Influence of Feeding β-Cyclodextrin to Laying Hens on the Egg Production and Cholesterol Content of Egg Yolk

B. S. Park*, H. K. Kang and A. Jang[1]
Animal Life Science Program, Kangwon National University, Chuncheon 200-701, Korea

ABSTRACT : The main objective of the present study was to determine the effect of dietary β-cyclodextrin (β-CD) on egg laying performance and cholesterol content of egg yolk. Feed intake, egg production and egg weight varied slightly, but not significantly, between hens fed either a control diet or a diet containing one of an increasing series of β-CD concentrations (2%, 4% or 6%). However, feed intake, egg production and egg weight were all lower in the hens fed on 8% β-CD ($p<0.05$). No difference in haugh unit values, egg yolk color and egg shell thickness were found between the different treatments. The cholesterol content of egg yolks (mg/g yolk) was significantly decreased by 0.71, 2.98, 4.00 and 4.24 mg in eggs from hens maintained on 2%, 4%, 6% and 8% β-CD, respectively ($p<0.05$). These observations indicate that appropriate supplementation of diets with β-CD can reduce the cholesterol content of egg-yolks, thus raising the prospect of the production of a healthier functional food. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 6 : 835-840)***

Key Words : β-Cyclodextrin, Egg Production, Egg Quality, Egg Yolk, Cholesterol

INTRODUCTION

It is known that an egg is nutritionally the most perfect natural food, but its intake is limited because it contains 200-250 mg of cholesterol (USDA, 1989; Weggemans et al., 2001). There are many reports that diets high in eggs produce a high total cholesterol/high density lipoprotein cholesterol ratio in the human blood (Shekelle and Stamler, 1989). This can, in turn, lead to an increased risk of cardiovascular disease. Current dietary advice is that the consumption of foods rich in cholesterol should be decreased (Palca, 1990). In order to resolve this problem, much research has been performed to find ways of lowering the cholesterol content of eggs. Many studies have been conducted to explore ways in which cholesterol can be lowered by varying the composition of the feed of laying hens (Hargis, 1988; Lien et al., 2003; Kehui et al., 2004). In this respect, technology has been developed to enable low-cholesterol eggs to be produced by absorbing the sterol using β-cylcodextrin, followed by centrifugal separation of the complex (Rao et al., 2000).

β-Cyclodextrin (β-CD) has the molecular structure of a ring form of an oligosaccharide in which seven glucose molecules are combined to form a β-(1,4) glycosidic structure, which is a substance produced and extracted from starch or malto-oligosaccharide by the enzymatic reaction of cyclodextrin glycosyl transferase (Szejtli, 1982). The inner surface of β-CD is strongly hydrophobic and, therefore, is able to bind hydrophobic molecules such as cholesterol (Horikoshi, 1979). When β-CD is used as an external adsorbent for removing cholesterol, it shows chemical stability and ease of isolation of its complex with cholesterol. Therefore, these properties make it a useful tool for the removal of cholesterol from foods (Yen and Chen, 2000). However, the use of such a method is limited by the fact that consumers would be limited to eating only processed eggs (Newsweek, 1992). It would be advantageous if various substances added to the feed of layers were to yield similar results, while obviating the need to break the eggs and mix their contents, thus achieving low-cholesterol fresh eggs (Newsweek, 1992; Chowdhury et al., 2002). However, there is a report that if the cholesterol content of egg-yolk is reduced greater than 30% by changing the composition of the feed, egg production is lowered (Hargis, 1988), while egg yolk colour and palatability are maintained (Birrenkott et al., 2000). Moreover, there are reports that when an animal is fed β-CD, then triacylglycerol and LDL-cholesterol content of the blood are lowered (Férézou et al., 1997; Park, 2003). This phenomenon is known to occur because the hydrophobic base of the inner core of β-CD maintains a high affinity for cholesterol *in vivo* to inhibit absorption of lipid, stimulate binding bile acids or neutral sterols in the gut and increase steroid excretion through the faeces (Férézou et al., 1997).

There is no report that feeding β-CD has any great effect on egg laying performance and egg cholesterol content from laying hens. The present study was performed to examine the effects of feeding hens with feed containing β-CD on egg laying performance and cholesterol content of egg yolks.

* Corresponding Author: B. S. Park. Tel: +82-33-250-8615, Fax: +82-33-244-2433, E-mail: bspark@kangwon.ac.kr
[1] School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, San 56-1, Seoul, 151-742, Korea.
Received July 12, 2004; Accepted January 15, 2005

Table 1. The ingredient and nutrient composition of the experimental diets

Ingredients (%)	β-cyclodextrin				
	0%	2.0%	4.0%	6.0%	8.0%
Yellow corn	50.03	50.03	48.03	50.03	50.23
Soybean meal	21.80	21.80	21.60	18.80	22.50
Wheat bran	11.70	9.70	9.70	4.50	-
Corn gluten meal	6.30	6.30	6.30	10.30	7.80
Soy bean oil[1]	1.00	1.00	1.20	1.30	2.30
β-cyclodextrin[2]	-	2.00	4.00	6.00	8.00
Oyster shell	5.70	5.70	5.70	5.70	5.70
Bone meal	2.00	2.00	2.00	2.00	2.00
Tricalcium phosphate	0.80	0.80	0.80	0.80	0.80
Common salt	0.30	0.30	0.30	0.30	0.30
DL-methionine (50%)	0.10	0.10	0.10	0.10	0.10
Vitamin and mineral mixture[3]	0.27	0.27	0.27	0.27	0.27
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrient content					
ME (kcal/kg)	2,862	2,833	2,838	2,810	2,815
Crude protein (%)	60	17.61	17.36	17.26	17.28

[1] Included 650 mg BHT per liter oil.

[2] Purity 99.4%, Cavamamax® w7, Wacker, USA.

[3] Provided per kilogram of diet: vitamin A (retinyl palmitate), 1,200 IU; cholecalciferol, 2,500 IU; vitamin E (dl-α-tocopheryl acetate), 20 IU; vitamin K3, 4.0 mg; thiamin, 1.5 mg; riboflavin, 50.0 mg; pantothenic acid, 10.0 mg; niacin, 30 mg; pyridoxine, 4.0 mg; choline chloride, 250 mg; folic acid, 0.5 mg; biotin, 220 mg; vitamin B12, 12 mg; BHT, 250 mg; manganese, 48 mg; zinc, 40 mg; iron, 24 mg; copper 16 mg; iodine, 0.6 mg; selenium, 0.12 mg; DL-methionine, 50 mg.

MATERIALS AND METHODS

Animals and diets

One hundred layers (Hy-Line Brown) that were 39 weeks old were randomly divided into five diet-groups of 20 layers each. Each group was housed in a laying cage and maintained for 10 weeks until they reached 48 weeks old. All the experimental diets were prepared by mixing components of the diets such that they met (or marginally exceeded) the nutrient requirements recommended by the NRC (1994) (Table 1). Layers in each dietary group ate an experimental diet containing 2%, 4%, 6% or 8% of β-CD added to the basic diet, mainly consisting of corn and soybean meal. The β-CD source (Cavamax® W7, Wacker, USA) was highly pure (99.4% or higher). The soy bean oil (to which BHT 0.07% was added) was supplemented to provide 15 g per kg of experimental diet. Both water and experimental diets were provided *ad libitum* during the whole experimental period. All scientific procedures, including those on animals, were conducted according to the guidance for scientific and ethical procedures provided in the European Experimental Animal Handling Licence (SCT-w94058).

Laying performance

Feed intake was measured weekly by subtracting the left-over feed from the quantity originally supplied to the animals. Eggs from individual layers were collected daily and weighed. The egg production and feed efficiency were calculated as rate of production per hen per day and feed intake/egg mass. Layers were fed the experimental diets between the ages 39 weeks and 48 weeks; egg quality was assessed every two weeks. Haugh unit was measured by using the quality control microscopes (QMC) as specified by the Technical Services Supplies Co. (UK). Egg yolk color was measured using the QMC color meter. Thickness of the egg shell was measured in the large end, middle region and the small end of the egg shell of each egg used for the above measurements by using the dial pipe gauge (Ozaki MFG Co., Ltd, Japan). All the data presented are means of these values.

Haugh unit: $100 \log (H+7.57-1.7W^{0.37})$

H: albumin height (mm), W: egg weight (g)

Measurement of yolk cholesterol contents

All eggs were boiled to enable the easy separation of egg albumin and yolk, and then frozen at -20°C, until used for analysis of lipid and cholesterol content. Total lipids of egg yolks were extracted with a chloroform/methanol mixture (2:1 vol/vol) (Folch et al., 1957). Lipid content was weighed after removing the solvent under N_2 gas. Determination of cholesterol in egg yolk was carried out by a direct saponification gas chromatographic method (Ulbrecht and Reich, 1992). A capillary column gas chromatographic system (Model GC-15A, Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector, an automatic sampler, model AOC-17, and a model Class-VP chromatography data system was used in this study.

Table 2. Egg production, egg weight, yolk weight, albumin weight, egg mass, feed consumption and feed efficiency for laying hens feed different levels of β-cyclodextrin

Item	β-cyclodextrin					PSE[3]
	0%	2.0%	4.0%	6.0%	8.0%	
Egg production (%)	92.85[a]	92.17[a]	93.01[a]	92.74[a]	91.07[b]	0.89
Egg weight (g)	65.07[a]	64.76[a]	65.13[a]	64.82[a]	63.16[b]	0.90
Yolk weight (g)	17.53	17.47	17.17	18.11	17.44	0.50
Albumin weight (g)	39.50	38.14	40.18	39.06	38.42	0.77
Egg mass (g/hen/day)[1]	60.42[a]	59.69[a]	60.58[a]	60.11[a]	57.52[b]	0.88
Feed consumption (g/hen/day)	116.7[a]	117.5[a]	118.4[a]	118.1[a]	110.0[b]	1.30
Feed efficiency (%)[2]	1.93	1.96	1.95	1.97	1.91	0.03

[1] Egg production×egg weight. [2] Feed consumption/egg mass (g:g). [3] Pooled standard error.
[a, b] Mean values within a same row with unlike superscript letters were significantly different ($p<0.05$).

Table 3. Haugh unit, egg yolk color and egg shell thickness for laying hens fed different levels of β-cyclodextrin

Item	β-cyclodextrin					PSE[1]
	0%	2.0%	4.0%	6.0%	8.0%	
Haugh unit	93.78	93.01	92.86	93.81	93.55	2.95
Egg yolk color	8.25	8.17	8.30	8.23	8.10	0.24
Egg shell thickness (mm)	0.39	0.40	0.41	0.38	0.37	0.01

[1] Pooled standard error.

Cholesterol for standards was obtained from Sigma Co. (St. Louis, MO). According to this method, an overall recovery of cholesterol was 96.78%. Egg yolk sample (0.2 g) was mixed with 5 ml of methanolic potassium hydroxide solution (0.5 M) and heated in an 80°C water bath for 15 min. After heating, the mixture was cooled, and 1 ml of water was added. Extraction of cholesterol was carried out using 5 ml of hexane. A 1 μl aliquot from the extract was submitted to gas chromatographic analysis on a fused silica capillary column, 15 m×0.32 mm i.d., coated with SPB-1 (Supelco Inc, Bellefonte, PA) with film thickness of 1.0 μm. The column temperature was programmed from 250°C to 275°C at increasing ratio of 2°C/min and held there for 12 min. Temperatures in both injection port and the flame ionization detector were set at 300°C. Flow rate of helium carrier gas was set at 2 ml/min, hydrogen at 30 ml/min, and air at 300 ml/min. All analyses were performed with a split ratio of 20:1.

Statistical analysis

Analysis of variance was conducted on the experimental data using GLM procedure of SAS and statistical significance between the mean values was tested ($p<0.05$) by Duncan's multiple range test (SAS, 2000).

RESULTS AND DISCUSSION

Groups of layers were fed one of five different experimental diets containing between 0% and 8% β-CD for a period of 10 weeks (see Methods). Table 2 shows the egg laying performance as investigated between the 4th and 10th weeks of this dietary regime. It can be seen that this period coincided with the lowering of yolk cholesterol content to its lowest (plateau) levels by the different β-CD-containing diets. There was no significant difference between the rate of feed intake of the control group (no β-CD in diet) and the groups consuming diets containing 2%, 4% and 6% β-CD. However, the group maintained on 8% β-CD showed a tendency towards a lower feed intake compared to controls and the other β-CD-groups ($p<0.05$). The egg production rate and the egg weight were lowered to the same extent in the animals receiving the 8% β-CD diet. The feed efficiency was not significantly different between control and β-CD diet animals. Overall, the egg laying performance of layers (Table 2) was only marginally affected by addition of β-CD to the diet (except 8% group - see below). However, several correlations were apparent between the egg laying performance and the feed characteristics. The egg production rate and egg weight were constant in all groups except those of the 8% diet group. Because the feed intake had a tendency to be decreased in the 8% β-CD group, their feed requirements per egg produced were lower. However, it is clear that this phenomenon would not be economically viable for the poultry farming business because this apparent increase in efficiency was offset by the decreased egg production rate and individual egg weight. The egg production rate and egg weight are the most important economic traits in the commercial egg farming business (Sheridan and Randall, 1977). It can be seen that egg weight, egg production rate and feed intake were decreased when layers were fed 8% β-CD treatment diet. Elkin et al. (2003) reported that when composition of the feed was varied in order to lower egg cholesterol content, the egg laying performance was not affected. However, Hargis (1988) reported that if the yolk cholesterol content decreases greater than 30%, the egg

Table 4. Cholesterol content and total lipid content of egg yolk

Item	β-cyclodextrin					PSE[1]
	0%	2.0%	4.0%	6.0%	8.0%	
Lipids (%)	37.68[a]	37.11[a]	37.14[a]	32.54[c]	35.71[b]	0.28
Cholesterol						
mg/g yolk	14.16[a]	13.45[a]	11.18[b]	10.16[b]	9.92[b]	0.32
mg/60 g egg	228.8[a]	217.7[a]	176.8[b]	179.7[b]	164.3[c]	8.15

[1] Pooled standard error.

[a, b, c] Mean values within a same row with unlike superscript letters were significantly different (p<0.05).

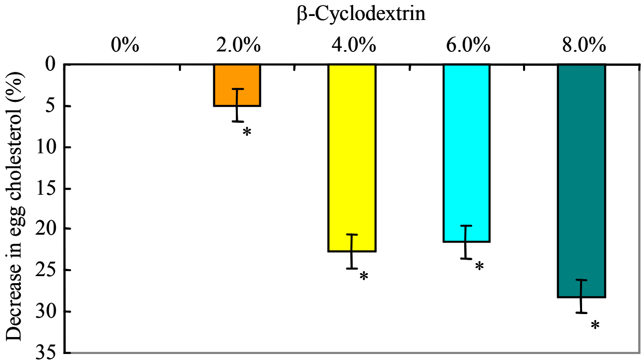

Figure 1. % Decrease in egg (an average large egg, 60 g) cholesterol from laying hens fed different levels of β-cyclodextrin. Bars represent standard error of mean values. Asterisks in each spot show statistical differences when compared with values of control group (0%) (p<0.05).

production rate drops sharply. It is possible that the decline in egg production rate in layers fed the 8% β-CD diet may have been due to a deficiency in protein and energy required for the maintenance of layer weight and production of eggs (Leeson and Summers, 1991) due to reduced feed intake (Table 2), and the physiological phenomenon (Hargis, 1988) that accompanies the lower egg cholesterol content.

There are no previous reports on the effects of adding β-CD to the diet of layer hens. By contrast there are several studies in which the effect of dietary β-CD has been tested on mammals e.g. a weight loss in the albino rat and an increase in feed intake in the hamster (Riottot et al., 1993) although other studies on the rat have shown no such changes (Oliver et al., 1991). Our results are generally consistent with reports that there was a decrease in feed intake and animal body weight when albino rats were fed the 20% β-CD diet (Riottot et al., 1993). This could be due to the chemical characteristics of β-CD as it is known that it is not absorbed by the small intestine but broken down by microbes (Nordskog and Cotterill, 1953) in the large intestine (Flourié et al., 1993). It is possible, therefore, that in animals fed large quantities of β-CD, a metabolic disorder occurs in the small intestine of the animal such that palatability of the diet is decreased.

There was no statistical significance in the HU, the egg shell thickness and egg yolk colour between the control and each of the β-CD-containing diets. Birrenkott et al. (2000) reported that although composition of the feed was varied in order to lower the yolk cholesterol content, the egg yolk colour showed no difference between the control and treatment groups. This is consistent with our experimental results. The HU value, which is an index of the internal quality of the egg, was consistently high (>93) in all diet groups. The shade of egg colour (classified on a scale of 1 to 14) was 8.21, meaning dark yellow, in both the control and all treatment groups.

The egg lipid content was significantly different between control and β-CD-fed groups (p<0.05). The egg cholesterol content was investigated for 7 weeks (42-48 weeks of age) from the time when the lowered yolk cholesterol was first evident, and was maintained at an almost constant level (plateau) for approximately four weeks after the commencement of the treatment period (Table 4). The cholesterol content per 1 g of yolk was 14.16 mg in the control group and 13.45 mg, 11.18 mg, 10.16 mg and 9.92 mg in the 2%, 4%, 6% and 8% β-CD-fed groups, respectively (p<0.05). The cholesterol content per egg (approx 60 g) also showed the same trend, and was 228.8 mg in the control group and 217.7 mg, 176.8 mg, 179.7 mg and 164.3 mg per egg in the layers fed 2%, 4%, 6% and 8% β-CD, respectively. Therefore, as the β-CD content of the diet was increased, the cholesterol content of the eggs was progressively decreased until it was only 28.2% of the control value in eggs produced by the 8% β-CD-fed hens (Figure 1).

The fact that addition of β-CD to the diet of layers could lower the yolk cholesterol content markedly was an important finding of this study (Table 4, Figure 1). The effect was maximized after 4 weeks and was maintained thereafter. Egg cholesterol content, as well as the endogenous cholesterol synthetic rate of cholesterol of the embryo are important determinants of growth and survival (Lesson and Summers, 1991; Favier et al., 1995). Therefore, cholesterol is an important component and if its content is lowered by greater than 30%, egg laying is discontinued by layers (Naber, 1983; Hargis, 1988; Elkin et al., 2003; Putina and Anurg, 2000). Studies on the effects of the inhibition of cholesterol synthesis in the liver through dietary manipulation have recently been reported. Chowdhury et al. (2002) suggested that yolk cholesterol could be lowered to 28% of control by feeding layers with 10% sun-dried garlic-containing feed, and Elkin et al. (2003) reported that the yolk cholesterol content could be reduced by between 31 and 46% in animals maintained on a diet of 0.06% atorvastatin.

However, the present data are the first reports of the effects of the addition of β-CD to the diet of layers, although studies on feeding β-CD to mammalian species have been published previously, showing that plasma cholesterol concentrations could be decreased by such treatment (Balasubramaniam et al., 1997; Catala et al., 2000; Park, 2003). The mechanism for such an effect is likely to involve a decrease in the activity of hepatic HMG-CoA reductase (Fukushima et al., 1996). In addition, there could be an increase in the excretion of steroid in the faeces, through the inhibition of cholesterol absorption across the gut (Einarsson et al., 1991). The fact that egg cholesterol content was markedly and progressively reduced as the β-CD content of the diet was increased, in the present study, suggests that related mechanisms could be involved. This may be related to the fact that β-CD has a molecular structure that accommodates the cholesterol molecule within the hydrophobic inner surface of its doughnut shape and the tight insoluble complex thus formed prevents cholesterol absorption in the gut (Smith et al., 1995; Yen and Chen, 2000). However, more studies are needed to confirm this suggestion.

ACKNOWLEDGEMENT

This work was supported by Korea Research Foundation Grant (KRF-2001-002-G00066).

REFERENCES

Balasubramaniam, S., J. L. Goldstein, J. R. Faust, G. Y. Brunsched and M. S. Brown. 1997. Lipoprotein-mediated regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol ester metabolism in the adrenal gland of the rat. J. Biol. Chem. 252:1771-1782.

Birrenkott, G., G. E. Brockenfelt, M. Owens and E. Haplin. 2000. Yolk and blood cholesterol levels fed a garlic-supplemented diet. Poult. Sci. 79(Suppl.1):75.

Catala, I., C. Juste, N. Boehler, J. Ferezou, M. Andre, M. Riottot, C. Cutton, H. Lafront, F. Bornet and T. Corring. 2000. Cholesterol crystallization in gall-bladder bile of pigs given cholesterol-β-cyclodextrin-enriched diets with either casein or soybean concentrate as protein sources. Br. J. Nutr. 83:411-420.

Chowdhury, S. R., S. D. Chowdhury and T. K. Smith. 2002. Effect of dietary garlic on cholesterol metabolism in laying hens. Poult. Sci. 81:1856-1862.

Einarsson, K., S. Ericsson, S. Ewerth, E. Reihner, M. Rudling, D. Stahlberg and B. Angelin. 1991. Bile acid sequestrants: mechanisms of action on bile acid and cholesterol metabolism. Eur. J. Clin. Pharm. 40(suppl 1):553-558.

Elkin, R. G., E. J. Furumoto and C. R. Thomas. 2003. Assessment of egg nutrient compositional changes and residue in eggs, tissues, and excreta follow in oral administration of atorvastatin to laying hens. J. Agric. Food Chem. 51:3473-3481.

Favier, M. L., C. Remesy, C. Moundras and C. Demigne. 1995. Effect of cyclodextrin on plasma lipids and cholesterol metabolism in the rat. Metabolism. 44:200-206.

Férézou, J., M. Riottot, C. Sérougne, C. Cohen-solal, I. Catala, C. Alguier, M. Parguet, C. Juste, H. Lafont, D. Mathé, T. Corring and C. Lutton. 1997. Hypocholesterolemic action of β-cyclodextrin and its effects on cholesterol metabolism in pigs fed a cholesterol-enrich diet. J. Lipid Res. 38:86-100.

Flourié, B., C. Molis, L. Achour, H. Dupas, C. Hatat and J. C. Rambaud. 1993. Fate of β-cyclodextrin in the human intestine. J. Nutr. 123:676-680.

Folch, L., M. Lees and G. H. A. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-507.

Fukushima, M., S. Akiba and M. Nakano. 1996. Comparative hypocholesterolemic effect of six vegetable oils in cholesterol-fed rat. Lipids. 31:415-419.

Hargis, P. S. 1988. Modifying egg yolk cholesterol in the domestic fowl: A review. World's Poult. Sci. J. 44:17-29.

Horikoshi, K. 1979. Production and industrial applications of beta-cyclodextrin. Process Biochem. Int. 14:26-30.

Kehui, O., W. Wenjun, X. Mingshen, J. Yan and S. Xinchen. 2004. Effects of different oils on the production performances and polyunsaturated fatty acids and cholesterol level of yolk in hens. Asian-Aust. J. Anim. Sci. 17(6):843-847.

Lien, T., C. Wu and J. Lu. 2003. Effects of cod liver oil and chromium picolinate supplements on the serum traits, egg yolk fatty acids and cholesterol content in laying hens. Asian-Aust. J. Anim. Sci. 16(8):1177-1181.

Lesson, S. and J. D. Summers. 1991. Commercial poultry nutrition. Canada NIH 6N8. pp. 77-148.

Naber, E. C. 1983. Nutrient and drug effects on cholesterol metabolism in the laying hen. Federation Proc. 42:2486-2493.

National Research Council. 1994. Nutrients requirements of poultry. 9th Ed. National Academy Press, Washington DC, USA.

News week. 1992. Betting on a guilt-free egg. Newsweek. April 6:46.

Nordskog, A. W. and O. Cotterill. 1953. Breeding for egg quality. Poult. Sci. 32:1051-1054.

Olivier, P., F. Verwaerde and A. R. Hedges. 1991. Subchronic toxicity of orally administered beta-cyclodextrin in rats. J. Am. Coll. Toxicol. 10:407-419.

Palca, J. 1990. Getting to the heart of the cholesterol debate. Sci. 247:1170-1171.

Park, B. S. 2003. The biological effect of β-cyclodextrin on antithrombotic activity and plasma lipid metabolism in rats. Kor. J. Anim. Sci. Tech. 45:199-210.

Punita, A. and C. Anurag. 2000. Effect of feeding crude red palm oil (*Elaeis guineensis*) and grain amaranth (*Ameranthus paniculatus*) to hens on total lipid, cholesterol, PUFA levels and acceptability of eggs. Plant Food for Human Nutr. 55:147-157.

Rao, P., S. U. Kumar, D. N. Rao and S. Divakar. 2000. Optimization of digestion parameters for the elimination of residual β-cyclodextrin used for cholesterol reduction in egg using glucoamylase. Eur. Food Res. Tech. 210:231-236.

Riottot, M., P. Olivier, A. Huet, J. J. Caboche, M. Parguet, J. Khallou and C. Lutton. 1993. Hypolipidemic effect of β-cyclodextrin in the hamster and in the genetically hypercholesterolemic rico rat. Lipids. 28:181-188.

SAS. 2000. SAS® User's Guide: Statistics. Version 8th Edition. SAS Institute Inc., Cary, NC, USA.

Shekelle, R. B. and J. Stamler. 1989. Dietary cholesterol and ischaemic heart disease. Lanset. 1:1177-1178.

Sheridan, A. K. and M. C. Randall. 1977. Heterosis for egg production in white Leghorn-Australop cross. Br. Poult. Sci. 18:69-77.

Smith, D. M., A. C. Awad, M. R. Bennick and J. L. Gill. 1995. Cholesterol reduction in liquid egg using β-cyclodextrin. J. Food Sci. 60:691-694.

Szejtli, J. 1982. Types, formation and structures of inclusion complexes. In: β-cyclodextrins and their inclusion complex. Budapest Akademial Kiado 11:93-108.

Ulbrecht, F. and H. Reich. 1992. Gas chromatographic determination of cholesterol in processed foods. Food Chem. 43:387-391.

USDA. 1989. Composition of food, dairy and egg products, raw-processed-prepared. USDA Agric. Handbook 8-1(Suppl.); USDA Washington, DC.

Weggemans, R. M., P. L. Zock and M. B. Katan. 2001. Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: a metaanalysis. Am. J. Clin. Nutr. 73:885-891.

Yen, G. C. and C. J. Chen. 2000. Effects of fractionation and the refining process of lard on cholesterol removal by β-cyclodextrin. J. Food Sci. 65:622-624.