Indian J. Anim. Nutr. 2020. 37 (4): 352-357
doi: 10.5958/2231-6744.2020.00057.2

Indian Journal of Animal Nutrition

Effect of Dietary Supplementation of Ashwagandha Root Powder on Production Performance, Egg Quality and Blood Biochemical Constituents of Laying Hens

Sandeep Kumar*, Raj Singh Berwal, Ramsawroop and Sajjan Sihag
Department of Animal Nutrition, College of Veterinary Sciences,
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, India

ABSTRACT

Due to the emergence of the problem of anti-microbial resistance, the antibiotic growth promoters (AGP) are being replaced with alternatives like herbal growth promoters in poultry diets. The present study was undertaken to evaluate the effectiveness of dietary Ashwagandha root powder supplementation on egg production performance, egg quality and serum biochemical constituents in laying hens. A total of 120 laying hens (White leghorn, 22-week-old) were randomly allocated into five groups (T_1, T_2, T_3, T_4 and T_5) of 24 hens with four replications having six birds in each replication. A maize - soybean meal based laying hen basal diet having all the nutrients in required quantity as per BIS, 2007 specifications was prepared that was supplemented with antibiotic was prepared and fed to experimental laying hens of control group (T_1), while the treatment groups T_2, T_3, T_4 and T_5 were fed the basal antibiotic free ration supplemented with 0.25, 0.5, 0.75 and 1.0 per cent Ashwagandha root powder, respectively. Each of such diets was offered as mash *ad libitum* to laying hens for a period of 16 weeks (22-38 weeks of age). The findings of the study revealed that hen day egg percent and egg mass production increased significant (P<0.05) as the level of Ashwagandha root powder inclusion increased. The egg weight and internal egg parameters *viz.* albumen index, yolk index and Haugh unit were not affected by supplementing different levels of Ashwagandha root powder. Egg yolk total cholesterol and low density lipoprotein bound cholesterol decreased significantly (P<0.05), while high density lipoprotein bound cholesterol increased significantly (P<0.05) as level of Ashwagandha root powder inclusion increased. The results of the study showed that supplementation of 1% Ashwagandha root powder significantly (P<0.05) decreased the serum cholesterol, triglycerides and low-density lipoproteins, while significantly (P<0.05) increased the high-density lipoprotein in laying hen as compared to control birds. Internal egg quality parameters were not affected by inclusion of Ashwagandha root powder in the diet. It was concluded that inclusion of Ashwagandha root powder at 1% in the laying hen diet as a replace of antibiotic growth promoter not only improve egg production but also decreased the amount of bad cholesterol being deposited into egg.

Key words: Ashwagandha root powder, Cholesterol, Egg quality, Layers, Serum

INTRODUCTION

Egg being most nutritious and unadulterated natural food, has acquired an important place in human diet. As a source of essential nutrients, eggs have been used worldwide to support the nutritional needs of human societies. Unfortunately, eggs also contain a significant amount of cholesterol, a lipid molecule that has been associated with the development of cardiovascular diseases (Kuang *et al.,* 2018). A yolk contains more than two-thirds of the recommended daily intake of 300 mg of cholesterol. Because chicken eggs are rich source of cholesterol, anxiety has often been created against their use in human diet. There is increased concern among consumers for cholesterol intake due to its association with the cardiovascular diseases leading to public demand for designer egg. Serum- biochemical profile of birds could serve as an index in predicting the effect of any ration given to the birds. Biochemical changes have clinical importance in assessment of health, nutritional status and diagnosis of disease (Raju *et al.,* 2019).

Medicinal plants have been a major source of therapeutic agents since ancient times. The use of roots and leaves of medicinal plants as feed additives can

*Corresponding author; E-mail: drskverma05@gmail.com

avoid wide spread abuses and occurrence of residues of hormone, antibiotic *etc*. in eggs and meat. Ashwagandha is an important medicinal plant, which is used in traditional medicine to cure many diseases. Ashwagandha has long been used as an anti-oxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent, antibacterial and antifungal agent (Punetha *et al.,* 2010). Considering the antimicrobial property of Ashwagandha, it had been proposed as one of the phyogenic feed additives to replace AGP in poultry ration (Kale *et al.,* 2016). In addition, the extract of this plant is a potent immune-stimulator and anticarcinogenic (Sharma *et al.,* 2010). The biologically active chemical constituents of Ashwagandha include alkaloids, phenolic tannins, withanolides and saponins (Mukherjee *et al.,* 2020). These active principles have been reported to possess immunomodulatory, general tonic, hepato-protective, anti-stress, growth promoter and antioxidant properties (Dwivedi *et al.,* 2015 and Singh *et al.,* 2010). Flavonoids were determined in the extracts of Ashwagandha root which possess hypoglycaemic and hypolipidaemic properties (Pandey *et al.,* 2014) that may be beneficial to reduce the risk of cardiovascular diseases. Thus, the present study was conducted to characterize the effect of Ashwagandha root powder on performance, egg quality and lipid related serum metabolite of laying hens.

MATERIALS AND METHODS

A total of 120 White leghorn laying hens at 22 weeks of age with uniform body weight, production performance and genetic background were randomly assigned to five treatment groups with four replicates per group and six birds per replicate. Laying hens in the control group (T_1) were given a corn-soybean- based basal diet in mash form supplemented with antibiotics, but without Ashwagandha root powder additive. The basal diet was formulated to meet nutrient requirements of laying hens as per BIS (2007) specifications, (Table 1). The remaining four treatment groups T_2, T_3, T_4 and T_5 of experimental hens were given the same basal diet supplemented (but antibiotic free) with Ashwagandha root powder at 0.25, 0.50, 0.75% and 1% of ration, respectively. The experiment was carried out between 22 and 38 weeks of age. Hens were housed in battery cages equipped with trough feeders and drinkers. Feed and Water were offered *ad libitum* during the study. The laying hens were kept in optimal and standard bio-climatic and welfare conditions. In this work, the national guidelines for care and precautions for animal use were followed and all procedures were approved by Institutional Animal Ethics Committee (IAEC), 12/ CPCSEA Dated 13.07.2018.

Table 1. Ingredients and nutrient composition of layers basal diet

Feed Ingredient	Quantity (kg/100 kg)
Maize	56
Groundnut cake	11
Soybean meal	15
Wheat bran	5
Fish Meal	6
Mineral mixture	3
Salt	0.5
Shell grit	3.5
Nutrient composition (%)	
Crude protein	18.86
Crude fibre	3.86
Ether extract	3.84
Nitrogen-free extract	62.52
Ash	10.92
Metabolizable energy(kcal/kg)	2699.10

Additives (g/100 kg): Intermix regular (Vitamins A, D_3, B_2, K) -10 g, Intermix-BE (Vitamins B_1, B_6, B_{12}, E) -10 g, Antibiotics – 10g

Egg production was recorded daily for each treatment and an average egg production rate (hen-day percentage) was calculated for the experimental period. The egg weight, egg mass production and internal egg quality in terms of Albumen Index, Yolk Index and Haugh Unit were recorded at the weekly interval. Egg weights were measured by using electronic weighing balance. Albumen and yolk height- width were measured using tripod spherometer and Vernier caliper. Haugh Unit (HU) was calculated using the values obtained for the egg weight and the albumen height as expressed by Haugh (1937) using the formula HU

=100log (H+7.5-1.7W0.37), where, H =albumen height in mm, W = egg weight in gram.

To estimate cholesterol, HDL and LDL in egg yolk, at the end of study 20 eggs were collected randomly, one from each replication of each treatment. Total lipids from sample were extracted according to the method of Angelo *et al.* (1987). Yolk cholesterol, LDL and HDL concentration were measured using commercial kit.

At the end of the study, four birds from each treatment, thus a total of 20 birds were randomly selected for blood sample collection. Blood samples (5.0 ml) were collected from the jugular vein from all hens into non-heparinised tubes using sterilized syringes and needles. After keeping in slanting position for 1 h at room temperature, serum was harvested by centrifuge at 3000 rpm for 10 min. Serum samples were stored at -20°C for determination of serum parameters. Serum concentrations of triglyceride, cholesterol, LDL and HDL were measured using commercial kit (ERBA Kit) in ERBA-EM-200 Automatic Analyzer.

The resultant data were statistically analysed according to the procedure laid down by Snedecor and Cochran (1994). One-way ANOVA was performed using SPSS 20.0 version of Microsoft. The mean differences among treatments were separated by Duncan's multiple range tests. Consequently, a significance level of 5% (P<0.05) was used as the criterion for statistical significance (Duncan, 1955).

RESULTS AND DISCUSSION

The experimental diets fed to the laying hens during first production cycle contained 18.86 % crude protein, 3.86% crude fibre, 3.84 % ether extract, 62.52% nitrogen-free extract, 10.92% total ash and 2699 kcal/kg ME (Table 1).

The effects of experimental diets on egg production and quality parameters are summarized in Table 2 and 3, respectively.

The results of the study unveiled that there was significant (P<0.05) positive effect of supplementation of different levels (0.5, 0.75 and 1%) of Ashwagandha root powder in the diet of layers on percent hen day egg production. Replacement of AGP with inclusion of Ashwagandha root powder increased the percent hen day egg production and egg mass production, the best response was observed in group T_3 in which AGP was replaced with inclusion of 0.75% Ashwagandha root powder. The results of present findings are in consonance with the results of Tahmasbi *et al.* (2012), who showed that supplementation of *Withania somnifera* in layers diet increased egg production than diet without supplementation. The results of the present study are also in agreement with the findings of Bhoyar *et al.* (2003), 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 and 10 g/kg feed for 10 weeks in 32 weeks old White leghorn birds. The results are in close agreement with Bhardwaj and Gangwar (2011) who found a positive effect of Ashwagandha root powder on egg mass production. The improvement in hen day egg production might be due to improved nutrient metabolizability and average feed intake.

The mean egg weights did not differ significantly among the groups (Table 2). Similarly, Ibrahim *et al.*

Table 2. Effect of dietary treatments on production performance of laying hens

Treatments	Hen day egg production percent	Egg mass production (g/day/hen)	Egg weight (g)
T_1	71.43^{a}±0.52	35.44^{a}±0.27	49.63±0.18
T_2	72.62^{ab}±0.66	36.02^{ab}±0.39	49.60±0.28
T_3	73.64^{bc}±0.83	36.16^{ab}±0.47	49.11±0.34
T_4	75.23^{c}±0.73	37.42^{c}±0.41	49.74±0.26
T_5	74.19^{bc}±0.69	37.05^{bc}±0.35	49.95±0.22

$^{a-c}$Mean values in same column with different superscripts differ significantly (P<0.05)

Table 3. Effect of dietary treatments on egg quality parameters of hens

Treatments	Albumen indexpercent	Yolk index percent	Haugh unit
T_1	9.64±0.16	46.39±0.32	88.43±0.62
T_2	9.70±0.16	46.73±0.31	89.01±0.54
T_3	9.86±0.16	46.69±0.30	90.41±0.48
T_4	9.85±0.15	47.05±0.35	90.34±0.39
T_5	9.84±0.16	46.93±0.39	90.06±0.34

(2016) who observed that supplementation of Ashwagandha did not influence egg weight in Japanese quails. On the contrary, Bhardwaj and Gangwar (2011) reported that egg weight was significantly (P<0.05) higher in diet containing 1 percent Ashwagandha than control group in laying quails. Tahmasbi *et al.* (2012) found that hens fed on a diet supplemented with the highest *Withania somnifera* had significantly (P<0.05) lower egg weight than those fed on the diet without *Withania somnifera* in layers during late phase of production. The differences in response could be due to significant improvement in egg mass and hen day egg production. Results of this experiment demonstrate that supplementation of Ashwagandha root powder as a replacement for AGP increase hen day egg production and total egg mass production without showing any adverse impact on egg weight.

Data pertaining to internal egg quality parameters *viz.* albumen index percent, yolk index percent and haugh unit are presented in Table 3. The results of the study depicted that there were no significant differences among different dietary treatments during the whole period with respect to these parameters. The present results are in agreement with Bhardwaj and Gangwar (2011) who reported that albumen index and yolk index of eggs had not been found to be affected significantly due to Ashwagandha root powder supplementation. Ibrahim *et al.* (2016) also found a non-significant effect on albumen height, yolk index and haugh unit of groups fed diets supplemented with different concentrations of *Withania somnifera.*

Contents of cholesterol, HDL and LDL (mg/g egg yolk) under different dietary treatments are presented in Table 4. Results of the study showed that egg yolk concentration of total cholesterol, LDL and HDL were significantly (P<0.05) affected by the supplementation of Ashwagandha root powder in the diet of laying hens. The values of total cholesterol and LDL were significantly (P<0.05) lower in T_5 (1.0% Ashwagandha root powder) group as compared to T_1 (control) and T_2 (0.25% Ashwagandha root powder) groups but T_3, T_4 and T_5 did not show any significant difference among themselves. The value of HDL was significantly (P<0.05) higher in T_5 group as compared to T_1 group but the groups T_2, T_3, T_4 and T_5 did not differ statistically among themselves. Thus, it is evident that dietary inclusion of Ashwagandha root powder can cause a reduction in egg yolk cholesterol and LDL content with an increase in the HDL. These finding are in agreement with Pandey *et al.* (2014) who reported the hypocholesterolemic activity of *Withania somnifera* in terms of lipid profile in eggs of birds. Supplementation

Table 4. Lipid profile of egg yolks of hens fed Ashwagandha root powder supplemented diets

Treatments	Cholesterol (mg/g egg yolk)	HDL(mg/g egg yolk)	LDL(mg/g egg yolk)
T_1	14.18^{b}±0.66	6.55^{a}±1.09	8.12^{c}±0.44
T_2	13.88^{b}±0.66	7.01^{ab}±0.47	7.81^{bc}±0.46
T_3	13.20^{ab}±0.66	7.88^{ab}±0.33	7.48^{bc}±0.43
T_4	12.93^{ab}±0.70	8.13^{ab}±0.20	6.64^{ab}±0.46
T_5	11.08^{a}±0.75	8.60^{b}±0.31	5.62^{a}±0.44

[a-c]Mean values in same column with different superscripts differ significantly (P<0.05)

Table 5. Effect of Ashwagandha root powder supplementation on selected serum parameters of laying hen

Treatments	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
T_1	165.51^{b}±3.88	975.00^{c}±46.68	31.33^{a}±1.85	90.58^{c}±2.04
T_2	163.25^{b}±1.89	948.00^{bc}±36.05	31.58^{a}±0.90	89.97^{c}±1.48
T_3	159.75^{b}±2.56	880.50^{abc}±40.40	36.60^{a}±1.45	87.33^{c}±1.78
T_4	144.25^{a}±2.96	833.50^{ab}±23.33	50.28^{b}±2.47	74.49^{b}±1.83
T_5	139.75^{a}±2.29	767.25^{a}±45.78	54.20^{b}±1.82	67.91^{a}±1.78

$^{a-c}$Mean values in same column with different superscripts differ significantly (P<0.05)

of *Withania somnifera* root powder (2% in diet) showed a significant reduction in total lipids, total cholesterol and yolk triglycerides. Mahajan *et al.* (2010) demonstrated that polyherbal preparation containing *Emblica officinalis*, *Ocimum sanctum, Withania somnifera* and *Tinospora cordifolia* showed a significant hypocholesterolemic effect and can be used effectively for reducing lipid contents in egg yolk. The cholesterol lowering effect of *W. Somnifera* could be due to elevated excretion of cholesterol and bile acids through fecal sterol excretion. It could also be attributed to higher phytosterol contents, which might lead to decrease in intestinal transit time for cholesterol and carbohydrate absorption from gut (Ebihara and Schneeeman, 1989). *Withania somnifera* root powder might have indirect inhibitory effects on 3 hydroxy-3-methyl-glutaryl-co-A reductase, a key enzyme in cholesterol biosynthesis (Ansari *et al.,* 2013).

Data pertaining to serum biochemical parameters of the experimental birds under are presented in Table 5. Data indicate serum concentrations of cholesterol, triglycerides and LDL decreased significantly (P<0.05) with increased level of Ashwagandha root powder in the diet. On the other hand, concentration of HDL increased significantly (P<0.05) from 31.33 mg/dl in control group to 54.20 mg/dl in T_5. Thus, it is evident that dietary inclusion of Ashwagandha root powder reduced serum total cholesterol, triglycerides and LDL and increased HDL concentration in a dose dependent manner. Our results corroborate well with those of Kanduri *et al.* (2013) who reported that, addition of hypocholesterolemic herbal agent at1.0, 1.5 and 2.0 kg per ton of feed in the basal diet was proved effective in reduction of serum total cholesterol (20-22 %) and egg yolk total cholesterol (18-22 %). Similarly, Kale *et al.* (2016) observed lowered serum concentration of triglyceride and cholesterol with a concomitant increase in concentration of HDL due to feeding of *Withania somnifera* root powder.

CONCLUSION

It can be concluded from this study that supplementation of 1 % Ashwagandha root as a replacement of AGP increased hen-day egg production and egg mass production. Addittionally supplementation of Ashwagandha root powder improved serum and egg yolk lipid profile in a dose dependent manner.

REFERENCES

Angelo, A.J. St., Vercellotti, J.R., Legendre, M.G., Vinnett, C.H., Kuan, J.W. and James, C. Jr. 1987. Chemical instrumental analysis of warmed over flavor in beef. *J. Food Sci.* 52: 1163-1168.

Ansari, J., Khan, S.H., Haq, A.U., Ahmad, T. and Abbass, M.I. 2013. Effect of Supplementation of *Withania somnifera* (Linn.) Dunal Roots on Growth Performance, Serum Biochemistry, Blood Haematology and Immunity of Broiler Chicks. *J. Herbs spices med. plants*. 19: 144-158.

Bhardwaj, R.K. and Gangwar, S.K. 2011. Effect of dietary supplementation of *Withania somnifera* on egg production and egg quality parameters in Japanese quails. *Int. J. Adv. Biol. Res.* 1: 32-34.

BIS 2007. *Requirement for Chicken Feeds*. IS: 1374-2007, Manak Bhawan, 9 Bahadurshah Zafar Marg, New Delhi-110001.

Bhoyar, A., Nimbulkar, M.V., Jagtab, D.G., Wankhade, D.K., Chaudhari, D.A. and Thakare M.M. 2003. Effect of supplementing Aswagandha (*Withania somnifera*) on performance of layers. *Indian Vet. Med. J.* 27: 153-155.

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics*, 11: 1-42.

Dwivedi, V., Singh, V.K., Tewari, D., Gautam, S., Singh, V.B. and Dwivedi, D. 2015. Growth performance, blood constituents and carcass traits of broiler chicken as affected by supplementation of Ashwagandha (*Withania somnifera*) and Mangrail (*Nigella sativa*). *Indian J. Anim. Nutri.* 32: 427-432.

Ebihara, K. and Schneeman, B.O. 1989. Interaction of bile acids, phospholipids, cholesterol and triglyceride with dietary fibers in the small intestine of rats. *J. Nutr.* 119: 1100-1106.

Haugh, R.R. 1937. The Haugh unit for measuring egg quality. United States Egg. *Poult. Magazine,* 43: 552-555.

Ibrahim, D., Ahmad, S., and Hussain, S. 2016. Effects of supplementation *Withania somnifera* L. roots on some egg production and quality traits of heat stressed japanese quails. *Int. Session Scient. Comm. Facult. Anim. Sci.* 59: 200-205.

Kale, V.R., Wankhede, S.M. and Karle, S.D. 2014. Effect of Dietary supplementation of Ashwagandha (*Withania somnifera*) on carcass quality of broiler chicken. *Indian. J. Anim. Nutr*. 31: 81-85.

Kale, V.R., Wankhede, S.M., Patil, C.S. and Share, A.A., 2016. Effect of supplementation of *Withania somnifera* (Ashwagandha) root powder as feed additive on performance and blood biochemicals of broilers. *Indian J. Anim. Res*. 50: 53-56.

Kanduri, A.B., Saxena, M.J., Ravikanth, K., Maini, S. and Dandale, M. 2013. Hypocholesterolemic effect of herbal supplement on serum and egg yolk cholesterol in layer poultry. *Intl. J. Biol. Pharm. Allied Sci.* 2: 1199-1202.

Kuang, H., Yang, F., Zhang, Y., Wang, T. and Chen, G. 2018. The impact of egg nutrient composition and its consumption on cholesterol homeostasis. *Cholesterol*. doi: 10.1155/2018/6303810.

Mahajan, C., Gehlaut, B.S., Quadri, M.A., Gupta, A. and Tiwari, R. 2010. Effect of polyherbal preparation on the lipid profile of egg yolk in various genotypes of poultry. *Livest. Res. Rural Dev.* 22(9).

Mukherjee, P.K., Banerjee, S., Biswas, S., Das, B., Kar, A. and Katiyar, C.K. 2020. *Withania somnifera* (L.) Dunal-Modern perspectives of an ancient Rasayana from Ayurveda. *J. Ethnopharmacol*. 264: 113157.

Pandey, S., Sahni, Y., Gautam, V. and Jain, S.K. 2014. Efficacy of indigenous herbs *Withania somnifera* and *Emblica officinalis* on egg yolk cholesterol in birds. *J. Vet. Pharmacol. Toxicol.* 13: 13-15.

Punetha, H., Sing, S. and Gaur, A.K. 2010. Antifungal and antibacterial activities of crude withanolides extract from the roots of *Withania somnifera* (L.) Dunal (Ashwagandha). *Environ. Conserv. J.* 11: 65-69.

Raju, S., Gurram, S., Bora, S. and Kumar, B.S. 2019. Effect of herbal immunomodulators on immune status, haematological and serum biochemical parameters of Japanese quails (*Coutrinix japanica*) during summer stress. *Indian J. Anim. Sci.* 89: 575-577.

Sharma, A., Deo, A.D., Riteshkumar, S.T., Chanu, T.I. and Das, A. 2010. Effect of *Withania somnifera* (L. Dunal) root as a feed additive on immunological parameters and disease resistance to Aeromonashydrophila in Labeorohita (hamilton) fingerlings. *Fish and Shell Fish Immunol.* 29: 508-512.

Singh, B., Chandan, B.K. and Gupta, D.K. 2010. Chemistry of garlic (*Allium sativum*) with special reference to allin and allicin- a review. In: Effect of dietary inclusion of plant extracts on the growth performance of male broiler. *Poult. Sci (Suppl).*S 43- S 44.

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical Methods*. 6th edition. The Iowa State University Press, Ames, Iowa, USA.

Tahmasbi, A.M., Mirakzehi, M.T., Hosseini, S.J., Agah, M.J. and Fard, M.K. 2012. The effects of phytase and root hydroalcoholic extract of *Withania somnifera* on productive performance and bone mineralisation of laying hens in the late phase of production. *Br. Poult. Sci.* 53: 204-214.

Received on 18-01-2021 and accepted on 19-04-2021