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

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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 both Cobb and 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.

Key words: 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 (Baneriee, 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, hematological 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

Table 1: Ingredients composition of experimental diet on percent basis

	Diet (1)		Diet (2)			
Ingredient	Starter (%)	Finisher (%)	Starter (%)	Finisher (%)		
Sorghum	61	61.5	61.5	68.5		
Groundnut cake	15.8	12	12	7.3		
Sesame cake	13	11.3	11.3	9		
Wheat bran	4	9	9	9		
Super concentrate	5	5	5	5		
Limestone	0.9	0.9	0.9	0.9		
NaCl	0.25	0.25	0.25	0.25		
Lysine	0.04	0.04	0.04	0.04		
Methionine	0.01	0.01	0.01	0.01		
Total	100	100	100	100		

\*Composition of supper concentrates BRO-5 (1504.10).

Fishmeal, vegetable protein, dicalciumphosphate, limestone, vitamins, trace-elements, antioxidant.

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 methodglucose 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

<sup>\*</sup>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, 2002, 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 (MCH) 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 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

Parameter	Ross		Cobb		Hubbard		
	Summer	Winter	Summer	Winter	Summer	Winter	SEM
PCV (%)	44.929°	37.917⁵	29.625 <sup>d</sup>	38.046⁵	26.117 <sup>d</sup>	34.042°	±1.8999
Hb (g/dl)	11.925 <sup>bc</sup>	12.760 <sup>ab</sup>	11.030°	13.883ª	11.721 <sup>bc</sup>	11.846 <sup>bc</sup>	±0.5683
H/L	0.5519°	0.5281⁴	0.5797⁵	0.5680₺፡	0.60528	0.5153⁴	±0.00913
TRBC(million cell/mm³)	2.243	2.67ª	2.87ª	2.49°	2.16°	2.36°	±560347
MCV (fL)	299.45°	144.47 <sup>b</sup>	157.95⁵	151.61 <sup>b</sup>	151.56⁵	130.83 <sup>b</sup>	±60.511
MCH (pg)	48.457b	70.392ª	44.481 <sup>b</sup>	70.588ª	51.210ab	58.879ab	±10.489
MCHC (q/dl)	27.407 <sup>d</sup>	36.159bc	30.908 <sup>cd</sup>	49.891ª	36.276b	45.830°	±2.6846

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

	Ross	Ross		Cobb		Hubbard		
Parameter	Summer	Winter	Summer	Winter	Summer	Winter	SEM	
Glucose(Mg/dl)	147.10 <sup>d</sup>	198.79ª	175.63b	183.03b	164.44 <sup>€</sup>	180.33b	±5.1332	
Albumin (g/dl)	1.5763 <sup>€</sup>	1.8150°	1.7508ab	1.7750°	1.6542 <sup>bc</sup>	1.7625ab	±0.0559	
Na (Mg/dl)	122.046 <sup>d</sup>	134.108bc	132.318°	137.121 <sup>a</sup>	115.524°	135.008⁵	±1.0330	
K (Mg/dl)	3.3979 <sup>d</sup>	3.9950⁰	3.6571⁵	4.0054°	3.8746 <sup>b</sup>	4.0200°	±0.0456	
Ca (Mg/dl)	9.2625°	9.3258°	7.9333b	9.7667°	9.1771ª	9.7196°	±0.3407	
P (Mg/dl)	5.8842b	8.5246°	5.9000₺	8.2204*	6.1083 <sup>b</sup>	8.0975°	±0.2699	

<sup>Ձ,Ե,c,৩,e</sup>: Mean values within the same row with different superscripts letter are significantly different at P<0.05. SEM: Standard error of means.

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

	Ross		Cobb	Cobb		Hubbard	
Parameter	High CP	Low CP	High CP	Low CP	High CP	Low CP	SEM
PCV (%)	42.638°	40.208ª	32.438bc	35.233₺	31.208°	28.950⁰	±1.8999
Hb (g/dl)	12.535°	12.150°	12.394°	12.520°	11.488°	12.079ª	±0.5683
TRBC(million cell/mm³)	2.46°	2.45°	3.15°	2.22°	2.22ª	2.30°	±560347
MCV (fL)	273.54ª	170.37 <sup>ab</sup>	146.91 <sup>b</sup>	162.65ab	147.61 <sup>b</sup>	134.78b	±60.511
MCH(pg)	67.890°	50.960°	55.802ª	59.268°	53.9448	56.145ª	±10.489
MCHC (g/dl)	31.267₺	32.300 <sup>b</sup>	41.935°	38.864ª	39.198ª	42.908ª	±2.6846

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

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 glycogen. 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 consistence with previous results (Devhim et al., 1990; Belay and Teeter, 1993; Ait-Boulahsen et al., 1995; Borges, 1997; Salvador et al., 1999) who demonstrated that the serum Na and K concentrations were lower during the heat stress. The results indicated that in Cobb strain there was significant (P<0.05) decrease in serum calcium concentration during the summer compared to the winter. Whereas, in Ross and Hubbard strains there was slight but not significant, decrease in serum Ca concentration during the summer compared to the winter. The results also demonstrated that in all strains there was significant decrease (P<0.05) in serum phosphorus concentration during the summer compared to the winter. This result is in an agreement with that conducted by Kohne and Jones (1975); El-Husseiny and Creger (1981); Bogin et al. (1981); Kamar et al. (1987) who found that high ambient temperature decrease the plasma Ca and P levels.

The effect of broiler strain and dietary protein level on haematological parameters and indices are shown in Table 4. The results showed that in all strains there was no significant difference (P<0.05) in PCV value between the birds fed high or low dietary protein levels. This result 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

Table 5: Effect of broiler strain and dietary protein level on blood biochemical compositions

	Ross	Ross		Cobb		Hubbard	
Parameter	High CP	Low CP	High CP	Low CP	High CP	Low CP	SEM
Glucose(Mg/dl)	175.48 <sup>ab</sup>	170.41 <sup>b</sup>	177.88ab	180.78°	168.48b	176.29ab	±5.1332
Albumin (g/dl)	1.7129°	1.6783°	1.7546°	1.7713°	1.7200°	1.6967 <sup>a</sup>	±0.0559
Na (Mg/dl)	128.379b	127.775b	135.307°	134.132a	125.512°	125.020⁰	±1.0330
K (Mg/dl)	3.6275 <sup>d</sup>	3.5654 <sup>d</sup>	3.8675₺₺	3.7950⁰	3.9937°	3.9008b	±0.0456
Ca (Mg/dl)	9.4342°	9.1542ab	9.0242ab	8.6758b	9.4842°	9.4125°	±0.3407
P (Mg/dl)	7.2113°	7.1975°	6.8992°	7.2213°	7.0938°	7.1121 <sup>a</sup>	±0.2699

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

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 consistence 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 Egbunike *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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