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Influence of ashwagandha and citrus bioflavonoids supplementation on haematological parameters in broiler chicken

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Abstract
An experiment was carried out to assess the effect of Ashwagandha and Citrus bioflavonoids as feed additives on haematological profile in broiler. A total of Eighty (80)-day old broiler chicks (Vencobb-400) were reared commonly and given similar managemental inputs during acclimatization period of two weeks. On 15th day birds were divided randomly and equally into four groups. Control (Group-I) basal diet. For the supplement groups basal diet was supplemented with ashwagandha extract @ 1000 mg/kg diet (Group-II), citrus bioflavonoids @ 1000 mg/kg of diet (Group-III) and ashwagandha extract @ 500 mg/kg + citrus bioflavonoids @ 500 mg/kg of diet as combination group (Group-IV) was fed from 15th day onwards up to 35 days of age. Blood samples were collected at weekly intervals till the end of experiment. Hematological parameters were measured manually as per standard protocol. Although numerically higher TEC, PCV were noted but the differences were found to be non-significant. Significantly (p<0.05) higher Hb concentration were noted in ashwagandha supplemented groups (Group II and IV). Significant (p<0.05) improvements in TLC levels were noted in Group III and IV during last week of study. Significantly (p<0.05) lower heterophil (%) and lower H:L ratios were noted in group II, III and IV. The results suggested that the supplementation of ashwagandha and citrus bioflavonoids supplementation alone and in combination in the diet of broilers has positive influences on all haematological parameters and rather has shown noticeable improvement in Hb content and lymphoproliferative property in broilers.

Keywords: Broilers, ashwagandha, citrus bioflavonoids, haematology

1. Introduction
India's poultry industry represented a major success story. While agricultural production has been rising at the rate around 2 percent per annum over the past two to three decades, poultry production has been rising at the rate of around 8 percent per annum.
The forces that are sustaining this growth are many. High per capita income growth and relatively low prices have played a catalytic role. A moderate shift in the consumption pattern from vegetarianism to non-vegetarianism is also helping the industry by increasing the demand for poultry products.
The growing concerns of consumers on the use of antibiotic as a growth promoter in livestock feed have fuelled the interest in alternative products. Use of herbal plants as feed ingredient for broilers is more acceptable due to its growth promoting quality and reducing the antimicrobial resistance among broilers and is preferred by health-conscious consumers (Singh *et al.*, 2016) [35]. The researchers are looking for natural alternatives to feed additives due to the emergence of drug resistance, residual toxicity and other side effects of synthetic growth promoters and health maintaining drugs like antibiotics.
Withania somnifera also known as Ashwagandha (Smell of Horse) or Indian ginseng, is a medicinal plant belonging to the family Solanaceae and used as feed additive to enhance the growth rate (Srivastava *et al.*, 2012 and Singh *et al.*, 2008) [38, 33]. The constituents of this plant are alkaloids and steroidal lactone, but the withanine, the main alkaloid found in its roots and leaves is thought to be responsible for its biological activity.
In recent years, natural antioxidants are receiving attention due to their assured safety and cheap availability. These are primarily phenolic and polyphenolic compounds, which generally are found in all plants with frequent abundance in medicinal plants and herbs. Among plant antioxidants, bioflavonoids are receiving noticeable attention due to their multifunctional biological activities (Middleton *et al.*, 2000) [23].

In this study we have focused on the application and protective role of two important antioxidant dietary agents, namely, Ashwagandha and Citrus Bioflavonoids are being used. The information on effect of different dietary antioxidants is available mostly related to growth performance. However, scientific information available on supplementation of and effect on haematological parameters in broiler chickens is limited. This necessitated to conduct the present study in broiler chickens by supplementing ashwagandha extract and citrus bioflavonoids with the objective to study the effect of Ashwagandha and Citrus Bioflavonoids supplementation on hematological profile in broilers.

2. Material and Methods

2.1. Experimental design

The present study was carried out in the Department of Veterinary Physiology and biochemistry and Department of Livestock Production and Management, KVAFSU, Veterinary College Bidar, for a period of 35 days (5 weeks). Day old commercial broiler chicks (80 Nos) weighing around 45-55 g were procured from commercial hatcheries and reared in common for 14 days for acclimatization period on broiler diet (as per NRC 1994). After 14 days of rearing, the broilers were divided randomly into four groups with each group comprising of 20 birds. They were allotted to different dietary supplements as mentioned below.

They were allotted to different dietary supplements as mentioned below

Groups	Description of Diet
Group-I	Basal diet
Group-II	Basal diet + Ashwagandha @ 1000 mg/kg diet
Group-III	Basal diet + Citrus bioflavonoids @ 1000 mg/kg diet
Group-IV	Basal diet + Ashwagandha @ 500 mg/kg+ Citrus bioflavonoids @ 500 mg/kg diet

All the broiler chicks were immunized against Marek's disease in hatchery; chicks were also vaccinated against Ranikhet disease (Newcastle disease) and Gumboro disease (infectious Bursal disease) on 7th and 20th day of age, respectively. During first five days of brooding period, Terramycin – WS powder was added in drinking water @ 2.5 g/ 4.5 litres as preventive medication against coliform bacterial infections. "Vimeral" (Vit-A, D3, E, B12) was also added in drinking water @ 4 ml/ 4.5 litre during first five days. All the broiler chicks during acclimatization period for first 14 days were kept for brooding by providing light source of 4×100-watt incandescent bulb for 24 hrs. Climatic parameters such as Temperature and Absolute humidity were recorded twice a day (morning at 10 AM and afternoon at 3 PM) by using Dry and Wet bulb thermometer and Hygrometer which were fixed in pen. The weekly average climatic factors were as given below.

The weekly average climatic factors were as given below.

Parameter	1 week	2 weeks	3 weeks	4 Weeks	5 Weeks
Dry Bulb Temperature	27.84 ºC	26.78 ºC	27.78 ºC	26.64 ºC	26.44 ºC
Wet Bulb Temperature	26.35 ºC	25.90 ºC	26.78 ºC	25.78 ºC	25.63 ºC
Absolute Humidity	58.50%	55.62%	46.28%	54.78%	53.82%

One milliliter of blood sample was collected in EDTA coating tubes from wing vein in ten (10) birds in each group at weekly intervals (from 2nd to 5th week) during the experimental period for estimation of hematological parameters.

Animal ethics and welfare

The study was conducted as per the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration number 164/GO/ReBi/S/99/CPCSEA25/11/1999 and the research was approved by Institutional Animal Ethical Committee vide approval number 06/2021/VCBVPY dated 29-06-2021 of Veterinary College Bidar, KVAFSU Bidar.

2.2. Estimation of Hematological parameters

Haemoglobin concentration was measured by Sahli's Haemoglobin meter. PCV was measured by a standard manual technique using micro haematocrit capillary tubes. TEC and TLC counts were determined manually by using Natt and Herrick solution at 1:200 dilution on Neubauer's haemocytometer. DLC counts were made on monolayer blood films, fixed and stained with Giemsa-Wright's stain. MCV, MCH and MCHC were calculated using standard formulas.

2.3. Statistical Analysis

The data obtained were analysed statistically by two-way ANOVA with the application of Bonferroni post-test using 'GraphPad Prism' version 5.01 (2007) computerized software. The values were expressed as Mean ± Standard Error and the level of significance or non-significance was determined at P value of 0.05.

3. Results and Discussion

3.1. Total Erythrocyte count

In the present study, Ashwagandha supplemented groups (Group-II and IV) had numerically higher TEC count however, the differences were found to be statistically non-significant compared to control (Group-I) at 4th and 5th week of age. (Table 1). These findings are in accordance with work performed by Abdallah *et al.* (2016) [1], Singh *et al.* (2016) [35], Pedhavi *et al.* (2017) [28], Tikore *et al.* (2018) [40] and Ganguly *et al.* (2020) [14] who reported similar findings with significantly (p<0.05) higher TEC counts in ashwagandha supplemented groups. The significantly higher TEC count in ashwagandha supplemented groups might be due to haematopoietic effect of ashwagandha which might leads to improvement in haematological parameters including TEC and haemoglobin in these groups. (Gupta *et al.* 2008) [16].

In the present study, the Citrus bioflavonoids supplemented groups (Group-III and IV) had numerically higher TEC when compared to control (Group-I) at 4th and 5th week of study period (Table 1). These observations are in accordance with the studies of Al-Harthi and Attia (2015)[4], Seidavi *et al.* (2015) [31] in broiler chicken. Also, similar influences on TEC were reported by Elwan *et al.* (2019) [12], Alagbe *et al.* (2020) [3] in rabbits and Stephen *et al.* (2019) [39] in rats. Mahmoud (2013) [21] opined that citrus juice is rich in antioxidant principle which might have protected erythrocytes from damage induced by free radicals and improved the diabetic-induced anaemia in streptozotocin induced diabetic rats. Also, Mahmoud and Hussein, (2016) [20] reported that the presence of active ingredients in citrus bioflavonoids *viz.*, naringin and its aglycone have an established antioxidant effect which might play a role in this.

3.2. PCV/ Haematocrit

PCV levels were numerically higher (non-significant) in ashwagandha supplemented groups (Group-II and IV) compared to Group-I (control) at 4th and 5th weeks of age (Table 1). These findings are in accordance with work performed by Mushtaq *et al.* (2012) [26], Singh *et al.* (2016) [35] in broiler chicken. In addition, work done by Priyanka *et al.* (2020) [29] in equines reported similar findings with significantly higher PCV in ashwagandha supplemented groups. The improvement in PCV levels observed in these groups might be due to the hematinic property of ashwagandha (Mushatq *et al.,* 2012, Bhardwaj *et al.,* 2012 and Ansari *et al.,* 2013) [26, 7, 5].

Similarly in citrus bioflavonoids supplemented groups (Group-III and IV) had numerically higher PCV levels compared to those in control (Group-I) at 4th and 5th week of study period (Table 1). These findings are in accordance with the studies of Seidavi *et al.* (2015) [31], Solanki *et al.* (2020) [37] in broiler chicken, Elwan *et al.* (2019) [12], Alagbe *et al.* (2020) [3] in rabbits and Stephen *et al.* (2019) [39] in diabetic rats who reported similar influences on PCV with supplementation of citrus fruits/extracts in diets. However, PCV levels in their reports were significantly higher. The reason for higher PCV levels in citric acid supplemented group might be due to the fact that broilers being fast growing birds with chances of more physiological as well as oxidative stress, leading to a sort of hypoxic state, and upon supplementation of a source of citric acid, its metabolism is catalyzed by cytosolic isoforms of kreb's cycle enzymes that are present in active and mature erythrocytes, which increases the glycolytic fluxes in erythrocytes exposed to hypoxia which might in turn leads to hypoxic storage of packed red blood cells and increase in PCV percentage in these groups.

3.3. Haemoglobin

Hemoglobin concentration were significantly higher in ashwagandha supplemented groups, Group-II and IV compared to Group-I (Control) during 4th and 5th week of age (Table 1). These results are in agreement with the studies of Choudhari *et al.* (2006) [8], Dhenge *et al.* (2009) [10], and Mushtaq *et al.* (2012) [26] in broiler chicken who also reported similar findings. The significantly higher haemoglobin concentration in the present study might be due to the higher micro-mineral composition of Ashwagandha, being a rich source of iron, which might be responsible for higher haemoglobin in these groups (Gupta *et al.,* 2003) [17]. Also, as concluded by Kumar *et al.* (2006) [19] ashwagandha had haemo-protective effect and this might lead to higher TEC and heamoglobin concentrations. Whereas, Raghavan *et al.* (2011) [30] who noticed non-significant, but numerically higher hemoglobin concentration in broiler chicken supplemented with ashwagandha.

Hemoglobin concentration were significantly higher in the citrus bioflavonoids supplement groups (Group-III and IV) compared to Control (Group-I) during last two weeks of growth period (Table 1). These findings are in concurrence with the reports of Seidavi *et al.* (2015) [31] who observed similar findings in broiler chicken supplemented with sweet orange peel extract. The enhancement in most haematological parameters might be due to the presence of many active compounds such as vitamin C, flavonoids, iron, and pyridoxine (Azra *et al.,* 2014) [6]. These active bioflavonoids present in citrus bioflavonoids might improve hematogical parameters including haemoglobin. However, Gultepe *et al.* (2019) [15] and Fikry *et al.* (2021) [13] who supplemented lemon juice in laying hens and citric acid in Japanese quail respectively reported no significant effect on haemoglobin concentration. Since the lemon juice and citric acid may not supplement bioflavonoids present in lemon peel and that might be the reason for non-significant improvements in haemoglobin concentrations in their studies.

Table 1: Erythrogram in different groups of Broilers (N=10)

Parameter	Week	Group-I	Group-II	Group-III	Group-IV
Total Erythrocyte Count ($\times 10^6$/µL)	2 Weeks	2.08±0.17	2.15±0.14	2.33±0.22	2.30±0.14
	3 Weeks	2.44±0.12	2.55±0.09	2.67±0.12	2.61±0.06
	4 Weeks	2.86±0.08	3.01±0.06	3.18±0.10	3.05±0.10
	5 Weeks	3.09±0.07	3.21±0.07	3.41±0.09	3.31±0.09
Packed Cell Volume (%)	2 Weeks	21.68±1.51	21.89±1.15	21.86±1.25	21.99±1.96
	3 Weeks	25.20±0.48	27.44±0.55	27.92±0.57	29.02±0.40
	4 Weeks	28.50±1.30	30.12±0.80	30.63±0.56	30.69±0.61
	5 Weeks	30.28±1.39	32.30±0.81	32.92±1.10	32.53±0.89
Haemoglobin concentration (g/dL)	2 Weeks	7.40±0.25	7.64±0.56	7.30±0.33	7.49±0.49
	3 Weeks	8.99±0.28	9.50±0.31	9.44±0.31	9.74±0.30
	4 Weeks	9.82±0.33 ^{a}	11.42±0.22 ^{b}	10.94±0.30 ^{ab}	11.32±0.33 ^{b}
	5 Weeks	10.49±0.36 ^{a}	11.78±0.31 ^{b}	11.25±0.33 ^{ab}	11.77±0.26 ^{b}
Mean Corpuscular Volume (fL)	2 Weeks	108.08±8.03	105.11±7.69	103.67±12.40	96.26±7.13
	3 Weeks	105.71±5.60	108.47±3.73	105.61±3.24	111.37±2.36
	4 Weeks	99.96±4.24	100.48±2.82	97.41±3.77	101.79±4.15
	5 Weeks	97.91±3.87	101.09±2.91	97.28±4.20	98.88±3.40
Mean Corpuscular Haemoglobin (Pg)	2 Weeks	37.62±3.00	37.78±4.64	34.28±3.68	33.01±2.10
	3 Weeks	37.65±2.11	37.61±1.75	35.92±2.10	37.36±1.18
	4 Weeks	34.38±0.80	38.17±1.16	34.58±0.96	37.60±1.95
	5 Weeks	33.98±1.13	36.86±1.12	33.21±1.25	35.87±1.41
Mean Corpuscular Haemoglobin Concentration (g/dL)	2 Weeks	37.62±3.00	37.78±4.64	34.28±3.68	33.01±2.10
	3 Weeks	37.65±2.11	37.61±1.75	35.92±2.10	37.36±1.18
	4 Weeks	34.38±0.80	38.17±1.16	34.58±0.96	37.60±1.95
	5 Weeks	33.98±1.13	36.86±1.12	33.21±1.25	35.87±1.41

The values with different superscripts (a, b) within a row for a particular parameter differ significantly (p<0.05).

3.4. Erythrocyte indices

Ashwagandha supplementation in Group-II and IV had showed no significant effect on Erythrocyte indices *viz.*, MCV, MCHC and MCH compared to Group-I and the levels were within normal range during different stages of growth period (Table 1). These observations are in agreement with studies of Dwivedi *et al.* (2015) [11], Abdallah *et al.* (2016) [1] and Ganguly *et al.* (2020) [14] who were noticed similar findings in broiler chicken.

Group-III and IV had non-significant effect on Erythrocyte indices *viz.*, MCV, MCHC and MCH compared to those in Group-I during different stages of growth period. These findings are in accordance with Fikry *et al.* (2021) [13] who observed similar influences on Erythrocyte indices in citric acid supplemented group in Japanese quail.

3.5. Total leukocyte count (TLC).

Ashwagandha supplemented groups, Group-II and IV, had numerically higher TLC count compared to those in Group-I at 4th and 5th week of age. Although the differences were found to be non-significant (Table 2). These observations are in agreement with the work done by Raghavan *et al.* (2011) [30] and Ansari *et al.* (2012) [5] who were reported similar findings however in their studies the increments in TLC were significantly higher in Ashwagandha extract supplement groups of broiler chicken. Also, similar influences on TLC were reported by Priyanka *et al.* (2020) [29] in equines. The study of Davis and Kuttan, (2000) [9] concluded that ashwagandha has stimulating effect on bone marrow and this led to improvements in TLC in these groups which could have been occurred in the presence of α-esterase positive cells in the bone marrow and showing the amplification of stem cell differentiation, because of this effect it has been concluded that ashwagandha had hematopoietic stimulatory effect.

Citrus bioflavonoid supplemented groups (Group-III and IV) had numerically higher TLC values when compared those in Group-I at 4th and 5th week of age (Table 2). These findings are in accordance with the studies of Alagbe *et al.* (2020) [3] who reported usage of sweet orange peel extract in the diet of Rabbit showed similar influences however, TLC count was significantly higher but it was within normal range. Higher WBC value is an indication for increase in antibody level and high resistance to diseases (Soetan *et al.*, 2013) [36].

3.6. Differential leukocyte count (DLC)

3.6.1. Heterophil

Heterophil percentage were significantly lower in ashwagandha supplemented groups (Group-II and IV) compared to heterophil percentage in control (Group-I) during 4th and 5th week of age (Table 2). These findings are in concurrence with study of Singh *et al.* (2009) [34] who reported similar findings in broilers. Higher heterophil percentage indicates an inflammatory condition and stress in broiler chicken and which might lead to reduced lymphocyte count and in turn increased H:L ratio and it may be increased by factors inducing any form of stress in broiler. However, because of presence of active antioxidant and anti-inflammatory principles in ashwagandha extract, it may reduce the heterophil percentage as well as H:L ratio and it may be an indication of ameliorated stress in broiler chicken. Besides, these findings are also in agreement with studies of Ganguly *et al.* (2020) [14] who also reported significantly lower heterophil counts in ashwagandha supplemented groups compared to control group.

Citrus bioflavonoids supplemented group (Group-III and IV) had numerically lower heterophil percentage however, the differences were found to be statistically significant compared to heterophil percentage in control (Group-I) during different stages of growth period (Table 2). This might be due to the presence of antioxidant and anti-inflammatory principles in citrus bioflavonoids.

3.6.2. Lymphocyte

Supplementation of ashwagandha numerically but non-significant increase lymphocyte percentages in Group-II and IV compared to those in Group-I during 4th and 5th weeks of age. The lymphocyte percentages in these groups were within normal range (Table 2). These observations are in accordance with Davis and Kuttan, (2000) [9] and Malik *et al.* (2007) [22] who reported higher lymphocyte percentage in ashwagandha supplemented broiler chicks. Singh *et al.* (2009) [34] supplemented broiler diets with herbal formulation (Zeetress®) containing Ashwagandha had resulted in increased lymphocyte counts. In studies of Mishra *et al.* (2000) [24] and Sharma *et al.* (2010) [32] they documented that ashwagandha extract has its intrinsic property to increase the phagocytic potential in avian and mammalian species. This increased phagocytic potential may be responsible for increase in number of phagocytic cells *viz.*, lymphocyte, monocyte, eosinophils and basophils in these groups (Davis and Kuttan, 2000; Malik *et al.*, 2007) [9, 22].

The result also revealed slight but non-significant rise in lymphocyte per centage in citrus bioflavonoids supplemented groups (Group-III and IV) compared to control (Group-I) during 4th and 5th weeks of age. (Table 2). These results are in agreement with the finding of Nobakht (2013) [27] who also opined that supplementation of dried lemon pulp in broiler chickens had no significant influence on TLC and lymphocyte percentages.

3.6.3. Monocyte

Group-II and IV had no significant influence on monocyte percentage compared to monocyte values in control (Group-I) (Table 2). The findings of other researchers are varied as Mushtaq *et al.* (2012) [26] reported non-significant but higher values of monocytes in the ashwagandha treated chicks. Whereas, Ganguly *et al.* (2020) [14] reported lower values of Monocytes upon Ashwagandha extract supplementation in IBDV infected birds. The significantly lowered monocyte count in IBDV infected birds treated with ashwagandha may be associated with to reduce the severity of inflammation and hence monocyte count was lowered and brought back to normal range.

Citrus bioflavonoid supplementation in Group-III and IV had no significant influence on monocyte percentage compared to monocyte values in Group-I (Table 2). These observations are in accordance with studies of Kadam *et al.* (2009) [18] and Aikpitanyi and Imaseum (2019) [2] who also reported similar results upon supplementation with citrus fruit products on monocyte percentage.

Table 2: Leucogram in different groups of Broilers (N=10)

Parameter	Week	Group-I	Group-II	Group-III	Group-IV
Total Leucocyte Count (×10^3/µL)	2 Weeks	20.90±1.21	20.81±1.09	20.57±0.92	21.47±0.98
	3 Weeks	20.82±0.20	22.03±0.31	21.47±0.20	22.27±0.36
	4 Weeks	22.04±0.51	24.34±0.58	22.83±0.53	24.38±0.65
	5 Weeks	23.35±0.65 ^{a}	25.63±0.63 ^{ab}	24.37±0.47 ^{ab}	26.05±0.26 ^{b}
Heterophil (%)	2 Weeks	26.80±0.61	25.80±1.25	25.10±0.89	26.70±1.03
	3 Weeks	26.90±0.53	26.30±0.47	26.30±0.97	25.20±0.76
	4 Weeks	27.60±0.82 ^{b}	24.30±0.99 ^{a}	24.40±0.81 ^{a}	24.30±0.47 ^{a}
	5 Weeks	26.60±0.72 ^{b}	23.60±0.52 ^{a}	23.40±0.65 ^{a}	23.70±0.63 ^{a}
Lymphocyte (%)	2 Weeks	62.70±1.18	63.20±1.23	63.40±0.95	63.00±1.24
	3 Weeks	62.60±0.86	64.00±0.67	64.70±0.91	64.50±0.78
	4 Weeks	61.90±0.86	63.30±0.37	63.60±0.83	63.00±0.75
	5 Weeks	60.10±1.83	63.10±0.99	62.90±1.32	62.50±1.18
Monocyte (%)	2 Weeks	6.90±0.41	6.60±0.52	6.80±0.42	6.20±0.49
	3 Weeks	7.20±0.39	6.20±0.36	6.60±0.60	7.10±0.38
	4 Weeks	6.50±0.31	7.50±0.45	7.30±0.30	7.30±0.42
	5 Weeks	7.00±0.52	7.30±0.65	7.10±0.46	7.40±0.37
Eosinophil (%)	2 Weeks	2.70±0.47	3.00±0.45	3.20±0.42	3.20±0.39
	3 Weeks	2.50±0.56	2.30±0.33	1.60±0.34	2.10±0.35
	4 Weeks	2.80±0.29	3.70±0.42	3.30±0.37	3.70±0.40
	5 Weeks	2.50±0.40	4.00±0.39	3.00±0.26	3.80±0.44
Basophil (%)	2 Weeks	1.30±0.21	1.40±0.27	1.10±0.31	0.90±0.23
	3 Weeks	1.00±0.33	1.20±0.25	0.80±0.33	1.10±0.31
	4 Weeks	1.00±0.21	1.20±0.25	1.20±0.33	1.50±0.27
	5 Weeks	1.80±0.33	1.60±0.34	1.80±0.33	1.40±0.31
Heterophil: Lymphocyte ratio	2 Weeks	0.43±0.02	0.41±0.03	0.40±0.02	0.43±0.02
	3 Weeks	0.43±0.01	0.41±0.01	0.41±0.02	0.39±0.02
	4 Weeks	0.45±0.02 ^{a}	0.38±0.02 ^{b}	0.39±0.02 ^{b}	0.39±0.01 ^{b}
	5 Weeks	0.44±0.02 ^{a}	0.37±0.01 ^{b}	0.37±0.01 ^{b}	0.38±0.02 ^{b}

The values with different superscripts (a, b) within a row for a particular parameter differ significantly (p<0.05).

3.6.4. Eosinophil

Supplementation of either ashwagandha or citrus bioflavonoids had no significant influence on eosinophil percentages in comparision to control group. (Table 2). However, Davis and Kuttan, (2000) [9] and Malik *et al.*, (2007) [22] reported that ashwagandha supplementation in broiler chicks had improved the number eosinophils. There are no reports to compare our results in citrus bioflavonoids supplemented groups.

3.6.5. Basophil

Supplementation of either ashwagandha or citrus bioflavonoids had no significant influence on basophil percentages in comparision to control group. (Table 2). There are no reports to compare our results either in ashwagandha or in citrus bioflavonoids supplemented groups.

3.6.6. H: L ratio

In the present study H: L Ratio was significantly lower in Group-II and IV compared to H: L ratio in Group-I to during last two weeks of study period. The values were well within normal range (Table 2). These findings are in accordance with the reports of Singh *et al.* (2009) [34], Raghavan *et al.* (2011) [30] who reported similar findings in ashwagandha extract supplement group in birds. The study performed by Mufeed, (2014) [25] concluded that these herbal plant extracts had potent antioxidant property, this might be the reason for lower H: L ratio in the herbal supplemented groups to ameliorate any form of stress in these birds.

In the present study H: L ratio was significantly lower in citrus bioflavonoids supplemented groups (Group-III and IV) compared to H: L ratio in control (Group-I) during last two weeks of study period (Table 2). The values were within normal range (Table 2). There are no reports to compare our results in citrus bioflavonoids supplemented groups. Though, the dosages of ashwagandha and citrus bioflavonoids in combination group (Group-IV) were reduced to be half the levels in Group II and III, the significantly lower influences on H:L ratio might be due to synergistic effect of both herbs.

4. Conclusions

Ashwagandha and citrus bioflavonoids extract showed positive influences on hematological parameters such as TEC, Hb and PCV, and lymphocyte population indicating improved health and physiological status of these birds. This may be due to the Haemato-poietic and lympho-proliferative property of these extracts in broiler chicken.

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