Influence of Indigenous Microorganisms (IMOs) Diets on Haematology and Serum Chemistry of Poultry Chickens

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Abstract: ***An experiment was conducted to study the haematological and serological parameters in Indigenous Microorganisms (IMOs) and control (caged) Chickens of different ages reared in Rayalaseema region of Andrapradesh India. 100 chickens of two breeds (50 Broilers and 50 Layers) were tested at different ages (1, 3, and 6 months) to observe the haematological and serological parameters were compared in the Indigenous Microorganisms (IMOs) inputs treated chickens and control commercial enzymatic (caged) farming Broiler Chickens and Layer chickens were evaluated. Haematological and serological characteristics were studied in 100 broilers and layers fed diets with varying levels the birds were assigned into two experimental groups; Broilers and Layers comprising 50 chickens each. The first group, Group A was given the control diets containing commercial enzymatic daits, while Group B IMOs diets respectively. Total protein, cholesterol and Triglycerides were significantly (p>0.05) affected by inputs. Blood Glucose, Blood Urea, Serum Creatinine, Total Protein, Total A/G ratio, Blood Uric Acid, and Alkaline Phosphatase were assessed and different among the two types. The haematological values in diet B at the 6th month was significantly higher (P<0.05) than the value recorded in the control diet, while the serological values in diet A in the 6th month significantly (P<0.05) higher when compared with diet B. Diets affect the Total cholesterol and Triglycerides difference among the various treatments.***

Keywords: IMOs Technology, Indigenous Microorganisms (IMOs), IMOs poultry, Poultry Hematology, Serum Chemistry, Natural farming

1. Introduction

In general, haematology and serum chemistry examination is performed for several reasons as a screening procedure to assess general health [1]. Because the clinical signs of illness in birds are frequently subtle, haematology and serum chemistry is necessary to evaluate cellular changes [2]. Haematological changes are routinely used to determine various statuses of the body and to determined stress due to environmental, nutritional and systematic factors [3]. Haematological values of chickens are influenced by breed, geographical season climate, age, sex, location, day length, nutritional status, life habit of species, present status of individual and such other physiological factors [4] [5]. It can be used as a diagnostic tool in order to assess the health status of an individual or group [6]. Analysis of normal haematological parameters of chickens is very much essential in diagnosing the various immunological and metabolic disorders [7] [8]. Because of these facts, during the recent decades the avian physiology is found to be of great importance to the scientists, researchers and veterinarians as well as poultry growers [9]. For proper management, feeding [10], breeding, prevention and treatment of diseases [11].it is desirable to know the normal physiological values under local conditions [12].

Indigenous microorganisms (IMOs) and their amendments powerfully perform when provide suitable temperature and climatic conditions with suitable nutrients at the suitable period [13]. Natural farming means sustainable microbial system and self-sufficient in equilibrium. The system is based on local and renewable resources. Organic farming builds on an integrated ethos, which encompasses the environmental, economic and social aspects in agricultural production both from a local and global perspective [14]. Thus organic farming perceives nature as an entity, which has value in its own rights. The sustainable agriculture system main aim is protecting life and integrity of natural environment. The basic principle of Nature Farming that has evolved that a farm should form a basic unit of self-sustainability [15]. The use of native materials can restore and enhance the fertility and vitality of the farm. The presence of a fundamental philosophy of sustainability and biodiversity will allow organic agriculture to transcend current trends to define it simply as a system of farming that restricts the use of certain chemical fertilizers and pesticides. Energy sources constitute the largest component of poultry diets, followed by plant protein sources and animal protein sources. Worldwide, Fish Meal and maize (corn) and soybean was the most commonly used energy source, Fish meal is a common protein source [16].

2. Material and Methods

Experimental design: 100 healthy chickens, one day old of age, were obtained from provinces region of Rayalaseema Andrapradesh in India. These Chickens were reared in the experimental Poultry form of IMOs Technology, Sri Venkateswara University, Tirupati for six months (fig 1). Blood profile were studied in 100 broilers and layers supplemented diets with varying levels the birds were assigned into two experimental groups; Broilers and Layers comprising 50 chickens each (fig 2 – 3). The first group, Group A was given the control diets containing commercial enzymatic daits (table 1), while Group B IMOs diets (table 2) respectively. Continuous feed and water were provided throughout the experiment. Three ml of blood was obtained from the wing vein using a 3 ml syringe and 23 gauge needle, then immediately transferred into 2 tubes; 1 ml into the first tube containing EDTA for haematological determination and 2 ml into the second tube without anticoagulant for other serum biochemistry determination. The samples were kept in an ice box, using icepacks and transferred to the laboratory for further assays.

Preparation of Indigenous Microorganisms (IMOs) from the natural materials:

Indigenous Microorganisms (IMOs):

Step-1: Hard cooked rice taken in a container and buried up to 75% depth under the soil in undisturbed condition (Locally found microorganisms are attracted).

Step-2: After 3 to 5 days culture turns into yellowish colour, mixed with brown sugar and stored in a clay pot. (Note: Sometimes the culture turned in black in colour it may be considered as contaminated and discorded.)

Step-3: Cultured IMO-2 mixed with rice husk and closed with paddy straws or wheat straws

Step-4: After 3 to 5 day's rice husk turns weightless and it adopted locally found microorganisms, later the culture was mixed with white ant soil [16].

Lactic Acid Bacteria (LAB):

Brown rice vinegar (BRV) and unpasteurised milk or Raw Milk (1:1) was allowed to ferment for 2 to 4 days, after fermentation extract were collected for further applications [17].

Indigenous Microorganisms (IMOs)-II&III

Lactic Acid Bacteria (LAB)

Fermented Plant Juice (FPJ):
Mugwart, Water amaranths and Bamboo shoots or locally fast growing plant leaves with IMO – 2 and Jaggery (1:1) were mixed vigorously and allowed to ferment for 3 to 5 days, after fermentation extract were collected for further applications [14].

Fermented Fruit Juice (FFJ):
Banana, Papaya, Mango, Grape, Melon, Apple etc., (chose any one fruit must be sweet) with IMOs – 2 and Jaggery (1:1) were mixed vigorously and allowed to ferment for 3 to 5 days, after fermentation extract were collected for further applications [17].

Oriental Herbal Nutrients (OHN):
Ginger or Garlic with IMO – 2 and Jaggery (1:1) were mixed vigorously and allowed to ferment for 3 to 5 days, after fermentation extract were collected for further applications [18].

Fish Amino Acid (FAA):
(Fish trash with IMOs – II) and Jaggery (1:1) were mixed vigorously and allowed to ferment for 3 to 5 days, after fermentation extract were collected for further applications [19].

Water Soluble potassium (WS-K):
Tobacco plant dried stem pieces soaked in water (1:5) were and allowed 5 to 7 days, then extract were collected for further applications.

Water Soluble Phosphoric Acid (WS-PA):
Charcoal obtained from dried Sesame stems were mixed with water (1:5) allowed to 5 to 7 days, then extract were collected for further applications.

Calcium (Ca):
Low roasted Eggshells or Seashells were mixed with Brown rice vinegar (BRV) and allowed to 2 to 3 days, then extract were collected for further applications [16].

Haematological technique

IMOs treated poultry chickens samples were collected from the brachial vein of 25 birds using a 3 mL sterile syringe and a 23-gauge needle. Each blood sample was transferred immediately into a sterile tube containing the anticoagulant, Ethylenediaminetetra acetic acid (EDTA) (**Campbell** *et al.*, 1995). The Total Erythrocyte count (TEC) or Total Red Blood Counts were performed in a 1:200 dilution of blood in Hayem's solution (**Pampori** *et al.*, 2003). The differential leukocyte counts were determined by preparation of blood smears stained with Wright's stain. The Hb concentration was evaluated by matching acid haematin solution against a standard coloured solution found in Sahl's hemoglobinometer (**Gaikwad** *et al.*, 2000) Packed cell volume (PCV) was measured by a standard manual technique after centrifugation of a small amount of blood using microhematocrit capillary tubes. RBC indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations MCHC and the serum was separated and analysed for glucose, cholesterol total protein and albumin creatinine, uric acid and alkaline phosphatase were calculated (Cell counter (pocH 100i) Sysmex- transasia) respectively.

Statistical Analyses

Mean of 50 replications, standard deviation (SD), analysis of variance (ANOVA), least significant differences (LSD) and co-efficient of correlation values (r) were computed using SPSS (version 11.0 for Windows). Data on various haematological profiles were subject to these statistical procedures to detect the significance of difference between the genetic groups of chicken under study.

3. Results and Discussion

Total Red Blood Cell Count (RBC)

The present findings that RBC number in IMOs treated Poultry broilers and Layers increasing with the advancement of age, being lowest in 1 month (Br. RBC **4.29 ± 1.2**) (La. RBC **4.32 ± 0.7**) and highest in 6 months (Br. RBC **4.98 ± 1.1** and La. RBC **4.71 ± 0.8**) of age (table 1and 2). In this study and compare with control, IMOs treated Poultry chicken shows better and equal to normal reference values or consistent with the findings.

Haemoglobin (Hb g %)

In this findings that haemoglobin concentration increases when treated with IMOs in the advancement of age, being lowest in 1 month (Hb **10.3 ± 0.73**) and highest in 6 months (Hb **13.2 ± 0.91**) of age (table 1). IMOs treated poultry chickens had greater haemoglobin whether compared to the control farming poultry chickens (table 1and 2).

Packed cell volume (PCV)

Low Packed Cell Volume (PCV) detected in IMOs treated early stage (**35.2±0.8**) and it is gradually increased with the advancement of age (**41.2±0.4**) (table 1). The PCV lower and higher values of IMOs treated and control poultry chicks were observed in 1 and 6 months of age respectively. The results of PCV of IMOs treated higher than the control based poultry chickens (table 1and 2).

Mean Corpuscular Volume (MCV)

The values of MCV in IMOs treated poultry chickens and chemical farming chickens were slightly related with the age and breed. MCV values gradually increased early age to advancement of age.

Mean Corpuscular Haemoglobin (MCH)

The values of MCH in IMOs treated poultry chickens and chemical farming chickens were related with the age and breed. MCH values gradually increased early age to advancement of age. The results are slightly lower increase of IMO inputs treated poultry chicken.

Platelet count (lacks/cu)

From these findings it is evident to that platelet count increase gradually with the advancement age (table 1 and 2). The results were IMO inputs treated chickens were slightly higher than the control poultry chickens. The highest and lowest Platelet count values were observed in 1 and 6 months of age respectively.

Serum Chemistry

The values for various serum constituents in IMOs treated poultry chickens and enzymatic feed treated poultry chickens were presented in Table 3 and 4. The values for Blood glucose, Blood urea, Total cholesterol, total protein, albumin globulin ratio, Blood uric acid, creatinine, and alkaline phosphatase recorded in present study differed significantly ($p<0.05$) among the both poultry chickens. The average values recorded were (Table 3 and 4). Significant difference was observed for glucose, uric acid, creatinine, calcium, phosphorus and alkaline phosphatase Triglycerides and Total cholesterol higher in control feed treated poultry chickens and lower in IMOs feed treated poultry chickens.

Total Cholesterol / Triglyceride

Blood samples were taken and the serum was harvested by centrifugation, and then the total cholesterol (TCH), Triglycerides (TG) were assed using an enzymatic kit. In conclusion, IMOs Treated Chicken affected serum biochemical indices. In addition, abdominal fat [Fig 1-3] was affected by the levels of serum biochemical indices. The fatty acid pattern of the abdominal fat was significantly influenced by the diets [Fig 4-6]. Fatness or obesity is related to several disturbances in metabolism. For instance, high concentrations of serum Total Cholesterol (**196.4±1.2)** and Triglyceride (**181.6±1.3)** in control Poultry Chicken and a low concentration of Total cholesterol (**129.5±1.2)** and Triglyceride (**139.8±1.2)** in IMOs Treated Poultry Chicken, whereas serum low-density Total cholesterol level is usually much less elevated in obesity.

Figures Control Poultry Chicken dissection. Results are shown in the figure 1 to 3.

Figure 1

Figure 2

Figure 3

Figures IMOs treated Poultry Chicken dissection. Results are shown in the figure 4 to 6.

Figure 4

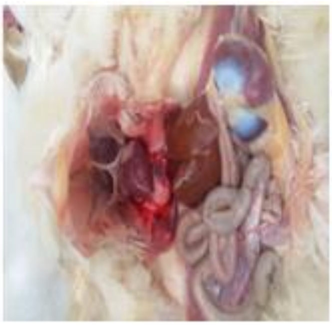

Figure 5

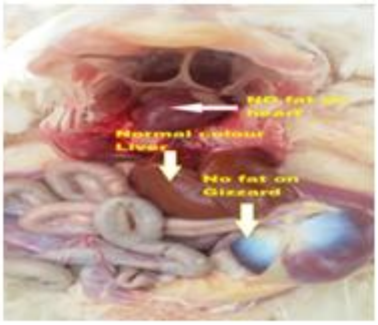

Figure 6

Comparison of IMOs treated and Control Poultry chicken blood profile

There are no published data on the haematology and serum chemistry of IMOs treated Broilers or Layers thus this study provides the first comprehensive study on these indices of health. These hematologic parameters and serological parameters showed significant differences between age and gender, especially RBC, Hb, PCV, total Cholesterol, Total Protein, and total A/G ratio concentrations. The hematologic data obtained in this study can be considered preliminary reference values, which are particularly important for Chicken production.

Haemoglobin (Hb g %) :In this findings that haemoglobin concentration increases when treated with IMOs in the advancement of age, being lowest in 1 month (Hb 10.3 ± 0.73) and highest in 6 months (Hb 13.2 ± 0.91) of age (table 1). IMOs treated poultry chickens had greater than equal to the normal healthy referral chicken blood profile haemoglobin whether compared to the control farming poultry chickens (table 3 – 4).

Packed cell volume (PCV)
Low Packed Cell Volume (PCV) detected in IMOs treated early stage (30.6 ± 0.98) and it is gradually increased with the advancement of age (39.2±1.2). The PCV lower and higher values of IMOs treated and control poultry chicks were observed in 1 and 6 months of age respectively. The results of PCV of IMOs treated higher than the control based poultry chickens (table 3 – 4).

Total Red Blood Cell Count (RBC)
The present findings that RBC number in IMOs treated Poultry broilers and Layers increasing with the advancement of age, being lowest in 1 month (Broiler RBC 3.7 ± 0.29) (Layer RBC 11.2 ± 0.78) and highest in 6 months (Br. RBC 4.98 ± 1.1 and La. RBC 4.71 ± 0.8) of age (table 1and 2). In this study and compare with control, IMOs treated Poultry chicken shows better and equal to normal reference values or consistent with the findings (table 3 – 4).

Mean Corpuscular Volume (MCV)
The values of MCV in IMOs treated poultry chickens and chemical farming chickens were slightly related with the age and breed. MCV values gradually increased early age to advancement of age.

Mean Corpuscular Haemoglobin (MCH)
The values of MCH in IMOs treated poultry chickens and chemical farming chickens were related with the age and breed. MCH values gradually increased early age to advancement of age. The results are slightly lower increase of IMO inputs treated poultry chicken (table 3 – 4).

Serum Chemistry
The values for various serum constituents in IMOs treated poultry chickens and enzymatic feed treated poultry chickens were presented in Table 4 and 5. The values for Blood glucose, Blood urea, Total cholesterol, total protein, albumin globulin ratio, Blood uric acid, creatinine, and alkaline phosphatase recorded in present study differed significantly ($p<0.05$) among the both poultry chickens.

The average values recorded were (Table 5 and 6). Significant difference was observed for glucose, uric acid, creatinine, calcium, phosphorus and alkaline phosphatase Triglycerides and Total cholesterol higher in control feed treated poultry chickens and lower in IMOs feed treated poultry chickens.

Total Cholesterol / Triglyceride

Blood samples were taken and the serum was harvested by centrifugation, and then the total cholesterol (TCH), Triglycerides (TG) were assed using an enzymatic kit. In conclusion, IMOs Treated Chicken affected serum biochemical indices. In addition, abdominal fat was affected by the levels of serum biochemical indices. The fatty acid pattern of the abdominal fat was significantly influenced by the diets. Fatness or obesity is related to several disturbances in metabolism. For instance, high concentrations of serum Total Cholesterol (196.4±1.2) and Triglyceride (181.6±1.3) in control Poultry Chicken and a low concentration of Total cholesterol (129.5±1.2) and Triglyceride (139.8±1.2) in IMOs Treated Poultry Chicken (table 5 – 6), whereas serum low-density Total cholesterol level is usually much less elevated in obesity [Fig 4 – 5].

4. Conclusion

Currently, Influence of Indigenous Microorganisms (IMOs) diet impact on poultry Chicken haematology and serum chemistry. The classes of Poultry of considered in this research (Broilers and Layers) show the benefits and efficiency of diet formulation utilization when Indigenous Microorganisms IMOs inputs were included in their diet effects on haematology and serum chemistry. One of the IMOs inputs like Lactic acid bacteria (LAB) contributes disease resistance in poultry chicken and Energy sources of IMOs inputs plant and fruit juice (FPJ, FFJ) had an important role to play in poultry diets through as mineral supplement and oriental herbal nutrients act as anti-oxidant. The balance of micronutrients in fish amino acid (FAA) may be superior to that in other proteins. The different IMOs inputs affect Total cholesterol and Triglycerides reducing. The mean haematological values among the IMO treated and control or caged poultry chicken Hb, PCV, RBC, WBC, MCV, MCH, MCHC, and DLC differ significantly (p<0.05). In terms of diet formulation the true value of IMOs inputs in poultry diets will be above its influence. The Poultry management with IMO inputs feed treated and control/enzymatic feed treated chicken haematology and serum biochemistry were compared. The values of haematology high in IMO inputs treated chickens and low in control/enzymatic feed treated chickens. Serum biochemistry parameters of IMO inputs treated chickens low, especially total cholesterol and triglycerides and high values observed in control/enzymatic feed treated chickens. This conclusion is drawn from extensive work carried out for alternative new technologies indicating that IMOs inputs produces response in poultry chicken management.

Table 1: Haematological Parameters in IMOs treated and control poultry Broilers

S.No	Parameter	Control poultry Broilers			IMO treated Broilers			
		1st Month	3rd Month	6th Month	1st Month	3rd Month	6th Month	Ref. values
1	**Hb (g %)**	12.1±0.29	14.7±0.45	15.3±0.55	10.3 ± 0.73	11.7 ± 0.43	13.2 ± 0.91	**11.6- 3.6**
2	**PCV (%)**	32.3±0.65	37.2±0.85	40.7±0.56	30.6 ± 0.98	35.7 ± 0.87	39.2 ± 1.2	**35.9- 41.0**
3	**RBC (10^6/mm^3)**	3.76 ± 0.4	3.95 ± 0.3	4.18 ± 0.7	4.29 ± 1.2	4.49 ± 0.9	4.98 ± 1.1	**4.21- 4.84**
4	**WBC (cells/ cu)**	4.26±0.4	4.92±0.9	4.42±0.8	4.27 ± 0.9	4.33 ± 0.5	4.41 ± 0.8	**4.07- 4.32**
5	**MCV(vol %)**	84.0±0.4	86.2±0.7	92.8±0.7	83.44 ± 0.5	85.63 ± 0.6	87.67 ± 0.4	**81.6- 89.1**
6	**MCH(pg.)**	27.7±0.7	30.7±0.9	32.2±0.5	26.2 ± 0.8	27.8 ± 0.7	29.7 ± 0.4	**27.2- 28.9**
7	**MCHcon(%)**	31.2±1.2	34.7±0.8	36.6±0.7	31.5 ± 1.3	32.8 ± 1.7	33.4 ± 1.2	**32.4- 33.3**

Table 2: Haematological Parameters in IMOs treated and control poultry Layers

S.No	Parameter	Control poultry Layers			IMO treated Layers			
		1st Month	3rd Month	6th Month	1st Month	3rd Month	6th Month	Ref. values
1	**Hb (g %)**	11.2±0.7	11.9±0.7	13.7±0.2	11.7±0.5	12.9±0.9	13.3±0.7	**11.6- 13.6**
2	**PCV (%)**	34.4±0.6	35.8±0.2	37.2±0.4	35.2±0.8	39.2±0.9	41.2±0.4	**35.9- 41.0**
3	**RBC (10^6/mm^3)**	3.12±0.8	3.40±0.7	4.17±0.7	4.32±0.7	4.35±0.9	4.71±0.8	**4.2- 4.8**
4	**WBC (cells/ cu)**	4.7±0.7	4.9±0.4	4.7±0.7	4.1±0.4	4.2±0.8	4.4±0.7	**4.0- 4.3**
5	**MCV (vol %)**	84.2±0.4	85.7±0.3	86.2±0.5	85.7±0.9	87.3±0.1	87.6±0.5	**81.6- 89.1**
6	**MCH(pg.)**	28.3±0.7	29.2±0.7	32.9±0.4	28.8±0.7	27.2±0.4	28.9±0.5	**27.2- 28.9**
7	**MCHcon (%)**	29.7±1.2	32.9±0.7	34.2±1.4	32.6±0.9	32.8±1.4	33.1±0.7	**32.4- 33.3**

Ref. values: https://en.wikivet.net/Chicken_Haematology. The above values are an average three independent experiments. Mean ± SD values differ significantly (P≤0.05) within the same row.

Table 3: Serum Chemistry Parameters in IMOs treated and control poultry Broilers

S. No	parameter	Control Poultry Broilers			IMO treated Poultry Broilers			Ref. values
		1st Month	3rd Month	6th Month	1st Month	3rd Month	6th Month	
1	**Blood Glucose (mg/dl)**	172.3±1.4	184.2±1.1	196.3±1.2	184.7±1.3	191.3±1.6	217.2±1.7	210 –230 mg/dl
2	**Blood Urea (mg/dl)**	3.9±0.2	4.1±0.2	5.5±0.1	2.6±0.1	3.7±0.3	4.2±0.2	2.0- 4.5 mg/dl
3	**Serum Creatinine (mg/dl)**	12.6±0.4	13.4±0.6	15.1±0.1	11.4±0.3	12.5±0.4	13.5±0.5	11.0-14.5 mg/dl
4	**Total Protein (g/dl)**	3.92±0.4	4.15±0.1	5.12±0.2	3.67±0.2	4.96±0.4	6.72±0.8	3.5-6.0 mg/dl
5	**Total A/G ratio (g/dl)**	2.9±0.1	3.5±0.5	4.2±0.5	3.4±0.1	3.9±0.4	4.1±0.2	2.5-5.5 mg/dl
6	**Blood Uric Acid (mg/dl)**	6.2±0.3	7.4±0.5	8.8±0.4	5.9±0.1	6.5±0.5	7.2±0.5	4.0-7.5 mg/dl
7	**Total cholesterol (mg/dl)**	110.9±1.5	136.3±1.6	158.6±1.9	86.3±1.3	112.1±1.1	129.4±1.2	90.0-140.0 mg/dl
8	**Triglycerides (mg/dl)**	117.3±1.5	132.3±1.4	147.2±1.9	52.3±1.2	59.6±1.3	115.4±1.5	70.0-120.0 mg/dl
9	**Alkaline Phosphatase (mg/dl)**	10.2±0.5	12.2±0.1	14.4±0.2	9.4±0.3	10.4±0.4	12.5±0.1	8.5-13.0 mg/dl

Table 4: Serum Chemistry Parameters in IMO treated and control poultry Layers

S. No	Parameter	Control Layers			IMO treated Layers			Ref. values
		1st Month	3rd Month	6th Month	1st Month	3rd Month	6th Month	
1	**Blood Glucose (mg/dl)**	219.6±1.3	226.1±1.2	239.3±1.4	211.2±1.4	220.5±1.8	226.1±1.7	**210 – 230 mg/dl**
2	**Blood Urea (mg/dl)**	2.8±0.6	3.0±0.1	3.4±0.4	2.8±0.4	3.1±0.8	3.9±0.7	**2.0- 4.5 mg/dl**
3	**Serum Creatinine (mg/dl)**	10.1±0.2	11.8±0.2	12.7±0.5	10.9±0.5	11.7±0.3	12.6±0.1	**11.0-14.5 mg/dl**
4	**Total Protein (g/dl)**	3.10±0.5	4.26±0.3	5.85±0.1	4.12±0.4	5.46±0.5	6.12±0.2	**3.5-6.0 mg/dl**
5	**Total A/G ratio (g/dl)**	3.7±0.4	4.9±0.4	5.2±0.5	3.5±0.4	4.5±0.5	5.1±0.2	**2.5-5.5 mg/dl**
6	**Blood Uric Acid (mg/dl)**	5.1±0.4	6.5±0.4	7.2±0.7	5.5±0.6	6.2±0.4	6.7±0.2	**4.0-7.5 mg/dl**
7	**Total cholesterol(mg/dl)**	126.4±1.1	139.3±1.5	196.4±1.2	119.4±1.5	122.5±1.4	129.5±1.2	**90.0-140.0 mg/dl**
8	**Triglycerides (mg/dl)**	106.8±1.4	129.7±1.6	181.6±1.3	109.4±1.5	124.2±1.2	139.8±1.2	**70.0-120.0 mg/dl**
9	**Alkaline Phosphatase (mg/dl)**	9.2±0.4	10.7±0.4	11.2±0.5	10.3±0.5	11.5±0.4	12.7±0.2	**8.5-13.0 mg/dl**

Ref. values: https://en.wikivet.net/Chicken_serum chemistry

The above values are an average three independent experiments. Mean ± SD values differ significantly ($P \leq 0.05$) within the same row.

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