# The effects of plant sterols on serum lipid profiles of laying hens

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**Abstract**
**Cardiovascular disease represents the major cause of death in developed countries. The main cause of heart problems is known to be due to higher circulating cholesterol. As cholesterol occurs only in animal products, and eggs are known as one of the major sources for this lipoprotein, therefore, egg consumption is considered one of the most controversial foodstuff. On the other hand, consumption of plant sterols in human and mammals have showed to have the ability to reduce circulating cholesterol significantly. The aim of this trial is to test this hypothesis that plant sterols could reduce circulating cholesterol in laying hens as well. Four feeding treatments, including basal diet as control (BC), and basal diet supplemented with 5 (BPS1), 10 (BPS2) and 15 (BPS3) mg/day/kg BW of free plant sterols were fed to Babcok laying hens in a repeated measures complete randomized block design with 12 hens per treatment for 60 days. Blood samples were taken in a two-week interval. There was an overall diet effect on serum total cholesterol (TC). After 15 days BPS3 in compared with control significantly ($p < 0.05$) reduced TC, 51.44 and 89.60 mg/dl, respectively. There was no diet effect on body weight (BW), triacylglycerols (TG), HDL-cholesterol, and glucose serum concentrations. There was a time effect on BW, total-cholesterol, TG and serum glucose concentrations, but no time by diet interaction on any of the tested factors. In conclusion, addition of plant sterols to laying hen diet affects circulating cholesterol and may reduce egg cholesterol as well.**

**Keywords**: cholesterol; plant sterols; laying hen; egg; cardiovascular

## Introduction

Cardiovascular disease represents the major cause of death in developed countries. It has been reported that in United States of America almost 40 percent of the deaths are related to cardiovascular diseases. A major risk factor for heart problems is known to be higher circulating cholesterol levels. One line of dietary advice to individuals with high cholesterol levels is reduction of daily cholesterol consumption. As cholesterol occurs only in animal products, and eggs are known as one of the major sources for this lipoprotein, therefore, egg consumption is considered to be a controversial foodstuff (Vorlova et al., 2001).

Within eggs, cholesterol and its esters are located mainly in egg yolk. These lipoproteins form an emulsion of LDL, VLDL and HDL particles. The "good cholesterol", HDL, accounts for almost 8% of dehydrated yolk (Stadelmann and Cotterill, 1995). The quality and biochemistry of yolk lipoproteins are similar to mammalian serum lipoproteins. Yolk lipoproteins are also synthesized in liver and secreted into blood, as in mammals (Voet and Voetova 1995). The average cholesterol content of one egg varies, depending on genetic factors, diet composition, lay intensity, layer age and medical treatment (Campo 1995; Al-Ankari et al., 1998; Elkin and Yan 1999), from 210 to 240 mg (Campo, 1995). In healthy individuals consumption of one egg per day may not increase the risk of heart diseases (Hu et al. 1999).

Consumption habits, high cholesterol content of eggs and the belief that cholesterol-enriched foods are important factors leading to heart disease, are principal reasons for limiting egg consumption (Zeidler, 1998). The desire of consumers for healthy diets has increased the demand for animal products containing low cholesterol and enriched with omega-3 fatty acids. Therefore, in recent years poultry research has focused on reducing yolk cholesterol content to satisfy the health conscious consumer (Basmacioglu and Ergul, 2005). To achieve this goal, alteration of the dietary composition of hens has been one of the targeted factors. For example, supplementing broiler diets with copper sulfate pentahydrate reduced plasma and yolk cholesterol contents by 20% and 14%, respectively (Al-Ankari et al., 1998). Diets enriched in omega-3 unsaturated fatty acids reduced yolk cholesterol content significantly (Jacob and Miles, 2005). Omega-3 fortified eggs are being produced commercially. Human subjects who consumed these eggs have not shown any changes in circulating cholesterol when consuming 7 eggs per week (California Poultry Letter 1996). Flaxseed is a rich source of omega-3 polyunsaturated alpha-linolenic acid (ALA) (Maddock et al,. 2005). Addition of flaxseed to poultry diet can have a positive effect on egg fatty acid profile (Maddock et al,. 2005). Fortified diets with flaxseed reduced chicken carcass fat and increased meat (Ajuyah et al., 1993) and egg ALA contents (Scheideler et al., 1994). Fish oil also significantly reduced plasma total and LDL-cholesterol of laying hens (Al-Sultan, 2005), and increased yolk n-3 fatty acid contents (Meluzzi et al., 2000).

In summary, it has been demonstrated that dietary modification can have positive effects on lowering egg cholesterol content and also enriching n-3 fatty acid profiles. It seems that dietary modification such as increasing vegetable portion of diet and also fortifying diets with plant sterols, which has been demonstrated to exert a beneficial effect on mammalian cholesterol and may be speculated to have benefit to poultry products as well.

Eggs are one the major source of essential amino- and fatty acids of human dietary, and cholesterol is an essential compartment of cell membrane. Vascular diseases are also major cause of the health problems. Therefore, a healthy diet might reduce the growing obesity problem and lower the risk factor of heart disease. For hypercholesterolemic and even mild-cholesterolemic populations, egg consumption is presently restricted. Accordingly, production of eggs with lower cholesterol contents would be desirable for individuals with vascular diseases. As plant sterols have the potential of reducing cholesterol contents of circulating blood and even liver in human and animal subjects, it is reasonable to hypothesize that the same effect would be realized on chicken and laying hen cholesterol pools. Introducing eggs with lower cholesterol levels will change the attitude of people and provide an improved market for eggs.

Plant sterols and their derivatives, plant stanols have similar structures as cholesterol (Jones and Raeini-Sarjaz, 2001;Vanstone et al. 2002). Plant sterols naturally occur in plants (Jones et al. 2000) and are part of the plant cell membrane, while cholesterol occurs only in animals. The actions of plant sterols are well known in humans and mammals (Jones et al. 1999; 2000), but not in avian species. In general, all consumers of plant products, humans, mammals and birds take some plant sterols in their daily diets, but this amount is not enough to reduce cholesterol level substantially.

Consumption of plant sterols in human (Vanhanen and Miettinen, 1992; Jones et al., 2000; Vanstone et al. 2002) and animal trials (Sugano et al., 1976; Ling and Jones, 1995; Vanstone et al., 2001) has shown that these natural products have the ability to reduce circulating cholesterol significantly, by a range of 5 to 26%. These substantial reductions in cholesterol lowering led to marketing of a range of functional foods and spreads, which are available now in Europe and North America markets. Indeed, addition of plant sterols and stanols to foods for the purpose of lowering plasma cholesterol concentrations presently reflects a major development in the functional foods area in Europe and North America. Therefore, the aim of this trial is to verify the potential of plant sterols in reducing circulating serum lipoproteins of laying hens.

## Materials and methods

In a 8-week experiment period, 48 Babcok laying hens (age 36 weeks) assigned to four treatment groups of 12 hens per treatment. Hens were housed in cages, each hen per one cage.

During the day 16 hours of light was provided, but temperature was not controlled. Therefore during the 8-week trial, from May 8 till July 7, housing temperature fluctuated as a function of ambient air temperatures (**Fig. 1**). Humidity also was not controlled during the trial.

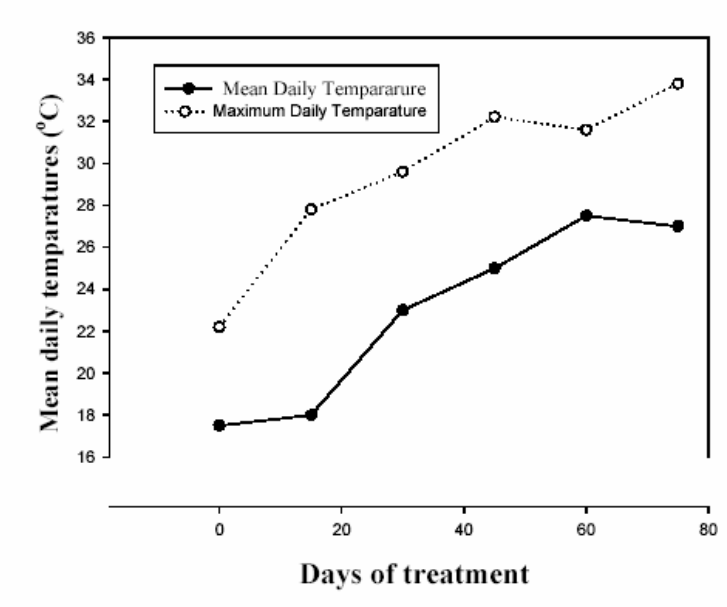


**Fig 1. Mean daily ambient temperatures during the trial**.

Laying hens during 8-week experiment period were fed with a basal laying hen mixture (Table 1) alone as control (BC), or basal diet supplemented with 5 (BPS1), 10 (BPS2) and 15 mg/day/kg BW (BPS3) of free plant sterols (20% beta-sitastanol and the rest of mixture composed of sitosterol and campesterol).

Due to spatial light and air temperature variations in experimental housing, because of cage allocations, hens of each treatment were randomly assigned to different blocks. Therefore, a complete randomized block design was employed.

**Table 1. composition of basal diet**

| Ingredient | Percentage |
|---|---|
| Corn | 65.29 |
| Soybean meal | 22.55 |
| Sunflower oil | 0.6 |
| Dicalcium phosphate | 1.5 |
| Salt | 0.03 |
| Vitamin mixtures | 0.25 |
| Mineral mixtures | 0.25 |
| vitamins | 0.1 |
| DL-mathionine | 0.15 |
| L-lysine | 0.05 |
| Lime stone | 9 |

Blood samples were collected from hens before fasting on days 0, 15, 30, 45 and 60 of the trial and 15 days after cutting the supplemented mixtures, day 75. Serum was obtained after 10 min of centrifugation at 2500 rpm at 4°C and stored at -20°C for further analysis. Serum total cholesterol, HDL-cholesterol, triacylglycerol (TG) and serum glucose concentrations were measured using spectrophotometer (UV/Vis Diode Array, WPA) and commercial enzymatic kits.

Serum lipid concentrations data were analyzed by using a three-factor repeated measures analysis of variance procedure with test for diet, block, time and time by diet interactions. Wilk's lambda test was employed to analyze time effects, while a SNK post-hoc test were used to analyze for diet effects.

## Results and discussion

During the trial no abnormal behavior was observed between 48 hens entered the experiment. Mean body weight (BW) did not differ between control and phytosterol-enriched diet groups during the trial, although it decreased over time significantly (Table 2). Endpoint means body weight on average decreased by 12% (10-13%) relative to day 0. In other studies with human and animals (Jones et al. 2000; Vanstone et al. 2001) no effect of plant sterols on BW was observed. Food intake of different groups did not show any significant differences.

The overall effect of diet on circulating total cholesterol (TC) of laying hens was statistically significant ($p \leq 0.05$). The mean TC of pre-trial, day 0, showed no differences between assigned groups. Fifteen days after onset of the trial, plant sterols (BSP3) relative to control (BC) significantly reduced TC (39%). In this group TC relative to day 0 also significantly decreased (35%). At day 30 no significant differences were observed between treatments, while at day 45 again BSP3 showed significant differences compared with control. Data of endpoint, day 60, showed no significant differences (Table 2 and Fig 2). This pattern of TC changes might be due to heat stress. During the trial the daily mean and maximum temperatures increased (from 22 to 34$^{o}$C) (Fig 1). Heat shock might have imposed a significant effect on cholesterol concentrations. Ozbey et al (2004) reported that high air temperature increased serum total cholesterol in Japanese quails. Sim and Bragg (1977) have also studied the effect of plant sterols on serum and yolk cholesterol concentrations. In their trial plant sterols reduced both serum and yolk cholesterol. While Dam et al. (1979) with a Japanese hen found no decrease in yolk cholesterol by consumption of plant sterols.

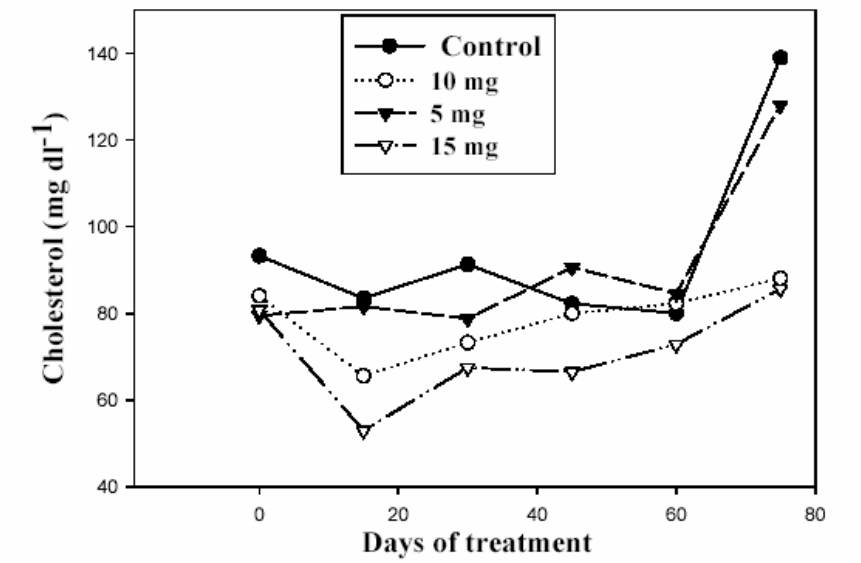


**Fig 2. The effect of free phytosterol-enriched diets on serum total cholesterol of Babcok laying hens. After day 60 hens were fed with pre-trial diet.**

The effect of plant sterols on triacylglycerols (TG) was not statistically significant. The mean TG concentrations of pre-trial were similar between the assigned groups (table 2). During the 60 day trial no effects of diet or diet-supplemented with plant sterols were found. At endpoint, overall TG mean reduced across treatments relative to day 0, and significant time effect was observed.

Serum glucose concentrations of pre-trial groups were not significantly different. The effect of diet enriched with plant sterols on serum glucose concentrations during 60 day trial was not significant. Serum glucose concentrations reduced (9% relative to day 0) by time and a significant difference was observed between day 0 and day 60. In summary, 15 mg PS per day per kg BW significantly reduced serum cholesterol, but did not affect other lipoproteins. Body weight, TG and serum glucose concentration reduced at endpoint relative to day 0.

We conclude that plant sterols of ≥15 mg per day per kg BW have the potential to reduce serum cholesterol.

**Table 2. Mean serum total cholesterol, triacylglycerol (TG), glucose concentrations (mg $dl^{-1}$) and body weight (BW) (g $hen^{-1}$) of laying Babcok hens across different dietary treatments: control (BC), 5 (BPS1), 10 (BPS2) and 10 (BPS3) mg $d^{-1}$ $kg^{-1}$ BW of free plant sterols. Rows that carry the same letter are not significantly different at 5% level. At last column, means across treatments, *, ** show overall time effects relative to day 0 (p = 0.05 and p ≤ 0.01, respectively).**

| Total cholesterol | Treatments | | | | Mean over time |
|---|---|---|---|---|---|
| | BC | BPS1 | BPS2 | BPS3 | |
| Day 0 | 93.3 ± 21.8 [a] | 79.5 ± 25.4 [a] | 83.2 ± 25.8 [a] | 80.8 ± 18.6 [a] | 84.2 |
| Day 15 | 83.5 ± 37.5 [a] | 81.6 ± 38.8 [a] | 65.9 ± 26.6 [ab] | 51.4 ± 13.0 [b] | 70.6* |
| Day 30 | 91.3 ± 49.4 [a] | 78.8 ± 38.2 [a] | 73.3 ± 21.0 [a] | 67.5 ± 14.5 [a] | 77.7 |
| Day 45 | 82.3 ± 22.5 [a] | 90.6 ± 29.9 [a] | 80.3 ± 34.1 [ab] | 66.5 ± 12.6 [b] | 79.9 |
| Day 60 | 80.0 ± 31.5 [a] | 84.6 ± 29.2 [a] | 82.5 ± 32.5 [a] | 72.9 ± 24.6 [a] | 80.0 |
| Mean | 86.1 | 83.0 | 77.0 | 67.8 | |
| Total triacylglycerols | | | | | |
| Day 0 | 1090.6 ± 449.8 | 1172.9 ± 492.7 | 966.1 ± 380.8 | 1379.2 ± 483.6 | 1152.2 |
| Day 15 | 616.9 ± 364.6 | 723.9 ± 215.0 | 741.2 ± 372.4 | 810.3 ± 256.7 | 723.0 |
| Day 30 | 834.1 ± 380.4 | 955.6 ± 264.8 | 953.1 ± 309.1 | 970.1 ± 484.9 | 928.2 |
| Day 45 | 935.5 ± 421.0 | 1023.7 ± 402.7 | 978.3 ± 459.9 | 1190.3 ± 536.1 | 1031.9 |
| Day 60 | 518.8 ± 157.3 | 570.6 ± 157.0 | 629.3 ± 40.8 | 587.5 ± 113.7 | 576.6* |
| Mean | 799.2 | 889.3 | 853.6 | 987.5 | |
| Glucose | | | | | |
| Day 0 | 180.1 ± 51.5 | 184.7 ± 55.8 | 206.0 ± 68.9 | 163.8 ± 19.9 | 183.6 |
| Day 15 | 178.2 ± 77.7 | 160.3 ± 48.0 | 195.1 ± 75.7 | 163.9 ± 49.3 | 178.4 |
| Day 30 | 176.3 ± 53.3 | 172.7 ± 55.0 | 181.7 ± 65.3 | 167.8 ± 55.1 | 174.6 |
| Day 45 | 181.1 ± 59.7 | 179.5 ± 61.4 | 191.2 ± 63.7 | 168.5 ± 65.2 | 180.1 |
| Day 60 | 166.9 ± 62.9 | 160.7 ± 33.3 | 184.1 ± 60.8 | 158.5 ± 52.1 | 167.6* |
| Mean | 176.5 | 171.6 | 191.6 | 164.5 | |
| Body weight | | | | | |
| Day 0 | 1632.1 ± 162.1 | 1558.4 ± 147.9 | 1657.8 ± 191.8 | 1558.1 ± 165.5 | 1601.6 |
| Day 15 | 1606.5 ± 228.8 | 1506.4 ± 139.9 | 1586.7 ± 147.1 | 1522.9 ± 156.9 | 1555.6* |
| Day 30 | 1535.1 ± 189.5 | 1515.1 ± 166.8 | 1573.0 ±165.4 | 1487.2 ± 203.6 | 1527.6* |
| Day 45 | 1543.6 ± 118.4 | 1508.6 ± 173.6 | 1596.9 ± 148.2 | 1517.3 ± 184.8 | 1541.6* |
| Day 60 | 1410.8 ± 131.9 | 1406.6 ± 201.7 | 1451.9 ± 135.5 | 1376.7 ± 213.9 | 1411.5* |
| Mean | 1545.6 | 1499.0 | 15733 | 1492.4 | |

# References


AJUYAH A.O., AHN, D.U., HARDIN T. and SIETAL J.S. (1993). Dietary anti-oxidants and storage affect chemical characteristics of w-3 fatty acid-enriched broider chicken meats. *J. Food Sci*. **61**: 43-46.

AL-ANKARI A., HAJIB H. and AL-HOZAB A. (1998) Yolk and serum cholesterol and production traits, as affected by incorporating a supraoptimal amount of copper in the diet of the leghorn hen. *Br. Poult. Sci*. **39**: 393-397.

AL-SULTAN S.I. (2005) Effect of dietary fish oil on production traits and lipid composition of laying hens. *International Journal of Poultry Science*, **4**: 586-588.

BASMACIOGLU H., and ERGUL M. (2005) Research on the factors affecting cholesterol content and some other characteristics of eggs in laying hens. *Turk J Vet Anim. Sci.*, **29**: 157-164.

California Poultry Letter. What is new in shell egg and egg product quality? Part II. Feb. 1996.

CAMPO, J. L. (1995) Comparative yolk cholesterol content in four Spanish breeds of hens an F2 cross, and a White Leghorn population. *Poult. Sci*. **74**: 1061-1066

ELKIN, R. G., and YAN, Z. (1999) Relation between inhibition of mevalonate biosynthesis and reduced fertility in laying hens. *J. Reprod. Fertil*. **116**: 269-75

HU, F. B., STAMPFER, M. J., RIMM, E. B., MANSON, J. E., ASCHERIO, A., COLDITZ, G. A., ROSNER, B. A., SPIGELMAN, D., SPEIZER, F. E., SACKS, F. M., HENNEKENS, CH., and WILLET, W. C. (1999) A prospective study of egg consumption and risk of cardiovascular disease in men and women. *JAMA*. **281**: 1387-1394.

JACOB J. and MILES R. (2005). Designer and specialty eggs. Factsheet PS-51. University of Florida. Institude of Food and Agricultural Sciences.
JONES P.J., NTANIOS F.Y., RAEINI-SARJAZ M., and VANSTONE C.A. (1999) Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. *Am J Clin Nutr*, **69**:1144-50.
JONES P.J., RAEINI SARJAZ M., NTANIOS F.Y., VANSTONE C.A., FENG J.Y. and PARSONS W.E. (2000) Modulation of plasma lipid levels and cholesterol kinetics by phytosterol- versus phytostanol-esters. *J Lipid Research*, **41**: 697-705.
JONES P.J., RAEINI SARJAZ M. (2001) Plant sterols and their derivatives: Spreading the results of recent studies. *Nutrition Reviews* **59***: 21-4*.
LING W.H. and JONES P.J.H. (1995) Enhanced efficacy of sitostanolcontaining versus sitostanol-free phytosterol mixtures in altering lipoprotein cholesterol levels and synthesis in rats. *Atherosclerosis* **118**: 319–331
MADDOCK T.D., ANDERSON V.L. and LANDRY G.P. (2005) Using flax in livestock diets. AS-1283.
MELUZZI A., SIRRI F., MANFREDA G., TALLARICO N. and FRANCHINI A. (2000). Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long-chain fatty acids. *Poultry Science*, **79**: 539-545.
Özbey,O., Yildiz, N., Aysöndü M.H. and Özmen Ö. (2004) The Effects of High Temperature on Blood Serum Parameters and the Egg Productivity Characteristics of Japanese Quails (Coturnix coturnix japonica). International Journal of Poultry Science, 3: 485-489.
SCHEIDELER S.E., CUPPETT S., and FRONING G. (1994) Dietary flaxseed for poultry: production effects, dietary vitamin levels, fatty acid incorporation into eggs and sensory analysis. *Proc. 55th flax institute*, Jan. 26-28, 1994, Fargo, N.D., pp. 86-95.
SIM, J.S. and BRAGG D.B. (1977). Effect of dietary factors on serum and egg yolk cholesterol levels of laying hens. *Poultry Sci*. **56**: 1616-1621.
SUGANO M. KAMO F., IKEDA, I. and MORIOKA H. (1976) Lipid-lowering activity of phytostanols in rats, *Atherosclerosis*, **24**: 301–309.
ZEIDLER G. (1998) Poultry products in the 21st century, the poultry industry towards the 21st century. WPSA-Israel Branch, *10th European Poultry Conference and Proceedings* Jerusalem, Israel. 502 pp.
VANHANEN HT, and MIETTINEN TA. (1992) Effects of unsaturated and saturated dietary plant sterols on their serum contents. *Clin Chim Acta*, **205**:97–107.
VANSTONE C.A., RAEINI-SARJAZ M., and JONES J.H.P. (2001) Injected phytosterol/stanol suppress plasma cholesterol levels in Hamsters. *The Journal of Nutritional Biochemistry* **12***:*565-574.
VANSTONE C.A., RAEINI-SARJAZ M., PARSONS W.E. and JONES P.J.H (2002) Unesterified plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. *Am J Clin Nutr*., **76**: 1272-1278.
VOET D. and VOETOVA J.G. (1995) Biochimie. Victoria Publishing, a.s. Praha, 1325 p.
VORLOVA L., SIEGLOVA E., KARPISKOVA R. and KOPROVA V. (2001) Cholesterol content in eggs during the laying period. *Act Vet, Brno*, **70**: 387-390.