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Full Length Article

# Haemato-Biochemical and Immunological Parameters of Naked Neck, Rhode Island Red, Fayoumi and Australorp Chickens in Pakistan

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# Abstract

This study aimed to investigate hematological, biochemical, and immunological parameters of four different breeds of 240day-old chicken reared under local conditions. The breeds included Naked Neck, Rhode Island Red, Fayoumi and Australorp. The primary objective was to compare the selected parameters among the four breeds and identify significant differences, if any. The study employed a four-group design (A, B, C and D) to represent each breed, and blood samples were collected from the birds to determine hematological, biochemical, and immunological parameters. The parameters considered in this study were red and white blood cell counts, hemoglobin level, Erythrocyte Sedimentation Rate (ESR), blood glucose and uric acid levels, cholesterol level, creatinine level, and antibody titer. Results indicated that significant differences existed among the breeds for some parameters. Australorp breed demonstrated the highest red blood cell count, lowest white blood cell count, highest hemoglobin levels, and lowest cholesterol levels. On the other hand, Naked Neck breed exhibited the highest white blood cell count, highest ESR, highest glucose and uric acid levels, as well as the highest antibody titer. Fayoumi breed demonstrated the highest antibody titer levels. Thus, this study showed that there were substantial differences in the measured parameters among the breeds of four different chickens under local rearing conditions. The findings of this study will be useful for future chicken breeding research. Moreover, it will enable hatcheries and poultry farmers to select the appropriate breed for their intended purpose based on the blood characteristics measured in this study. In conclusion, the current study informs the scientific community about the hematological, biochemical, and immunological features of four different chicken breeds under local rearing conditions. © 2024 Friends Science Publishers

Keywords: Hematology; Biochemical; Immunological; Antibody titers

## Introduction

The rural chicken are between the many limited resources of rural farmers living in the villages, which could be harnessed and are used in utilization for poverty easing (Njue *et al.* 2002). Though, selections in rural chickens have been adopted further in adapting the harsh environment and resistant to disease rather than improved productions (Minga *et al.* 2004). An evaluation of immunity responsive capacity of chickens becomes very important. Haemato-biochemical profiles have been described in providing important information's on the immunity condition of animal (Kral and Suchy 2000). These types of information's, apart from being useful for diagnosis and managemental purpose, can equally be included into breeding program for the genetical improvements of rural chicken.

One of the most significant regional breeds of chicken

raised in Pakistan is the naked neck. According to Mwacharo et al. (2007), Naked Neck chickens have an admirable resistance to several diseases and are well adapted to the harsh tropical climate and inadequate feeding circumstances. In the southern region of Tanzania, Black Australorp chickens are raised as egg birds and are superb scavengers (Mata and Mwakifuna 2012). Due to their rapid development rates and little variations in biochemical values between the three breeds, Rhode Island Red chickens have gained additional significance among the chickens introduced in Pakistan compared to other varieties (Khawaja et al. 2012). Furthermore, the breeds stay in Pakistan is long which has prepared it fully adjusted to the local environment and climatic conditions. Principally, this is a double-purpose chicken belonging to American class and gaining extra fame in villages as "Golden birds" (Ashraf et al. 2003). Since 1980, the Fayoumi chickens have been imported in our

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country Pakistan and are fully adjusted in local climatic and environment condition of villages. Because of their calm characters and excellent immune system against certain pathogens, farmer keeps these chickens at home and at farm fields (Rajput *et al.* 2005).

The mean value of serum triglyceride (528.0 mg/dL), glucose (221.8 mg/dL), total proteins (5.23 mg/dL) and uric acids (4.75 mg/dL) are in local birds (Bhatti *et al.* 2001). In some research experiments, there was no differences in blood cholesterol levels between the breed at egg laying stages, as is also noticed. There are no differences in Ca levels between three breeds at egg laying stage. The birds are known to be uniformly affected by the stages of eggs production during which there is mobilization of Ca for shells formations (Bhatti *et al.* 2002). When compared to blood profile, which may be used for nutritional research and the improvement of bird stock (Ladokun *et al.* 2008).

The body's transfer of nutrients, elimination of waste, and exchange of gases all depend on blood. The focus of the blood investigations is on the cellular and chemical components. The majority of the cellular components of the blood are examined by complete blood counts (Hrubec et al. 2002). The majority of nutritional research on birds employs haemato-chemical and serum biochemical profiles (Adeyemi et al. 2000). Additionally, blood parameters can provide fundamental knowledge for the research of immunological and comparative chicken diseases as well as aid in the diagnosis of certain poultry hen problems (Bonadiman et al. 2009). While reductions in blood cells suggest anemic conditions and put flocks at risk for infections, serum biochemical profiles of blood are used as supplementary assessments of dietary protein quality (Alikwe et al. 2010). A programme for the genetic development of birds with greater production and protection status can benefit significantly from an understanding of the genetics and relationship between different immune features and layer performance. According to Parmentier et al. (1998), birds with stronger antibody responses to sheep erythrocyte antigen solution develop more antibodies to a variety of antigens. Serum biochemical and Hematological response is used to measure the experimental and physiological sensitivity and as well as health of chicken (Sharma et al. 2015). Serum biochemical and Hematological parameters are influenced by feeds, medications, toxic compound, age, infection and gender of the bird (Huff et al. 2008; Schmidt et al. 2009).

The village farmers of Pakistan grow the indigenous Naked Neck, Rhode Island Red, Fayoumi and Australorp breeds extensively; however, there is very little information on the Haemato-biochemical and immunological qualities of these chickens. Similar restrictions apply to research on the heamatological and serum biochemical value of Naked Neck, Rhode Island Red, Fayoumi and Australorp during the various stages of growth. In this regard, to generate a base-line data on hematological and biochemical and immunological profile of these breeds accessible locally, some vital blood parameter of four rural breeds will be analyzed. The present studies would help evaluating chicken diseases, identifying of the healthy birds, improving of required breed, and scheming suitable breeding plans for poultry breed in Pakistan.

In order to analyze the blood under local environmental circumstances in Pakistan, the current research was carried out to compare the heamatological, biochemical, and immunological characteristics of Naked Neck, Rhode Island Red, Fayoumi and Australorp.

# **Materials and Methods**

## Experimental design and management

The current experimental trial was carried out at the Poultry Experimental Station Department of Poultry Husbandry Sindh Agriculture University Tandojam. Sixty (60) chicks from each of the four breeds-Fayoumi, Rhode Island Red, Naked Neck and Australorp-were obtained from the poultry research facility in Rawalpindi. The study took sixteen weeks to complete. The brooding and growing needs of the experimental birds were met in brooder-cum-grower buildings. Rice husk was used as litter, and it was adequately sterilized. Every week, the brooder homes were thoroughly cleaned and disinfected. For the first week, all of the experimental birds were hatched and raised at the standard temperature (95°F), which was managed by electric mechanism heaters. Thereafter, the temperature was lowered by 5°F each week until it reached 70°F. According to how the birds beneath the brooder behaved, the temperature under the brooder was changed and maintained. The birds were given access to artificial light for the first week for 24 h straight, with a weekly reduction of 1 h after that. The Department of Poultry Husbandry advised following the lighting schedule and temperature control. For the first two weeks, starter meal was offered ad libitum to chicks in plate feeders. Feed was supplied to them in linear feeders after the second week for up to four weeks of their age (Table 1). After four weeks, circular feeders were used to feed instead of linear feeders. At the end of the sixth week, growing birds were switched from starter to grower feed. Only daylight was supplied for the growing birds. The brooder-cum-grower house served as the birds' home until the end of the experiment.

## **Parameters** measured

Blood samples were collected from 5 chickens of every breed in the age of 16-week-old and analyzed for the valuation of heamatological, biochemical and immunological parameter. Samples were collected early in the morning before feeding and watering the birds. Samples were stored under proper management until the analysis was done. Haemato-biochemical samples were processed analyzed at Tabanis Veterinary Diagnostic Division DHA, Karachi while Immunological parameters were analyzed at Sindh Institute of Animal Health Karachi.

#### Heamatological parameters

Total Red Blood Cell count was evaluated by manual method using a hemocytometer. Pack cells volume was measured by the regular manual techniques by using of micro hematocrit capillary tube centrifuged at 2500 RPM for five minutes. Total White Blood Cells count was determined manually by method using hemocytometer. Differential White blood cell count was done on monolayer films of blood, were fixed, finally then stained with Giemsa-Wright stains. Hemoglobin concentrations were calculated by cyanmethemoglobin method from total RBC.ESR values were recorded in mm (mm in 1 h) by the Westergren method from the anti-coagulated blood samples.

#### **Biochemical parameters**

Glucose concentrations were quantitatively calculated on the basis of enzymatic colorimetric methods. The uric acids levels were calculated using the method of absorbency of the supernatant at 290 mu. Creatinine value was determined according to the procedure described by Boisness and Taussky. Total concentrations of cholesterol were quantitatively calculated on the basis of enzymatic colorimetric methods.

#### Immunological parameter

The Hemagglutination Inhibition (HI) tests were performed for the determination of the HI titer of the serum sample obtained from the birds. The HI titer of serum sample was calculated for measuring maternal antibodies and their persistence. These experiments were performed by the use of constant 4 HA unit antigen and decreasing serum methods ( $\beta$  procedure).

## Data analysis

SAS's JMP software and Microsoft Excel were used to tabulate and analyze the data that had been collected.

## Results

The results showed significantly highest red blood cell in Australorp breed  $(3.35 \pm 0.15)$  followed by Rhode Island Red  $(2.8 \pm 0.6)$ , Fayoumi  $(2.5 \pm 0.1)$  and lowest count was recorded in Naked Neck breed  $(2.4 \pm 0.2)$ . The results showed significantly the highest white blood cell in Naked Neck breed  $(19.5 \pm 0.5)$  followed by Rhode Island Red  $(17.5 \pm 0.5)$  and Fayoumi  $(15.5 \pm 1.5)$  and lowest white blood cell count was recorded in Australorp breed  $(13 \pm 1)$ . The results showed significantly highest Hemoglobin (Hb) levels in Australorp breed  $(16.4 \pm 0.4)$  followed by Rhode

Island Red (15  $\pm$  1), Fayoumi (12.85  $\pm$  0.85) and lowest Hemoglobin (Hb) levels were recorded in Naked Neck breed (11.85  $\pm$  0.65). The result shows significantly high ESR in Naked Neck breed (6.5  $\pm$  1.5) followed by Fayoumi (5.5  $\pm$  0.5), Australorp breed (2  $\pm$  1) and lowest ESR are recorded in Rhode Island Red breed (1.5  $\pm$  0.5) Table 2.

The results showed significantly high glucose levels in Australorp breed (192  $\pm$  4) followed by Naked Neck (177.5  $\pm$  1.5), Fayoumi breed (162.5  $\pm$  2.5) and lowest glucose levels were recorded in Rhode Island Red breed (140  $\pm$  2). The results showed significantly high uric acid levels in Australorp breed  $(6.1 \pm 0.1)$  followed by Naked Neck (5.75  $\pm$  0.55), Rhode Island Red breed (5.4  $\pm$  0.3) and lowest uric acid levels were recorded in Fayoumi breed (4.6  $\pm$  0.2). The results showed significantly high creatinine levels in Australorp breed  $(1.4 \pm 0.2)$  followed by Naked Neck breed  $(1.1 \pm 0.1)$  while there was non-significant difference between creatinine levels between Rhode Island Red (0.7  $\pm$ 0.1) and Fayoumi breed (0.7  $\pm$  0.1). The results show significantly high cholesterol levels in Rhode Island Red breed (238.5  $\pm$  3.5) followed by Australorp (227.5  $\pm$  2.5) and Naked Neck breeds (198  $\pm$  2) and lowest cholesterol levels were recorded in Fayoumi breed (183  $\pm$  3). The results showed significantly high antibody titer in Fayoumi breed (7.5  $\pm$  0.5) followed by Naked Neck (7  $\pm$  1) and Australorp breeds (5.5  $\pm$  0.5) and lowest antibody titer levels were recorded in Rhode Island Red breed  $(3 \pm 3)$ . The results showed significantly high antibody titer in Naked Neck breed  $(1.5 \pm 0.5)$  followed by other three breeds Rhode Island Red (0.5  $\pm$  0.36), Fayoumi (1.1  $\pm$  0.25) and Australorp breeds  $(0.8 \pm 0.05)$  with non-significant differences in antibody titers among these three breeds Table 3.

#### Discussion

According to the findings of the current study, the Australorp breed had the greatest red blood cell content at the age of 18 weeks (3.35), followed by the Rhode Island Red breed (2.8), the Fayoumi breed (2.5), and the Naked Neck breed (2.4). The same outcomes were noted by Khawaja *et al.* (2012). High ESR values in early ages in this research trial were in agreement with those values of Khawaja *et al.* (2012). Likewise, Mean Hemoglobin's concentration values are in accordance with those values observed by Khawaja *et al.* (2012). Hematological profiles in chickens have been showed to be influence by different factor such as sex, age, seasons and nutritional setups. Generally, hematological value of birds is affected by diurnal change in daily metabolic and physical activity (Piccione *et al.* 2001).

The results of the current experiment are consistent with those from Khawaja *et al.* (2012). According to their findings, anemia in birds would be indicated by hemoglobin (Hb) levels (07–18.6 g/dL) and values below normal limits. Similar to this, El-Safty *et al.* (2006) hypothesized that a

Table 1: Ration	formulation	and feed	ingredients
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Ingredient	Rate of inclusion		
-	Layer starter	Layer grower	
Rice	60.25	65.47	
Soybean meal	32.5	28	
Lime stone	1.2	1.2	
Feed premix	0.5	0.5	
L-lysine	0.1	0.1	
NaCl	0.3	0.3	
D1-methionine	0.15	0.13	
Dicalcium phosphate	2	1.8	
Corn gluten meal	3	2.5	
-	Calculated chemical composition of diet		
ME (kcal/kg)	2998	3023.9	
Available P (%)	0.531	0.485	
Ca (%)	0.988	0.949	
Methionine (%)	0.496	0.440	
Methionine + cystine (%)	0.80	0.72	
Lysine (%)	1.21	1.09	
Crude protein (%)	21.19	19.20	

Table 2: Mean Values of heamatological parameters in various breeds

Parameters	Breeds			
	Naked Neck	Rhode Island Red	Fayoumi	Australorp
Red blood cells $(10^{9/L})$	$2.4 \pm 0.2$	$2.8 \pm 0.6$	$2.5 \pm 0.1$	$3.35\pm0.15$
White blood cells $(10^{9/L})$	$19.5 \pm 0.5$	$17.5 \pm 0.5$	$15.5\pm1.5$	$13 \pm 1$
Hemoglobin (g/dL)	$11.85 \pm 0.65$	$15 \pm 1$	$12.85\pm0.85$	$16.4 \pm 0.4$
Erythrocyte sedimentation rate (mm in 1st h)	$6.5 \pm 1.5$	$1.5 \pm 0.5$	$5.5\pm0.5$	$2\pm 1$

Table 3: Mean values of biochemical and immunological parameters in various breeds

Parameters	Breeds			
	Naked Neck	Rhode Island Red	Fayoumi	Australorp
Glucose (mg/dL)	$177.5 \pm 1.5$	$140 \pm 2$	$162.5 \pm 2.5$	$192 \pm 4$
Uric acid (mg/dL)	$5.75\pm\!0.55$	$5.4 \pm 0.3$	$4.6\pm0.2$	$6.1\pm0.1$
Creatinine (mg/dL)	$1.1 \pm 0.1$	$0.7 \pm 0.1$	$0.7 \pm 0.1$	$1.4 \pm 0.2$
Cholesterol (mg/dL)	$198 \pm 2$	$238.5 \pm 3.5$	$183 \pm 3$	$227.5 \pm 2.5$
Antibody titer (New Castle disease)	$7 \pm 1$	$3\pm3$	$7.5 \pm 0.5$	$5.5\pm0.5$
Antibody titer (Infectious bronchitis)	$1.5 \pm 0.5$	$0.5 \pm 0.36$	$1.1 \pm 0.28$	$0.8 \pm 0.05$

higher hematocrit value for the Naked Neck genotype indicated a greater ability to transport oxygen to tissues at various temperatures. Various factors, including food, sex, age, management, and environmental conditions, may have an impact on blood profile fluctuation. According to our claims, blood profiles fluctuate as a result of daily metabolic and physical activity (Piccione *et al.* 2001). Additionally, blood profiles may be employed in numerous clinical research trials as a disease diagnosis tool.

The biochemical analysis of uric acid and creatinine shows no significant (P > 0.05) variations in blood serum and values were in normal range. The mean values of uric acid (5.4 mg/dL) and serum glucose (140 mg/dL) in Rhode Island Red in the current trial were similar to result of Khawaja *et al.* (2012), who quoted the uric acid (4.75 mg/dL) and serum glucose (121.80 mg/dL) in Rhode Island Red breed. Overall means of uric acids values and cholesterols level in three Sudanic breeds (Betwil, Large Beladi and Bare Neck) are found as 99.97 mg/dL and 7.42, respectively, that are similar to the results of the present experiment other than cholesterol levels which tend to be high (Elagib et al. 2012). Blood cholesterols values in various breeds (Fayoumi, Desi, crossbred (Fayoumi x RIR) and Naked Neck) in duration of post and pre-laying period are similar which imply that egg-laying conditions did not use any additional requirements on cholesterols level bio synthesis and its releases in the stream of blood directly (Bhatti et al. 2002). In the present research trial, blood cholesterol levels in Naked Neck, Rhode Island Red, Fayoumi and Australorp breeds were observed within the standard ranges. Though, some values are low than the value documented in crossbred (Fayoumi female x RIR male) cockerel (187.80 mg/dL) at the 12 weeks of age by Khan (1998). Chicken uric acid is a main nitrogen catabolism product, being the end product of proteins and metabolism of amino acid, and indicates same rates in proteins and metabolism of amino acid in various birds groups (Elagib et al. 2012). Diets and Age can control the concentrations of serum uric acids values in chickens. The uric acids value (4.23–4.61 mg/dL) of four breeds during the current research are almost similar as compared to value (5.7, 5.4, 4.6 and 6.1 mg/dL) observed by Bhatti et al.

(2001) in Naked Neck and Desi breeds. Though, all the levels are low in comparison to levels (7.425 mg/dL) noted by Elagib *et al.* (2012) in indigenous breeds of Sudan.

Immunity of an organism is mainly comprised of innate and adaptive compartments and the parameter of immunity can be measured as an antibodies titer and activities of the complement systems in the blood. Diseases which cause infections are one of the important factors constraining the sector of poultry birds. One of the main causes of mortality in poultry birds is Newcastle disease (Nguyen 1992) and various species of domestic and wild bird have been found infected to Newcastle disease Virus (Arshad *et al.* 1988; Wernery *et al.* 1992). The spreading of Newcastle disease in area is generally through new introduce chickens, giving away or selling carrier and sick chickens (Tu *et al.* 1998).

## Conclusion

On the basis of current experiment observations, it was concluded that Australorp breeds had high red blood cells, hemoglobin, and uric acid levels while Naked Neck breeds have low Red Blood Cell rates with high White Blood cell counts in local environmental conditions. Glucose and Creatinine levels were comparatively high in Australorp breed followed by Naked Neck and lowest Glucose levels were seen in Rhode Island breed. Naked Neck breed gives better performance regarding antibody titers of Newcastle disease as compared to other three breeds while, Rhode Island Red presented poor performance regarding Newcastle antibody titers and local environment adaptability as compared to others. Australorp breed is a good producer in semi-intensive housing with haematobiochemical traits, disease resistibility and antibody titers against certain diseases but their performance is linked with good management practices. Hence, these chickens' breeds could be useful in rural area of Pakistan.

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## **Author Contributions**

Waja Irfan and Nasir Rajput conducted and developed tests. Waja Irfan, Muhammad Naeem Jan, and Rahat Abbas carried out tests. Data that was examined: Rehana Shah Nawaz and Waja Irfan, Materials, techniques, and analysis provided by Waja Irfan and Nasir Rajput. Jan. Muhammad Naeem wrote the paper.

## **Conflicts of Interest**

All authors declare no conflicts of interest.

#### **Data Availability**

Data presented in this study will be available on a fair request to the corresponding author.

## **Ethics Approval**

This study received ethics approval from the Institutional Animal Ethics Committee (IAEC) of Sindh Agriculture University Tandojam. The chickens were maintained under standard living conditions, and blood samples were collected using standard procedures to prevent any harm or discomfort to the animals. The study followed the Guidelines for the Care and Use of Laboratory Animals by the National Institutes of Health (NIH) and ARRIVE guidelines.

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