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Haematological and Biochemical Changes in Japanese Quails *Coturnix coturnix Japonica* and Chickens Due to *Ascaridia galli* Infection

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Abstract: A study was carried out in Japanese quails *Coturnix coturnix japonica* and chickens (Rhode Island Red) for comparative haematological and biochemical changes occurring due to *Ascaridia galli* infection. Quails as well as chickens were divided into four small groups, each consisting six numbers of birds in each group and were marked as q1, q2, q3, qC and p1, p2, p3, pC, respectively. Haematological study showed that the total erythrocytic count (TEC), packed cell volume (PCV) and haemoglobin (Hb) percentage decreased significantly in infected groups of quails and chickens. The total leucocytic count (TLC) showed significant increase in infected groups of quails and chickens. Heterophils and eosinophils were increased significantly in all the infected groups of quails and chickens. Lymphocytes decreased significantly in all the infected groups. Biochemical study showed that total serum protein decreased significantly in the infected groups of quails and chickens. Serum albumin level was significantly lower in all the infected groups of quails and chickens. Serum globulin and albumin: globulin (A:G) ratio failed to show any significant difference between control and infected groups of quails and chickens.

Key words: Japanese quails, chickens, haematological changes, biochemical changes, *Ascardia galli*

Introduction

In most of the developing countries, including India, teeming with millions of poultry, are at a stage of perpetual protein hunger. Poultry meat and eggs, though the major source of animal protein, is still now unable to meet up the protein hunger of the world (Gaffer, 1986).

During the last two decades, India has had a remarkable growth in poultry industry. India's egg production was 2 million tones in the year 2002 and remained amongst the top 5 of egg producing countries in the world. The broiler meat production was 1.566 million tones in the same year (Poultry International, Vol-42, No. 11, Nov. 2003).

Nowadays, quail farming is cropping up as a new venture of diversification of poultry farming, not only to diverse the choice of taste but also to strengthen the meat production unit for fulfilling the shortage of animal protein demands amongst the 'Non-Veg' peoples of India.

In India, Japanese quail (*Coturnix coturnix japonica*) breeding for egg and meat production has been introduced very recently. Since 1974, research work on the various aspects of quail rearing such as breeding, feeding and management, disease control etc. have been taken up by the Central Avian Research Institute of Izatnagar and it is attracting the poultry farmers and consumers for quails egg and meat.

Quails and domesticated fowls belong to the same sub-family Phasioxidas. Quails possess an excellent disease resistance quality than those of chickens and have been chosen for its economical viability in farming. Japanese quails are rapidly gaining popularity for its commercial exploitation and in near future, may acquire an important segment in rapidly expanding Indian poultry industry. Among the helminth parasites, *Ascaridia galli* is most prevalent species occurring in most of the fowls. Due to the close association and frequent indirect contact between the quail and chicken rearing units in the same premises, there is a possibility that *Ascaridia galli* might establish themselves as a parasite for the quails as a new host (Singh *et al*., 1996). It will not only directly cause damages to the host but also may predispose the quails to other infections. The present experiment was carried out to observe a comparative haematological and biochemical changes in Japanese quails and chickens that occurs due to *Ascaridia galli* infection.

Materials and Methods

Source: A total of 24 day old chicks of Japanese quail and same number of Rhode Island Red (RIR) day old chicks were obtained from State Poultry Farm, Tollygunj, Kolkata, India.

Feeding and management: The Japanese quail chicks and poultry chicks were reared separately in brooder at experimental house for two weeks. Afterwards, the poultry and quail chicks were randomly distributed in four groups of each with three infected and one control group as PC, p1, p2 and p3 and Q_C, q1, q2 and q3 respectively. Each small group contained six numbers of birds and was maintained in separate wire mesh battery

cages. Each bird was marked with leg bands. Regular cleaning and aseptic measures were followed with regular feeding and watering. The quails were maintained on balanced broiler starter ration throughout the life. The fowl chicks were also offered broiler starter ration up to three weeks of age and then was shifted to balanced poultry ration till the end of the experiment.

Collection and culture of *Ascaridia galli* eggs: The adult female worms of *Ascaridia galli* were collected from the intestine of desi (local) fowl, procured from poultry slaughter houses. The worms were thoroughly washed in normal saline solution to remove the mucous and other debris's. The anterior portion of the worm was taken on a slide and was cut off near the vagina by a sharp scalpel. The worm was then held at the posterior end by a forceps and pressure was applied by a needle to takeout the gravid uterus containing mature eggs. The uterus was then disintegrated with the help of a needle to get the eggs free from the uterus. It was then cultured in 0.5% formalin solution in a petridish to prevent bacterial growth. The culture was shaken daily for aeration. Fresh media was added to replenish the volume. The development of eggs was examined regularly till they reach the infective stage. The second stage infective larvae developed in 15-20 days in the B.O.D. incubator. The temperature in B.O.D. was maintained at 28±1°C as described by Mallik (1981).

Counting of infective eggs: When infective stage was reached, the eggs were collected in a centrifuge tube containing saline and centrifuged at 1000 rpm for five minutes, after which the supernatant fluid was discarded. The process was repeated three times. Then a small quantity of distilled water was added to the sediment and mixed thoroughly. Finally the egg suspension was prepared in measured volume of distilled water. From this, 0.1 mL of homogenous suspension was taken on microslide and covered with coverslip. The numbers of infective *Ascaridia galli* egg in 0.1 mL of the suspension was counted under a compound microscope. Five such counts were made and the mean infective egg concentration per 0.1 mL of suspension was calculated (Choudhury, 1989). The infective doses of eggs i.e., 100, 500 and 1000 numbers of eggs were adjusted in 1, 1 and 2 mL of the suspensions respectively.

Experimental designs: Chickens as well as quails were divided into four small groups with each consisting 6 number of birds in each group. Chicken groups were marked as pC, p1, p2, p3 and quail groups were marked as qC, q1, q2 and q3.

Group pC and qC: The birds of these two groups were kept as control for comparison of all the parameters with infected groups.

Group p1 and q1: Each bird of these groups was infected orally with one hundred infective eggs at the age of twenty one days. Food and water was withdrawn for twelve hours before administration of infection (Shilaskar and Parasar, 1985). After giving infection birds were kept under observation to see the effect of infection.

Group p2 and q2: Each bird of these groups was infected orally with five hundred infective eggs on twenty one days age. The procedure adopted for giving infection was same as Group q1 and p1.

Group p3 and q3: Each bird of these groups was also infected orally with one thousand infective eggs at the same age and the procedure of giving infection was same as previous groups.
All the birds were sacrificed on 9th week post infection.

Methods

Haematological: Blood was collected from the individual birds of each group at the time of slaughter from jugular vein. Sterile vials with 20 µL of 10% EDTA were used as anticoagulant for collection of blood. Two milliliters of anti-coagulated blood was collected from each bird and was kept in refrigerator for haematological studies. TEC and TLC were done by Neubauer haemocytometer. The Rees and Ecker solution was used as diluting fluid as described by Sastry (1983). DLC was estimated by using Wright-Giemsa stain as per method described by Schalm *et al.* (1986). Hb concentration was estimated by cyanmethemoglobin method as described by Dacial (1985). PCV was determined by Wintrobe haematocrit method as described by Schalm *et al.* (1986).

Biochemical: Blood was collected from the individual birds of each group at the time of slaughter from jugular vein. Two milliliters of blood was collected from each bird in sterile test tubes without anticoagulant and allowed to clot. Serum was separated out and kept at 20°C until analysis. Total protein (g dL^{-1}) and albumin (g dL^{-1}) were estimated by Biuret and Dumas method as described by Dumas *et al.* (1971) by using SPAN diagnostic kit (Code No. 25931). Serum globulin (g dL^{-1}) was estimated as a difference between total protein and albumin. The A:G ratio was calculated by dividing the concentration of albumin in (g dL^{-1}) by concentration globulin (g dL^{-1}).

Statistical analysis: All the data obtained in respect to haematological and biochemical parameters studied during experiment were statistically analysed for test of significant (Table 3-6) as per statistical methods of Snedecor and Cochran (1967).

Results and Discussion

Haematological changes: In quails, TEC decreased significantly ($p<0.01$) in group q1 and q2, but group q3 were non significant with that of control group. Tanwar *et al.* (2001) also found similar observation in their experiment. TEC values were significantly lower by 1% level in all the infected groups of chicken than that of control group.

Lowered TEC in *Ascaridia galli* infected chickens and quails might be due to lowered erythropoesis. *A. galli* are usually associated with mild/acute enteritis which hampers the absorption of essential nutrients for blood cell formation. Group q3 showed no significant change in TEC.

In quails, PCV decreased significantly ($p<0.05$) in all the infected groups than that of control group. PCV percentage in chickens of group p1 and p2 decreased significantly ($p<0.01$) and in group p3 the same was decreased ($p<0.05$) to that of control group. Matta and Ahluwalia (1982) recorded the same finding in their experiment with fowls infected with *Ascaridia galli*. PCV may have decreased due to the lower concentration of erythrocytes per unit volume of blood in the infected group of chickens and quails.

The haemoglobin percentage lowered significantly ($p<0.05$) in all the infected groups of chicken in comparison to that of control group. In quail, Hb percentage reduced in group q1 and q2 ($p<0.01$) and in group q3 ($p<0.05$) to that of control group. This finding is in concordance with the finding of Matta and Ahluwalia (1982) and Kumar *et al.* (2003). They opined that lowered haemoglobin concentration in infected birds was correlated with the activities of early larval stage of *A. galli* in the process of penetration with resultant destruction of mucosa of small intestine and rupture of small blood vessels. Kumar *et al.* (2003) also cited that fall of Hb content might be due to metabolic disturbance caused by worms rather than a direct blood loss. In the present study, the TLC in quails were significantly ($p<0.01$) higher in all the infected groups to that of control group. The TLC in chickens of group p1 and p2 increased significantly ($p<0.05$) whereas group p3 did not show significant increase to that of control group. This is in agreement with the findings of Tanwar and Mishra (2001).

In chickens, the heterophil percentage increased significantly ($p<0.01$) in group p1 and p2 while in group p3 it was increased ($p<0.05$) to that of control group. The quails of group q1, q2 and q3 also showed the similar results with that of chicken and this finding simulated the findings of Tanwar and Mishra (2001). They expressed that heterophils are actively amoeboid and phagocytic in

Table 1: Haematological profiles of Japanese quails and chicke^{NS} infected with *ascaridia galli*

Groups	TEC (10^6/Cu mm)	Hb (%)	PCV (%)	TLC (10^3/Cu mm)	Heterophil (%)	Eosinophil (%)	basophil (%)	Lymphocytes (%)	Monocytes (%)
qC	4.040^{a}	11.120^{a}	35.083^{a}	24.448^{a}	30.333^{a}	7.5^{a}	2.5^{a}	54.000^{a}	5.667^{a}
	±0.120	±0.308	±1.235	±0.664	±0.558	±0.428	±0.224	±0.577	±0.422
pC	3.685^{a}	11.373^{a}	33.033^{a}	29.054^{a}	35.5^{a}	6.833^{a}	1.833^{a}	50.667^{a}	5.167^{a}
	±0.159	±0.608	±1.349	±0.966	±0.764	±0.477	±0.401	±0.615	±0.543
q1	3.087^{**b}	9.267^{**c}	3.087^{**b}	31.325^{**b}	35.833^{**c}	10.667^{**b}	1.333^{NS}	45.833^{**b}	6.333^{NS}
	±0.125	±0.480	±0.125	±0.763	±1.167	±0.495	±0.211	±0.946	±0.823
p1	2.480^{**b}	8.895^{*b}	24.657^{*c}	32.354^{*b}	41.0^{**c}	10.5^{**c}	2.667^{NS}	37.667^{**b}	8.166^{**b}
	±0.206	±0.630	±0.846	±0.725	±0.730	±0.847	±0.667	±1.230	±0.477
q2	3.257^{**b}	9.565^{**c}	3.257^{**b}	29.868^{**b}	35.833^{**c}	11.000^{**b}	1.833^{NS}	43.667^{**b}	7.333^{NS}
	±0.175	±0.324	±0.175	±0.527	±1.470	±0.633	±0.401	±1.283	±0.715
p2	2.863^{**b}	9.085^{*b}	27.833^{**c}	32.260^{*b}	39.667^{**c}	9.5^{*b}	1.777^{NS}	43.833^{**b}	6.446^{NS}
	±0.102	±0.602	±0.994	±0.629	±1.117	±0.847	±0.495	±1.887	±0.882
q3	3.783^{NS}	10.027^{*b}	10.027^{*b}	29.692^{**b}	34.833^{*b}	10.000^{**b}	1.667^{NS}	45.500^{**b}	8.000^{NS}
	±0.13	±0.335	±0.335	±0.513	±1.302	±0.577	±0.333	±1.336	±0.365
p3	3.014^{**b}	9.314^{*b}	29.008^{*b}	31.058^{aNS}	38.444^{*b}	9.167^{*b}	2.433^{NS}	42.167^{**b}	6.566^{NS}
	±0.150	±0.618	±0.815	±0.681	±0.667	±0.601	±0.422	±1.797	±0.428

**Mean±SE (row-wise) bearing different superscripts differs significantly ($p<0.01$); *Mean±SE (row-wise) bearing different superscripts differs significantly ($p<0.05$); ^{NS}Non significant

Table 2: Bio-chemical profiles of Japanese quails and chickens infected with Ascaridia galli [Mean±SE]

Groups	Total protein (gm/100 mL)	albumin (gm/100 mL)	Globulin (gm/100 mL)	A:G ratio
qC	5.446^{a}±0.237	2.506^{a}±0.099	2.973^{a}±0.279	0.904^{a} 0.164
pC	3.857^{a}±0.207	1.775^{a}±0.213	2.082^{a}±0.167	0.883^{a}±0.124
q1	4.604^{*b}±0.265	1.894^{**b}±0.071	2.544^{NS}±0.142	0.733^{NS}±0.038
p1	2.923^{**c}±0.109	1.065^{**c}±0.041	1.859^{NS}±0.107	0.592^{NS}±0.043
q2	4.641^{*b}±0.197	1.979^{**b}±0.062	2.661^{NS}±0.184	0.761^{NS}±0.054
p2	3.103^{**c}±0.133	1.146^{**c}±0.044	1.957^{NS}±0.159	0.611^{NS}±0.067
q3	4.970^{NS}±0.218	1.916^{**b}±0.125	3.053^{NS}±0.194	0.643^{NS}±0.066
p3	3.192^{*b}±0.165	1.194^{*b}±0.079	1.998^{NS}±0.163	0.619^{NS}±0.072

**Mean±SE (row-wise) bearing different superscripts differs significantly ($p<0.01$); *Mean±SE (row-wise) bearing different superscripts differs significantly ($p<0.05$); NS:Non significant

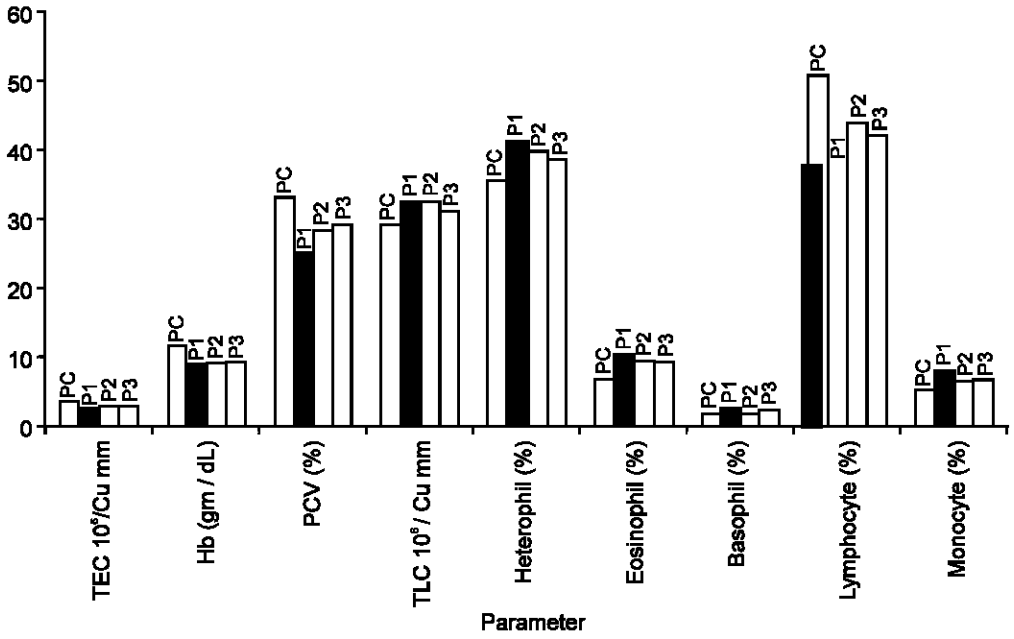

Fig. 1: Haematological profiles of the chickens infected with *A. galli*

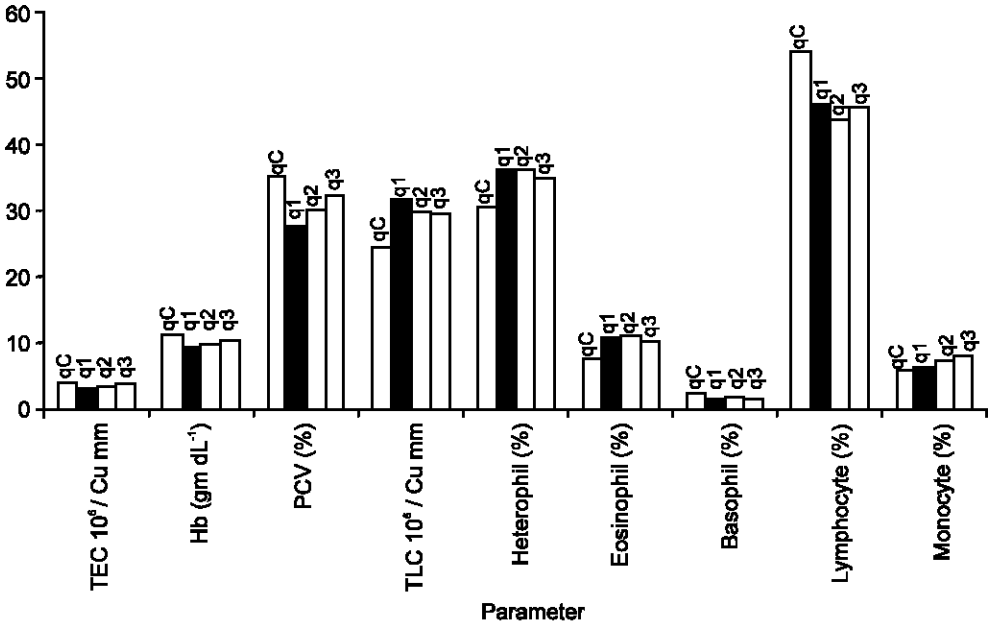

Fig. 2: Haematological profiles of the Japanese quails infected with *A. galli*

nature. The phagocytic action of heterophils may thus correlate with their increased number as a first line of defence of the host in the present study. Eosinophil percentage in chicken increased significantly ($p<0.01$) in group p1 and p2 while in group p3 the same was increased to $p<0.05$. In quails the significant increase of eosinophils in all the infected groups was ($P<0.01$) in comparison to that of control group.

Tanwar and Mishra (2001) and Kumar *et al.* (2003) had also observed the same result in their experiment. They observed that increased number of eosinophils in the blood is an indication of parasitic infection. Tanwar and Mishra (2001) reported that the net increase in the total leucocytic count might be due to the increase in heterophils and eosinophils.

In the present study, the lymphocyte percentage were significantly decreased ($p<0.01$) in all the infected groups of chickens and quails to that of control group. This finding was in accord with the findings of Tanwar and Mishra (2001).

Table 3: Analysis of variance of haematological parameters in chickens infected with *Ascaridia galli*

		TEC (10^6/Cu mm)		
SOV	df	SS	MS	F
Between groups	3	4.584	1.516	12.426**
Within groups (Error)	20	2.44	0.122	
		Hb (gm/dl)		
SOV	df	SS	MS	F
Between groups	3	23.224	7.741	3.489*
Within groups (Error)	20	44.394	2.219	
		PCV (%)		
SOV	df	SS	MS	F
Between groups	3	202.871	67.624	10.512**
Within groups (Error)	20	128.653	6.433	
		TLC (10^3/Cu mm)		
SOV	df	SS	MS	F
Between groups	3	33.282	11.094	3.196*
Within groups (Error)	20	69.426	3.471	
		Heterophil (%)		
SOV	df	SS	MS	F
Between groups	3	99.458	33.153	7.878**
Within groups (Error)	20	84.167	4.208	
		Eosinophil (%)		
SOV	df	SS	MS	F
Between groups	3	44.90	14.967	4.935**
Within groups (Error)	20	60.667	3.033	
		Basophil (%)		
SOV	df	SS	MS	F
Between groups	3	2.458	0.819	0.520^{NS}
Within groups (Error)	20	31.50	1.575	
		Lymphocyte (%)		
SOV	df	SS	MS	F
Between groups	3	523.449	174.50	13.406**
Within groups (Error)	20	260.334	13.017	
		Monocyte (%)		
SOV	df	SS	MS	F
Between groups	3	27.458	9.153	4.114*
Within groups (Error)	20	44.5	2.225	

**(p<0.01), *(p<0.05), NS:Non significant

Table 4: Analysis of variance of haematological parameters in quails infected with *Ascaridia galli*

		TEC (10^6/Cu mm)		
SOV	df	SS	MS	F
Between groups	3	3.570	1.19	10.085**
Within groups (Error)	20	2.360	0.118	
		Hb (gm/dl)		
SOV	df	SS	MS	F
Between groups	3	11.058	3.686	5.098**
Within groups (Error)	20	14.468	0.723	
		PCV (%)		
SOV	df	SS	MS	F
Between groups	3	185.575	61.858	8.749**
Within groups (Error)	20	141.414	7.07	
		TLC (10^3/Cu mm)		
SOV	df	SS	MS	F
Between groups	3	163.467	54.489	23.629**
Within groups (Error)	20	46.115	2.306	
		Heterophil (%)		
SOV	df	SS	MS	F
Between groups	3	124.125	41.375	4.990**
Within groups (Error)	20	165.833	8.292	
		Eosinophil (%)		
SOV	df	SS	MS	F
Between groups	3	45.125	15.042	8.635**
Within groups (Error)	20	34.833	1.742	
		Basophil (%)		
SOV	df	SS	MS	F
Between groups	3	4.333	1.444	2.625^{NS}
Within groups (Error)	20	11.000	0.550	
		Lymphocyte (%)		
SOV	df	SS	MS	F
Between groups	3	380.833	126.944	18.179**
Within groups (Error)	20	139.667	6.983	
		Monocyte (%)		
SOV	df	SS	MS	F
Between groups	3	19.333	6.444	2.929^{NS}
Within groups (Error)	20	44.000	2.200	

**(p<0.01), *(p<0.05), NS:Non significant

Chickens of group p1 showed a significant (p<0.01) increase in monocyte percentage whereas group p2 and p3 were non significant to that of control group. In all the infected groups of quail, the percentage of monocytes were non significant to that of control group. The data's are summarized in Table 1.

Biochemical changes: In the present study, the total protein showed a significant decrease (p<0.05) in group q1 and q2 while group q3 failed to show any significant difference with control group. In chickens, the total protein was significantly lower in group p1 and p2 (p<0.01) rather than in group p3 (p<0.05). This finding was in agreement with the findings of Tanwar and Mishra (2001).

Hypoprotenaemia might occur due to increased motility of intestine as in diarrhoea. In that case the proteins might get lost from the bowel. Coles (1967) reported that a considerable loss of tissue protein may occur through leakage into gut with loss of digestive secretion and mucous due to intestinal parasitism in anaemic birds, which also cause inefficient protein absorption and utilization in the system to the extent of leading to marked decrease in serum protein.

In quails, the entire three infected group showed a significant (p<0.01) lower albumin level than that of the control group. The level of albumin was significantly lower in chickens of group p1 and p2 (p<0.01) while in group p3 the same was decreased (p<0.05).

The decrease in albumin concentration is a common

Table 5: Analysis variance of bio-chemical parameters in chickens infected with *Ascaridia galli*

		Total protein (gm/100 mL)			Albumin (gm/100 mL)			Globulin (gm/100 mL)			A : G ratio		
SOV	df	SS	MS	F	SS	MS	F	SS	MS	F	SS	MS	F
Between groups	3	2.993	0.998	6.696**	1.894	0.631	8.892**	0.155	0.052	0.082^{NS}	0.346	0.115	2.883^{NS}
Within groups (Error)	20	2.988	0.149		1.423	0.071		12.698	0.635		0.804	0.040	

**(p<0.01), NS:Non significant

Table 6: Analysis variance of bio-chemical parameters in quails infected with *Ascaridia galli*

		Total protein (gm/100 mL)			Albumin (gm/100 mL)			Globulin (gm/100 mL)			A : G ratio		
SOV	df	SS	MS	F	SS	MS	F	SS	MS	F	SS	MS	F
Between groups	3	2.976	0.992	3.109*	1.520	0.510	10.000**	1.07	0.357	1.405^{NS}	0.212	0.071	1.341^{NS}
Within groups (Error)	20	6.385	0.319		1.028	0.051		5.072	0.254		1.067	0.053	

**(p<0.01), *(p<0.05), NS:Non significant. SOV: Source of variation. df: degree of freedom

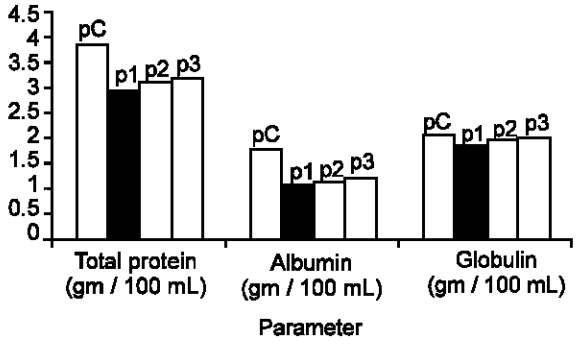

Fig. 3: Biochemical profiles of chickens infected with *A. galli*

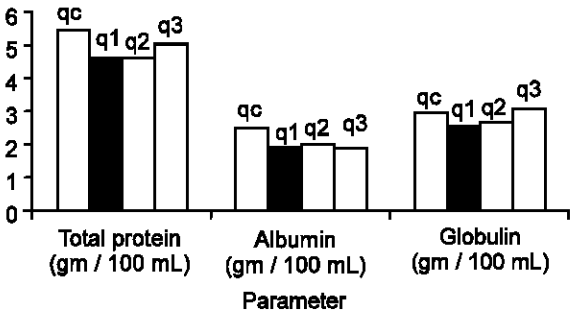

Fig 4: Biochemical profile of Japanese quails infected with *A. galli*

form of hypoproteinaemia due to its small size and osmotic sensitivity to fluid movement. The albumin is selectively lost in intestinal parasitism. The hypoalbuminaemia of intestinal parasitism is aggravated by increased albumin catabolism (Tanwar and Mishra, 2001).

Globulin percentage in the infected groups of chicken and quail did not show significant changes with that of control groups and are in conformity with the findings of Tanwar and Mishra (2001).

In the present study, the protein profile of both chicken and quails indicated hypoproteinaemia with no change in globulin and in A:G ratio. The results are summarized in Table 2.

Conclusion: Haematological study showed that the total erythrocytic count (TEC), packed cell volume (PCV) and haemoglobin (Hb) percentage decreased significantly in infected groups of quails and chickens except that group q3, which did not show any significant difference in TEC and PCV when compared to the control group. The total leucocytic count (TLC) showed significant increase in infected groups of quails and chickens whereas group p3 failed to show significant difference. Heterophils and eosinophils increased significantly in all the infected groups of quails and chickens whereas monocytes increased only in group p1. Lymphocytes decreased significantly in all the infected groups.

Biochemical studies showed that total serum protein decreased significantly in the infected groups of quails and chickens except the group q3, which was non significant. Serum albumin level was significantly lower in all the infected groups of quails and chickens. Serum globulin and albumin:globulin (A:G) ratio failed to show any significant difference between control and infected groups of quails and chickens.

From the above study, it was observed that quails were also susceptible to helminth parasites which are usually prevalent in chicken, but the quails are slightly more resistant to infections than chickens. As the quails possess an excellent disease resistance quality against the parasitic infection than those of chickens, they can be chosen for economical viability in farming.

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