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Effect of Dietary Inclusion of Ashwagandha Root Powder on Production Performance, Hematological Parameters and Immune Response of White Leghorn Hens

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ABSTRACT

This study aimed to investigate the efficacy of supplementation of Ashwagandha root powder as feed additive on production performance, hematological parameters and immune characteristics in laying hens. A 16-week feeding trial was conducted with 120 White Leghorn layers of 22 weeks of age which were distributed randomly into five experimental groups, having 4 replicates with 6 birds in each. The laying hens of control group (T1) were fed the maize- soybean based basal diet with antibiotic, while in treatment groups T2, T3, T4 and T5 basal diet was supplemented with Ashwagandha root powder at levels of 0.25, 0.50, 0.75 and 1 percent of feed, respectively. The results of study revealed that the feed intake was significantly ($P<0.05$) improved by supplementation of 0.75 and 1.00 percent Ashwagandha root powder in feed of layers. FCR was not significantly affected by supplementation of Ashwagandha root powder in diets. Dry matter, nitrogen and gross energy metabolizability were significantly ($P<0.05$) higher in 0.75 and 1.00 percent Ashwagandha root powder supplemented group as compared to control group. Hematological parameters including RBC and WBC were found significantly ($P<0.05$) higher in T5, whereas significantly ($P<0.05$) lower heterophil and higher lymphocytes were found in T3, T4 and T5 groups resulting in lower H:L ratio in T3, T4 and T5 groups as compared to control. The relative mRNA expression of TLR2 was found to be enhanced ($P<0.05$) in T4 and T5 while, TLR4 showed significant downregulation as compared to control. Whereas, A non-significant difference was observed on expression of relative mRNA levels of TLR7 in the blood. Thus, it can be concluded that the inclusion of Ashwagandha root powder @1% in dietary ration could enhance the nutrient metabolizability, feed intake, hematological parameters and immune status of birds.

Key Words: Ashwagandha root powder, Hematology, Immune response, Layers, Nutrient metabolizability

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INTRODUCTION

For decades, antibiotic growth promoters (AGP) have been used in poultry production to maintain intestinal health, stimulate growth and prevent diseases (Dibner and Richards, 2005). However, as a result of long-term use of AGP, public concerns have increased regarding antibiotic drug residues and resistant bacteria, which pose a potential risk to animal and human health (Yang et al., 2019). Consequently, the use of AGP in chicken diets has been restricted in several countries, particularly in Europe (Castanon, 2007) which has increased pressure to develop safe and effective alternatives that can maintain intestinal health and performance in poultry. An ideal alternative should have the same beneficial effects as AGP, ensure optimum animal performance, and increase nutrient availability (Huyghebaert et al., 2011). Considering the proposed mechanism of action of AGPs (microbiome and immune-modulating activities), a practical alternative should possess both of these properties in addition to having a positive impact on feed conversion and/or growth (Huyghebaert et al., 2011; Seal et al., 2013).In the pig and poultry industry, phytogenic feed additives (PFAs) have been used as natural growth stimulants in recent years. (Franz et al., 2010). Phytogenic feed additives, also known as phytobiotics or botanicals, are natural bioactive substances obtained from plants that are added to animal feed to improve productivity (Windisch et al., 2008).

Ashwagandha (*Withania somnifera* Dunal, common name: Winter Cherry) is a medicinal plant belonging to the Solanacea family. It has been safely used in traditional medicine for the treatment of a variety of human diseases. Although studies have reported various biological and biochemical properties of Ashwagandha including antioxidative, anti-inflammatory and anti-stressor, very few studies have been performed on the immunomodulatory roles of Ashwagandha. It is endowed with several health benefits, for example, steroidal lactones (with anolides) which is extracted from its roots, have been reported to have a wide range of therapeutic activities. The biologically active constituents that have been recovered so far include withanolides, withanolide D, withaferin A, isopelletierine, anaferine and withasomnine. The immune-modulatory activity of Withania somnifera may be due to the presence of glycowithanolides (Mishra et al., 2000). Hence, the current study was designed to analyze the effect of Ashwagandha on feed intake, nutrient metabolizability, haematological and immunological profile of laying hens.

MATERIALS AND METHODS

Birds, experiment design, and management

A total of one hundred and twenty single comb White Leghorn hens of commercial strain (22 weeks of age), in the first phase of their production cycle with an average weight of 1109 ± 16.2 g were randomly distributed into five treatment groupsi.e.T1, T2, T3, T4 and T5, having four replications with six birds in each following completely randomized design.The laying hens of control group (T1) were fed corn-soybean- based basal diet (18.8% CP) in mash form supplemented with antibiotic, formulated as per BIS (Bureau of Indian Standards, 2007) specifications as shown in Table 1.

Table 1. Ingredient and chemical composition of ration for layers of control group

Feed ingredients	Quantity (kg/100kg)
Maize	56
Groundnut cake	11
Soybean meal	15
Wheat bran	5
Fish meal	6
Mineral mixture	3
Salt	0.5
Shell grit	3.5
Chemical composition	% DM basis
Crude protein	18.8
Crude fibre	3.86
Ether extract	3.84
Nitrogen free extract	62.5
Ash	10.9
Metabolizable energy (Kcal/Kg)	2699

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

The remaining four treatment groups T2, T3, T4 and T5 of experimental hens were given the same basal diet supplemented (without antibiotic) with Ashwagandha root powder at 0.25, 0.50, 0.75 and 1% of ration, respectively. Hens were fed the experimental diet for sixteen weeks of experimental period beginning at 22 weeks of age and continued up to 38 weeks of age.The laying hens were reared under deep litter system equipped with hanging automatic feeder and plastic waterer. Feed and water were offered *ad libitum* during the study. The laying hens were kept in optimal and standard bio-climatic and welfare conditions.Group wise feed consumption per bird was taken for 16weeks. Feed conversion ratio (FCR) was expressed as kilogram of feed consumed per dozen egg produced and per kg of egg mass produced. About 2 ml of blood was collected from each bird via jugular vein using sterilized syringes and needles into EDTA (vacutainer) tubes for hematology using auto analyser and for TLR mRNA expression and stored at -20°C until further analysis.

Reverse transcription (cDNA Synthesis); RNA extraction and preparation of cDNA

Total RNA was isolated from blood samples by using TRIZOL® as per the manufacturer's instruction. Total RNA extracted was dissolved in 20 ìl NFW and quantified using Qubit® 2.0 fluorometer (Invitrogen). Reverse transcription was carried out with total reaction volume of 20 ìl using Onescript® cDNA synthesis kit. The RT-PCR cyclic conditions were as: initial incubation at 25°C for 10 min, reverse transcription at 42°C for 50 min and deactivation at 85°C for 5 min in thermal cycler (Applied Biosystem). The cDNA was stored at "20°C till further use.

Real time PCR

For the analysis of temporal expression profile of different genes, real-time PCR was carried out using Step I plus real-time PCR system. For the real-time PCR reaction, SYBR green dye based universal PCR master mix (Luna) was used, and all the instructions were followed as per the manufacturer. The reaction for the target gene, TLRs (TLR 2, TLR 4 and TLR 7) and the endogenous control, â-actin gene was carried out in triplicate along with non template control as a negative control for each sample. The reaction mixture used to carry out the real-time PCR reaction for TLRs 2, 4 and 7; and â-actin gene contains 1X SYBR green PCR mastermix (Luna, 5ìl), forward primer (0.3µl), reverse primer (0.3µl), NFW (1.4µl) and template (3ìl). Amplification was done with initial denaturation for 60sec at 95°C, followed by 40 cycles of denaturation for 15 s at 95°C and extension for 30 s at 60°C, and a final melting curve analysis at 60ºC. The set of primers used for the real time PCR are as shown in Table 2.

Table 2. Oligonucleotide sequences of sense and antisense primers for real-time PCR products determined

Gene[1]	Primer	Primer sequence[2]	Accession No.	Product size
β-Actin	Sense	5′-GAGAAATTGTGCGTGACATCA-3′	L08165	152
	Antisense	5′-CCTGAACCTCTCATTGCCA-3′		
TLR 2	Sense	5′-CATTCACCATGAGGCAGGGATAG-3′	AB046533	157
	Antisense	5′-GGTGCAGATCAAGGACACTAGGA-3′		
TLR 4	Sense	5′-TTCAGAACGGACTCTTGAGTGG-3′	AY064697	131
	Antisense	5′-CAACCGAATAGTGGTGACGTTG-3′		
TLR 7	Sense	5′-TTGCTGCTGTTGTCTTGAGTGAG-3′	AJ627563	182
	Antisense	5′-AACAACAGTGCATTTGACGTCCT-3′		

[1]TLR 2 = Toll-like receptor 2; TLR 4 = Toll-like receptor 4; TLR 7 = Toll-like receptor 7.
[2]Primers for Toll-like receptors and â-actin were described by Sato *et al*. (2009) and Bai *et al*. (2008), respectively.

Relative quantification by comparative Ct method (DDCt Method)

The average Ct (Threshold cycle) value obtained for the TLRs 2, 4 and 7 (target) gene was normalized to b- actin (endogenous control). The data obtained was subjected to comparative Ct method(Livak and Schmittgen, 2001) for the analysis of the expression levels of targeted TLR gene and an endogenous control. The sample at 26 hour of incubation was selected as calibrator.

Sequencing of product

Amplicons were sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) on an automatic ABI 3130 xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). The sequence obtained shows 100% nucleotide identity with the TLR sequence of chicken available in the global database.

Ethical committee approval

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee (IAEC), 12/CPCSEA in the Department of Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar.

Statistical analysis

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).

Feed intake, feed conversion ratio and Egg mass production

The results of study revealed that the feed intake was significantly (P<0.05) improved by supplementation of 0.75% and 1% Ashwagandha root powder in feed of layers (Table 3).

Table 3. Effect of different dietary treatments on feed consumption and feed conversion ratio

Treatments	Feed consumption(g/hen/day)	FCR		Egg mass production (g/day/hen)
		Feed intake (kg) per dozen egg production	Feed intake (kg) per kg egg mass production	
T1	115.2^{a}±1.31	1.94±0.02	3.25±0.03	35.4^{a} ±0.27
T2	116.2^{ab}±1.03	1.92±0.02	3.23±0.04	36.0^{ab} ±0.39
T3	118.5^{bc}±1.20	1.94±0.02	3.29±0.04	36.1^{ab} ±0.47
T4	119.6^{c}±0.98	1.91±0.01	3.20±0.02	37.4^{c} ±0.41
T5	119.4^{c}±0.94	1.94±0.01	3.23±0.03	37.0^{bc} ±0.35

The mean values in same column with different superscripts differ significantly (P< 0.05)

It was also noticed that as the inclusion level of Ashwagandha root powder increased in the diet of layers, the feed intake was significantly (P<0.05) improved as compared to control group. Several researchers reported that there was (P<0.05) significant increase in feed intake in different Ashwagandha fed groups. Vasanthakumar et al.(2015) reported that the feed intake (g) was significantly (P<0.05) more in 1% Ashwagandha root powder (T2) and 0.15% root extract (T3) supplemented groups as compared to the control group (T1). Jyotsna et al.(2018) also observed that feed intake was significantly (P<0.05) high in broilers birds supplemented with 1% ashwagandha root powder in diet. However, Bhardwaj and Gangwar (2011) found that cumulative feed intake (g/d) for 8- 23 weeks feeding period was significantly (P<0.05) higher in control and lower in the entire Ashwagandha treated groups in Japanese quail hens. The improvement in feed intake might be related to main active constituent withanine and withanolide of *Withania somnifera* root powder that could act not only as antibacterials and antioxidants but as a stimulant of digestive enzymes in the intestinal mucosa and pancreas that improved the digestion of dietary nutrients (Nobakht, 2011).

As per results of study feed intake per dozen egg production and per kg egg mass production were not significantly affected by the supplementation of different levels of Ashwagandha root powder in diet of layers. These findings indicate that the dietary supplementation of Ashwagandha root powder at different levels had no effect on the feed intake per dozen egg production and per kg egg mass production. These results are similar to the findings of Tahmasbi et al.(2012) who reported that feed conversion ratio was not significantly different among birds fed on different concentrations of *Withania somnifera* in diet of quails. Similarly, non-significant difference in feed conversion ratio was recorded by Shisodiya et al. (2008) and Thange et al. (2009). Findings of these experiments were contrary to the observation of Ibrahim et al. (2016) who showed that, Ashwagandha root powder 1g/kg diet resulted in a significant ($P<0.05$) improvement in feed conversion ratio in Japanese quails. Bhardwaj and Gangwar (2011) also observed that FCR (feed intake/egg mass) and net FCR were significantly ($P<0.05$) better in Ashwagandha supplemented groups than control group in laying quail hens.

The research's findings had demonstrated that egg mass production was significantly ($P<0.05$) increased in laying hens fed diets supplemented with different levels (0.75 and 1%) of Ashwagandha root powder as compared to laying hens fed control diet (Table 3). Egg mass production values in treatment groups T4 and T5 were significantly ($P<0.05$) higher from T1, T2 and T3 treatment groups but did not show significant difference among themselves. The results are in close agreement with Bhardwaj and Gangwar (2011) who found a positive effect of Ashwagandha root powder on egg mass production. Similarly, Ibrahim et al. (2016) showed that supplemented hens with 1g/kg diet *Withania somnifera* root powder gave higher values ($P<0.05$) of egg mass compared to other treatments.The increased egg mass production may be the result of better nutrient metabolizability and average feed consumption.

Metabolizability of nutrients

The results indicated that dietary supplementation at the rate of 0.75% and 1.0% Ashwagandha root powder resulted in significantly ($P<0.05$) higher dry matter (DM) metabolizability than all other dietary treatments (Table 4).

Table 4. Metabolizability dry matter, nitrogen and gross energy in birds under different dietary treatments

Treatments	Dry Matter Metabolizability (%)	Nitrogen Retention (%)	Gross Energy Metabolizability (%)
T1	$68.4^{a}\pm0.34$	$65.6^{a}\pm0.16$	$66.5^{a}\pm0.27$
T2	$68.6^{a}\pm0.31$	$65.7^{a}\pm0.14$	$66.3^{a}\pm0.37$
T3	$69.4^{ab}\pm0.28$	$67.0^{b}\pm0.18$	$67.9^{b}\pm0.35$
T4	$70.5^{c}\pm0.41$	$68.9^{c}\pm0.30$	$69.5^{c}\pm0.31$
T5	$70.3^{c}\pm0.40$	$68.4^{c}\pm0.30$	$68.9^{c}\pm0.31$

The mean values in same column with different superscripts differ significantly ($P< 0.05$)

Nitrogen metabolizability was significantly ($P<0.05$) higher in T4 (0.75% Ashwagandha root powder) and T5 (1.0% Ashwagandha root powder) groups as compared to T1 (control). The group supplemented with 0.75% Ashwagandha root powder (T4) had significantly ($P<0.05$) higher gross energy metabolizability followed by 1.0% (T5) and 0.50% (T3) Ashwagandha supplemented groups as compared to control, 0.25% (T2) Ashwagandha supplemented groups. It was noticed that as the inclusion level of Ashwagandha root powder increased in the diet of layers, the nutrient and energy metabolizability was significantly ($P<0.05$) improved as compared to control group. The results of study are similar with the findings of Jyotsana (2018) who concluded that dietary inclusion of Ashwagandha root powder as herbal feed supplement @ 0.75% and 1.0% Ashwagandha root powder led to significant increase in the dry matter metabolizability, nitrogen metabolizability and gross energy metabolizability in broiler chickens.Saini et al. (2017)also found that dry matter digestibility of chicken dietincreased with increase in level of Ashwagandhaup to 1.0% and thereafter decreased. Further, he also suggested that inclusion of Ashwagandha as feed additive at the rate of 1.0% in chicken ration, exerted maximum nitrogen retention and thereafter at 0.5% level of inclusion. Similarly, Raghavan et al. (2011) also reported significant effect on nitrogen balance due to supplementation of herbs.

Hematological parameters

The mean values of Hb (g/dl) were significantly (P<0.05) higher in T3, T4 and T5 as compared to control group (Table 5).

Table 5. Hematological parameters of birds under different dietary treatments

Treatments	Hemoglobin (g/dl)	RBC×10^6/µl	WBC×10^3/µl	Heterophil (%)	Lymphocyte (%)	H: L
T1	10.4^a±0.09	2.59^a±0.21	29.4^a±0.03	29.6^c±0.21	60.4^a±0.40	0.49±0.00
T2	10.6^a±0.03	2.81^a±0.24	29.3^a±0.01	29.3^{bc}±0.22	60.5^a±0.16	0.48±0.00
T3	10.7^b±0.03	3.07^{ab}±0.18	29.3^a±0.02	28.8^b±0.17	61.4^{ab}±0.44	0.47±0.00
T4	11.1^c±0.03	3.21^{ab}±0.19	30.5^b±0.02	28.7^b±0.22	61.4^{ab}±0.42	0.47±0.00
T5	11.2^c±0.03	3.53^b±0.17	30.5^c±0.02	28.0^a±0.20	62.0^b±0.28	0.46±0.00

The mean values in same column with different superscripts differ significantly (P< 0.05)

Mean values of RBC was found significantly (P<0.05) higher in 1.0% (T5) Ashwagandha supplemented group and mean values of WBC was found significantly (P<0.05) higher in T4 (0.75%) and T5 (1.0%) Ashwagandha supplemented group as compared to other treatments and control group. Lowest heterophil count was observed in T5 group followed by T3 and T4 group whereas, highest lymphocyte % was observed in T5. Our results are in consonance with findings of Shefali et al. (2008), who observed increased hemoglobin and hematocrit, in birds fed *Withania somnifera* aqueous roots (WSR). Micro-mineral composition of WSR in their study showed that WSR was a rich source of iron, which might have increased the level of hemoglobin in broilers. Singh et al. (2016) observed that the groups treated with both Ashwagandha and selenium showed significantly higher values of hematological parameters when compared to that of other groups. Mushtaq et al.(2012) observed that the TLC values were significantly higher in the group receiving 20g plant extract water compared with control. Increase in the TLC values may be due to stimulating effect of *Withania somnifera* on the bone marrow cells as has been reported by Davis and Kuttan (2000) in the mice injected (intraperitoneally) with powdered roots of *Withania somnifera* at 20 mg/dose/animal for 10 days. The increase in the presence of á-esterase positive cells in the bone marrow showing the enhancement of stem cells differentiation due to *Withania somnifera* was another indicator of its hematopoietic stimulatory effects (Davis and Kuttan, 2000).

Gene expression of Toll Like receptors (TLRs)

The nutrigenomic expression analysis as presented in Table 6, revealed that relative mRNA expression of TLR 2 of layers was found to be significantly (P<0.05) enhanced in the treatment groups T4 and T5 supplemented with 0.75% and 1.0% of the Ashwagandha root powder, respectively.

Table 6. Relative quantification expression analysis of the toll like receptors (TLR 2, TLR 4 and TLR 7) with the reference to the endogenous reference gene β actin

Sample Name	Target Name	C_T Mean	C_T SE	ΔC_T Mean	$\Delta\Delta C_T$	RQ
T1	TLR 2	23.4	0.121	4.83	0	1.00
T2		23.2	0.067	4.41	-0.42	1.34
T3		23.1	0.026	4.46	-0.37	1.29
T4		21.6	0.026	3.36	-1.47	2.77
T5		22.1	0.088	3.31	-1.52	2.87
T1	TLR 4	22.6	0.055	4.02	0	1.00
T2		23.7	0.090	4.88	0.86	0.55
T3		23.5	0.050	4.87	0.85	0.55
T4		23.1	0.014	4.94	0.92	0.53
T5		24.2	0.020	5.41	1.39	0.38
T1	TLR 7	28.5	0.088	9.85	0	1.00
T2		28.8	0.064	10.03	0.18	0.88
T3		27.7	0.111	9.12	-0.73	1.66
T4		27.5	0.088	9.26	-0.59	1.51
T5		28.8	0.021	10.01	0.16	0.90
T1	β actin	18.6	0.07	-	-	-
T2		18.8	0.10	-	-	-
T3		18.6	0.06	-	-	-
T4		18.2	0.06	-	-	-
T5		18.8	0.03	-	-	-

While, at the end of the 38 weeks of experimental period layers had significant down regulation pattern of relative mRNA expression of TLR 4 in the plasma of layers fed diet supplemented with 0.25, 0.50, 0.75 and 1.0% of Ashwagandha root powder in the treatment groups T2, T3, T4 and T5, respectively as compared to control (T1) group. However, the data pertaining to the relative mRNA levels of TLR 7 in the plasma of layers revealed non-significant differences in the experimental groups T2, T3, T4 and T5 as compared to the control group (T1). The experimental treatments containing Ashwagandha root powder in the layers diet had potent immune modulating activity by showing stimulatory effect on relative mRNA expression of TLR 2 and down regulation pattern of TLR 4 of the layers. Our study for TLR 2, 4 and 7 are in agreement with finding of Jyotsana (2019), who concluded that that the supplementation of Ashwagandha root powder at higher levels 0.75% and 1% results in up regulation of TLR 2, down regulation of TLR 4 and non-significant difference in TLR 7 . The results of study for TLR2 are also consonance with finding of Sheoran et al. (2017), reported that the addition of garlic powder and holy basil leaf powder at higher level of 1% of the feed either alone or in combination in the diet of the broilers increased the relative mRNA expression of TLR 2, 4 and 7. Our study in case of expression of TLR4 and TLR 7 is in contrary to Sheoran et al. (2017). Medicinal herbs had shown to possess multiple immune modulatory actions like phagocytosis, modulation of immunoglobulin and cytokine secretion, cellular co-receptor expression, class switching, lymphocyte expression, and histamine release (Mahima et al., 2012). In current work, it was observed that dietary inclusion of Ashwagandha root powder significantly ($P<0.05$) modulated the relative mRNA expression of TLR cell markers; this confirmed that these herbal feed additives could stimulate the T cell immune system in the plasma of layers birds. In the present investigation, we found that there was a significant increase in the relative mRNA expression of TLR 2 in the plasma of the layers fed diet supplemented with 0.75% and 1% Ashwagandha root powder. TLR 2 recognizes a variety of microbial components. These include lipoproteins/lipopeptides from various pathogens, peptidoglycan and lipoteichoic acid from Gram-positive bacteria (Takeda and Akira, 2005). TLR 4 is the principal receptor for lipopolysaccharide. This is a major component of outer membrane of gram-negative bacteria (Kannaki et al., 2010). Several studies have shown that the essential oils and biologically active compounds in herbs are effective against bacteria such as *E. coli, Shigella* spp. *Salmonella typhi,* and *Pseudomonas aeruginosa* (Prakash and Gupta, 2005). The antimicrobial actions of essential oils in herbs are due to phenolic compounds present in them. They exert membrane damaging effects to microbial strains and stimulate leakage of cellular potassium this is responsible for a lethal action related to cytoplasmic membrane damage (Mahamood et al., 2008). They show its immunomodulatory effect by increase in interferon-ã, interleukin-4, T-helper cells, NK cells (Mondal et al., 2011), thus reducing total bacterial count, increasing neutrophil and lymphocyte count and enhancing phagocytic activity and phagocytic index. Another study revealed that the ethanol and methanol extracts of *O. sanctum* had the ability to inhibit the growth of all test bacteria including *E. coli and P. aeruginosa* (Pathmanathan et al., 2010). Herbs can influence selectively the microorganism by an antimicrobial activity thus favors better nutrient utilization and absorption or the stimulation of the immune system (Wenk, 2003). From the above reported studies and our result findings, it can be inferred that, supplementation of diet with 0.75% Ashwagandha improved performance, suppressed the growth of harmful organisms like *Coliforms,* thereby creating a conducive environment for the growth of the beneficial microbes like *Lactobacillus, Bifidobacteria* spp. and thereby, aided in digestion and gave better performance. From the result findings related to the relative mRNA gene expression of TLR 2 in the present study, it could be inferred that Ashwagandha given at 0.75% and 1% of feed showed better results as compared to control group. TLR 7 family is implicated in intracellular recognition of nucleic acids. The TLR 7 recognizes some antiviral compounds and single-stranded viral RNA. In this study, supplementation of diet with Ashwagandha root powder did not significantly increase the relative mRNA expression of TLR 7 in the plasma of the layer birds. Based on above study it can be concluded that the supplementation of Ashwagandha root powder at higher levels 0.75% and 1% results in up regulation of TLR 2 and down regulation of TLR 4. This might be due to enhanced growth of beneficial gram-positive bacteria and decreased gram negative bacteria in birds.

CONCLUSION

From the present investigation, we can conclude that dietary supplementation of Ashwagandha root powder enhanced performance and immune status of layers.

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