart disease was established from early observations that cholesterol was the major component of atherosclerotic plaque (1). Recently, much attention has been drawn to the effects of dietary components on hypercholesterolemia and atherosclerosis, and the concern has led to publication of a number of reports resulting in changes in human diet. These have included recommendations for the restriction to less than 300 mg cholesterol per day in human diet (2). However, most cholesterol found in the body is synthesized in the liver (1) and the 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase is the rate-limiting enzyme in cholesterol biosynthesis (3). So several studies with microsomal HMG-CoA reductase have been conducted *in vitro* to screen the materials for lowering the activity of the enzyme.

Garlic (*Allium sativum*) is widely distributed and used in all parts of the world as a spice and herbal remedy for the prevention and treatment of a variety of diseases (4). Recent studies have shown that garlic contains various active components which lowers lipid levels in humans (5,6) and animals (7,8). This effect has been attributed to aliphatic disulfides of allicin existing in garlic (8). Garlic contains alliins (S-allyl L-cysteine sulfoxide, $CH=CH-CH_2-SO-CH_2CHNH_2-COOH$), which are converted to allicin (a disulfide oxide) by alliinase when the vegetable cells are crushed. These compounds have been shown to inactivate thiol groups and cause the oxidation of NADPH which are required for cholesterol and fatty acid synthesis (9,10). However, the information about the effects of garlic on cholesterol metabolism is quite limited presently and the mechanism of the hypocholesterolemic effect of garlic is unclear.

Dietary copper has been demonstrated to alter lipid metabolism (11). Metabolic changes by dietary copper include those of the rate of cholesterol biosynthesis and hepatic glutathione concentrations (12). Glutathione is known to stimulate the enzyme HMG-CoA reductase in cholesterol biosynthesis (13). Copper deficiency appears specifically to increase hepatic and plasma reduced glutathione (GSH), while oxidized glutathione (GSSG) concentrations remain unaffected (14). The relationship between plasma copper and plasma cholesterol in humans has not been clearly established (15).

Lovastatin is a competitive inhibitor of HMG-CoA reductase and thus inhibits cholesterol synthesis (16). In humans with heterozygous familial or nonfamilial hypercholesterolemia, lovastatin treatment usually reduces plasma cholesterol and low density lipoprotein (LDL) cholesterol concentrations by 25~40% (17).

In the present experiments, we selected the materials show-

†Corresponding author. E-mail: kchsung@hanbat.chungnam.ac.kr

ing the inhibitive effect of HMG-CoA reductase *in vitro,* and report that the influence of garlic, lovastatin and copper on cholesterol concentrations in plasma, liver and muscle of pullets.

MATERIALS AND METHODS

Screening of HMG-CoA reductase inhibitors *in vitro* (Exp 1)

Samples were extracted with either methanol or water, and stored at 4°C before enzyme assay using the method of Lee et al. (18). Selected materials are shown in Table 1. First, yeast (*Saccharomyces cerevisiae* ATCC 96519) was incubated in a medium including glucose 1%, polypeptone 0.5%, yeast extract 1% in 30°C semianaerobic condition for 24 hours. Then the 1% extracts were incubated in a new medium including glucose 3%, polypeptone 0.5%, yeast extract 0.5%, KH_2PO_4 0.5% in 30°C, for 15 h. For the enzyme assay, yeast extracts were collected, centrifuged at 4,000 rpm for 15 min at 4°C and washed with distilled water, 4°C. The pellet was suspended in 0.1 M Triethanolamine buffer, pH 7.4 (5~15% W/V) including 20 mM EDTA (ethylene diamine tetraacetate). The supernatant was homogenized with a hand homogenizer and centrifuged at 8,000 rpm for 15 min at 4°C to exclude mitochondria. Then, the supernatant was ultracentrifuged at 34,000 rpm for 90 min at 4°C to isolate the microsome. Microsomal protein was washed with 0.1 M triethanolamine buffer, pH 7.4 including 2 mM DTT (dithiothreitol) and stored at -70°C prior to assay for enzymatic activity. Protein concentration was determined by the method of Lowry et al. (19). Inhibition activitiy against HMG-CoA reductase was measured using the modified method of Hulcher and Olesson (20). Microsomal protein 1mg, HMG-CoA 150 nM, NADP 2 μM, glucose-6-phosphate 3 μM, glucose-6-phosphate dehydrogenase 2 units and test samples (100 mg/ml) 100 μl were mixed and reacted at 37°C for 30 min and added to 10 mM, sodium arsenite 20 μl. Then the solution was left alone for 1 min and incubated at 37°C for 10 min by adding 0.1 ml 2 mM citrate buffer (pH 3.5) including 3% sodium tungstate to stop the reaction. 1 ml supernatant centrifuged at 15,000 rpm was added to 2 M Tris buffer (pH 10.6) 0.2 ml and 2 M Tris buffer (pH 8.0) 0.1 ml. In this solution, 0.4 M sodium arsenite 50 ml incubated for 5 min and the reaction mixture 1 ml was formed by adding 20 μl 3 mM DTNB (5,5'-dithio-bis(2-nitrobenzoic acid)) and finally the CoA-SH concentration was measured by spectrophotometer at 420 nm.

Effect of HMG-CoA reductase inhibitors *in vitro* on cholesterol concentration of pullets (Exp 2)

Animals, management, and diets

Field study was conducted with commercial 13-wk-old ISA brown pullets. The hens were alloted to individual wire-floored metabolism cages (one hen per cage) and subjected to four dietary treatments : 1) control 2) 3% garlic 3) 300 mg lovastatin/kg 4) 200 mg copper/kg. A commercial pullet diet was used as control treatment, and garlic, lovastatin and copper were added to the control diet. Garlic powder and lovastatin were provided by Ottogi Co., and Jongkeundang Co., respectively. Copper was supplemented as feed grade cupric sulphate pentahydrate. Feed and water were provided ad libitum throughout the 30 day experimental period. The experiment was conducted at an environmentally controlled house.

Sample preparation

At the start and end of the experiment, all pullets were not fed for 15 hours and individual blood samples were drawn from wing veins using heparinized syringes. Then, pullets were killed by decapitation. Plasma was separated by centrifugation at 3,000 rpm for 10 min at 4°C and stored at -70°C. Liver and breast muscle samples were frozen at -70°C until analysis. After thawing, cholesterol and triglyceride of liver and breast muscle were extracted by the method of Folch et al. (21) modified by Bligh and Dyer (22). Briefly, samples were thoroughly homogenized with 2 : 1 chloroform : methanol (2 : 1, v/v) mixture and centrifuged at 40,000 rpm for 20 min at 4°C. Then, 1 ml of chlorform at the bottom was carefully pippetted out, concentrated with nitrogen, mixed with 100 μl Triton X-100/chloroform (1 : 1, v/v) mixture and 900 μl chloroform, and stored at -20°C until analyses.

Table 1. Samples used in screening of HMG-CoA reductase inhibitors

Botanical name	English name	Korean name	Used part
Phellodendron amurens[1)]	Amurcork tree	Hwangbaek	Bark
Eugenia caryophyllus[1)]	Clove	Jeonhyang	Seeds
Paulownia corena[1)]	Paulownia	Odongnamu	Leaves
Pinus densiflora[1)]	Pine tree	Jeoksong	Leaves
Lonicera japonica Thunb[1)]	Honey suckle	Kumeunhwa	Flower
Allium satium var. pekinense[2)]	Garlic	Manul	Bulb
Platycodon glaucum[1)]	Platycodon	Doragee	Root
Bombyx mori [1)]	Bombycidae	Baekkangzam	Body
Scrophularia buergeriana Miguel[1)]	Radix scrophulariae	Hyunsam	Root
Aloe arborescens[1)]	Aloe	Aloe	Leaves
Copper[2)]	Copper	Guri	
Lovastatin[1)]	Lovastatin	Lovastatin	

[1)]Methanol extracts
[2)]Water extracts

Sample analyses

Total cholesterol, LDL cholesterol, triglyceride concentrations of plasma, liver and breast muscle samples were estimated by an enzymatic colorimetry method using the chemical automatic analyzer, Hitachi-7150 (Hitachi medical Co., Japan).

Total cholesterol concentrations were determined by using BM reagent (Boehringer Mannhiem Co., Germany). LDL cholesterol and triglyceride concentrations were measured by Daichi reagent (Daichi Chemical Co., Ltd. Japan)

Statistical analysis

Data obtained from blood were subjected to analysis of covariance using the General Linear Model procedure of SAS[00] (23) and comparisons of means between treatment were conducted by the Student Newman Keuls test (24) when significant difference ($p<0.05$) was found. Data obtained from liver and breast were analysed by one-way analysis of variance test. Statistical significances among treatment means were determined by the method of new multiple range test of Duncan (25) when the F value was significant at 5% level.

RESULTS AND DISCUSSION

Screening of HMG-CoA reductase inhibitors *in vitro* (Exp 1)

The results of the screening of HMG-CoA reductase inhibitors from several materials *in vitro* are shown in Table 2. Lovastatin had the highest inhibition rate 87.51% against HMG-CoA reductase, the inhibition of water extracts of copper was 82.0% and water-soluble garlic extract also had a degree of inhibition of 51.3%. Methanol extracts of platycodon and pine tree showed a degree of inhibition of 46.9% and 41.3%, respectively.

HMG-CoA reductase is responsible for the conversion of HMG-CoA to mevalonate and thus plays a key role as an enzyme in cholesterol biosysthesis (26). Therefore, many attempts have been conducted to reduce cholesterol levels by inhibiting the activity of the HMG-CoA reductase (27).

Table 2. Inhibition rate of HMG-CoA reductase activity by samples *in vitro*

Treatment[1)]	Specific activity (CoA-SH pmoles/min/mg protein)	Degree of inhibition (%)
Phellodendron amurense	65.9	6.9
Eugenia caryophyllus	61.0	13.8
Paulownia corena	59.3	16.3
Pinus densiflora	41.6	41.3
Lonicera japonica Thunb	58.4	17.5
Allium satium var. pekinense	34.5	51.3
Platycodon glaucum	37.6	46.9
Bombyx mori	50.0	29.4
Scrophularia buergeriana Miguel	57.1	19.4
Aloe arborescens	49.1	30.0
Copper	12.7	82.0
Lovastatin	8.8	87.5
Control	70.8	0

[1)]100 ppm

Qureshi et al. (26) reported that the activity of HMG-CoA reductase was decreased in pullets fed solvent extracts of garlic and consequently reduced plasma cholesterol levels. The activity of HMG-CoA reductase was decreased by 3% dietary garlic (4) and significantly reduced by supplementing 50 μg/ml of garlic extracts (28). The results of these experiments are in agreement with those of our experiments, in which water-soluble garlic extracts lowered the activity of HMG-CoA reductase (Table 2).

It is reported that lovastatin the pharmacological characteristics of reducing cholesterol levels by inhibiting the activity of HMG-CoA reductase (29) and stimulating receptor-mediated uptake and degradation of LDL cholesterol in the liver (30). The results of the present study shows that lovastatin inhibits the activity of HMG-CoA reductase *in vitro* (Table 2).

Copper also depressed the activity of HMG-CoA reductase *in vitro* (Table 2), however Konjufca et al. (4) reported that it could not inhibit the activity of the enzyme in the liver *in vivo*. On the other hand, Kim et al. (12) demonstrated that dietary copper deficiency caused hypercholesterolemia by elevating hepatic reduced glutathione levels and increasing hepatic HMG-CoA reductase activity in rats.

Effect of HMG-CoA reductase inhibitors *in vitro* on cholesterol concentration of pullets (Exp 2)

Garlic, lovastatin and copper, which showed significant inhibition of HMG-CoA reductase *in vitro* were added to the pullets' diet in order to determine their effect on cholesterol levels in pullets. Supplementation of 3% garlic powder, 300 mg lovastatin/kg or 200 mg copper/kg did not affect feed consumption (Table 3).

Plasma cholesterol and LDL cholesterol were significantly decreased in pullets fed 3% garlic powder ($p<0.05$). The decrease in plasma cholesterol by garlic is very similar to what has been repeatedly observed in broiler chickens (4). On the other hand, plasma triglyceride concentrations were not influenced by 3% garlic. The cholesterol concentrations of liver and breast muscle did not appear to be affected by garlic, suggesting that overall transport of lipids was probably not decreased. These findings may indicate that feeding garlic could down-regulate hepatic LDL receptors to lower total plasma cholesterol and LDL cholesterol. Similarly, Bordia (5) reported that garlic could ameliorate patients with coronary heart disease and decrease LDL cholesterol levels in chickens (26) and in humans (31). Although there are many reports that dietary garlic supplements alter lipid metabolism in rats (9,28) and birds (4,8,32), the evidence about the effects of garlic on cholesterol metabolism is quite limited presently and the mechanism of the hypocholesterolemic effect of garlic is not yet clear. Konjufca et al. (4) reported that garlic affected lipid and cholesterol metabolism without any side effects and cholesterol levels in broilers fed 3% garlic were significantly re-

Table 3. Influence of dietary garlic, lovastatin and copper on feed intake, and plasma total cholesterol, LDL cholesterol, plasma triglyceride, liver cholesterol and breast muscle cholesterol concentrations of pullets

	Treatment			
	Control	3% garlic	300 mg lovastatin/kg	200 mg cu/kg
Feed intake (g/hen/day)	62.7± 3.3[1)]	67.2± 7.5	70.3±11.7	64.5± 7.2
Plasma total cholesterol (mg/100 ml)	119.4±19.2[b2)]	99.7±19.6[c]	114.4±11.7[b]	138.4±15.4[a]
LDL cholesterol (mg/100 ml)	55.1±10.5[a]	30.1±15.2[b]	44.5±15.2[ab]	51.1±11.3[a]
Plasma triglyceride (mg/100 ml)	29.7± 4.7	27.0± 8.0	25.4±5.8	35.0±11.9
Liver cholesterol (mg/100 g)	50± 5.4	56± 6.2	52±5.9	52± 2.1
Breast muscle cholesterol (mg/100 g)	9.8± 0.5	9.3± 1.5	11.5±1.4	11.3± 1.0

[1)]Values are mean±SD.
[2)]Means having same superscripts do not significantly differ ($p<0.05$).

duced, which are in agreement with our results. In pullets fed 3% garlic powder, the suppressive action of garlic is clear for the levels of plasma cholesterol. Both the suppression of HMG-CoA reductase *in vitro* study and the increase of hepatic LDL cholesterol receptors *in vivo* study manifested decreasing plasma concentrations of total cholesterol and LDL cholesterol in our experiments. It is very similar to the change reported in White Leghorn pullets (26). However, Kang and Kang (33) reported that cholesterol and triglyceride in plasma and liver were decreased only in rats fed cholesterol-rich diets containing garlic while no changes were found in rats fed normal diet containing garlic.

Although there were no significant differences in plasma total cholesterol, triglycerides, and liver and breast muscle cholesterol in groups fed lovastatin compared to the control group (Table 3). LDL cholesterol were numerically decreased in groups fed lovastatin without having statistical significances among treatments such as the reports of Kari et al. (34) that lovastatin enhances hepatic uptake of low density lipoprotein in humans. Especially, supplementing lovastatin to the laying hen's diet decreased plasma cholesterol and egg yolk cholesterol (35), however the doses may need to be greater than those found to be effective for humans (36). Luhman et al. (36) and Vargas et al. (37) found that low amounts of lovastatin did not reduce cholesterol in plasma, liver, and muscle because laying hens must synthesize much more cholesterol per kilogram of metabolic body weight for deposition in the egg yolk when consuming a diet containing little cholesterol. Plasma cholesterol belongs to the "fast turnover cholesterol pool" (27) while the changes of cholesterol in muscle and egg yolk are not sensitive to dietary treatment so they may be affected by a longer feeding period (4,16). These may demonstrate that the liver and muscle cholesterol concentrations were not influenced by lovastatin diet for 30 days in our study.

In this experiment, dietary copper did not decrease levels of plasma total cholesterol, LDL cholesterol, triglyceride, liver cholesterol and breast muscle cholesterol (Table 3). Especially, the increase in total plasma cholesterol in pullets did not agree with the degree that dietary copper could decrease in cholesterol in chickens (38) and swine (39). The copper requirement for the hen is not known, however it has been known that copper deficiency in diet may cause hypercholesterolemia in poultry (38, 40). So the researches about mechanism of copper deficiency are more concentrated than copper supplements. The influence of copper deficiency appears to specifically increase hepatic and plasma reduced glutathione (GSH) as oxidized glutathione (GSSG) concentrations remained unaffected to increase HMG-CoA reductase activity and plasma cholesterol levels (12).

Bakalli et al. (41) and Konjufca et al. (4) reported that supplementation of 180 mg copper/kg as cupric sulphate pentahydrate could reduce levels of plasma and breast muscle cholesterol in young broiler chickens. Pesti and Bakalli (40) observed that plasma cholesterol and egg yolk cholesterol concentration were decreased by feeding 125 mg copper/kg to laying hens. However, our experiment demonstrated that excess of copper supplementation may lead the results which cholesterol concentration in pullets were higher than control.

Taking together all of these data suggest that it is not easy to reduce cholesterol levels in pullets with HMG-CoA reductase inhibitors obtained *in vitro* assay due to the complicate metabolism of cholesterol. However, garlic administration can lower the levels of plasma cholesterol in pullets. Therefore the results of these experiments indicated that under normal dietary conditions, pullets may be capable of regulating cholesterol levels in excess of its needs for preparing yolk deposition.

REFERENCES

1. Hargis, P. S. : Modifying egg yolk cholesterol in the domestic fowl - a review. *World's Poultry Sci. J.*, **44**, 17 (1988)
2. Brown, W. V. : Dietary recommendations to prevent coronary heart disease. *Ann. New York Acad. Sci.*, **598**, 376 (1990)
3. Hunter, C. F. and Rodwell, V. W. : Regulation of vertebrate liver HMG CoA reductase via regulation modulation of its catalytic activity. *J. Lipid Res.*, **21**, 399 (1980)
4. Konjufca, V. H., Pesti, G. M. and Bakalli, R. I. : Modulation of cholesterol levels in broiler meat by dietary garlic and copper. *Poultry Sci.*, **76**, 1264 (1997)
5. Bordia, A. : Effect of garlic on blood lipid in patients with coronary heart disease. *Am. J. Clin. Nutr.*, **34**, 2100 (1981)
6. Jain, R. C. : Effect of garlic on serum lipids, coagulability and fibrinolytic activity of blood. *Am. J. Clin. Nutr.*, **30**, 1380 (1977)
7. Myung, S. C., Enusoonk, T. K. and Stewart, T. J. : Effect of Garlic on lipid metabolism in rats fed cholesterol and lard. *J. Nutr.*, **112**, 241 (1982)
8. Sklan, D., Berner, Y. N. and Rabinowitch, H. D. : The effect of dietary onion and garlic on hepatic lipid concentrations and activity of antioxidative enzymes in chicks. *J. Nutr. Biochem.*, **3**, 322 (1992)

9. Adamu, L., Joseph, P. K. and Augusti, K. T. : Hypolipidemic action of onion and garlic unsaturated oils in sucrose fed rats over a two months period. *Experimenta*, **38**, 899 (1982)
10. Katzen, H. M. and Tietz, F. : Studies on the specificity and mechanism of the action of glutathione insulin transhydrogenase, *J. Biol. Chem.*, **241**, 3561 (1966)
11. Klevay, L. M. : Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested. *Am. J. Clin. Nutr.*, **26**, 1060 (1973)
12. Kim, S., Chao, P. Y. and Allen, G. D. A. : Inhibition of elevated hepatic glutathione abolishes copper deficiency cholesterolemia. *FASEB J.*, 6, 2467 (1992)
13. Valsala, P. and Kurip, P. A. : Investigations on the mechanism of hypercholesterolemia of copper deficiency in rats. *J. Biosci. (Bangalore)*, **12**, 137 (1987)
14. Ziegler, D. M. : Role of reversible oxidation-reduction of enzyme thiols-disulfides in metabolic regulation. *Ann. Rev. Biochem.*, **54**, 305 (1985)
15. Ankari, A. A., Najib, H. and Hozab, A. A. : Yolk and serum cholesterol and production traits, as affected by incorporating a super-optimal amount of copper in the diet of the leghorn hen. *Brit. Poultry Sci.*, **39**, 393 (1998)
16. Elkin, R. G. and Rogler, J. C. : Reduction of the cholesterol content of eggs by the oral administration of lovastatin to laying hens, *J. Agric. Food Chem.*, **38**, 1635 (1990)
17. Henwood, J. M. and Heel, R. C. : Lovastatin: A preliminary review of its pharacodynamic properties and therapeutic use in hypercholesterolemia. *Drugs*, **36**, 429 (1988)
18. Lee, Y. H., Shin, Y. M., Lee, J. E., Chio, Y. S. and Lee, S. Y. : *In vitro* screening of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor from plants extracts. *Kor. J. Biotechnol. Bioeng.*, **6**, 55 (1991)
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. L. : Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265 (1951)
20. Hulcher, F. H. and Olesson, W. H. : Simplified spectrophotometric assy for microsomal-3-hydroxy-3-methylglutaryl CoA reductase by measurement of coenzyme. *Am. J. Lipid Res.*, **14**, 625 (1987)
21. Folch, J. M. L. and Sloane-Stanley, G. H. : A simple method for the determination of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497 (1957)
22. Bligh, E. G. and Dyer, W. J. : A rapid method of total extraction and purification. *Can. J. Biochem. Physiol.*, **37**, 911 (1959)
23. SAS Institute : *SAS User's Guide* : Statistics. SAS Inst, Inc., Cary, NC (1986)
24. Steel, R. G. D. and Torrie, J. H. : *Principles and procedures of statistics*. McGraw Hill Book Co., NY. (1986)
25. Duncan, D. B. : Multiple range and multiple F-test. Biometrics, 11, 1 (1955)
26. Qureshi, A. A., Din, Z. Z., Abuirmeileh, N., Burger, W. C., Ahmad, Y. and Elson, C. E. : Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: impact on serum lipids. *J. Nutr.*, **113**, 1746 (1983)
27. Chobanian, A. V. and Hollander, W. : Body cholesterol metabolism in man. I. The equilibration of serum and tissue cholesterol. *J. Clin. Invest.*, **41**, 1732 (1962)
28. Gebhardt, R. : Inhibition of cholesterol biosynthesis by a water-soluble garlic extract in primary cultures of rat hepatocytes. *Arzneimittelfrschung*, **41**, 800 (1991)
29. Alberts, A. W. : Lovastatin and simvastatin-inhibitors of HMG-CoA reductase and cholesterol biosynthesis. *Cardiology*, suppl. 4, 14 (1990)
30. Maher, V. M. and Thompson, G. R. : HMG-CoA reductase inhibitors as lipid-lowering agents: five years experience with lovastatin and an appraisal of simvastatin and pravastatin. *Q. J. Med.*, **274**, 165 (1990)
31. Jain, A. K., Vagars, R., Gotzkowsky, S. and McMahon, F. G. : Can garlic reduce levels of serum lipids? A controlled clinical study Am. *J. Med.*, **94**, 632 (1993)
32. Qureshi, A. A., Din, Z. Z., Abuirmeileh, N. and Burger, W. C. : Inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes by polar fractions of garlic. *Lipids.*, **18**, 343 (1983)
33. Kang, J. A. and Kang, J. S. : Effect of garlic and onion on plasma and liver cholesterol and triacylglycerol and platelet aggregation in rats fed basal or cholesterol supplemented diets. *The Kor. Nutr. Soc.*, **30**, 132 (1997)
34. Kari, K., Markku, J. S., Juhani, I. H. and Antero, Y. K. : Lovastatin enhances hepatic uptake of low density lipoprotein in humans. *J. Lipid Res.*, **34**, 1975 (1993)
35. Elkin, R. G., Rogler, J. C. : Effect of lovastatin on laying hen performance and egg cholesterol content. *Poultry Sci.*, **68** (Suppl. 1), 49 (Abstr.) (1989)
36. Luhman, C. M., Miller, B. G. and Beitz, D. C. : Research Note: The effect of feeding lovastatin and colestipol on production and cholesterol content of eggs. *Poultry Sci.*, **69**, 852 (1990)
37. Vargas, R. E., Allred, J. B., Biggert, M. D. and Naber, E. C. : Effect of dietary 7-ketocholesterol, pure or oxidized cholesterol on hepatic 3-hydroxy-3-methyl-glutary-coenzyme A reductase activity, energy balance, egg cholesterol concentration, and ^{14}C-acetate incorporation into yolk lipids. *Poultry Sci.*, **65**, 1333 (1986)
38. Bakalli, R. I., Pesti, G. M., Ragland, W. L. and Konjufca, V. : Dietary copper in excess of nutritional requirement reduces plasma and breast muscle cholesterol of chickens. *Poultry Sci.*, **74**, 360 (1995)
39. Amer, M. A. and Elliot, J. I. : Effects of supplemental dietary copper on glyceride distribution in the backfat of pigs. *Can. J. Anim. Sci.* **53**, 147 (1973b)
40. Pesti, G. M. and Bakalli, R. I. : Studies on effect of feeding cupric sulfate pentahydrate to laying hens on egg cholesterol content. *Poultry Sci.*, **77**, 1540 (1998)
41. Bakalli, R. I. and Pesti, G. M. : Studies on the feeding of cupric sulfate pentahydrate and cupric citrate to broiler chickens. *Poultry Sci.*, **75**, 1086 (1996)

(Received January 25, 1999)