



Polyherbal hypocholesterolemic supplement lowers egg yolk cholesterol without affecting performance and egg quality in layers

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ABSTRACT : This study was designed to evaluate efficacy of polyherbal hypocholesterolemic supplement AV/HLP/16 (test compound) on serum and egg yolk cholesterol levels and egg quality parameters in layers. One hundred and twenty healthy White Leghorn (BV 300 strain) of 54 weeks age were randomly divided into four treatments (T_0 , T_1 , T_2 , T_3); subdivided into three replicates with ten birds in each replicate for a period of five weeks (54th to 58th weeks of age). T_0 was the control supplied with commercial basal diet without addition of test compound. T_1 , T_2 and T_3 were supplemented with test compound in basal diet @ 1.0, 1.5 and 2.0 kg/ton of feed, respectively. Weekly feed intake, daily egg production, egg weights, egg yolk total cholesterol, egg weight, shape index, yolk weight, albumen weight, shell weight, shell thickness was recorded. Significant reduction in serum cholesterol and egg yolk cholesterol ($P < 0.05$) was observed in treatment groups. Polyherbal AV/HLP/16 supplementation in layers did not impart any beneficial or deleterious effect on feed intake, feed efficiency, hen day egg production or egg quality traits viz., egg weight, shape index, yolk weight, and albumen weight except shell thickness. Polyherbal hypocholesterolemic supplement AV/HLP/16 @ 1.0, 1.5 and 2.0 kg per ton of feed reduced egg yolk cholesterol with better egg shell thickness without affecting the performance as well as egg quality parameters in layers.

KEY WORDS : Layer, Hypocholesterolemic supplement, Cholesterol

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INTRODUCTION

There is increased concern among consumers for

: cholesterol intake due to its association with the cardiovascular
 : diseases leading to public demand for designer egg. Dietary,
 : genetic, and pharmacological manipulations aimed at reducing
 : the cholesterol content of eggs has resulted in marginal
 : declines in yolk cholesterol levels. Fatty acid composition of
 : the yolk is readily altered by dietary modification of layers.
 : Research into lowering egg cholesterol is already in progress
 : either with dietary or pharmacological intervention. However,
 : pharmacological interventions in poultry may have some side
 : effects like reduced egg production, egg weight or egg quality
 : etc. (Kim *et al.*, 2004). Oral administration of phytosterols to
 : treat hypercholesterolemia was first achieved in chickens over
 : 50 years ago (Peterson, 1951) while the first report of their
 : effectiveness in humans appeared shortly thereafter (Pollak,
 : 1953). Administration of plant sterol interferes with intestinal
 : absorption of dietary and enterohepatically circulating
 : cholesterol, thus, it lowers cholesterol levels (Haust and

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Beveridge, 1996). Harmful effects of plant sterols have not been observed even after prolonged administration of excessive amounts to humans and animals (Herrmann and Samawi 1962; Schan, 1959). Several indigenous plants like *Ashwagandha*, *Allium sativum*, *Embllica officinalis*, *Ocimum sanctum*, *Trogonella foenum graecum*, *Allium cepa*, *Cucurma longa*, *Picrorrhiza curroa*, *Terminalia arjuna* etc. have been claimed to possess hypolipidemic and hypocholesterolemic properties that may be beneficial to reduce the risk of cardiovascular diseases.

Garlic (*Allium sativum*) has potential hypolipidemic, hypoglycemic and hypoatherogenic properties (Chowdhury *et al.*, 2002). Amla (*Embllica officinalis*) is an effective hypolipidemic agent and can be used as a pharmaceutical tool in hyperlipidemic subjects (Mathur *et al.*, 1996) Fenugreek (*Trigonella foenum-graecum*) seeds and leaves have hypolipidemic properties (Soumya and Rajalakshmi, 1999). Guggul (*Commiphora mukul*) became one of those herbs holding huge promises for the development of hypolipidemic and antiatherogenic effect. Several workers has reported that, certain herbs supplementation lowered egg yolk cholesterol (Chowdhury *et al.*, 2002; Premkumar *et al.*, 2002; Mottaghitalab and Taraz, 2004). Present study was undertaken to evaluate effect of polyherbal hypocholesterolemic supplement AV/HLP/16 on egg cholesterol, egg production and egg quality in layers.

MATERIAL AND METHODS

One-hundred and twenty healthy White Leghorn (BV 300 strain) laying hens of 54 weeks age were randomly divided into four groups *viz.*, T₀, T₁, T₂ and T₃ subdivided into three replicates with ten birds in each replicate. Birds were housed in California cages under standard managerial conditions with commercial layer diet. Trial was conducted for a period of five weeks (54th to 58th weeks of age). Commercial layer basal diet was provided to control (T₀). Subsequently, the polyherbal hypocholesterolemic agent AV/HLP/16 (supplied by M/s Ayurved Ltd., Baddi, India) was supplemented @ 1.0, 1.5 and 2.0 kg/ton of feed to treatment T₁, T₂ and T₃, respectively. Major constituent's herbs of this test compound were namely *Embllica officinalis*, *Commiphora mukul*, *Allium sativum* and *Trogonella foenum graecum*.

Weekly feed intake, daily egg production and egg weights were recorded to arrive at hen day egg production and feed

efficiency. Nine representative samples of eggs were collected from all groups at the end of 54, 55, 56, 57 and 58th weeks of age for estimation of egg yolk total cholesterol (mg/g of yolk) and egg quality parameters *viz.*, egg weight, shape index yolk weight, albumen weight, shell weight was calculated as described earlier (Carter, 1968), while shell thickness excluding shell membranes was measured using a micrometer at three locations on the egg (air cell, equator and sharp end).

Weekly egg yolk cholesterol (54th to 58th weeks of age) was estimated as described earlier (Wybenga *et al.*, 1970). For the estimation of egg yolk cholesterol about 2 g of yolk was mixed in 15 ml 2:1 chloroform-methanol solution. After mixing, 5 ml distilled water was added and centrifuged at 2500 rpm for 10 minutes, filtered through glass wool to obtain clear filtrate. Then cholesterol was measured by using the test kit (Span Diagnostics Ltd., India) following the manufacturer's directions. Nine representative blood samples were collected from all groups at weekly interval to estimate serum cholesterol (mg/dl) by using the test kit (Span Diagnostics Ltd. India). The data generated were subjected to statistical analysis using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Data of mean values for weekly serum cholesterol (mg/dl) of layers for a period of five weeks (54th to 58th week of age) were statistically significant (P<0.05) between different groups (Table 1). Serum cholesterol of layers in group T₁, T₂, T₃ were found to be lowered than control except for 57th week where higher serum cholesterol values were observed in T₁ and T₂ than control. Among all treated groups, significant (P<0.05) reduction of serum total cholesterol was observed in group T₃ than T₂, T₁ and T₀ at 54, 55, 56 and 58th weeks of age. However, serum cholesterol values at 57th and 58th weeks of age were numerically lowered in group T₃.

Statistical analysis of data on egg yolk cholesterol (Table 2) for T₂ and T₃ were significantly (P<0.05) lowered. Results of the present study indicated that, significant lower values for egg yolk cholesterol were observed in groups supplemented with polyherbal AV/HLP/16 @ 1.5 and 2.0 kg per ton of feed. Egg yolk cholesterol was lowered linearly as the dose of supplement increased in the study.

Average cumulative feed intake (kg) and feed efficiency from 54th to 58th weeks of age in layers is presented in Table 3.

Table 1 : Average weekly serum total cholesterol in layers supplemented with polyherbal hypocholesterolemic supplement

Weeks	T ₀	T ₁	T ₂	T ₃
54 th	267.90±7.42 ^b	261.76±8.22 ^b	254.32±8.74 ^{ab}	223.76±17.10 ^a
55 th	258.98±10.94 ^b	256.70±28.63 ^b	190.56±4.76 ^a	161.76±10.32 ^a
56 th	173.18±14.23 ^b	132.38±14.24 ^{ab}	120.42±1.32 ^a	114.60±13.83 ^a
57 th	209.50±30.95	234.42±20.75	221.24±9.42	167.94±55.09
58 th	249.50±8.25 ^b	226.62±14.44 ^{ab}	221.24±9.42 ^{ab}	194.94±18.73 ^a

Means bearing different superscript in a row differed significantly (P<0.05). Mean ± SE

Statistical analysis of data on average feed intake indicated that, there was no significant difference between all test groups supplemented with polyherbal hypocholesterolemic supplement and the control. Similarly, feed efficiency in treatment groups did not differ significantly with control. Results indicated that supplementation of hypocholesterolemic supplement in layers from 54th to 58th weeks of age did not have any effect on hen day egg production.

Egg quality parameters (Table 3) viz., egg weight, shape index, yolk weight, albumen weight and egg shell weight in all treatment groups were comparable with control. Average shell thickness of eggs from different treatments differed significantly ($P < 0.05$). Shell thickness observed to be higher in T₁, while T₂ and T₃ are middle of these two.

Results of study indicated that, polyherbal hypocholesterolemic supplementation in layer birds does not impart any beneficial or deleterious effect on feed intake and feed efficiency, hen day egg production or egg quality traits viz., egg weight, shape index, yolk weight, and albumen weight. However, the birds supplemented with hypocholesterolemic supplement @ 1.0 kg/ ton of feed shown significantly ($P < 0.05$) increased shell thickness.

Serum cholesterol was reduced by 0.88-23.6 per cent, 5.1-30.5 per cent and 12.14-37.54 per cent in hens supplemented with test compound @ 1.0, 1.5 and 2.0 kg per ton of feed, respectively. Egg yolk cholesterol was reduced (5.20-24.81 %, 18.9-35.4 % and 25.96-40.94 % in T₁, T₂ and T₃, respectively) in hens supplemented with polyherbal hypocholesterolemic supplement. Similar results were reported in previous study (Kanduri *et al.*, 2013). They demonstrated that, addition of hypocholesterolemic herbal agent @ 1.0, 1.5 and 2.0 kg per ton of feed in the basal diet has proved effective with highly significant reduction in serum total cholesterol (20-22 %) and egg yolk total cholesterol (18-22 %). Supplementation of certain

herbs like arjuna bark powder, guggul resin powder, cinnamon bark powder, amla pulp powder singly or in combination caused significant decline in both plasma and egg yolk cholesterol in White Leghorn hens (Sharma *et al.*, 2009). In present study, polyherbal hypocholesterolemic agent (Containing herbs *Emblica officinalis*, *Commiphora mukul*, *Allium sativum* and *Trogonella foenum graecum*) reduced serum cholesterol within a week of supplementation and continued till the end of experiment. *Allium Sativum* (Garlic) effectively reduced serum or plasma and egg yolk cholesterol level in poultry (Elangovan *et al.*, 2011; Khan *et al.*, 2008; Lonkar *et al.*, 2009; Raj *et al.*, 2013). Principle behind the action of garlic was that the sulphur containing compounds decreased cholesterol biosynthesis by inhibiting rate limiting HMG-CoA reductase enzyme (Ferry *et al.*, 2003; Konjufca *et al.*, 1997; Merat and Fallahzadeh, 1996). Cholesterol lowering effect of HMG-Co-A reductase enzyme (rate limiting enzyme in cholesterol synthesis) inhibitors on egg cholesterol have successfully demonstrated earlier (Elkin *et al.*, 1999; Mori *et al.*, 1999). Dietary inclusion of *Trogonella foenum graecum* (fenugreek) also found to lower the concentration of serum and egg yolk cholesterol (Elangovan *et al.*, 2011; Raj *et al.*, 2013, Abbas, 2010). Lipid lowering and anti-atherosclerotic effects of *Emblica officinalis* (Amla) fresh juice were evaluated in cholesterol fed rabbits (rendered hyperlipidemic by atherogenic diet and cholesterol feeding) by (Mathur *et al.*, 1996) and reported that amla fresh juice administered at 5ml/kg body weight per rabbit per day for 60 days could lower serum cholesterol level by 82 per cent. Hypolipidemic effect of guggulipid and guggulsterone from *Commiphora mukul* (Guggul) has been consistently demonstrated in various animal species, including rat, mouse, rabbit (Satyavati *et al.*, 1996) and chicken (Baldwa *et al.*, 1981). Guggulsterone upregulates the expression of the bile salt export pump, a rate-limiting efflux transporter for eliminating

Table 2 : Average egg yolk cholesterol (mg/g of yolk) in layers supplemented with polyherbal hypocholesterolemic supplement

Weeks	T ₀	T ₁	T ₂	T ₃
54 th	43.34±2.40 ^b	37.90±1.50 ^b	28.95±1.41 ^a	29.04±2.49 ^a
55 th	35.45±1.50 ^c	31.99±1.22 ^c	27.75±1.11 ^b	23.44±1.26 ^a
56 th	36.94±0.78 ^c	33.09±1.56 ^b	29.95±0.92 ^{ab}	27.35±1.55 ^a
57 th	60.35±1.54 ^c	45.38±2.30 ^b	38.99±1.18 ^a	35.64±2.8 ^a
58 th	50.61±2.25 ^c	47.98±1.51 ^c	39.01±1.40 ^a	37.00±2.05 ^a

Means bearing different superscript in a row differed significantly ($P < 0.05$). Mean ± SE

Table 3 : Laying hens performance and egg quality (average value of five weeks) during supplementation polyherbal hypocholesterolemic supplement

Treatments	Feed intake (kg)	Feed efficiency	Hen day egg production (%)	Egg weight (g)	Shape index	Yolk weight %	Albumen weight %	Shell weight %	Shell thickness (mm)
T ₀	30.17± 1.41	2.36±0.33	69.04± 6.94	55.43±1.15	74.72±0.56	32.29±0.74	55.80±0.73	11.90±0.46	0.364 ^a ±0.074
T ₁	29.99±1.31	2.03±0.17	76.53±4.12	57.22±0.61	74.75±0.25	31.71±0.66	55.86±0.83	12.42±0.34	0.384 ^b ±0.005
T ₂	30.53±1.48	2.06±0.22	73.14±5.38	56.41±0.89	75.03±0.38	30.87±0.47	56.85±0.57	12.27±0.23	0.376 ^{ab} ±0.066
T ₃	30.35±0.96	2.02±0.20	74.00±5.41	58.13±1.18	74.94±0.24	31.36±0.70	56.29±0.61	12.33±0.15	0.371 ^{ab} ±0.051

Means bearing different superscript in a column differed significantly ($P < 0.05$). Mean ± SE

cholesterol metabolites bile acids from the liver. Such upregulation is possibly mediated through the activating protein 1 signalling pathway (Deng *et al.*, 2007). Enhanced bile salt export pump expression has been proposed as possible mechanisms for the hypolipidemic effect of guggulsterone (Deng *et al.*, 2007; Urizar *et al.*, 2001). Diosgenin, the primary furostanol saponin in fenugreek has been proven to have various effect on cholesterol metabolism (Sauvaire *et al.*, 1991), the most important being its capacity to lower plasma cholesterol concentration. This hypocholesterolemic effect appears to be dependent upon the capacity of diosgenin to inhibit cholesterol absorption, to decrease liver cholesterol, to increase biliary cholesterol secretion and fecal excretion of neutral sterols. *Emblica officinalis* contains flavonoids which reduce the level of serum and tissue lipid by degeneration and elimination of cholesterol (Anila and Vijayalakhmi, 2002). The findings of the present study are in agreement with the above workers which clearly indicated that polyherbal hypocholesterolemic supplement AV/HLP/16 significantly decreased serum cholesterol.

Addition of hypocholesterolemic herbal agent @ 1.0, 1.5 and 2.0 kg per ton of feed has no significant effect on feed intake, feed efficiency (per dozen of egg), egg production, egg weight, shape index, egg yolk weight and albumen weight. The present findings are in agreement with (Kanduri *et al.*, 2013) who showed addition of hypocholesterolemic herbal agent @ 1.0, 1.5 and 2.0 kg per ton of feed has no significant effect on body weight gain, feed consumption, feed efficiency (per dozen of egg), egg production, egg weight, egg yolk weight, egg yolk index and haugh unit. Sharma and colleagues (Sharma *et al.*, 2009) reported dietary supplementation of various herbs either alone or in combination to hens did not exert any beneficial or detrimental effect on egg production, feed intake, feed efficiency or egg quality traits which are in agreement with the present findings. Some workers (Kim *et al.*, 2004; Chowdhury *et al.*, 2002; Balevi and Coskun, 2004; Lien *et al.*, 2004) could not find any significant effect on egg production of laying hens due to supplementation of garlic (2, 4, 6 and 8 %). In concordance with present findings, supplementation of certain herbal agents like garlic powder, tulsi oil and fenugreek seeds did not showed any significant effect on shell weight (Elangovan *et al.*, 2011). Investigation of Khan and others (Khan *et al.*, 2008) also found that supplementation of garlic powder had a non-significant ($P < 0.05$) effect on the feed consumption and feed efficiency in native Desi laying hens. No change in egg quality parameters after supplementation of fenugreek was also revealed previously (Abbas, 2010). Pharmacological interventions in poultry may have some side effects like reduced egg production, egg weight or egg quality etc. (Kim *et al.*, 2008) however, the herbs *Emblica officinalis*, *Commiphora mukul*, *Allium sativum* and *Trogonella foenum graecum* did not showed any detrimental effect on egg quality

and egg production parameters.

It was concluded that the, Supplementation of polyherbal hypocholesterolemic supplement AV/HLP/16 lowered egg yolk and serum cholesterol with better shell thickness of eggs at 1, 1.5 and 2.0 kg per ton of feed without altering the performance parameters like feed intake, feed efficiency and egg production and egg quality parameters like egg weight, shape index, yolk weight, albumen weight and shell weight in laying hens. Reduction in egg yolk cholesterol was observed within a week of supplementation of polyherbal hypocholesterolemic supplement. Cholesterol lowering effect of hypocholesterolemic agent might be due to presence of flavonoids in *Emblica officinalis*, guggulipid and guggulsterone in *Commiphora mukul*, Allicin in *Allium sativum* and diosgenin in *Trogonella foenum graecum*.

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