HEMATOLOGICAL STUDY IN THREE LINES OF QUAIL AND THEIR CROSSES

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ABSTRACT:
The present study was conducted at poultry laboratories, department of animal production, Agriculture College, Duhok University, Kurdistan region, Iraq, (2016). Three lines of quail (White, light brown and dark brown) and their crosses that resulted from diallel cross design; aged 8 weeks old were used. A total of 54 blood samples by about 5 ml, from both sexes within each genotype aged 8 weeks old, were collected directly after slaughtering and bleeding for 3 second. PVC %, hemoglobin (Hb), Hetrophyl (H), Lymphocyte (L), H/L ratio, total protein, globulin, albumen and cholesterol were studied. The results showed insignificant differences among genotypes and between sexes for both PVC and Hb, while H, L, H/L, total protein, globulin, albumen and cholesterol appeared high significant (p<0.01) differences among the nine studied genotypes. The effect of sex was significant (p<0.05) for H, L and cholesterol. Interaction between genotype and sex was just significant for albumen. There was a significant negative correlation between H/L ratio and globulin (-0.39). Dependence of H/L ratio on globulin, resulted in derives a prediction equation.

Keywords: Quail, Hematological parameters, Genotypes, Crosses.

1. INTRODUCTION

Crossing in poultry plays an important role in changing the values of some blood parameters; On the other hand, the blood parameters affected by different factors such as genotype, sex, physiological conditions and laying period (Suchy *et al.* 2000; Gyenis *et al.* 2006; Pavlik *et al.* 2009). Poultry genotypes resulted from crossing need to be tested physiologically through blood samples, in older to know how the hematological parameters may change by crossing.
Dowidar *et al.* (1999) reported significant differences in total protein and cholesterol of chicken's blood samples. Hematological studies on some poultry species exposed to cross breeding were investigated by several studies (Elnaggar *et al.* 2001; El-Kaiaty &Hassan, 2004; El-Tahawy, 2005 and Abd El-Aziz, 2006). A study on J. quail conducted by Farahat *et al.* (2010), showed that all studied hematological parameters (Ht %, Hb, RBC, WBC, Hetrophyl and Lymphocytes) were not statistically significant between both sexes, they added that all these parameters correlated significantly to each other, and they concluded that blood parameters in J. quail may use as selection criteria for genetic improvement in the future. However, several research workers indicated changing in some blood biochemical parameters in chicken's breeds and their cross, such as Hetrophyl to Lymphocytes ratio (H/L ratio) (Fathi *et al.*, 2005) total protein, albumen and globulin (Mahrous *et al.*, 2008); total cholesterol (Mahrous and El- Dlebshany, 2011). Moreover, (Peters *et al.* 2011) reported significant (*p<0.05*) difference between pure breeds and their crosses in respect to PCV, serum glucose, cholesterol and globulin in Nigerian native fowl. Aly *et al*. (2006), mentioned significant differences among all studied genotypes (pure strains and their crosses) of Egyptian local strain of chickens concerning all studied blood hematology parameters, they added that to concerning blood biochemical parameters, the pure strains had

nearly significantly similar averages of serum protein, while one strain had the highest serum albumin average and the other strain had the highest cholesterol content, which mean that the hematological and biochemical parameters may differed from strain to another. So, the present investigation aimed to study some hematological and biochemical parameters in blood samples of three quail lines and their crosses.

2. MATERIAL AND METHODS

The present study was conducted at poultry laboratories, department of animal production, Agriculture College, Duhok University, Kurdistan region, Iraq in 2016. Three lines of quail (White, light brown and dark brown) were reared and crossed using full diallel cross design; nine genotypes were obtained as progeny (three genotypes from each of pure breeds, crosses and their reciprocal crosses). The experiment was done on the progeny. Management and rearing conditions were same as possible throughout the whole trial periods, where the light was provided as 15 h/d. The amount of energy and protein in the ration submitted to the layer show in table (1).

Table 1. Amount of energy and protein of the Layer ration which submitted to the birds during the trial period

Calculated Chemical composition	
ME (Kcal/kg)	2838.4
Crude protein (%)	17.83

2.1 Blood sample collection and analysis

A total of 54 blood samples by about 5 ml, from both sexes within each genotype of quail birds aged 8 weeks old, were collected directly after slaughtering and bleeding for 3 second. 2 ml of the blood was dropped into the test tube containing ethylene diamine tetra-acetic acid (EDTA) for hematological parameters; packed cell volume (PCV) or hematocrit,

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hemoglobin concentration (Hb)**,** Heterophil (H) / lymphocyte (L) ratio (H/L ratio) analysis. The other 3 ml was placed in sterilized glass test tubes without anticoagulant and left at room temperature for two hours, then centrifuged (3000 RPM) for 15 min and the serum was discrete by micropipette and put into tubes. After that it stored at -20 ℃ until the analysis for biochemical parameters; total protein (TP), albumin (A), globulin (G) and cholesterol (Ch).

The (PCV) was determined by the micro hematocrit method (Archer, 1965). The capillary tube was filled with blood then closed with rubber mud immediately, after that put in the micro-hematocrit centrifuge for15 minutes, then calculate the percentage of the size of blood cells by using a special ruler. The hemoglobin concentration estimated by using Drabkin's reagent which converts hemoglobin to a Cyanomethemoglobin, 20μl of blood was mixed with 5 ml of reagent then placed in the centrifuge speed (5000 r / min) for 15 minutes to get rid of nuclei and membrane of red blood cells, then redd by spectrophotometer according to Varley et al, (1980).

Granular (heterophils) and non-granular (lymphocyte) ratio was calculated by using a drop of blood on the slide and distributed by another slide at an angle of 45 without the pressure, then pigmented with Gimsa stain for 15 mints and washed with distell water after the smear was drying and the numbers were read Heterophil and lymphocyte under microscope on the strength enlarges ×100 (put a drop of oil on the slide).

Serum cholesterol concentration, total protein and albumin were determined by using analyzing material (kit) produced by BIOLAB SA, France by using spectrophotometers. Globulin was calculated as the difference between total protein and albumin.

2.2 Statistical analysis

The collected data were analyzed statistically by SAS software (SAS, Institute, 2010) using GLM procedure and according to the following models:

$$Y_{ijk} = \mu + G_i + S_j + (G*S)_{ij} + e_{ijk}$$

Where: Y_{ijk} = the studied parameters in k bird of j sex and i genotype; μ = the overall mean; G_i = the effect of genotype*; S_j* = the effect of sex; $(G*S)_{ij}$ = the interaction between the genotype and sex *and e_{ijk}* = the experimental error.

The correlation coefficients between studied parameters and the dependence of H/L ratio on globulin parameter were analyzed within the same software. The differences between sexes mean and among genotypes means were tested by Duncan multiple ranges test (Duncan, 1955).

3. RESULTS AND DISCUSSION

3.1 Hematological parameters:

3.1.1 PCV % and Hemoglobin:

The results of both Hematocrit (PCV %) and Hemoglobin (g/dl) as affected by the studied genotypes are represented in Table (2). There are insignificant differences among all genotypes for both studied parameters (*P>0.05*), but it could be noticed that LD cross genotype recorded the highest PCV (44.25 %) with the lowest CV (8.7 %), while the lowest percentage of PCV was recorded by WD cross genotype (34.3 %) with intermediate CV (11.8 %). Also, the other genotypes were resulted in relatively medium to high CV % for the PCV %, which reflect the presence of some extreme values that showed insignificant differences among the genotypes. The same trend was found for Hemoglobin values, because the latter one derived from the former. The present results are in agreement with the findings of Dief *et al.* (2007) who reported insignificant differences in Hematocrit % between two broiler strains. On the other hand, the present results are disagreement with the findings that reported by Galal *et al*. (2008) on laying hens.

Table 2. Effect of genotype on both hematocrit and hemoglobin

Genotype		Hematocrit (PCV %)		Hemoglobin (g/dl)	
		Mean ± SE	CV%	Mean ± SE	CV%
Purebred	WW	33.5±2.3	13.4	11.15±0.74	13.5
	LL	33.75±1.5	8.8	11.2± 0.5	8.7
	DD	37.75±3.1	16.4	12.58±1.0	16.4
Crosses	WL	37.33±5.0	23.4	12.4±1.7	23.3
	WD	34.3±2.3	11.8	11.4±0.75	11.5
	LD	44.25±1.9	8.7	14.7±0.6	8.7
Reciprocal crosses	LW	33.5±4.3	25.8	11.12±1.4	25.8
	DW	37±3.4	18.3	12.27±1.1	18.4
	DL	40.5±2.3	11.5	13.48±0.8	11.3
	Sig.	ns		ns	

The means with different letters differ significantly.

Regarding to the effect of sex on both PCV % and Hemoglobin, the results showed insignificant effect (*P>0. 05*) as illustrated in Fig. 1. However, the interaction between genotype and sex was insignificant. On the contrary of the, Kosshak *et al*. (2014) who found that females of quail had higher values of hemoglobin than males, and they attributed their result to the fact that before sexual maturity age, the physiological parameters changes in metabolism of female of J. quail, while PCV % had no significant differences between both sexes.
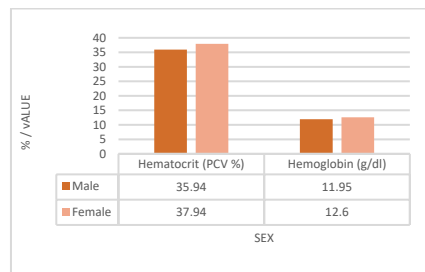

Figure 1. Effect of sex on both PCV and hemoglobin in genotypes quail.

3.1.2 Heterophil and Lymphocyte

The results of heterophil, lymphocyte and their ratio as affected by studied genotypes are representing in table (3). As it is shown, there are a significant (*P<0.01*) differences among the studied genotypes for H, L and H/L. Regarding to H, the results

showed that the pure line W had the highest value (59.25) with the lowest CV %, while the LL and LD crosses had the lowest H value with (25 and 29.5, respectively) with relatively high CV %. The all other genotypes had intermediate values. This result indicates that W line may have a high immunity response to bacterial infections, while L line and LD crosses have the lowest response. In respect to lymphocyte, the opposite results were found, where the pure line L line and the cross LD have the highest values (75 and 70.5, respectively), while the lowest value was recorded for the pure line W (40.8), and the other genotypes were recorded intermediate values. This finding may illustrate the possible high response of both L and LD

genotypes to viruses, while the opposite is right for W pure line, which was affective against bacteria. In regard to H/L ratio, the results showed that the highest ratio was recorded for the W pure line (1.48) and the lowest one was recorded for the L pure line (0.33), this insure the possibility of W pure line to response more to bacteria than all the other genotypes, while the L pure line more response to viruses. The remaining crosses (including LD cross) and reciprocal crosses had intermediate response to both bacteria and viruses, because H/L ratio is determining the immunity response not H or L alone. Makram *et al.* (2014) reported significant differences between duck strains for H and L.

Table 3. Effect of genotype on hetrophil, lymphocyte and hetophil/lymphocyte ratio

Genotype		Hetrophile		Lymphocyte		Hetrophil / lymphocyte	
		Mean ± SE	CV%	Mean ± SE	CV%	Mean ± SE	CV%
Pure breed	WW	59.25±2.8 [a]	9.6	40.8±2.8 [e]	13.9	1.48±0.2 [a]	26.4
	LL	25±1.8 [e]	14.2	75±1.8 [a]	4.7	0.33±0.03 [e]	20.6
	DD	30.75±3.9 [de]	25.6	69.25±3.9 [ab]	11.4	0.46±0.07 [de]	34.3
crosses	WL	33.75±2.4 [cde]	14.2	66.25±2.4 [abc]	7.2	0.52±0.05 [cde]	22.1
	WD	38.75±4.3 [bcd]	22.0	61.25±4.3 [bcd]	13.9	0.66±0.1 [bcd]	37.4
	LD	29.5±3.8 [e]	25.7	70.5±3.8 [a]	10.8	0.43±0.1 [de]	39.6
Reciprocal cross	LW	44.25±2.2 [b]	9.8	55.75±2.2 [d]	7.8	0.80±0.1 [b]	18.1
	DW	40.25±2.3 [bc]	11.2	59.75±2.3 [cd]	7.5	0.68±0.1 [bcd]	18.6
	DL	42±3.6 [bc]	17.3	58±3.6 [cd]	12.5	0.75±0.1 [bc]	29.1
Sig. (*p*)		0.0001		0.0001		0.0001	

The means with different letters differ significantly.

Regarding to the effect of sex on H, L and H/L ratio, the Fig. (2). It was illustrated that males were significantly (*P<0.05*) surpassed females for H, while the opposite was true for L, where females were significantly (*P<0.05*) surpassed males. This may mean that the quail male response to bacteria more than female, while the latter is more response to viruses**) Uduak** et al. 2015**).** The present results are disagreement with that found by Farahat *et al*. (2010) who reported insignificant differences between both sexes for H and L. However, Muhammad (2013) illustrated that H/L ratio of adult females of golden feathers quail was higher than those of males. The interaction between genotype and sex was insignificant.
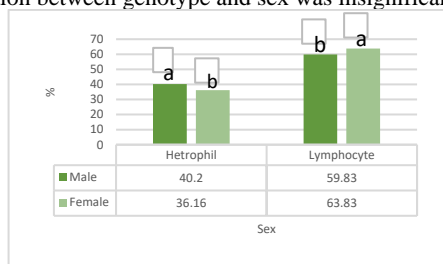

Figure 1. Effect of sex on both Hetrophil and Lymphocyte

3.2. Biochemical parameters:

The results of biochemical parameters are represented in Table (4). It could be noticed that all studied parameters were different significantly (*p<0.01*) among the studied genotypes. Total protein parameter in the cross LD and both reciprocal crosses LD & DW had the highest values (46.25-47.25 g/l) with small CV % compared to the other genotypes. However, the lowest value was recorded to W line (30.25 g/l). Regarding to Albumen, the same trend of total protein for the highest values was found, but the lowest values were recorded for D line and WL cross. In respect to globulin, it could be noticed that the reciprocal cross LW had the highest value (34.02 g/l), while the lowest ones was recorded by W line and cross WL (15.8- 18.9 g/l). This finding may illustrate that when L line used as a sire and W line used as a dam, resulted in the best immunity response, and the opposite is true when the former used as a dam and the later used as a sire. The result of cholesterol in the previous table (4), showed the superiority of the cross WD (103.8 mg/dl) on the remaining genotypes, while the lowest one was recorded for the L line (71.75 mg/dl), this result may reflect the interaction between both purposes of production in respect to raising cholesterol, because both meat and egg yield are sharing to elevate the triglycerides contents, while the other genotypes may include L line (which considered as dual purpose line) as a sire or as a dam. The present results are in agreement with Aly *et al*. (2006) findings on chickens and Uduak *et al*. (2013) findings on Turkey for the most biochemical studied parameters.

Table 4. effect of genotypes on biochemical parameters

Genotype		Total protein(g/l)		Albumin(g/l)		Globulin (g/l)		Cholesterol(mg/dl)	
		Mean ± SE	CV%	Mean ± SE	CV%	Mean ± SE	CV%	Mean ± SE	CV%
Pure breed	WW	30.25±1.4 [c]	9.5	14.5±1.3 [ab]	18.2	15.8±2.6 [d]	32.5	88±3.02 [bc]	6.9
	LL	37±2.1 [bc]	9.7	14.4±0.3 [ab]	3.8	22.56±2.4 [cd]	18.4	71.75±4.2 [d]	11.7
	DD	43.5±5.5 [ab]	25.3	12.5±0.2 [c]	3.4	31±5.4 [abc]	34.7	87.5±1.8[bc]	4.2
crosses	WL	31.75±1.4 [c]	8.6	12.8±0.1 [c]	1.7	18.9±1.3 [d]	13.5	97±0.7 [ab]	1.4
	WD	37.5±3.4 [bc]	18.2	13.5±0.33 [bc]	4.5	24±3.6 [bcd]	30.2	103.8±5.5 [a]	10.6
	LD	46.25±0.8 [a]	3.4	15.15±0.2 [a]	3.2	31.1±1.1 [abc]	6.9	78.75±1.5 [cd]	3.8
Reciprocal	LW	49.25±1.4 [a]	5.6	15.22±0.3 [a]	3.5	34.02±1.5 [a]	8.8	80±3.5 [cd]	8.8
	DW	47.25±0.9 [a]	3.6	15.05±0.3 [a]	3.3	32.2±1.1 [ab]	6.6	74±5.1[d]	13.8
	DL	36.25±1.4 [bc]	7.6	14.1±0.3 [ab]	4.2	22.15±1.5 [cd]	13.6	82.25±4.0 [cd]	9.8
Sig.(*p*)		0.0005		0.0001		0.001		0.0001	

The means with different letters differ significantly.

Effect of sex on the studied biochemical parameters was significant (*p<0.05*) only for cholesterol, where males had higher cholesterol level than females, the other parameters were not significantl (*p>0.05*). The present results are in agreement with the findings of Uduak *et. al.* (2013) in respect to all biochemical parameters except cholesterol level which was insignificant.
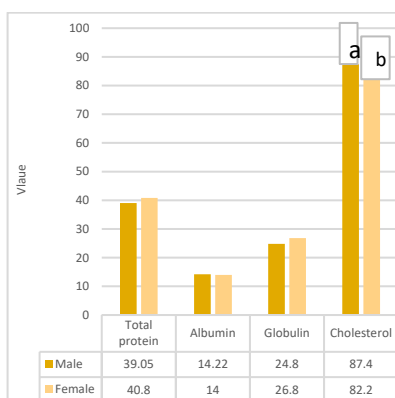

Figure 2. Effect of sex on biochemical parameters

However, the interaction between genotype and sex was insignificant for all biochemical parameters except albumen (Fig. 3), where the male of W line resulted in the highest value (16.5 g/l).
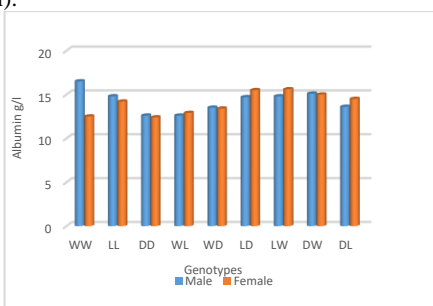

Figure 3. The interaction between genotype and sex on quail's albumin.

3.3. The relationship between hematological and biochemical parameters

The correlation coefficients between the studied hematological and biochemical parameters are shown in Table (5). It could be seen that most correlation coefficients are significant, and the highest positive significant (*p<0.01*) coefficient was recorded between total protein and globulin (0.98), this result is logical because the later derived from the former, while the highest negative significant (*p<0.05*) correlation coefficient was

recorded between total protein and cholesterol (-0.41), this result insure that protein play a reverse role to cholesterol (because cholesterol represent one of the most important compound of fat in the body). Also, it could be noticed that H/L ratio is correlated negatively and significantly (*p<0.05*) to globulin (-0.39), which mean that globulin value increase as H/L ratio decrease, this result indicate that globulin may work more as immunity defense system in case of virus attack more than the bacteria attack in the quail body. As it noticed from the Table (5) also, that H/L ratio correlated negatively and significantly (*p<0.05*) to total protein (-0.35), this result reflects the importance of protein in immunity response especially in case of virus attack. On the contrary, the correlation coefficient between H/L ratio and albumen is positive and significant (0.33), because albumen is the second component of total protein beside the globulin which was negative. Contrary to the relationship between H/L ratio and protein components, cholesterol seem to be play a reverse role with H/L ratio, where the correlation coefficient was negative and significant (*p<0.05*) between cholesterol and albumen (-0.36), while the correlation coefficient between cholesterol and globulin was positive and significant (*p<0.05*) and estimated as (0.34). This result is logical because of negative significant correlation between both total protein and cholesterol. Unfortunately, and according to global research, there were no references about the relationship between hematological and biochemical parameters in blood of quail resulted from diallel cross.

Table 5. Correlation coefficients between different blood parameters in quail

	PCV	HL ratio	total protein	albumin	globulin
HL ratio	-0.21	1			
total protein	0.29 *	-0.35 *	1		
Albumin	0.05*	0.33 *	0.21	1	
Globulin	0.28	-0.39 *	0.98 **	0.05	1
cholesterol	-0.11	0.18	-0.41 *	-0.36*	0.34*

*= significant at level (*p<0.05*); **= significant at level (*p<0.01*)

However, the linear regression analysis (Table 6) shows the dependence of H/L ratio on the globulin (g/l), in another word, it could be predicted with H/L ratio (as indicator to the hematological immunity response) from the globulin parameter value (biochemical immunity indicator). But unfortunately, the coefficient of determination (R^2) value for the prediction equation is limited (0.15), which prevent the present research to suggest this result as single indicator of immunity in the quail. The final prediction equation will be as following:

H/L ratio = -0.02 * (Globulin value) + 1.17

If the final resulted value is higher than 1, then the immunity of the quail is responding to the bacteria more than viruses, but in the opposite case the response will be more active against viruses. This way will help to predict the immunity response without measuring both H and L, in addition to eliminate the calculation of H/L ratio. Unfortunately, and despite to a wide survey there was no similar research which deal the same problem concerning with the prediction of H/L ratio from globulin in quail.

Table 6. Regression analysis for the dependence of H/L ratio on globulin

Multiple R	0.392645			
R Square	**0.15417**			
	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	**1.164542**	0.205511	5.666575	2.34E-06
b	**-0.01901**	0.007637	-2.48942	**0.017849**

4. CONCLUSION

It could be concluded that most hematological parameters of quail have changed according to the genotype.

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