Effect of Season and Dietary Protein Level on Some Haematological Parameters and Blood Biochemical Compositions of Three Broiler Strains

E.A.A. Mohamed¹, O.H.A. Ali², Huwaida, E.E. Malik¹ and I.A. Yousif³

¹Department of Poultry Production, Faculty of Animal Production, University of Khartoum, Khartoum North, Sudan
²Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum, Khartoum North, Sudan
³Department of Genetics and Animal Breeding, Faculty of Animal Production, University of Khartoum, Khartoum North, Sudan

Abstract: The objectives of the present study were to investigate the effect of season (summer versus winter) and dietary protein level (high versus low) using three broiler strains (Ross, Cobb and Hubbard) on some physiological parameters; haematological parameters, haematological indices, serum metabolites and serum inorganic elements. Three hundred and sixty, one-day-old unsexed broiler chicks, were used during the summer and winter seasons, 120 from each strain. The number of chicks of each strain was divided into two groups, with six replicates (10 chicks per each). Group A of each strain was fed on a starter diet containing 23% crude protein for the first four weeks of age, replaced by a finisher diet containing 21% crude protein. Group B was fed on a starter diet containing 21% crude protein replaced by a finisher diet containing 19% crude protein. In both Cobb and Hubbard strains, the Packed Cell Volume (PCV) decreased significantly (P<0.05) during the summer season, whereas, it was significantly (P<0.05) increased in Ross strain during the same season. The haemoglobin concentration (Hb) decreased significantly (P<0.05) in Cobb strain during the summer. It was not significantly affected by the season in both Ross and Hubbard strains. The total red blood cells count (TRBC count) did not significantly affected by the season in the three experimental strains. The Mean Cell Volume (MCV) increased significantly (P<0.05) during the summer in Ross strain but it was not significantly affected by the season in both Cobb and Hubbard strains. The Mean Cell Haemoglobin (MCH) decreased significantly (P<0.05) during the summer in both Ross and Cobb strains but it was not significantly affected by the season in Hubbard strain. The Mean Cell Haemoglobin Concentration (MCHC) decreased significantly (P<0.05) during the summer in the three experimental groups. Serum glucose concentration decreased significantly (P<0.05) during the summer in both Ross and Hubbard strains but was not affected by the season in Cobb strain. The serum albumin concentration decreased significantly (P<0.05) during the summer in Ross strain but was not significantly affected by the season in Hubbard strains. The results reflected that, serum sodium (Na), potassium (K) and phosphorus (P) concentrations were decreased significantly during the summer in all strains. The serum calcium (Ca) concentration decreased significantly during the summer in Cobb strain. Whereas, it was not significantly affected by the season in both Ross and Hubbard strains. Although there was broiler strain X protein level interaction effect on all of following physiological parameters; PCV, MCV, MCHC, serum glucose, serum albumin, serum Na, K, Ca and serum P, the level of dietary protein appeared to be has no significant effect on any of physiological parameters under investigation.

Keywords: Broiler strain, season, protein level, haematological and biochemical parameters

INTRODUCTION

Poultry industry showed rapid development worldwide, especially in developing countries, importation of temperate-zone high performance stocks to hot regions is on the rise. However, the use of unsuitable genotypes in hot regions results in large economic losses due to depression in general performances and higher mortality (Cahaner, 1990). Economically, the poultry should be supplied with cheaper feed to get maximum return with minimum cost. The recent trend among the poultry nutritionist to explore the possibilities of using different protein concentrates to reduce the cost of poultry feed. Nowadays, most of the poultry farmers have been using different protein concentrates in poultry diets as replacement of fish meal. The requirement of dietary protein depends on species, age and breed (Alam et al., 2004).

Like the other poultry industries, the successful broiler production depends on many factors such as; availability of quality feed ingredients at reasonable cost, proper
management and quality chicks. Among these factors feed itself constitutes about 65 to 70% of the total cost of production. The price of protein ingredients is comparatively higher than that of the other ingredients i.e., protein cost involves about 15% of the total feed cost (Banerjee, 1992; Singh, 1990).

Hematological parameters and its knowledge can be used to assess the health status of broiler chicks. Normally, the values of blood constituents are affected by many factors such as genotype, age, physiological condition, gender, diet, micro- and macro-climatic conditions, the method of rearing, season and pathological factors. Under tropical conditions, birds are exposed to marked seasonal changes in the thermal environment. The heat load imposes severe stress and results in reduced physiological and productive performance of birds (Abdalla and Nawal, 2009).

In the past years, hemotalogical analysis has been used as a guide in the diagnosis of many diseases and in evaluating the responses to the therapy in both animals and human beings. However, recently, the hematological changes have been routinely used to assess the level of stresses due to environmental and nutritional factors (Mmereole, 2004). Moreover, nowadays there is great interest in using plasma metabolites as diagnostic tools of animals and human’s diseases and health status.

MATERIALS AND METHODS

Two experiments were carried out in the premise of poultry research unit, department of poultry production. Faculty of Animal Production University of Khartoum, Shambat (Khartoum North, Sudan). The laboratory analyses were carried out at the Department of Physiology, Faculty of Veterinary Medicine -University of Khartoum.

Experimental birds: Three hundred and sixty (360) one-day-old unsexed broiler chicks of three broiler strains (Ross, Cobb and Hubbard) were used during the summer and winter seasons. The number of chicks of each strain was divided into two groups, with six replicates (10 chicks per each).

Experimental plan and diets: Group A of each strain was fed on a diet containing 23% crude protein and 3000 kcal/kg ME as starter diet for the first four weeks of age and then replaced by a diet containing 21% crude protein and 3000 kcal/kg ME as finisher diet. Group B for each strain was fed a diet containing 21% crude protein and 3000 kcal/kg ME as starter diet for the first four weeks and then shifted by a diet containing 19% crude protein and 3000 kcal/kg ME as finisher diet. The formulation of experimental diets is shown in Table 1.

Collection of blood samples: The blood samples were collected from the heart of the birds using A sterile disposable syringe (5ml) in the early morning at both third and seventh week of age. Three samples from each replicate were randomly taken. A sample of 3ml of blood was collected from each bird and immediately preserved into two test tubes. One with anticoagulant (EDTA) and the other one without. The blood in the tube that with anticoagulant was used for determination of packed cell volume, hemoglobin concentration, total erythrocyte counts and differential leukocyte count, while, the second tube was left for about 3 hours at room temperature, then centrifuged at 3000 r.p.m for 15 minutes. Haemolysis-free serum samples were separated and transferred to clean plastic vials and immediately frozen at -20°C for the determination of serum glucose, albumen, calcium, phosphorus, sodium and potassium.

Laboratory analysis: Packed Cell Volume (PCV) was measured using a microhematocrit centrifuge. Hemoglobin concentration (Hb) was measured by colorimeter using cyanmethaemoglobin method. Total Red Blood Cells (TRBC) count were estimated using hemocytometer. The haematological indices; Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were calculated from PCV, Hb and TRBC count. Glucose concentration was determined by the enzymatic method-glucose oxidase reaction using a kit (SPINREACT, S.A. SPAIN). Albumin was determined by the bromocresol green method using a kit (SPINREACT, S.A. SPAIN). Serum calcium (Ca) and phosphorus (P) were determined by colorimetric method using a kit (SPINREACT, S.A. SPAIN). Serum sodium (Na) and potassium (K) concentrations were determined by flame photometer technique.

Statistical analysis: The recorded data was subjected to the analysis of variance for factorial experiment in a completely randomized design by general linear model using (Statistix program, version 9). Means separation

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Table 1: Ingredients composition of experimental diet on percent basis

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet (1)</th>
<th>Diet (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td>61</td>
<td>61.5</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>15.8</td>
<td>12</td>
</tr>
<tr>
<td>Sesame cake</td>
<td>13</td>
<td>11.3</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Super concentrate</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

*Composition of supper concentrates BRO-5 (1504.10).
Fishmeal, vegetable protein, dicalciumphosphate, limestone, vitamins, trace-elements, antioxidant.
*Vitamins and premix minerals per kg of diet.
was done by LSD multiple range tests and the values were expressed as Means and Standard Error. The means were considered significantly different when P<0.05.

**RESULTS AND DISCUSSION**

The effects of broiler strain and season on hematological parameters and indices are presented in Table 2. The results showed that there was significant (P<0.05) decrease in PCV value in both Cobb and Hubbard strains during the summer compared to winter season, whereas, in Ross strain, the PCV was significantly (P<0.05) increased during the summer compared to the winter. The decrease in PCV in Cobb and Hubbard strains during summer could be attributed to reduction in number of erythrocyte, caused by a rise in erythrocyte destruction or haemodilution. This finding is in an agreement with that ones reported by (Yahav and Hurwitz, 1996) who observed that the exposure of male broiler chickens to high temperatures caused a decrease in PCV values. Whereas, the increase in PCV during the summer in Ross might be due to hemoconcentration because of high temperature (Huston, 1960; Moye et al., 1969; Khan et al., 2002). The results reflected that there was significant (P<0.05) decrease in haemoglobin concentration in Cobb strain during the summer compared to winter season, whereas, when compared the Hb concentration in the summer to that of the winter in both Ross and Hubbard strains, there was slight but not significant decrease. Lower levels of haemoglobin in the chicken blood during the summer can be related to the effect of high environmental temperature on changing the distribution of iron in the organisms of broilers, these changes are described by Jamadar and Jalnapurkar (1995). The decrease in Hb concentration is in accordance with that reported by Deaton et al. (1969); Zimmerman et al. (1973, 1975); Vecerek et al. (2002); Khan et al. (2002); Comito et al. (2007) who reported that the exposure of birds to high temperature cause significant decrease in Hb concentration. The current findings demonstrated that there was slight but not significant decrease in Total Red Blood Cells count (TRBC) during the summer compared to the winter season in the three strains. This result is in harmony with that of Khan et al. (2002) who found that high ambient temperature decreases TRBC, which might be due to a decrease in oxygen consumption by the chicks, as a result of high ambient temperature, which might be associated with a concurrent reduction in the production of red blood cells (depressed hemopoetic activity) as a consequence of lower basal metabolic rate (Huston et al., 1962). The results highlighted that the Mean Cell Volume (MCV) was significantly (P<0.05) increased in Ross strain. Whereas, in Cobb and Hubbard strains there was no significant (P<0.05) difference in MCV values during the summer compared to the winter seasons. The increase in MCV in Ross strain is in the line with the previous findings (Aengwanich and Chinrasri, 2003, 2004; Aengwanich, 2007) who observed that the exposure of broiler birds to heat stress increases their MCV. The results showed that the Mean Cell Haemoglobin Concentration (MCHC) was significantly (P<0.05) decreased in Ross and Cobb strains but there was slight but not significant decrease in Hubbard strain during the summer compared to the winter season. The significant (P<0.05) decrease in MCH could be attributed to the high bilirubin concentration, relative bile volume and bile contamination in the feces during the heat stress, which indicates that chickens under heat stress lost haemoglobin (Aengwanich and Chinrasri, 2002). This result is in consistence with the previous ones (Aengwanich and Simaraks, 2003, 2004; Aengwanich, 2007) which indicated that heat stress decreases MCH. In regards to Mean Cell Haemoglobin Concentration (MCHC), the results revealed that in all strains there was significant (P<0.05) decrease during summer compared to winter seasons. The observed decrease in MCHC value during the summer could be attributed to the decrease of salt content in blood plasma. In addition it could be justified by the reduction of RBC counts during the summer. The differences between strains in measured parameters can be related to the fact that a lot of variations exist among the different breeds of broiler chickens (Mmereole, 2009).

**Table 2: Effect of broiler strain and season on hematological parameters and indices**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ross</th>
<th>Cobb</th>
<th>Hubbard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summer</td>
<td>Winter</td>
<td>Summer</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>44.929*</td>
<td>37.917*</td>
<td>29.625*</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.925*</td>
<td>12.760*</td>
<td>11.030*</td>
</tr>
<tr>
<td>H/L</td>
<td>0.5519*</td>
<td>0.5281*</td>
<td>0.5797*</td>
</tr>
<tr>
<td>TRBC(million cell/mm³)</td>
<td>2.24*</td>
<td>2.67a</td>
<td>2.87a</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>299.45</td>
<td>144.47</td>
<td>157.95</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>48.457</td>
<td>70.392</td>
<td>44.481</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>27.407</td>
<td>36.159</td>
<td>30.908</td>
</tr>
</tbody>
</table>

*a,b,c,d*: Mean values within the same row with different superscripts letters are significantly different at P<0.05.

**SEM: Standard error of means.**
Table 3: Effect of broiler strain and season on some blood biochemical compositions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ross</th>
<th>Cobb</th>
<th>Hubbard</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>147.10</td>
<td>196.79</td>
<td>175.63</td>
<td>183.03</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.5763</td>
<td>1.8150</td>
<td>1.7508</td>
<td>1.7750</td>
</tr>
<tr>
<td>Na (mg/dl)</td>
<td>122.046</td>
<td>134.108</td>
<td>132.318</td>
<td>137.121</td>
</tr>
<tr>
<td>K (mg/dl)</td>
<td>3.9797</td>
<td>3.9950</td>
<td>3.6571</td>
<td>4.0054</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>9.2625</td>
<td>9.3258</td>
<td>7.9333</td>
<td>9.7667</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td>5.8842</td>
<td>8.5246</td>
<td>5.9000</td>
<td>8.2204</td>
</tr>
</tbody>
</table>

a,b,c,d: Mean values within the same row with different superscripts letter are significantly different at P<0.05.

Table 4: Effect of broiler strain and protein level on hematological parameters and indices

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ross High CP</th>
<th>Ross Low CP</th>
<th>Cobb High CP</th>
<th>Cobb Low CP</th>
<th>Hubbard High CP</th>
<th>Hubbard Low CP</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>42.638a</td>
<td>40.208a</td>
<td>32.438abc</td>
<td>35.233a</td>
<td>31.208a</td>
<td>28.950a</td>
<td>±1.8999</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.535a</td>
<td>12.150a</td>
<td>12.394a</td>
<td>12.520a</td>
<td>11.488a</td>
<td>12.079a</td>
<td>±0.5683</td>
</tr>
<tr>
<td>TRBC (million cell/mm³)</td>
<td>2.46a</td>
<td>2.45a</td>
<td>3.15a</td>
<td>2.22a</td>
<td>2.22a</td>
<td>2.30a</td>
<td>±0.560347</td>
</tr>
<tr>
<td>MCV (lL)</td>
<td>273.54a</td>
<td>170.37e</td>
<td>146.91h</td>
<td>162.65j</td>
<td>147.61k</td>
<td>134.78l</td>
<td>±60.511</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>67.890a</td>
<td>50.960a</td>
<td>55.802a</td>
<td>59.268a</td>
<td>53.944a</td>
<td>56.145a</td>
<td>±10.489</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>31.267c</td>
<td>32.300c</td>
<td>41.935bc</td>
<td>38.864c</td>
<td>39.198bc</td>
<td>42.908c</td>
<td>±2.6846</td>
</tr>
</tbody>
</table>

a,b,c,d,e: Mean values within the same row with different superscripts letter are significantly different at P<0.05.

Result in Table 3 shows the effect of broiler strain and season on blood biochemical. The results indicated that in Ross strain there was significant (P<0.05) decrease in serum glucose concentration during the summer compared to the winter season. Whereas, in both Cobb and Hubbard strains there was slight but not significant decrease in serum glucose during the summer compared to the winter season. The significant (P<0.05) decrease in glucose concentration during the summer could be due to the reduction in feed intake, in addition to, it could be also due to the increase in the rate of water consumption accompanied by haemodilution in response to thermal stress and consequently a decrease in carbohydrate consumption and probably the hepatic storage of glycolgen. This finding is in the line with the previous research carried out by Arad et al. (1983); Abdalla and Nawal (2009).

The results indicated that in Ross strain there was significant decrease (P<0.05) in serum albumin concentration during the summer compared to the winter. Meanwhile, in Cobb and Hubbard strains there was slight but not significant decrease in serum albumin concentration in the summer compared to the winter. This decrease in serum albumin during the summer could be justified by reducing amount of protein consumed and consequently deficiency of essential amino acids as a result of decreased amount of feed consumed by birds, accompanied by reduction in digestibility of proteins as a result of exposure of broilers to high environmental temperature (Bonnet et al., 1997). This result is in harmony with previous results reported by Sahin et al. (2001a,b); Ozbey et al. (2004); Faisal et al. (2008). All strains showed a significant (P<0.05) decrease in the concentrations of serum Na and K during the summer compared to the winter. This could be due to hemodilution following the increase in water consumption. This finding is in full accordance with the old and recent ones (Ahmed et al., 1994; Donkoh et al., 1999; Alam et al., 2004; Alabi et al., 2008; Ogbuewu et al., 2010). The results also indicated that the dietary protein level had no significant effect (P<0.05) on Hb concentration. This can be justified by the fact that supplementation of
dietary protein level may leads to increase the globin part of the haemoglobin but not the haem part (Shahidullah et al., 2008). This result is in consisten with that of (Ahmed et al., 1994; Donkoh et al., 1999; Alam et al., 2004; Shahidullah et al., 2008 and Adeyemo, 2010). In all strains the level of dietary protein did not significantly affect the total red blood cells count. This finding could be confirmed that of (Ahmed et al., 1994; Donkoh et al., 1999; Alam et al., 2004; Adeyemo, 2010). Furthermore, the level of dietary protein had no significant effect on MCV, MCH and MCHC values. This finding is not in contradiction with the previous ones reported by Egbenike et al. (2009) who found that the level and the source of dietary protein have no any effect on haematological indices.

The effect of broiler strain and dietary protein level on blood biochemical composition is shown in Table 5. The results clearly indicated that, in all strains the level of dietary protein had no significant (P<0.05) effect on serum glucose concentration. It has been well documented that the level of dietary protein does not affect the concentration of blood glucose level on poultry (Swennen et al., 2005; Kamran et al., 2010). The present study reflected that in all strains the level of dietary protein had no significant (P<0.05) effect on serum albumin concentration. Our result agrees with those obtained by Agbede and Aletor (2003) and Bunchasak et al. (2005). Moreover, in all strains the level of dietary protein had no significant (P<0.05) effect on serum Na concentration. This result disagrees with the result obtained by Nworgu (2004) and Nworgu et al. (2007) who reported a significant increase in serum Na and K in broilers fed mimosa leaf meal and supplemented in water with different levels of Fluted Pumpkin Leaves Extract. This disagreement could be justified by the differences in the source and type of protein.

Moreover, in Hubbard strain there was significant increase (P<0.05) in serum K concentration in birds fed high dietary protein level. Whereas, there was no significant difference (P<0.05) in K concentration of Ross and Cobb strains fed high dietary protein compared to birds fed low dietary protein level. The significant increase (P<0.05) in Hubbard strain fed high dietary protein is in agreement with findings obtained by previous studies of (Nworgu et al., 2007, 2004 and Kermanshahi et al., 2011) who stated that the serum K increases when dietary protein level is increased. In all strains neither the concentration of serum Ca nor serum P had been significantly (P<0.05) affected by the dietary protein level.

**Conclusion:** It can be concluded that there was significant broiler strain X season interaction affecting the haematological parameters, haematological indices and blood biochemical compositions. The physiological responses of genetically improved broiler strains to environment (heat stress) and nutritional (low protein) stresses appeared to be significantly different from each other. Reducing crude protein level in rations supplemented with limited essential amino acids to meet the NRC requirements (mainly methionine and lysine), possibly has no significant effect on most of physiological parameters.

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