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Withania somnifera reduces egg yolk total lipids, cholesterol and triglycerides in birds

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ABSTRACT

Cardiovascular disease, currently the leading cause of death and illness in developed countries, will soon become the pre-eminent health problem worldwide. Several indigenous plants have been claimed to possess hypolipidemic and hypocholesteremic properties that may be beneficial to reduce the risk of cardiovascular diseases. Root powder of *Withania somnifera* possesses hypocholesteremic, hypoglycaemic and diuretic properties. The study was thus conducted on twenty four healthy coloured dwarf birds for forty two days. Group T_1 was kept as a control while group T_2 was the treatment group. Roots of *Withania somnifera* were dried, crushed, powdered and used for supplementation in the diet of birds. Supplementation was done at the rate of 2% in the feed of birds. Eight eggs were collected from each dietary treatment group of birds on day 0, 14, 28 and 42 of the experiment. Yolk was collected from each egg for the extraction of lipids and was analyzed for estimation of Total lipids, Cholesterol and Triglycerides. *In vitro* determination of cholesterol and triglycerides was done by using diagnostic reagent kits. Control group did not show any significant change in egg yolk lipids, cholesterol and triglycerides. The mean values of egg yolk lipids, cholesterol and triglycerides in dietary group of birds supplemented with 2 percent *W. somnifera* was 216.86 mg/g, 12.18 mg/g and 134.68 mg/g yolk respectively on day 42 post treatment. The reduction was significant at all intervals. Dietary herbal supplementation with *Withania somnifera* exhibited a significant reduction in levels of egg yolk lipids, cholesterol and triglycerides of birds. Production performance traits and Egg quality traits were also analysed to check the untoward effects if any but the supplementation was found to be safe.

Keywords: Egg yolk, Hypolipidemic, *Withania somnifera,* Cholesterol, Herbal supplementation

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INTRODUCTION

Hypercholesterolemia or Hyperlipidemia is the aetiology behind most of the Cardio vascular diseases & Atherosclerosisis is one among them. It is a progressive disease characterized by the accumulation of cholesterol, low density lipoprotein and fibrous elements in the large arteries, which constitutes the single most important contributor to the growing burden of cardiovascular diseases. These diseases are currently the leading cause of death and illness in developed countries, and will soon become the pre-eminent health problem worldwide. These diseases are more lethal than the cancers and the worst part is that they are asymptomatic and are not detected by most diagnostic methods, the first symptom of atherosclerotic cardiovascular disease is a heart attack or a sudden cardiac death. Cholesterol has always been a constituent of concern for the mankind.

Chicken eggs are rich sources of cholesterol and anxiety has often been created against their use in human diet, egg being one of the most nutritious and unadulterated natural food, is a rich source of all the essential amino acid, minerals and vitamins, however, in addition to these essential dietary components; egg contains about 200-250 mg of cholesterol which is more than 2/3rd recommended intake of cholesterol. In an adult human, cholesterol levels greater than 250 mg/dl in blood may enhance the risk of cardiovascular diseases like atherosclerosis, hypertension, coronary heart diseases, myocardial infaract, ischemic stroke and even Alzheimer's disease in humans.

Cholesterol has always been a constituent of concern for us. Cholesterol and its esters are found in egg yolk, where they form emulsion of low density lipoproteins (LDL), very low density lipoproteins (VLDL) and high density lipoproteins (HDL). The HDL, so called "good cholesterol" accounts for 8 % of dehydrated yolk. Cholesterol content in the eggs is influenced by genetic factors, diet composition, lay intensity, layer age and medical treatment. High intake of cholesterol in foods affects the blood cholesterol levels in humans. Therefore the relation of cholesterol intake and the consumption of eggs were studied by many authors. Levy studied a diet with two eggs daily during 3 weeks and detected both an increase of plasma LDL cholesterol and a decrease of HDL cholesterol by 11%.

Several indigenous plants have been claimed to possess hypolipidemic, hypocholesteremic, and immune stimulating properties that may be beneficial to reduce the risk of cardiovascular diseases. *Withania somnifera,* an indigenous medicinal plant commonly known as Ashwagandha, Indian ginseng and Winter cherry, has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory, astringent and antiulcer agent [1]. Root powder of *W. somnifera* possesses hypocholesteremic, hypoglycaemic and diuretic properties [2]. Thus the present study was formulated with an objective to decrease the lipid content of the eggs with dietary herbal supplementation.

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MATERIAL AND METHODS

The study was conducted on twenty four healthy coloured dwarf birds of forty two week's age. Birds were randomly divided into two groups with twelve birds in each group having two replicates of six birds each. Group T_1 was kept as a control while group T_2 was the treatment group. Roots of *Withania somnifera* (Ashwagandha) were dried, crushed, powdered and used for supplementation in the diet of birds. Control diet consisted of basal diet without any herbal supplements. The treatment diet consisted of basal diet along with herbal supplement. Group T_1 was kept as a control while group T_2 was supplemented with dried root powder of W.somnifera. Supplementation was done at the rate of 2% in the feed of birds. Treatment was done for a period of 42 days.

Egg yolk lipid profile

Eight eggs were collected from each dietary treatment group of birds on day 0, 14, 28 and 42 of the experiment. Yolk was collected from each egg for the extraction of lipids and lipids were analyzed for estimation of Total lipids (mg/g), Cholesterol (mg/g) and Triglycerides (mg/g). The diagnostic reagent kits from Transasia Bio- medicals Ltd., were used for *in-vitro* determination of cholesterol and triglycerides in egg yolk. The estimation was done using semi auto-analyzer (ERBA CHEM-5).

Extraction of Lipids from egg yolk

Lipid was extracted from the egg yolk by using chloroform: methanol mixture (2:1 v/v) [3]. Lipid extract (20 µl) was taken for the estimation of total lipids by the method of Fringe [4]. Quantitative estimation of total cholesterol in egg yolk was done by employing method as suggested by Roeschlau [5] while as Quantitative estimation of Triglycerides was done by the method outlined by Wako and modified by Mc Gowan [6].

Production performance traits and Egg quality traits in birds

Body weight: All the birds were weighed individually for observing change in body weights on day 0, 14, 28 and 42 of the experimental period with hanging double dial balance.

Egg production: Daily egg production of birds was recorded on individual hen basis. Eight eggs were collected from each group on day 0, 14, 28 and 42 of experiment to study the egg quality traits.

Shape index: Shape index was calculated as per the formula suggested by Singh [7].

$$\text{Shape index} = \frac{\text{Width of egg}}{\text{Length of egg}} \times 100$$

Albumen index: The albumen index was measured by using the following formula [8]. The egg shell was broken at the middle portion with the help of blunt end of knife. The egg contents were poured on a perfectly leveled glass plate. Maximum length and the maximum

width of thick albumen were measured with the help of vernier caliper. The height of thick albumen was taken between the yolk and the outer border of thick albumen avoiding the chalaza. Albumen height was measured with the help of spherometer with a least count of 0.001 mm after adjusting for the zero error on the plain glass plate.

$$\text{Albumen index} = \frac{\text{Average height of albumen}}{\text{Average width of albumen}} \times 100$$

Yolk index: Yolk index was calculated by the following formula [9]. The height of the yolk was measured with the help of tripod spherometer and width by vernier caliper. The formula used to calculate yolk index was:

$$\text{Yolk index} = \frac{\text{Average height of yolk}}{\text{Average width of yolk}} \times 100$$

Shell thickness: The egg shell thickness was measured by taking three pieces of shell from narrow, broad and mid portion of egg. Shell membrane was removed from egg shell and measurement was taken with the help of screw gauge. The average shell thickness was taken as the final reading.

The research plan was screened by the **Institutional Animal Ethics Committee** and was approved.

Statistical Analysis

Means were obtained as per standard procedure. All the parameters were analyzed by using the method for complete randomized design with the treatments allotted to group of 12 birds each. The differences within each treatment were tested statistically for their significance [10].

RESULTS AND DISCUSSION

Effect of W. somnifera on egg yolk total lipids

The findings with regards to the effect of *W. somnifera* on egg yolk lipid values in laying hens have been summarized in Table 1. Total lipids were calculated in terms of mg/g of egg yolk on day 0, 14, 28 and 42 of the experimental period in different groups of birds. Untreated control (T_1) did not show any significant change in egg yolk lipids and was 287 mg/g yolk on day 42, whereas group of birds with dietary supplementation of herbs revealed significant reduction in egg yolk lipids post treatment. The mean values of egg yolk lipids in dietary group of birds supplemented with 2 percent *W. somnifera* (T_2) was 216.86 mg/g yolk while the percent reduction was calculated to be 25 percent on day 42 post treatment. The reduction was significant at all intervals.

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Table 1: Effect of W. somnifera on egg yolk total lipids

Group	Inclusion Level	Egg yolk total lipids (mg/g)							SEM	CD at P<0.01
		Pre Treatment	Post Treatment			Percent Reduction				
		Day 0	Day 14	Day 28	Day 42	Day 14	Day 28	Day 42		
T_1 Control	-	292.60	295.00	285 .00	287 .00	-	-	-	4.48	NS
T_2 W. somnifera	2 %	289.35[a]	269.90[b]	244.57[c]	216.86[d]	6.7	15.4	25	4.09	12.61

Values are mean of eight observations.
The mean values with different alphabet as superscript differ significantly from each other.
SEM : Standard Error Mean; CD : Critical Difference; NS : Non Significant

Total lipids content of egg yolk in the control group was found to be 30 percent of the total egg yolk weight. This value corresponds with that reported by El Bagir [11] and Cunningham and Lee [12]. Cholesterol concentration of egg yolk in control group was approximately 5 percent of total lipids which is in close agreement to the values reported by Van Elswyk [13]. The triglycerides comprised 30 percent of egg yolk total lipids, which is similar to the values reported by Leskanish and Noble [14].

Effect of W. somnifera on egg yolk cholesterol (CHO)

Table 2: Effect of W. somnifera on egg yolk cholesterol (CHO)

Group	Inclusion Level	Egg yolk cholesterol (mg/g)							SEM	CD at P<0.01
		Pre Treatment	Post Treatment			Percent Reduction				
		Day 0	Day 14	Day 28	Day 42	Day 14	Day 28	Day 42		
T_1 Control	-	17.68	18.00	17.40	17.90	-	-	-	0.49	NS
T_2 W. somnifera	2 %	17.40[a]	15.73[a]	13.58[b]	12.18[b]	9.50	21.0	30.0	0.56	1.71

Values are mean of eight observations.
The mean values with different alphabet as superscript differ significantly from each other.
SEM : Standard Error Mean; CD : Critical Difference; NS : Non Significant

The mean values of egg yolk CHO as influenced by dietary supplementation of *W. somnifera* on laying hens have been presented in the Table 2. Untreated control group did not reveal any significant variation on day 0, 14, 28 and 42 of the experimentation and was 17.9 mg/g yolk on day 42. However, 2 percent *W. somnifera* (T_2) showed a significant reduction in the egg CHO levels was 12.18 mg/g yolk and the percent reduction was 30 percent respectively. Moreover the reduction was significant at all intervals.

In laying hens, egg cholesterol is synthesized in the liver and secreted into the blood as very low density lipoprotein particles, the main yolk cholesterol carrying macromolecules. Plasma very low density lipoprotein particles are then internalized by the oocyte vitellogenin receptor in the rapidly growing follicles and deposited to yolk [15]. Thus, cholesterol is mainly excreted through the egg in the hen. Faecal neutral and acidic sterol represents a second major pathway for elimination of cholesterol. It has therefore been suggested that either selective inhibition of liver cholesterol biosynthesis or increased excretion of cholesterol from the body would result in reduction of egg cholesterol. The report of Visavadiya and Narasimhacharya indicated hypocholesteremic effect of root powder of *Withania somnifera* at the dose of 1.5 g/day, added to the diet of rats for 28 consecutive days [16]. They further observed a significant reduction in total lipids by 50.69 percent, cholesterol by 53.01 percent, triglycerides by 44.85 percent, LDL cholesterol by 62.7 percent and VLDL cholesterol by 44.8 percent in plasma. The reports of Visavadiya and Narasimhacharya are in close conformation to our findings where *Withania somnifera* in dose level of 2 percent caused a gradual and significant reduction in total lipids, cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol on day 28 and 42 of experimentation.

The cholesterol lowering effect of *Withania somnifera* could be due to elevated excretion of cholesterol and bile acids through fecal sterol excretion. In the present study, *Withania somnifera* was used in form of root powder and the property could be attributed to higher fiber and phytosterol contents, which may lead to decrease in intestinal transit time for cholesterol and carbohydrate absorption from gut [17], that ultimately decreased hepatic lipogenesis and reduction in hepatic and plasma triglyceride concentrations [18].

Effect of W. somnifera on egg yolk triglycerides (TG)

Table 3: Effect of W. somnifera on egg yolk triglycerides (TG)

		Egg yolk triglycerides (mg/g)								
Group	Inclusion Level	Pre Treatment	Post Treatment			Percent Reduction			SEM	CD at P<0.01
		Day 0	Day 14	Day 28	Day 42	Day 14	Day 28	Day 42		
T_1 Control	-	185.00	192.00	189.00	190.00	-	-	-	4.99	NS
T_2 W. somnifera	2 %	182.00[a]	169.20[a]	151.00[b]	134.68[c]	7	17	26	5.07	15.62

Values are mean of eight observations.
The mean values with different alphabet as superscript differ significantly from each other.
SEM : Standard Error Mean; CD : Critical Difference; NS : Non Significant

The mean values of egg yolk TG as altered by dietary supplementation of *W. somnifera* have been presented in the Table 3. Untreated control group did not reveal any significant variation on day 0, 14, 28 and 42 of the experiment and was 190 mg/g yolk on day 42. However, 2 percent *W. somnifera* (T_2) showed a significant reduction in the egg yolk triglyceride with the course of treatment and was 134.68 mg/g yolk on day 42 post treatment and the corresponding

percent reductions was 26 percent. The reduction was significant at all intervals and in all treatments.

Udayakumar, determined hypolipidemic activity of *Withania somnifera* root extract (200 mg/kg body weight) as feed supplement daily for eight weeks [19]. The results exhibited a significant reduction in serum triglycerides of rats. Similarly, Roughani reported that oral administration of *Withania somnifera* mixed pelleted food at the dose of 6.25 percent for 2 months, produced significant reduction in triglycerides and serum cholesterol level in rats and significant reduction in LDL cholesterol was also obtained [20]. The above reports are in close conformation to our findings where *Withania somnifera* in dose level of 2 percent caused a gradual and significant reduction in total lipids, cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol on day 28 and 42 of experimentation.

Effect of W. somnifera on Production performance traits and Egg quality traits

Table 4: Effect of W. somnifera on body weight

		Body weight (g)					
Group	Inclusion Level	Pre Treatment	Post Treatment			SEM	CD at P<0.01
		Day 0	Day 14	Day 28	Day 42		
T_1 Control	-	1430	1440	1460	1480	16.89	NS
T_2 W.somnifera	2 %	1455[a]	1465[a]	1515[b]	1565[c]	9.90	32.27

Values are mean of twelve observations.
The mean values with different alphabet as superscript differ significantly from each other.
SEM: 6 Standard Error Mean; CD: Critical Difference; NS: Non Significant

Table 5: Effect of W. somnifera on egg production

Egg Production (No. of eggs/bird)		
	T_1 (Control)	T_2 (W. somnifera)
Phase 1 (Day 0-14)	4.08	3.92[a]
Phase 2 (Day 15-28)	4.16	5.57[b]
Phase 3 (Day 29-42)	4.91	6.67[b]
SEM	0.11	0.61
CD at P<0.05	NS	1.77

Values are mean of twelve observations.
The mean values with different alphabet as superscript differ significantly from each other.
SEM: Standard Error Mean; CD: Critical Difference; NS: Non Significant

Body weight of birds increased as the age advanced in both the groups. However, increase in body weight was not significant in control group, whereas significant increase in body weight was recorded in the group of birds supplemented with 2 percent *W. somnifera*

(Table 4). There was a significant increase in the egg production of both groups however the increase was more pronounced in case of the treatment group (Table 5).

Mean values of Shape Index, Albumen Index, Yolk Index and egg shell thickness did not differ significantly on day 0, 14, 28 and 42 of the experiment. Group of birds supplemented with dietary herbal supplementation showed significant increase in production performance traits namely, body weight and egg production during last four weeks of the experiment. The results of the present study are in agreement with the findings of Bhoyar, who studied the effect of *Withania somnifera* root powder on growth and egg production in layers and reported improvement in egg production on dietary supplementation of *Withania somnifera* root powder at the dose of 5 g/kg feed and 10 g/kg feed for 10 weeks in 32 weeks old White leghorn birds [21]. Further, dietary supplementation of *Withania somnifera* is helpful in maintaining body weight and can be used as an anti-stressor. Samarth also reported that supplementation of 0.5 percent *Withania somnifera* to the diet of day-old broiler chicks for a period of 6 weeks, produced significant increase in feed consumption and gain in body weight [22].

Table 6: Effect of W. somnifera on egg traits

		Shape Index					
Group	Inclusion Level	Control	Post Treatment			SEM	CD at P<0.05
		Day 0	Day 14	Day 28	Day 42		
T_1	-	75.14	74.69	75.04	74.14	0.43	NS
T_2	2 %	72.98	73.19	74.03	74.72	0.60	NS
		Albumen Index					
Group	Inclusion Level	Control	Post Treatment			SEM	CD at P<0.05
		Day 0	Day 14	Day 28	Day 42		
T_1	-	9.96	10.14	10.11	9.80	0.26	NS
T_2	2 %	9.96	10.24	9.9	10.61	0.29	NS
		Yolk Index					
Group	Inclusion Level	Control	Post Treatment			SEM	CD at P<0.05
		Day 0	Day 14	Day 28	Day 42		
T_1	-	40.99	41.78	43.15	42.40	0.33	NS
T_2	2 %	42.02	42.78	42.86	43.23	0.52	NS
		Shell Thickness					
Group	Inclusion Level	Control	Post Treatment			SEM	CD at P<0.05
		Day 0	Day 14	Day 28	Day 42		
T_1	-	0.33	0.33	0.33	0.33	0.01	NS
T_2	2 %	0.33	0.33	0.32	0.33	0.01	NS

Values are mean of eight observations.
The mean values with different alphabet as superscript differ significantly from each other.
SEM: Standard Error Mean; CD: Critical Difference; NS: Non Significant

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Egg quality traits are the characteristics of an egg that affect its acceptability to the consumer. The economic success of a laying flock solely depends on the total number of quality eggs produced. In the present study, different egg quality traits such as shape index, albumen index, yolk index and shell thickness in groups supplemented with two percent *Withania somnifera* did not show any significant changes (Table 6). Rehman also could not find any significant effect on albumen index, yolk index and Haugh unit when diet of layers supplemented with 1, 2 and 3 percent garlic powder for 12 weeks [23]. Similarly, Elangovan did not find any significant effect on albumen index, yolk index and shell thickness in the eggs of Japanese quails on dietary supplementation of 1 percent garlic powder, 0.25 percent Ocimum sanctum oil and 2 percent fenugreek seeds [24]. In the present study, dietary supplementation with *Withania somnifera* for 42 consecutive days did not reveal any significant change in shape index, albumen index, yolk index and shell thickness of eggs which is indicative of non- toxic effect of *Withania somnifera* on egg quality traits.

CONCLUSION

Dietary herbal supplementation with *Withania somnifera* exhibited a significant reduction in levels of egg yolk total lipids, egg yolk cholesterol and egg yolk triglycerides of birds. This significant finding could be employed in the production of designer eggs with lower Cholesterol levels and the amount of anxiety around the consumption of eggs could be reduced. It could also lower the risk of cardiovascular diseases like atherosclerosis, hypertension, coronary heart diseases, myocardial infaract, ischemic stroke and even Alzheimer's disease in humans.

Production performance traits such as egg production and body weight of birds treated with dietary supplementation of *Withania somnifera* showed a significant increase in the aforementioned traits and dietary supplementation with *Withania somnifera* for 42 consecutive days did not reveal any toxic effects on egg quality traits of birds.

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