



Asian Journal of **Biochemistry**

ISSN 1815-9923



Academic
Journals Inc.

www.academicjournals.com

**Aqueous Extract of *Hibiscus sabdariffa* Calyces Reduces Serum
Triglycerides but Increases Serum and Egg Yolk
Cholesterol of Shika Brown Laying Hens**

¹S.A. Habibullah, ²L.S. Bilbis, ²M.J. Ladan, ¹O.P. Ajagbonna and ²Y. Saidu

¹Department of Veterinary Physiology and Pharmacology

²Department of Biochemistry, Usmanu Danfodiyo University,
Sokoto, P.M.B. 2346 Sokoto State-Nigeria

Abstract: Reducing cholesterol content of table egg may be important in curbing the risks of atherosclerosis. In this study, forty, (32 week old) Shika Brown laying hens were randomly distributed into five groups to investigate the effect of aqueous extract of *Hibiscus sabdariffa* calyces on serum lipid profile and egg yolk cholesterol. Groups 2 - 5 received 1, 2, 4 or 8 g L⁻¹ solution of the extract. Group 1 (control) received tap water. All the animals were fed *ad libitum* with layers mash for twelve weeks. Blood samples and eggs were collected after every three weeks and sera and egg yolk were analyzed for total cholesterol, triglycerides (TAG), high-density lipoprotein cholesterol (HDL-C) and egg yolk cholesterol respectively. *Hibiscus sabdariffa* extract caused a significant (p<0.05) non dose dependent increase in serum total cholesterol and egg yolk cholesterol but significant (p<0.05) dose-dependent decrease in serum TAG and HDL-C content. No strong correlation (0.2273) was found between serum and yolk total cholesterol. These results suggest that aqueous extract of *Hibiscus sabdariffa* may possess hypotriglyceridemic effect in Shika Brown laying hens but caused increase in serum and egg yolk cholesterol.

Key words: Lipid profile, cholesterol, Shika Brown Laying hens, *Hibiscus sabdariffa*

Introduction

Eggs have been described as nature's convenience food since they come in a hygienic pack, one easily stored and readily opened and cooked (Ladan and Lawal, 2002). Eggs are valuable and readily acceptable in the diet of older people whose caloric needs is lower and who sometimes have difficulty in chewing certain types of food (Passmore and Eastwood, 1986). Fats and cholesterol represent a higher proportion of lipids in eggs with the amount of fats in many cases approaching that of protein (Gunstone *et al.*, 1986). Dietary cholesterol raises serum LDL-cholesterol levels and very high intakes causes atherosclerosis in numerous animal models (Stamler and Shekelle, 1988). Addition of egg yolk to the daily diet in a study (1.3 egg yolks/day) was associated with an increase of 8-11% in LDL-cholesterol concentrations (Lichtenstein *et al.*, 1994). Because of recent understanding of the association between total plasma cholesterol and the incidence of heart disease, people are being advised to consume not more than 300 mg cholesterol daily and limit consumption of eggs, which contain about 213mg cholesterol per egg (National Cholesterol Education Programme, 1991; Krauss *et al.*, 1996).

Corresponding Author: S.A. Habibullah, Department of Veterinary Physiology and Pharmacology, Usmanu Danfodiyo University, Sokoto, P.M.B. 2346 Sokoto State-Nigeria Tel: +2348035052957

Despite that eggs are among the most concentrated sources of cholesterol, yet egg consumption is highly recommended for the sustenance of good health due to its nutritional value (especially protein). For example, an egg provides 6 g of protein and substantial amounts of several important vitamins such as vitamins A and B12, folate, thiamin and riboflavin and minerals such as phosphorus and zinc. The protein is of the highest quality, higher even than that of milk, meat and fish (Kurtzweil, 1998).

Because of their importance in nutrition and the implication in cardiovascular diseases there are strong agitations to lower the cholesterol content of eggs. Although several attempts have been made in that regard little or no success has so far been recorded. Guclu *et al.* (2004) reported significant decrease in egg yolk cholesterol of laying quails, after feed supplementation with alfalfa, a known hypocholesterolemic agent in rabbits (Horlick *et al.*, 1967). Similarly, Wang and Pam (2003) reported significant decrease in the levels of egg yolk cholesterol after feeding laying hens with red mold rice. Contrarily, Beyer and Jensen (1993) observed significant increase in the levels of egg yolk cholesterol after feeding laying hens with sorbose.

Meanwhile, studies on the effect of *Hibiscus sabdariffa* extract on lipid profile in animals including chicken have been recommended (Sofowora, 1992). Despite the fact that aqueous extract of *Hibiscus sabdariffa* calyces have been reported to have hypocholesterolemic effects in rats (Ajagbonna and Adebayo, 2001; Hussaini *et al.*, 2004) and rabbits (Chen *et al.*, 2003), no literature is available yet on any attempt to test the said activity in avian species. This study however, was conducted to test the effect of aqueous extract of *Hibiscus sabdariffa* calyces on serum lipid profile as well as egg yolk cholesterol of Shika Brown laying hens.

Materials and Methods

Preparation of Extract

Dry calyces of *Hibiscus sabdariffa* purchased from Sokoto Central Market was identified at the Botany unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. A voucher specimen has been kept at the herbarium for reference purpose. The extraction of the calyx was as described by Ajagbonna and Adebayo (2001) with some modifications. Five hundred gram of the dry calyces were pulverized using pestle and mortar. The resulting powder was dissolved into 4 L of boiling distilled water and allowed to continue boiling for ten minutes. The hot decoction was allowed to cool and then filtered using double wrapped muslin cloth. The filtrate was evaporated in aeration oven at 40°C. The dried residue was scrapped and kept in a capped bottle and stored in a desiccator. From the dried extract, a fresh solution was prepared on each day of the experiment.

Treatment of Animals

Forty Shika brown laying hens weighing (1.50-1.90 kg) and about 32 weeks old were obtained from the poultry house of the Department of Veterinary Public Health and Animal Production, Usmanu Danfodiyo University, Sokoto. The birds were randomly grouped into five; A, B, C, D and E (8 birds each) and allowed to acclimatize for three weeks. Water and feed (layers mash, Vital Feeds, Jos, Plateau State, Nigeria) were provided *ad libitum*.

Group A was earmarked as control, so were fed with layers mash and tap water *ad libitum* throughout the period of the study. Groups B, C, D and E were however fed with layers mash and graded concentrations of the aqueous extract of *Hibiscus sabdariffa* solutions; 1, 2, 4, or 8 g L⁻¹, respectively, *ad libitum* for 12 weeks.

Sample Collection and Analyses

Six birds from each group are randomly selected for blood sample collection (after overnight fast) via venepuncture, using wing veins. The specimens are collected in 5 mL sample bottles. Six eggs from each group are also randomly collected on the day of sampling. The blood samples are centrifuged at 2000 rpm for 20 min and the serum separated using Pasteur pipette. Each egg is weighed, carefully broken and the egg white (albumen) separated from the yolk. Serum and egg yolk are used to assay for serum lipid profile and egg yolk cholesterol respectively. The sampling was done on the 3rd, 6th, 9th and 12th weeks of treatment.

Assay Methods Used

The kits used were procured from Randox Ltd. UK and the reagents used are of analytical grade. Serum cholesterol was determined using enzymatic procedure described by Allain *et al.* (1974). Colorimetric method described by Tietz (1990), was used to determine the TAG after enzymatic hydrolysis with lipases. Method for direct measurement of HDL-C without sample pre-treatment was used to assay serum high density lipoprotein. Low-density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the HDL cholesterol fraction, which remains in the supernatant, was determined using enzymatic procedure described by Allain *et al.* (1974).

The egg total cholesterol was analyzed as described by Handelman *et al.*, 1999. A, 50 μ L sample of each yolk was diluted with 1 mL buffer pH 7.4 (10 mmol/l sodium phosphate, 100 mmol/l NaCl). A 20 μ L aliquot of the dilute egg yolk was analyzed with 1 mL enzymatic cholesterol reagent.

Statistical Analyses

Analysis Of Variance (ANOVA) was employed to analyze the data, while, post-hoc LSD multiple range test was used to test for mean differences between individual treatment groups ($n = 6$), using SPSS for windows version 10. $p < 0.05$ was considered statistically significant.

Results

As shown in Fig. 1, the mean \pm SEM level of serum total cholesterol in this study ranges from 59.15 ± 0.33 to 175.76 ± 3.03 mg dL^{-1} . After three weeks of treatment with the extract, there was significant ($p < 0.05$) increase in the values obtained in group E when compared to group A (control). After the sixth week, however, there was increase in all the groups but significant ($p < 0.05$) in B, D and E. At the ninth week, significant ($p < 0.05$) difference was seen in groups D and E only when compared with the control. On the 12th week however, E differed significantly ($p < 0.05$) from the control.

Figure 2 shows the mean serum triglycerides values recorded after twelve weeks of feeding laying chickens with graded concentrations of aqueous extract of *Hibiscus sabdariffa*. The results indicated that the value ranges from 266.67 ± 11.18 to 884.05 ± 28.88 mg dL^{-1} . Throughout the period of the study however, significant ($p < 0.05$) dose dependent decrease was recorded for all the groups except group B where the difference was not significant at weeks three, sixth and twelfth. Similarly, the difference was not significant ($p > 0.05$) for group C at the third week.

Figure 3 shows the mean \pm SEM values obtained for serum HDL-C. The value ranges from 5.51 ± 1.28 to 27.28 ± 0.41 mg dL^{-1} . The value indicates significant ($p < 0.05$) dose dependent increase in all the treatment when compared to the control. The increase in group B at the third week was not however significant ($p > 0.05$).

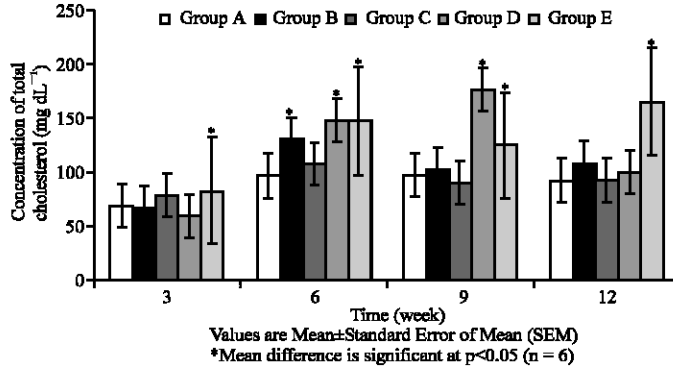


Fig. 1: Effect of aqueous extract of *Hibiscus sabdariffa* calyx on serum total cholesterol of shika brown laying hens

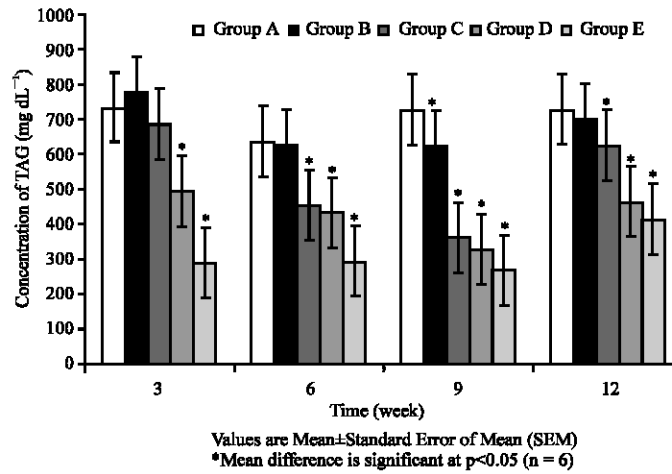


Fig. 2: Effect of aqueous extract of *Hibiscus sabdariffa* calyx on serum triglycerides (TAG) of shika brown laying hens

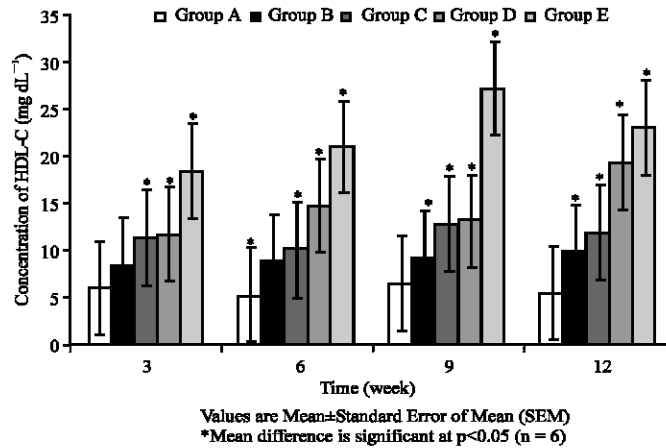


Fig. 3: Effect of aqueous extract of *Hibiscus sabdariffa* calyx on serum HDL cholesterol of shika brown laying hens

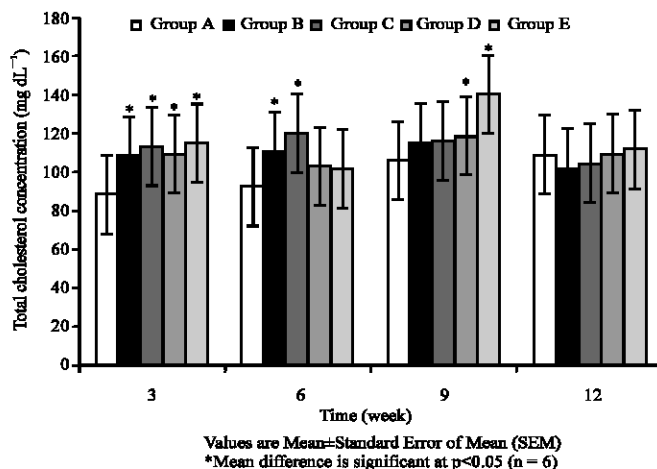


Fig. 4: Effect of aqueous extract of *Hibiscus sabdariffa* calyx on egg yolk total cholesterol

The result for the egg yolk cholesterol content presented in Fig. 4 indicates that, after three weeks of the treatment with the extract, there was none dose dependent significant ($p < 0.05$) increase in all the treatments when compared with the control. Significant ($p < 0.05$) increase was also recorded for groups B and C after the sixth week. There was dose dependent increase at the ninth week but only significant in groups D and E. The dose dependent increase recorded at the twelfth week was not however significant ($p > 0.05$).

Discussion

Atherosclerosis can be influenced by dietary cholesterol. Excessive ingestion of fats is attributed to the initial deposition of cholesterol, which could lead to initial lesion of atherosclerosis (Sharaf and Ali, 2004; Stamler and Shekelle, 1988). Several clinical studies have shown that modification of serum cholesterol level by diet and drugs can reduce this risk (Sharaf and Ali, 2004; Hu *et al.*, 1997). Hypocholesterolemic activities of *Hibiscus sabdariffa* extract have been reported in rats (Onyenekwe *et al.*, 1999; Ajagbonna and Adebayo, 2001; Hussaini *et al.*, 2004) and rabbits (Chen *et al.*, 2003). In the present study however, significant increase ($p < 0.05$) in the level of serum cholesterol is indicated (Fig. 1). This observation may be due to increased intestinal absorption and/or increased endogenous synthesis of cholesterol since certain biological activities in plants may vary with the animal species (Sofowora, 1992). The extract may contain a substance that potentiates the activity of 3-hydroxymethylglutaryl-CoA (HMG-CoA) reductase, an enzyme that catalyzes the committed step of the cholesterol biosynthetic pathway. Studies on cholesterol, triglycerides and lipoproteins in domestic animals have clearly shown that species variations exist and that even within the same species significant differences may occur (Nazifi *et al.*, 2005). These differences may be due to nutritional and metabolic specificity of these organisms. Chen *et al.* (2003) earlier speculated that *Hibiscus sabdariffa* extract may inhibit intestinal absorption of lipids in rabbits.

Hypotriglyceridemic activity of *Hibiscus sabdariffa* extract has been reported in rabbits (Chen *et al.*, 2003). The current study also indicated significant ($p < 0.05$) dose-dependent decrease in the value of TAG (Fig. 2). *Hibiscus sabdariffa* calyx has been reported to contain very high level of vitamin C, riboflavin and some major minerals (Babalola, 2000). Previously, dietary supplementations

with vitamin C (Seyrek *et al.*, 2004), Manganese and Chromium (Sands and Smith, 2002), vitamin C and folic acid (Gursu *et al.*, 2004) and red mold rice (Wang and Pam, 2003) have been reported to decrease triglycerides levels in chickens. Additionally, Vitamin C reduces corticoid secretion and so lipoprotein and tissue lipases are consequently not stimulated as a result of which lipids is not mobilized from tissues. Vitamin C also increases the rate of beta-oxidation by stimulating carnitine synthesis leading to reduction of serum triglycerides concentration (Groff *et al.*, 1995).

Heinecke and Lusic (1998) have reported that HDL has a role in preventing LDL oxidation *in vitro*. The HDL transports cholesterol from the peripheral tissues (Adkins *et al.*, 1993) to the liver for conversion into bile (Lenfant *et al.*, 1997). The present study recorded significant dose dependent increase ($p < 0.05$) in the HDL-C level (Fig. 3). This agrees with previous studies (Sands and Smith, 2002; Kim *et al.*, 1995; Lien *et al.*, 2004). On the contrary however, Wang and Pam (2004) reported no significant change in the level of HDL following supplementation of red mold rice in diets of laying hens.

Hypercholesterolemia, a frequent finding in human atherosclerosis have been reported following egg yolk consumption (West *et al.*, 1966; Stamler and Shekelle, 1988; Hopkins 1992; Garry *et al.*, 1999; Clarke *et al.*, 1997; Howell *et al.*, 1997; McGill, 1979). In an attempt to model heart friendly egg, reduced egg yolk cholesterol content was achieved by Guclu *et al.* (2004) and Wang and Pam (2003). The present study, in contrast, indicated significant ($p < 0.05$) increase in the levels of egg yolk cholesterol (Fig. 4). This finding is in agreement with the work of Beyer and Jensen (1993), who reported significant increase in the egg yolk cholesterol despite the significantly reduced serum VLDL and cholesterol levels.

Despite that there was significant increase in both serum and egg yolk total cholesterol no strong correlation (0.2273) was found.

References

- Adkins, S., K.N. Gan, M. Mody and B.N. La Du, 1993. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase; glutamine or arginine at position 191, for respective A or B allozymes. *Am. J. Hum. Genet.*, 52: 598-608.
- Ajagbonna, O.P. and A.E. Adebayo, 2001. Safety evaluation of acute and chronic treatment of aqueous calyx extract of *Hibiscus sabdariffa* L. in rats. *Sokoto J. Vet. Sci.*, 3: 20-26.
- Allain, C.C., L.S. Poon, C.S.G. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic determination of total serum cholesterol. *Clin Chem.*, 20: 470.
- Babalola, S.O., 2000. Chemical analysis of roselle leaf (*Hibiscus sabdariffa*). In: proceedings of 24th Annual Conference of NIFST,
- Beyer, R.S. and L.S. Jensen, 1993. Reduced plasma cholesterol and lipoprotein in laying hens without concomitant reduction of egg cholesterol in reposes to dietary sorbose. *Poult. Sci.*, 72: 88-97.
- Chen, C.C., J.D. Hsu, S.F. Wang, H.C. Ching, M.Y. Yang, E.S. Kao, Y.C. Ho and C.J. Wang, 2003. *Hibiscus sabdariffa* extracts inhibits the development of atherosclerosis in cholesterol-fed rabbits. *J. Agric. Food Chem.*, 51: 5472-5477.
- Clarke, R., C. Frost, R. Collin, P. Appleby and R. Peto, 1997. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. *BMJ.*, 314: 112-117.
- Garry, J.H., D.N. Zachary, H.L. Alice, J.S. Ernst and B.B. Jeffery, 1999. Lutein and Zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. *Am. J. Clin. Nutr.*, 70: 247-251.
- Groff, J.L., S. Groppers and S.M. Hunt, 1995. The Water Soluble Vitamins. In advanced Nutrition and Human Metabolism. Minneapolis West Publishing Company, pp: 222-237.

- Guclu, B.K., K.M. Iscan, F. Uyanik, M. Eren and A.C. Agca, 2004. Effect of alfalfa meal in diets of laying quails on performance, egg quality and some serum parameters. *Arch. Animal Nutr.*, 58: 255-63.
- Gunstone, F.D., J.L. Harwood and F.B. Padlgy, 1986. *The Lipid Handbook*. Chapman and Hall, London, pp: 50.
- Gursu, M.F., M. Onderci, F. Gulcu and K. Sahin, 2004. Effects of vitamin C and Folic acid supplementation on serum paraoxonase activity and metabolites induced heat stress *In vivo*. *Nutr. Res.*, 24: 157-164.
- Handelman, G.J., Z.D. Nightingale, A.H. Lichtenstein, E.J. Schaefer and J.B. Blumberg, 1999. Lutein and zeaxanthine concentrations in plasma after dietary supplementation with egg yolk. *Am. J. Clin. Nutr.*, 7: 247-251.
- Heinecke, J.W. and A.J. Lusis, 1998. Paraoxonase-gene polymorphism associated with coronary heart disease: support for the oxidative damage hypothesis. *Am. J. Hum. Genet.*, 62: 20-24.
- Hopkins, P.N., 1992. Effects of dietary cholesterol on serum cholesterol: a meta-analysis and review. *Am. J. Clin. Nutr.*, 55: 1060-1070.
- Horlick, L., F.B. Cookson and S. Feodoroff, 1967. *Circulation* In: *Biochemistry of Nutrition 1*. by Neuberger A. and T.H. Jukes. 1979 University Park Press, 27: 199.
- Howell, W.H., D.J. Mc Namara, M.A. Tosca, B.T. Smith and J.A. Gaines, 1997. Plasma lipid and Lipoprotein responses to dietary fat and cholesterol: a meta analysis. *Am. J. Clin. Nutr.*, 65: 1747-1764.
- Hu, F.B., M.J. Stampfer, J.E. Manson, E. Rimm, G.A. Colditz, B.A. Rosner, C.H. Hennekens and W.C. Willett, 1996. Dietary fat and risk of coronary heart disease in women. *N. Eng. J. Med.*, 337: 1491-1499.
- Hussaini, D.C., O.E. Orisakwe, D.N. Akunyili, A.A. Njan, D.D. Akumka and O.O. Udemezue, 2004. Subchronic administration of Nigerian species of aqueous extract of *Hibiscus sabdariffa* Calyx in rats did not produce cardiotoxicity. *Eur. Bull. Drug Res.*, Vol. 12.
- Kim, S.W., I.K. Han, K.J. Chol, I.S. Shin and B.J. Chae, 1995. Effects of Chromium picolinate on growth performance, carcass composition and serum traits of broilers fed dietary different levels of crude protein. *Asian Au. J. Anim. Sci.*, 8: 463-470.
- Krauss, R.M., R.J. Deckelbaum, N. Ernst and E. Fisher, 1996. Dietary guidelines for healthy American adults: A statement for health professionals from the nutrition committee. American Heart Association. 1996. *Circulation*, 94: 1795-1800.
- Kurtzweil, P., 1998. Safer egg: Laying the groundwork. U.S. Food and Drug Administration. FDA Consumer Magazine: September-October 1998.
- Ladan, M.J. and M. Lawal, 2002. Total lipid and cholesterol profile in the egg of some domestic birds: *Sok. J. Vet. Sci.*, 4: 1-3.
- Lenfant, C.J., A. Shepherd, P. Gotto, A. Kwiterovich, R. Scam and O. Superk, 1997. Lowering LDLs plays only a part in preventing heart disease. *Modern Med.*, 65: 14-17.
- Lichtenstein, A.H., L.M. Ausman and W. Carrasco *et al.*, 1994. Hypercholesterolemic effect of dietary cholesterol in diets enriched in polyunsaturated and saturated fat; Dietary cholesterol, fat saturation and plasma lipids. *Arterioscler Thromb.*, 14: 168-175.
- Lien, T.F., K.L. Chen, C.P. Wu and J.J. Lu, 2004. Effects of copper and chromium on the serum and egg traits of laying hens. *Br. Poult. Sci.*, 45: 535-539.
- McGill, H.C., 1979. The relationship of dietary cholesterol to serum cholesterol concentration and to atherosclerosis in man. *Am. J. Clin. Nutr.*, 32: 2664-2702.
- National Cholesterol Education Programme: 1991. Report of the Expert Panel on a population Strategies for Blood Cholesterol reduction: Executive Summary. *Arch. Intl. Med.*, 151: 1071-1084.

- Nazifi, S., M. Saeb, S. Rategh and A. Khojandi, 2005. Serum lipids and lipoproteins in clinically healthy Caspian miniature horses. *Veterinarski Arhiv*, 75: 175-182.
- Onyenekwe, P.C., E.O. Ajani, D.A. Ameh and K.S. Gamaniel, 1999. Anti hypertensive effect of *Hibiscus sabdariffa* infusion in spontaneously hypertensive rats and a comparison of its toxicity with that in Wister rats. *Cell Biochem.*, 17: 199-206.
- Passmore, P. and M.A. Eastwood, 1986. *Human Nutrition and Dietetics*. 8th Edn. Longman Group (FE) Ltd. Hong Kong, pp: 100-120.
- Sands, J.S. and M.S. Smith, 2002. Effects of dietary manganese protienate or chromium picolinate supplementation on plasma insulin, glucagons, glucose and serum lipids in broiler chickens reared under thermoneutral or heat stress conditions. *Intl. J. Poult. Sci.*, 1: 145-149.
- Seyrek, K., C. Yenisey, M. Serter and K. Kiral, *et al.* 2004. Effects of dietary vitamin C supplementation on some serum biochemical parameters of laying Japanese quails exposed to heat stress (34.8 °C) *Revue. Med. Vet.*, 155: 339-342.
- Sharaf, K.H. and J.S. Ali, 2004. Hypolipidemic effect of Kuub (*Gundelia tournefortii* A.) oil and clofibrate on lipid profile of atherosclerotic rats. *Veterinarski Arhiv*, 74: 359-369.
- Sofowora, A., 1992. *Medicinal Plants and Traditional Medicine in Africa*. 2nd Edn., New York: John Wiley and Sons', pp: 8.
- Stamler, J. and R. Shekelle, 1988. Dietary Cholesterol and Human Coronary Heart Disease. *Arch. Pathol. Lab. Med.*, 112: 1032-1040.
- Tietz, N.W., 1990. *Clinical Guide to Laboratory tests*, 2nd Edn., W.B. Saunders Company, Philadelphia, USA., pp: 554-556.
- Wang, J.J. and T.M. Pam, 2003. Effect of red mold rice supplements on serum and egg yolk cholesterol levels of laying hens. *J. Agric. Food Chem.*, 30: 4828-4829.
- West, E.S., W.R. Todd, H.S. Mason and J. Van Bruggen, 1966. *Textbook of Biochemistry*. 4th Edn., Macmillan New York, pp: 1370.