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Effect of dietary supplementation of Quercetin and Lycopene on Haematological profile in broilers

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Abstract
An experiment was carried out to investigate the effects of dietary supplementation of quercetin and lycopene on hematological parameters in broilers. Day old commercial broiler chicks (80 Nos) were reared commonly and given similar managemental inputs during acclimatization period of two weeks. On 15th day the broilers were divided randomly and equally into four groups. Group I (Control) provided with basal diet, in Group II basal diet were supplemented with quercetin @ 100 mg/ kg of diet, in Group III basal diet were supplemented with lycopene @ 100 mg/ kg of diet and in Group IV basal diet were supplemented with quercetin @ 50 mg/ kg + lycopene @ 50 mg/ kg of diet. Blood samples were collected at weekly intervals till the end of the experiment. Haematological parameters were analyzed as per the standard protocol. The results showed that supplementation of quercetin and lycopene significantly (p<0.05) increased Hb, PCV values; significantly (p<0.05) decreased Heterophil (%), and Heterophil: Lymphocyte ratios, during last week of the study. There was significant (p<0.05) increase in TEC values during last two weeks of the study. However, treatments showed no significant (p>0.05) difference in the values of MCV, MCH, MCHC, TLC, Lymphocytes, Monocytes, Eosinophils and Basophils during the study period. It is concluded that the supplementation of quercetin, lycopene and their combination in the diet of broilers have positive influence on heamotological parameters.

Keywords: Quercetin, lycopene, haematology, broilers

1. Introduction
In a developing country like India, poultry plays an important role in improving nutritional status of masses, who are mostly suffering from malnutrition due to inadequate and inferior quality protein in their diet. The poultry industry has now emerged as a highly structured and market-oriented enterprise. Thus, the major objectives of poultry farming are to increase the profit margin in poultry business by improving feed efficiency and growth rate.

At farm level birds are under constant pressure to maximize production via improvement in body weight gain and FCR, which leads to stress. Stresses caused by nutritional, environmental, microbiological, and managemental factors negatively affect poultry health and production, and induces oxidative stress, which is a major concern in poultry production.

Feed supplements and feed additives has served the purpose of best production efficiencies over the years. However, due to public health concerns regarding antibiotic resistance and residue issues, the use of antimicrobial agents to improve productivity has been banned (Ertaş *et al*., 2005) [11]. Various alternative feed additives have been tested to improve the feed efficiency and to enhance the health status of poultry, and are expected to exert antimicrobial, antioxidant, and immune-enhancing activities and be retained in the poultry products (Abdel-Rahman *et al.,* 2013; Kamboh *et al.,* 2015) [1, 17]. Among all, Flavonoids (Quercetin) and Carotenoids (Lycopene) are better to maximize production efficiency. These herbal feed additives having no side effects on the health of birds.

Quercetin is a naturally occurring polyphenolic bioflavonoid abundantly distributed throughout the plant kingdom and is found in variety of foods including edible plants, mainly apples, berries, guavas, avocados, herbs, spices, brassica vegetables (broccoli), lettuce, grapes, onions, tomatoes, grains, and beverages, mostly red wines, tea and coffee (Manach *et al.*, 2004) [24].

Quercetin has been shown to possess a wide spectrum of physiological and pharmacological properties *viz*. anti-inflammatory, anti-proliferative, anti-oxidant, anti-bacterial, antineoplastic, antiviral, anti-atherogenic, neuroprotective, antihypertensive and anti-allergenic properties (Dajas, 2012) [10].

Lycopene ($C_{40}H_{56}$) is a natural carotenoid that imparts red color to various fruits and vegetables such as tomato, watermelon and pink grape fruit (Shi, 2000) [33]. Tomatoes are known to be the major source of lycopene with the content of 3100–8600 µg per 100 g of tomatoes or their products (Stahl and Sies, 1996) [34]. Lycopene has been shown to possess antineoplastic, anti-proliferative, anti-diabetic, anti-oxidant, anti-atherogenic and cardioprotective properties. It is also used to cure infertility.

Perusal of literature revealed that there has been paucity of work carried out on the supplementation of quercetin and lycopene in broilers. Hence, keeping in view the importance of Quercetin and Lycopene as supplements in the broiler ration, the present study was undertaken with the objective of studying the effect of dietary supplementation of Quercetin and Lycopene on haematological parameters in broilers. In a study conducted by Petruška *et al*., (2021) [29] rabbits receiving three different doses of quercetin for 90 days, three times a week, exhibited slight changes in some haematological parameters (WBC, RBC, Hb, Lymphocytes, Granulocytes and PCV).

however, the differences were not significant. MCV, MCH, MCHC and Platelets were not influenced (P>0.05) after quercetin treatment. Abimbola (2017) [3] observed no significant differences concerning PCV and Hb in addition to red blood cells in broilers which were fed with 50mg quercetin/kg diet at 28, 35 and 42 days of age. Abid, A.R and Ahmed, S.K., (2019) [2] conducted an experiment to investigate the influence of different levels of quercetin on physiological status of layer hens and found a significant increase in PCV and Hb in T2 group supplemented with 400mg quercetin /kg of diet. Selim *et al.*, (2013) [31] conducted evaluations of some natural antioxidant sources in broiler diets and found that group treated with tomato puree showed significant increase in Hb, RBCs, WBCs and Lymphocyte values while showed significant decrease in Heterophil and Heterophil/ Lymphocyte ratio when compared to control group. Ali and Al-massad (2015) [6] in their study on effect of adding lycopene to the broiler ration found that the addition of lycopene by 250 and 500mg/kg feed to broiler diet led to significant improvement in red and white blood cell counts, Hematocrit and Hemoglobin concentration and significant decrease in Heterophil to Lymphocyte ratio.

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, Veterinary College Bidar, for a period of 35 days (5 weeks). Day old commercial broiler chicks (80 Nos) weighing around 45-55g were procured from commercial hatcheries and reared in common for 14 days for acclimatization period on broiler pre-starter diet (NRC, 1994) [25]. 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.

Description of Diet

Groups	Description of Diet
Group-I	Basal diet
Group-II	Basal diet + Quercetin @100mg/kg diet
Group-III	Basal diet + Lycopene @100mg/kg diet
Group-IV	Basal diet+ Quercetin @ 50mg/kg + Lycopene @ 50mg/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 were provided ad-libitum feed and fresh water (as per NRC 1994).

2.2 Climate / Weather parameters

All the broiler chicks during the first 14 days of acclimatization period were kept for brooding by providing light source of 4×100-watt incandescent bulb for 24 hrs. Climatic parameters such as temperature and humidity index were recorded twice a day (morning at 10 AM and afternoon at 3PM) by using dry and wet bulb thermometer and hygrometer which were fixed in the pen. The weekly average climatic factors were as given below.

The weekly average climatic factors

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%

About 1 mL of blood sample was collected in EDTA coating tubes from wing vein in Ten (10) birds in each group at weekly intervals (from 2nd week upto 5th week) during the experimental period, for estimation of hematological parameters.

2.3 Estimation of Hematological parameters

Haemoglobin concentration was measured by Sahli's Haemoglobinometer. 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 amd MCHC were calculated using standard formulas.

2.4 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 Haemoglobin

In the present study, in quercetin supplemented groups (Group II and Group IV) the mean values of haemoglobin concentration (g/dL) were significantly (P<0.05) higher at 5th week of age as compared to those of control group (Group I) (Table 1). The present observations are in agreement with the results of Koriem (2009) [22], Parabathina *et al.* (2011) [27], Selvakumar *et al.* (2013) [32], Al-Uboody (2015) [7], Keskin *et al.* (2016) [20], Al-Omair *et al.* (2017) [5], Bahar *et al.* (2017) [9] and Asmaa *et al.* (2018) [8] who reported significant increase in haemoglobin concentration, near to normal levels in rats

which were intoxicated by Lead, Doxorubicin, Polychlorinated biphenyls, O-anisidine hydrochloride, streptozotocin induced diabetes, Manganese and Carbon tetrachloride. From the results of their study, Mahmoud, A.M., (2013) [23], Keskin *et al.* (2016) [20] in rats and Ohlsson and Aher (2017) [26] in human infants, opined that quercetin stimulates hemopoietic system by stimulating secretion of erythropoietin and thereby stimulates stem cells to increase the production of red blood cells. Therefore, the concentration of hemoglobin is positively correlated with the number of red blood cells (Sturkie, 1986) [35].

In lycopene supplemented groups (Group III and Group IV) the mean values of haemoglobin concentration (g/dL) were significantly (P<0.05) higher at 5th week of age as compared to those of control group (Table 1). These results were in accordance with the observations of Selim *et al.* (2013) [31], Ali and Al-massad (2015) [6] and Alhelbawy *et al.* (2019) [4] in broilers, who reported significant improvement in haemoglobin concentration. The rise in haemoglobin concentration was positively correlated with the increase in red blood cells, which was attributed to body's need to meet new requirements for the transfer of nutrients and oxygen to cells due to increased metabolic rate in lycopene supplemented groups and also to the strong antioxidant nature of lycopene, which effectively protected blood cells from oxidative damage by quenching free radicals and inhibiting oxidation of lipid membranes. (Ali and Al-massad, 2015) [6].

3.2 PCV/ Haematocrit

The results obtained in the present study revealed significantly (P<0.05) higher PCV (%) values in quercetin supplemented groups (Group II and Group IV) at 5th week of age as compared to control group (Table 1). The present findings are in agreement with the results of Koriem (2009) [22], Parabathina *et al.* (2011) [27], Selvakumar *et al.* (2013) [32], Al-Uboody (2015) [7], Keskin *et al.* (2016) [20], Al-Omair *et al.* (2017) [5], Bahar *et al.* (2017) [9] and Asmaa *et al.* (2018) [8] who reported significant increase in PCV values, near to normal levels in rats which were intoxicated by Lead, Doxorubicin, Polychlorinated biphenyls, O-anisidine hydrochloride, streptozotocin induced diabetes, Manganese and Carbon tetrachloride. From the results of their study, Mahmoud, A.M., (2013) [23], Keskin *et al.* (2016) [20] in rats and Ohlsson and Aher (2017) [26] in human infants, opined that quercetin stimulates hemopoietic system by stimulating secretion of erythropoietin and thereby stimulates stem cells to increase the production of red blood cells. Therefore, the increase in PCV values are positively correlated with the increase in number of red blood cells (Sturkie, 1986) [35].

In lycopene supplemented groups (Group III and Group IV) the mean values of Packed cell volume (%) were significantly (p<0.05) higher at 5th week of age as compared to those of control group (Table 1). These results were in accordance with the observations of Ali and Al-massad (2015) [6] in broilers, who reported significant improvement in PCV values. The increase in PCV was positively correlated with the increase in red blood cells, which was attributed to body's need to meet new requirements for the transfer of nutrients and oxygen to cells due to increased metabolic rate in lycopene supplemented groups and also to the strong antioxidant nature of lycopene, which effectively protected blood cells from oxidative damage by quenching free radicals and inhibiting oxidation of lipid membranes. (Ali and Al-massad, 2015) [6].

3.3 Total Erythrocyte Count (TEC)

In the present study, in quercetin supplemented groups (Group II and Group IV) the mean values of Total Erythrocyte Count (x10^6/µL) were significantly (P<0.05) higher at 4th and 5th week of age as compared to those of control group (Table 1). The present observations are in agreement with the results of Koriem (2009) [22], Parabathina *et al.* (2011) [27], Selvakumar *et al.* (2013) [32], Al-Uboody (2015) [7], Keskin *et al.* (2016) [20], Al-Omair *et al.* (2017) [5], Bahar *et al.* (2017) [9], Asmaa *et al.* (2018) [8], Pasdar *et al.* (2020) [28] and Golabi-Habashi *et al.* (2021) [13] who reported improvement in TEC values back to normal levels in rats, mice and humans, which were intoxicated by Lead, Doxorubicin, Polychlorinated biphenyls, O-anisidine hydrochloride, streptozotocin induced diabetes, Manganese, Carbon tetrachloride and Benzene. From the results of their study, Mahmoud, A.M., (2013) [23], Keskin *et al.* (2016) [20] in rats and Ohlsson and Aher (2017) [26] in human infants, opined that quercetin stimulates hemopoietic system by stimulating secretion of erythropoietin and thereby stimulates stem cells to increase the production of red blood cells.

In lycopene supplemented groups (Group III and Group IV) the mean values of Total Erythrocyte Count (x10^6/µL) were significantly (P<0.05) higher at 4th and 5th week of age as compared to those of control group (Table 1). These results were in accordance with the observations of Selim *et al.* (2013) [31] and Ali and Al-massad (2015) [6] in broilers, who reported significant improvement in haemoglobin concentration on lycopene supplementation. Ali and Al-massad (2015) [6] attributed the increase in TEC to body's need to meet new requirements for the transfer of nutrients and oxygen to cells due to increased metabolic rate in lycopene supplemented groups and also to strong antioxidative as well as haemo-protective nature of lycopene, which prevented RBCs from oxidative damage and lysis by scavenging ROS.

Table 1: Erythrogram in different groups of broilers (n=10)

Parameters	Weeks	Group-I	Group-II	Group-III	Group-IV
Haemoglobin concentration (g/dL)	2 Weeks	7.12 ± 0.26	7.25 ± 0.20	7.05 ± 0.22	7.06 ± 0.25
	3 Weeks	7.52 ± 0.28	7.96 ± 0.29	8.09 ± 0.24	8.04 ± 0.36
	4 Weeks	7.83 ± 0.30	8.46 ± 0.39	8.75 ± 0.32	8.33 ± 0.39
	5 Weeks	8.09 ± 0.26[a]	9.28 ± 0.22[b]	9.58 ± 0.20[b]	9.36 ± 0.37[b]
Packed cell volume (%)	2 Weeks	23.30 ± 1.16	23.00 ± 0.42	23.10 ± 0.57	23.40 ± 0.99
	3 Weeks	23.70 ± 1.04	25.90 ± 1.62	25.80 ± 1.21	24.70 ± 1.02
	4 Weeks	24.20 ± 0.99	27.40 ± 0.88	28.00 ± 1.65	27.50 ± 0.60
	5 Weeks	26.10 ± 1.19[a]	30.90 ± 1.49[b]	31.60 ± 1.34[b]	30.90 ± 1.05[b]
Total Erythrocyte Count (x10^6/µL)	2 Weeks	1.87 ± 0.04	1.89 ± 0.04	1.92 ± 0.05	1.90 ± 0.04
	3 Weeks	2.00 ± 0.03	2.05 ± 0.03	2.08 ± 0.02	2.00 ± 0.03
	4 Weeks	2.04 ± 0.03[a]	2.28 ± 0.04[b]	2.32 ± 0.07[b]	2.21 ± 0.02[b]

	5 Weeks	2.10 ± 0.05[a]	2.37 ± 0.06[b]	2.42 ± 0.08[b]	2.30 ± 0.07[b]
Mean Corpuscular Volume (fL)	2 Weeks	124.77± 5.50	122.47 ± 3.30	121.22 ± 4.71	123.05 ± 4.01
	3 Weeks	118.90 ± 5.35	126.00 ± 7.27	124.31 ± 5.96	124.06 ± 5.56
	4 Weeks	118.87 ± 5.03	120.10 ± 3.62	120.97 ± 6.41	124.57 ± 2.81
	5 Weeks	125.06 ± 7.17	132.08 ± 8.47	131.77 ± 7.23	135.60 ± 6.71
Mean Corpuscular Hemoglobin (pg)	2 Weeks	38.32 ± 1.86	38.51 ± 0.97	36.86 ± 1.22	37.31 ± 1.54
	3 Weeks	37.88 ± 1.77	38.88 ± 1.53	38.93 ± 1.06	40.42 ± 2.03
	4 Weeks	38.61 ± 1.93	37.16 ± 1.83	38.02 ± 1.63	37.70 ± 1.69
	5 Weeks	38.73 ± 1.75	39.46 ± 1.32	39.90 ± 1.57	40.81 ± 1.71
Mean Corpuscular Haemoglobin Concentration (g/dL)	2 Weeks	31.05 ± 1.62	31.60 ± 0.97	30.73 ± 1.41	30.77 ± 1.96
	3 Weeks	32.48 ± 2.25	31.54 ± 1.92	31.84 ± 1.43	33.30 ± 2.44
	4 Weeks	32.91 ± 1.90	31.33 ± 2.07	32.42 ± 2.53	30.31 ± 1.32
	5 Weeks	31.80 ± 2.21	30.83 ± 2.01	31.00 ± 1.89	30.55 ± 1.47

Means with different superscripts (a, b) within a row for a particular parameter differ significantly (P<0.05).

3.4 Erythrocyte Indices

In the present study, in quercetin supplemented groups (Group II and Group IV) the mean values of MCV, MCH and MCHC were non-significant at 2nd, 3rd, 4th and 5th week of age as compared to those of control group (Table 1). The present observations are in agreement with the results Petruška *et al.* (2021) [29] in rabbits, Uzun and Kalender (2013) [36] and Keskin *et al.* (2016) [20] in diabetic rats who reported non-significant changes in MCV, MCH and MCHC values. Asmaa *et al.* (2018) [8] in rats reported non-significant changes only in MCV and MCH values.

In lycopene supplemented groups (Group III and Group IV) the mean values of MCV, MCH and MCHC were non-significant at 2nd, 3rd, 4th and 5th week of age as compared to those of control group (Table 1). There are no studies or reports on broilers to compare our results. However, these observations are in accordance with the results of Yonar (2017) [37] in fish, who reported non-significant changes in MCH and MCHC values.

3.5 Total Leukocyte Count (TLC)

In the present study in quercetin supplemented groups (Group II and IV), the mean values of Total Leukocyte Count ($x10^3/\mu L$) were numerically higher at 4th and 5th week of age as compared to those of control group (Table 2). However, the differences were not significant (P>0.05). These observations are in accordance with the results of Kanashiro *et al.* (2009) [18], Heinz *et al.* (2010) [14], Konrad *et al.* (2011) [21], Parabathina *et al.* (2011) [27], Petruška *et al.* (2021) [29], Selvakumar *et al.* (2013) [32], Uzun, and Kalender (2013) [36], Keskin *et al.* (2016) [20], Al-Omair *et al.* (2017) [5], Bahar *et al.* (2017) [9], Kasmi *et al.* (2018) [19], Pasdar *et al.* (2020) [28] and Golabi-Habashi *et al.* (2021) [13] who reported protective effects of quercetin on leukocytes and non-significant changes in TLC values in rats, mice, rabbits, hamsters and humans. The numerical increase in TLC values at 4th and 5th week of age in quercetin supplemented groups might be due to lymphoproliferative effects and protection of leukocytes from oxidative damage by inhibiting the formation of excessive free radicals and preventing lipid peroxidation in broilers which are under production stress.

In lycopene supplemented groups (Group III and Group IV) the mean values of Total Leukocyte Count ($x10^3/\mu L$) were numerically higher at 4th and 5th week of age as compared to those of control group (Table 2). However, the differences were not significant (P>0.05). These results are in accordance with the observations of Pozzo *et al.* (2013) [30] in broilers, who reported dietary lycopene supplementation did not showed any significant changes in TLC values. However, numerical increase in TLC values at 4th and 5th week of age can be attributed to lycopene which stimulate leukocyte proliferation and synthesis (Fachinello *et al.*, 2018) [12]. Yuksek *et al.* (2013) [38] in their study on rats observed increase in TLC and lymphocytes which they attributed to lycopene, stimulating lymphocytes by increasing the production of interleukin-2 and interferon gamma, a potent activator of T-lymphocytes. The differences may be due to different species of animal and different dose of lycopene used in the study. The higher dose of lycopene might have increased leukocyte levels significantly.

3.6 Differential Leukocyte Count (DLC)

3.6.1 Heterophil

In the present study, in quercetin supplemented groups (Group II and Group IV) the mean values of Heterophil count (%) were significantly (P<0.05) lower at 5th week of age as compared to those of control group (Table 2). The present observations are in agreement with the results of Bahar *et al.* (2017) [9] who reported decrease in neutrophil values back to normal levels in quercetin treated manganese intoxicated rats. Heterophils contain a variety of granules together with macrophages, are a source of active oxygen species. Acute or chronic inflammatory disease is the predominant cause of monocytosis and heterophilia in birds (Irizaary-Rovira, 2004) [16]. The decrease in neutrophil count might be due to antioxidant and antibacterial nature of quercetin, which decreased stress and infection level in birds supplemented with quercetin.

In lycopene supplemented group (Group III) the mean values of Heterophil count (%) were significantly lower at 4th week of age as compared to those of control group (Table 2). Also, at 5th week of age in lycopene supplemented groups (Group III and Group IV) the mean values of Heterophil count (%) were significantly lower as compared to those of control group. These results are in accordance with the observations of Selim *et al.* (2013) [31] in broilers, Ibrahim and Banaee (2014) [15] in fish, Fachinello *et al.* (2018) [12] in pigs and Alhelbawy *et al.* (2019) [4] in broilers, who reported decrease in heterophil count on lycopene supplementation. The decrease in Heterophil count might due to powerful antioxidative and antimicrobial nature of lycopene which prevented infections and oxidative stress by scavenging ROS.

3.6.2 Lymphocyte

In the present study, in quercetin supplemented groups (Group II and Group IV) the mean values of Lymphocyte count (%) were non-significant at 2nd, 3rd, 4th and 5th week of age as compared to those of control group (Table 2). However, numerical higher values were observed at 4th and 5th week of age in Group II and Group IV. The present

observations are in agreement with the results of Kanashiro *et al.* (2009) [18] in hamsters, Heinz *et al.* (2010) [14] in humans, Keskin *et al.* (2016) [20] in diabetic rats, Bahar *et al.* (2017) [9] in rats and Pasdar *et al.* (2020) [28] in humans, who reported non-significant changes in lymphocyte values. The numerical increase in lymphocyte values at 4th and 5th week of age in quercetin supplemented groups might be due to lymphoproliferative effects and protection of cells from oxidative damage in broilers which are under oxidative and production stress.

In lycopene supplemented groups (Group III and Group IV) the mean values of Lymphocyte count (%) were numerical higher values at 4th and 5th week of age as compared to those of control group (Table 2). These observations are in accordance with the observations of Selim *et al.* (2013) [31] in broilers, Yuksek *et al.* (2013) [38] in rats, and Fachinello *et al.* (2018) [12] in pigs who reported improvement in lymphocyte count on lycopene supplementation. The improvement in lymphocyte count can be attributed to lycopene, stimulating lymphocytes by increasing the production of interleukin-2 and interferon gamma, a potent activator of T-lymphocytes. (Yuksek *et al.*, 2013) [38].

3.6.3 H:L ratio

In the present study, in quercetin supplemented groups (Group II and Group IV) the mean values of Heterophil: Lymphocyte ratio were significantly lower at 5th week of age as compared to those of control group (Table 2). There were no studies or reports to compare our results with respect to quercetin supplementation. H/L ratio is used as a reliable indicator of stress in birds. The decrease in H:L ratio indicates good health and low stress on body. This decrease in H:L ratio might be due to lymphoproliferative effects and strong antioxidant nature of quercetin, which decreased heterophils and increased lymphocytes by decreasing stress and infection level in birds supplemented with quercetin.

In lycopene supplemented group (Group III) the mean values of H:L ratio were significantly lower at 4th week of age as compared to those of control group (Table 2). Also, at 5th week of age in lycopene supplemented groups (Group III and Group IV) the mean values of H:L ratio were significantly lower as compared to those of control group. These results were in accordance with the observations of Selim *et al.* (2013) [31] in broilers, Ali and Al-massad (2015) [6] in broilers and Fachinello *et al.* (2018) [12] in pigs who reported significant decrease in H:L ratio on lycopene supplementation. The decrease in H:L ratio might due to stimulation of leukocyte proliferation and synthesis by lycopene (Fachinello *et al.*, 2018) [12]. The powerful antioxidative and antimicrobial nature of lycopene prevented infections and oxidative stress by scavenging ROS, therefore the decrease in heterophils count and increase in lymphocytes count were observed.

3.6.4 Monocyte

In the present study, in quercetin supplemented groups (Group II and Group IV) the mean values of Monocyte count (%) were non-significant at 2nd, 3rd, 4th and 5th week of age as compared to those of control group (Table 2). The present observations are in agreement with the results of Kanashiro *et al.* (2009) [18] in hamsters, Keskin *et al.* (2016) [20] in diabetic rats, Asmaa *et al.* (2018) [8] in rats and Pasdar *et al.* (2020) [28] in humans, who reported non-significant changes in monocyte count values.

In lycopene supplemented groups (Group III and Group IV) the mean values of Monocyte count (%) were non-significant at 2nd, 3rd, 4th and 5th week of age as compared to those of control group (Table 2). These findings are in accordance with the observations of Pozzo *et al.* (2013) [30] in broilers and Fachinello *et al.* (2018) [12] in pigs who reported non-significant changes in monocyte count values.

3.6.5 Eosinophil

In the present study, in quercetin supplemented groups (Group II and Group IV) the mean values of Eosinophil count (%) were non-significant at 2nd, 3rd, 4th and 5th week of age as compared to those of control group (Table 2). The present observations are in agreement with the results of Kanashiro *et al.* (2009) [18] in hamsters, Petruška *et al.* (2021) [29] in rabbits, Keskin *et al.* (2016) [20] in diabetic rats, Asmaa *et al.* (2018) [8] in rats and Pasdar *et al.* (2020) [28] in humans, who reported non-significant changes in Eosinophil values.

In lycopene supplemented groups (Group III and Group IV) the mean values of Eosinophil count (%) were non-significant at 2nd, 3rd, 4th and 5th week of age as compared to those of control group (Table 2). These results are in accordance with the observations of Pozzo *et al.* (2013) [30] in broilers and Fachinello *et al.* (2018) [12] in pigs who reported non-significant changes in Eosinophil values.

Table 2: Leukogram in different groups of broilers (n=10)

Parameters	Weeks	Group-I	Group-II	Group-III	Group-IV
Total Leukocyte Count (x10^3/µL)	2 Weeks	21.53 ± 0.78	21.65 ± 1.08	21.79 ± 1.02	21.56 ± 0.96
	3 Weeks	22.63 ± 1.11	23.06 ± 1.29	22.25 ± 1.08	22.00 ± 0.96
	4 Weeks	23.60 ± 1.14	26.04 ± 1.23	25.87 ± 0.73	25.37 ± 0.94
	5 Weeks	24.98 ± 0.78	27.43 ± 1.20	27.01 ± 1.11	26.86 ± 1.21
Heterophil Count (%)	2 Weeks	27.40 ± 1.08	27.10 ± 1.59	27.00 ± 1.26	26.90 ± 1.40
	3 Weeks	29.70 ± 0.92	27.70 ± 0.63	27.30 ± 0.93	28.00 ± 1.67
	4 Weeks	30.20 ± 1.30^{b}	26.50 ± 0.92^{ab}	25.80 ± 1.05^{a}	27.70 ± 0.80^{ab}
	5 Weeks	31.80 ± 1.26^{b}	24.10 ± 0.89^{a}	23.90 ± 0.85^{a}	25.40 ± 0.82^{a}
Lymphocyte Count (%)	2 Weeks	56.70 ± 1.34	56.90 ± 1.39	57.90 ± 1.39	56.70 ± 1.16
	3 Weeks	57.10 ± 1.02	57.60 ± 1.18	57.90 ± 1.12	56.80 ± 0.93
	4 Weeks	59.10 ± 1.19	61.00 ± 0.94	61.20 ± 1.68	60.30 ± 1.20
	5 Weeks	61.20 ± 0.85	63.40 ± 1.17	64.20 ± 1.21	64.10 ± 1.10
Heterophil: Lymphocyte ratio	2 Weeks	0.48 ± 0.02	0.48 ± 0.03	0.47 ± 0.03	0.48 ± 0.03
	3 Weeks	0.52 ± 0.02	0.48 ± 0.02	0.48 ± 0.02	0.50 ± 0.03
	4 Weeks	0.51 ± 0.03^{b}	0.44 ± 0.02^{ab}	0.43 ± 0.03^{a}	0.46 ± 0.02^{ab}
	5 Weeks	0.52 ± 0.03^{b}	0.38 ± 0.01^{a}	0.37 ± 0.02^{a}	0.40 ± 0.01^{a}
Monocyte Count (%)	2 Weeks	7.10 ± 1.16	8.40 ± 0.73	7.60 ± 1.10	8.30 ± 0.68

	3 Weeks	6.20 ± 0.81	7.00 ± 0.54	7.10 ± 0.55	6.70 ± 0.70
	4 Weeks	5.50 ± 0.79	5.10 ± 0.71	6.60 ± 0.78	5.90 ± 0.94
	5 Weeks	3.70 ± 0.65	6.30 ± 0.79	6.40 ± 0.72	6.20 ± 0.83
Eosinophil Count (%)	2 Weeks	7.20 ± 1.22	6.30 ± 1.5	6.10 ± 0.97	6.70 ± 1.18
	3 Weeks	5.90 ± 1.00	6.70 ± 1.15	6.80 ± 1.17	7.50 ± 1.00
	4 Weeks	4.20 ± 0.90	6.20 ± 1.22	5.40 ± 0.81	4.90 ± 0.98
	5 Weeks	2.70 ± 0.68	5.00 ± 0.91	4.50 ± 0.82	3.70 ± 0.70
Basophil Count (%)	2 Weeks	1.60 ± 0.22	1.30 ± 0.26	1.40 ± 0.31	1.30 ± 0.15
	3 Weeks	1.10 ± 0.35	1.00 ± 0.26	0.90 ± 0.31	1.00 ± 0.33
	4 Weeks	1.00 ± 0.21	1.20 ± 0.25	1.00 ± 0.26	1.20 ± 0.25
	5 Weeks	0.60 ± 0.16	1.20 ± 0.25	1.00 ± 0.21	0.60 ± 0.16

Means with different superscripts (a, b) within a row for a particular parameter differ significantly ($p<0.05$)

3.6.6 Basophil

In the present study, in quercetin supplemented groups (Group II and Group IV) the mean values of Basophil count (%) were non-significant at 2nd, 3rd, 4th and 5th week of age as compared to those of control group (Table 2). The present observations are in agreement with the results of Kanashiro *et al.* (2009) [18] in hamsters, Petruška *et al.* (2021) [29] in rabbits, Keskin *et al.* (2016) [20] in diabetic rats, Asmaa *et al.* (2018) [8] in rats and Pasdar *et al.* (2020) [28] in humans, who reported non-significant changes in Basophil values.

In lycopene supplemented groups (Group III and Group IV) the mean values of Basophil count (%) were non-significant at 2nd, 3rd, 4th and 5th week of age as compared to those of control group (Table 2). These observations are in accordance with the findings of Pozzo *et al.* (2013) [30] in broilers and Fachinello *et al.* (2018) [12] in pigs who reported non-significant changes in Basophil values.

4. Conclusions

Dietary supplementation of quercetin, lycopene and their combination significantly improved hematological parameters like Hb, PCV, TEC and H:L ratio and thus the overall heath in broilers. However, from economic point of view, we found that lycopene being cheaper and easily available compared to quercetin, dietary supplementation of lycopene @ 100 mg/ kg of diet can be recommended for field level use for improving the health status in broilers.

5. References

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