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Full Length Research Paper

Influence of electrolytes and ascorbic acid supplementation on serum and erythrocytic indices of broiler chickens reared in a hot environment

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Effect of supplementing drinking water with ammonium chloride, sodium bicarbonate, calcium chloride and ascorbic acid on haematology and selected serum indices of broiler chicks reared during high temperature and humified period was evaluated. A total of 200 one-day old Arbor acre broiler chicken strain was randomly allotted to five treatments: T1 (control-water without any supplement), T2 (0.5% ammonium chloride), T3 (0.5% sodium bicarbonate), T4 (0.5% calcium chloride), T5 (300 ppm ascorbic acid) in a completely randomized design. The packed cell volume, haemoglobin and albumin values obtained at day 28 and 49 varied significantly ($p<0.05$) between treatments with consistently higher ($p<0.05$) values for birds on T5. The serum total proteins at day 28 and alanine amino transferase at day 49 were also significantly different ($p<0.05$) among treatments. Glucose values (g/dl) of groups at day 28 were not significantly ($p>0.05$) different, while glucose levels of control group was significantly ($p<0.05$) higher than those on T3 at day 49. Ascorbic acid supplementation enhanced the haematological profile of birds while supplemental electrolytes ameliorated heat stress with more profound effects on serum indices of birds on sodium bicarbonate.

Key words: Heat stress, ascorbic acid, haematological indices, serum metabolites, electrolytes, tropic environment.

INTRODUCTION

In the tropics, high environmental temperature is one of the most important stressors affecting production performance of broilers and compromises the ability of birds to maintain homeostasis. Reduction in feed intake, growth rate, egg production and feed efficiency are immediate consequences of heat stress in poultry production (Siegel, 1995).

It has been reported by Cier et al. (1992) that supplemental ascorbic acid alleviates the effect of heat stress on the performance of broiler chicks and increased immunoglobulin G levels (Tras et al., 2001). Though, ascorbic acid was reported to be synthesized by poultry and therefore not required to be supplemented in the diet under normal conditions, there has nevertheless been considerable interest in its possible role in maintaining homeostasis. The requirements of ascorbic acid may be elevated under hot environmental conditions (Pardue and Thaxton, 1986) when endogenous synthesis may not be adequate to meet the physiological needs of the birds. Also, electrolytes of various sources may have different effects on the physiology and blood characteristics of heat stressed birds, depending on whether they were included in the feed or water (Teeter and Smith 1985; Branton et al., 1986; Borges et al., 2007).

The blood system is particularly sensitive to changes in temperature, being an important indicator of physiological responses in birds to stressing agents. Borges et al. (2007) surmised that quantitative and morphological

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Table 1. Gross composition of diets fed to birds given supplemental electrolytes and ascorbic acid.

Feed ingredients (%)	Starter diet	Finisher diet
Maize	52.00	52.00
Wheat offals	7.73	7.73
Soyabeans	35.00	30.00
Palm kernel meal	-	4.50
Palm oil	2.50	3.00
Oyster shell	0.50	0.50
Dicalcium phosphate	1.50	1.50
Salt	0.25	0.25
Methionine	0.15	0.15
Lysine	0.06	0.06
Broiler premix	0.25	0.25
Avatec	0.06	0.06
	100	100
Calculated analysis		
Crude protein	22.97	19.06
Metabolizable energy (kcal/kg)	3007.7	3018.9
Calorie:protein ratio	136.4:1	152.8:1

1 kg of premix contains: Vitamin A-10,000,000 IU; Vitamin D3-2,000,000; Vitamin E-20,000 IU; Vitamin K-2,250 mg; Thiamine B1-1,750 mg; Riboflavin B2- 5,000 mg; Pyridoxine B6-2,750 mg; Niacin-27,500 mg; Vitamin B12-15 mg; Pantothenic acid-7,500 mg; Folic acid-7500 mg; Biotin-50 mg; choline chloride-400 g; Antioxidant-125 g; Magnesium-80 g; Zinc-50 mg; Iron-20 g; Copper-5 g; Iodine-1.2 g; Selenium-200 mg; Cobalt-200 mg.

changes in blood cells were associated with heat stress and were translated by variations in haematocrit value, leukocyte counts, erythrocyte and haemoglobin contents. Vecerek et al. (2002) reported decreased haemoglobin levels and increased total blood leukocytes counts in chicken due to gradually increasing temperature. Maxwell et al. (1990) studied the effect of feed restriction on erythrocyte characteristics and reported significant changes in hematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and leukocyte count. Junqueira et al. (1999) evaluated sodium bicarbonate supplementation together with ammonium chloride on the drinking water and found no effect of treatments on the hematological parameters.

The effect of electrolytes and ascorbic acid supplementation on the erythrocytic and serum indices of heat stressed broiler is not adequately documented. This study therefore evaluated the effect of electrolytes and ascorbic acid supplementation in drinking water on erythrocytic and selected serum indices of broiler chickens exposed to heat stress.

MATERIALS AND METHODS

The study was undertaken at the Teaching and Research Farm of the University of Ibadan, Nigeria during the hot dry season of the year for a period of seven weeks. The site is located between latitudes 6°10” and 9°10” North of the equator and longitudes 30 and 60 of the Greenwich.

Experimental birds and management

Two hundred one day-old Arbor acre broiler chicken strain was used for the experiment. After one week of brooding, they were weighed and randomly allotted to five treatments. Each treatment was replicated four times with 10 birds per replicate in a completely randomized design (CRD). The birds were raised on conventional deep litter open sided house. Ambient temperature and humidity of the poultry house were recorded daily at 8.00, 13.00 and 20.00 h using thermo hygrometers.

Formulated broiler starter and finishers’ diets contained 3000 Kcal/kg ME and 23% CP; 3000 kcal/kg ME and 19% CP respectively were offered to birds ad libitum in the course of the experiment. Composition of the experimental diets is shown in Table 1. Clean water in which test electrolytes or ascorbic acid has been added was provided *ad libitum* from day 15 to day 49 of the experiment. Treatment 1 (control) was without any supplement added, Treatment 2 (0.5% ammonium chloride), Treatment 3 (0.5% sodium bicarbonate), Treatment 4 (0.5% calcium chloride), and Treatment 5 (300 ppm ascorbic acid). Routine vaccinations and other medications were administered.

Blood collection

At day 28 and 49 of the experiment, three birds per replicate were randomly selected and bled at the jugular vein from which blood was drained and collected into heparinized bottles for haematological study. Blood for serum indices determination was collected into plain vacutainer tubes without EDTA for serum separation. Blood samples were centrifuged, serum separated out, decanted, and deep frozen for analysis.

Estimation of haematological variables

Packed cell volume (PCV) was determined by micro-hematocrit method (Schalm et al., 1975). Haemoglobin (Hb) concentration was measured spectrophotometrically using cyanomet haemoglobin method (Schalm, 1975). Red blood cell (RBC) count was estimated using haemocytometer (Schalm et al., 1975). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from Hb, PCV and RBC (Jain, 1986)

Determination of serum parameters

Serum total protein (STP) was determined using Biuret method as described by Kohn and Allen (1995). Albumin was determined using Bromocresol green (BCG) method (Peter et al., 1982). The globulin concentration was obtained by subtracting albumin from the total protein. Cholesterol determination was as described by Roschlan et al. (1974). Aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were determined by spectrophotometric methods (Rej and Hoder, 1983)

Statistical analysis

Data generated were analysed using analysis of variance (SAS, 1999). Significant treatment means were compared using Duncan’s option of the same software. P<0.05 level was accepted statistically significant level.

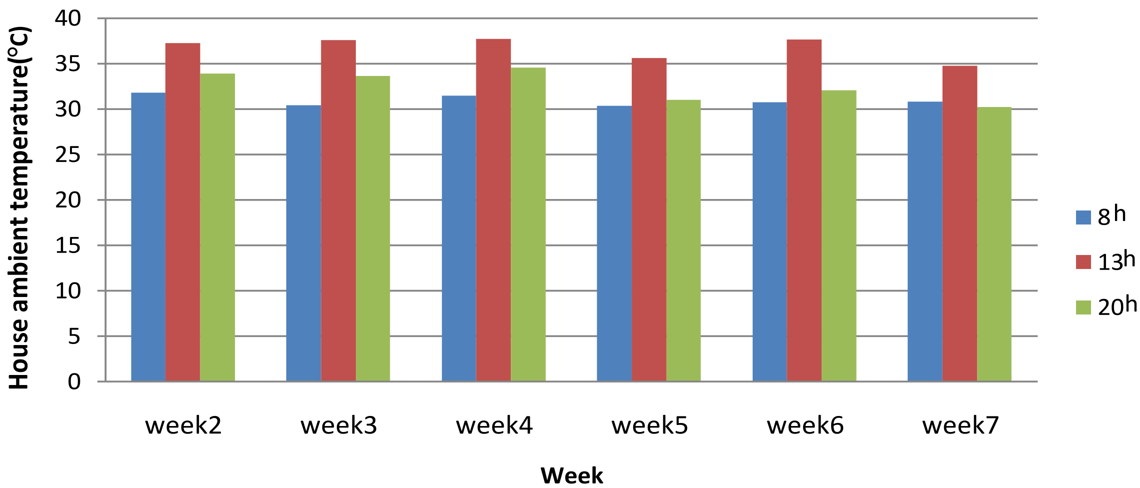

Figure 1. Weekly poultry house ambient temperature.

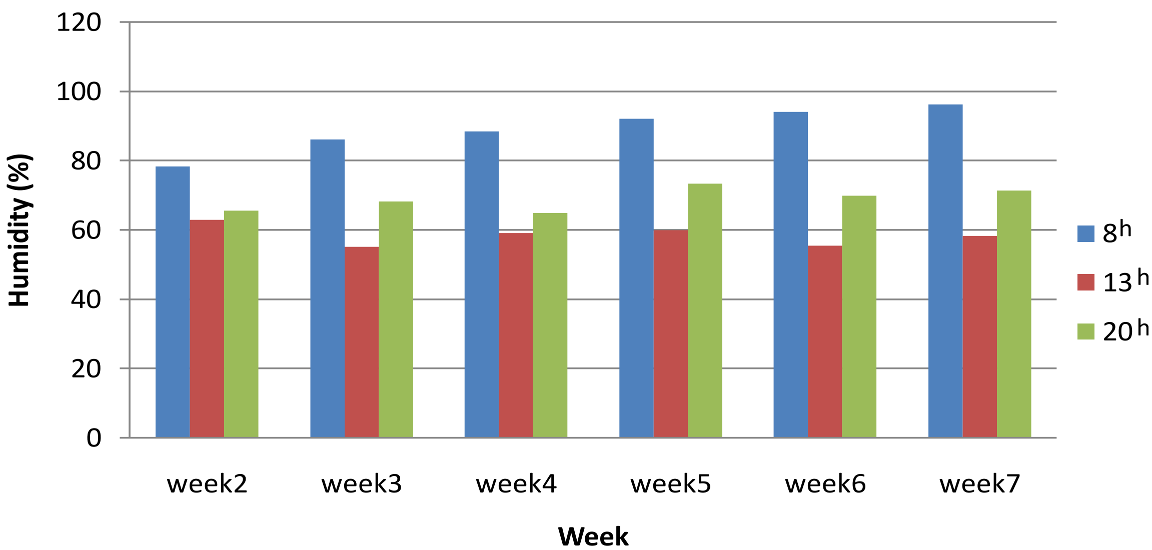

Figure 2. Weekly poultry house humidity.

RESULTS AND DISCUSSION

Environmental condition

Figure 1 shows the average weekly house ambient temperature. The recorded ambient temperature in the experimental period indicated a range (30.90 to 36.73°C) well above the thermo neutral zone (18 to 22°C) for broiler (Charles, 2002) indicating exposure of birds to perpetual heat stress. Behavioural responses such as panting, couching near cool surfaces and wide spreading of the wings were observed during the experimental period. According to Gray et al. (2003) panting would normally be expected to occur when the ambient temperature is near or above 30°C.

Figure 2 shows the average weekly relative humidity of the poultry house. A range of 58.48 to 89.24% relative humidity was observed which was quite high. High humidity above 60% has been reported to impair heat transmission from body core to the peripheral at 35°C but facilitated it at 30°C in broiler chicken of 4-week age (Lin et al., 2006). The prevailing weather conditions were clearly indicative that the birds were perpetually under stress.

Poultry house temperature and relative humidity

Table 2 shows the haematological profile of heat stressed broiler given water supplemented with electrolytes and ascorbic acid at day 28. PCV (%) and haemoglobin levels of treatment 5 were higher (p<0.05) higher than treatments

Table 2. Haematological parameters of heat stressed broiler given water supplemented with electrolytes and ascorbic acid at day 28.

Parameter	T1	T2	T3	T4	T5	SEM
Packed cell volume (%)	27.5[b]	27.3[b]	29.0[ab]	27.7[b]	29.7[a]	0.18
Haemoglobin (g/dl)	9.16[b]	9.11[b]	9.66[ab]	9.24[b]	9.91[a]	0.06
Red blood cell (×10^6/mm^3)	2.36	2.07	2.14	2.20	2.26	35.6
MCV(μ^3)	117	136	155	128	137	3.54
MCHC (%)	33.3	33.3	33.3	33.3	33.3	0.00
MCH (μμg)	39.2	45.3	51.8	42.7	45.7	1.18

[a,b]Means on the same row with different superscripts are significantly different (p<0.05). MCV, Mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular volume. SEM, Standard error of mean; T1, control; T2, ammonium chloride; T3, sodium bicarbonate; T4, calcium chloride; T5, ascorbic acid.

Table 3. Haematological parameters of heat stressed broiler given water supplemented with electrolytes and ascorbic acid at day 49.

Parameter	T1	T2	T3	T4	T5	SEM
Packed cell volume (%)	31.1[ab]	30.5[ab]	28.6[b]	28.5[b]	34.6[a]	0.62
Haemoglobin (g/dl)	10.3[ab]	10.16[ab]	9.54[b]	9.49[b]	11.4[a]	0.20
Red blood cell (x10^6/mm^3)	2.16	1.96	2.21	2.24	2.28	0.05
MCV(μ^3)	146	157	132	130	156	4.05
MCHC (%)	33.3[a]	33.3[a]	33.3[a]	33.3[a]	33.1[b]	0.02
MCH (μμg)	48.8	52.5	44.0	43.4	51.6	1.33

[a,b]Means on the same row with different superscripts are significantly different (p<0.05) MCV, Mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular volume. SEM, Standard error of mean. T1, control; T2, ammonium chloride; T3, sodium bicarbonate; T4, calcium chloride; T5, ascorbic acid.

1, 2 and 4. The haematological response of heat stressed broiler at day 49 is shown in Table 3. Increased (p<0.05) PCV and haemoglobin values of treatment 5differ significantly (p<0.05) from treatments 3 and 4.

The Hb, PCV and MCHC values obtained in the present study were consistent with the reported range for broiler (Mitruka and Rawnsley, 1977). Iheukwumene and Herbert (2003) reported values of 6.0 to 13.0%, 29.0 to 38.0% and 33.0 to 35.0 pg, respectively for these parameters. Islam et al. (2004) noted that commercial and local chickens reared inSylhet region in Bangladesh recorded Hb value of 7.06 to 9.37%, PCV value of 26.56 to 34.60% and MCV value of 84.27 to 163.56 fl which conformed with the report of this study. The PCV range of 26.25 to 34.62% obtained for birds in this study was lower than the average value of 38.52% reported by Awotuyi (1990) for adult domestic chicken. The observed difference in the value of PCV may be due to breed and age difference. As earlier documented (Kubena et al., 1972; Oyewale, 1987) that birds in the tropics tend to have lower haemoglobin values compared to those reared in the temperate.

The noted increase in the values of PCV and hemoglobin of birds on Treatment 5 could be attributed to the effect of vitamin C in protecting the membrane integrity of the erythrocytes as earlier reported (Candan et al., 2002; Adenkola et al., 2010). This function directly affected the haemoglobin concentration of birds on Treatment 5 which was higher than the values obtained for other groups. The increase in haemoglobin concentration could also be attributed to the role of vitamin C in increasing the absorption of iron from digestive system (Harper et al., 1979)

Table 4 shows the serum metabolites and enzymes of heat stressed broiler given water supplemented with electrolytes and ascorbic acid at day 28. The values obtained for serum enzymes did not vary significantly (p>0.05). However, significant variations (p<0.05) were observed in the serum total protein and albumin values. Birds on treatment 3 had the higher total protein value which was not significantly different (p>0.05) from values obtained for birds on treatments 1, 2 and 5. Significant variations (p<0.05) were also observed in the values obtained for albumin. Birds on treatment 5 had higher value of albumin but not significantly different (p>0.05) from the values obtained for birds on treatments 1, 2 and 3.

Serum metabolites and enzymes of heat stressed broiler treated with electrolytes and ascorbic acid at day 49 is shown in Table 5. Cholesterol, triglyceride, total protein, globulin and alanine aminotransferase values of birds on different treatments were not significantly affected by supplemental electrolytes and ascorbic acid. Significant difference (p<0.05) was observed in the

Table 4. Serum metabolites of heat stressed broiler treated with electrolytes and ascorbic acid at week four.

Parameter	T1	T2	T3	T4	T5	SEM
Glucose (g/dl)	196	181	182	187	184	3.18
Cholesterol (mg/dl)	96.4	96.3	106	103	98.0	3.02
Triglycerides (mg/dl)	299	295	306	282	309	0.08
Total protein (g/dl)	3.29^{ab}	3.42^{ab}	3.78^{a}	2.91^{b}	3.47^{ab}	0.04
Albumin (g/dl)	2.42^{ab}	2.43^{ab}	2.53^{a}	2.22^{b}	2.65^{a}	0.08
Globulin (g/dl)	0.87	0.99	1.25	0.69	0.82	1.85
Aspartate Aminotransferase (AST) (i.u/l)	102	104	106	111	110	0.19
Alanine aminotransferase (ALT) (i.u/l)	7.04	7.34	7.84	7.58	7.50	5.23

[a,b]Means on the same row with different superscripts are significantly different(p<0.05). SEM, Standard error of mean. T1, control; T2, ammonium chloride; T3, sodium bicarbonate; T4, calcium chloride; T5, ascorbic acid.

Table 5. Serum metabolites of heat stressed broiler treated with electrolytes and ascorbic acid at week 7.

Parameter	T1	T2	T3	T4	T5	SEM
Glucose(g/dl)	180^{a}	174^{ab}	143^{b}	172^{ab}	157^{ab}	3.80
Cholesterol(mg/dl)	128	135	134	124	113	4.73
Triglyceride(mg/dl)	243	250	271	260	263	11.7
Total protein(g/dl)	4.19	3.83	3.89	3.58	3.79	0.13
Albumin(g/dl)	1.89^{a}	1.78^{ab}	1.58^{ab}	1.25^{b}	1.59^{ab}	0.07
Globulin(g/dl)	2.29	2.05	2.31	2.32	2.20	0.13
Aspartate aminotransferase AST (i.u/l)	132	125	129	136	130	2.11
Alanine aminotransferase ALT (i.u/l)	9.78^{ab}	7.68^{b}	9.46^{ab}	10.3^{ab}	11.8^{a}	0.35

[a,b]Means on the same row with different superscripts are significantly different (p<0.05), SEM, Standard error of mean. T1, control; T2, ammonium chloride; T3, sodium bicarbonate; T4, calcium chloride; T5, ascorbic acid.

glucose values of birds on treatments 1 and 3. Birds on treatment 1 had higher (p<0.05) glucose value while birds on treatment 3 had the lower glucose value which was similar (p>0.05) to those obtained for treatments 2, 4 and 5. According to Borges et al. (2007) increase in glucose concentration was one of the direct responses of birds to greater adrenaline, noradrenalin and glucocorticoid secretion in stressful conditions which was needed to prepare birds for a “fight and flight” response. The lower values of serum glucose observed for birds on treatments 2, 3, 4 and 5 compared with control group indirectly connoted reduced level of stress among the birds brought about by the effect of supplemental electrolytes and ascorbic acid. The lowest value of glucose obtained for birds on treatment 3 indicated that the said treatment had greater mitigating effect on heat stress.

Significant variation (p<0.05) was also observed in the albumin values, with birds on Treatment 1 having higher value which was similar (p>0.05) to the values obtained for birds on treatments 2, 3 and 5. Significant difference (p<0.05) was observed in the values of alanine amino transferase (ALT) with treatment 5 having higher value which was significantly different (p<0.05) from the value obtained for birds on treatment 2. ALT catalyzes the transfer of an amino group from alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. This index has been an indication of liver function test and elevated levels monitored liver malfunction (Murray et al., 1990).

Conclusion

Erythrocytic indices of birds reflected the positive enhancing effects of supplemental ascorbic acid. Also, the obtained pattern of serum glucose concentration was indicative of the mitigating effect of both supplemental electrolytes and ascorbic acid on stressed birds which was more pronounced in birds on sodium bicarbonate.

REFERENCES

Adenkola AY, Kaankuka FG, Ikyume TT, Ichaver IF. Yaakugh IDI (2010). Ascorbic acid effect on erythrocyte osmotic fragility, haematologicalparameters and performance of weaned rabbits at the end of rainy season in Makurdi Nigeria. J. Anim. plant Sci. 9(1):1077-1085

Awotuyi EK (1990). A study of baseline haematological values of domestic and commercial chickens in Ghana. Bull. Anim. Health. Prod. Afr. 38:453-458.

Borges SA, Fischer da Silva, Maiorka A (2007).Acid- base balance in broilers. World’s Poultry Sci. J. 63:73-81

Branton SL, Reece FN, Deaton JW (1986). Use of ammonium chloride and sodium bicarbonate in acute heat exposure of broilers. Poult. Sci. 65(9):1659-1663.

Candan F, Gultekin F, Candan F (2002). Effect of Vitamin C and Zinc on fragility and lipid peroxidation in Zinc-deficient haemodialysispatent. Cell Biochem. Function 20:95-98.

Charles DR (2002) Responses to the thermal environment.In: Poultry Environment Problems, A guide to solutions (Charles, D.A. and Walker, A.W. Eds.), Nottingham University Press, Nottingham, United Kingdom. pp. 1-16.

Cier D, Rimsky Y, Rand N, Polishuk O, Gur N, Shoshan AB, Frish Y, Moshe AB (1992). The effect of supplementing ascorbic acid on broiler performance under summer conditions. Proc. 19thWorld Poult. Cong. Amsterdam, Netherlands, September 19-24(1):586-589.

Gray D, Butcher DV, Richard M (2003). Heat stress management in broilers.Veterinary Medicine large Animal clinical science department, Florida cooperative Extension Service.Institute of food and Agricultural Science, University of Florida. http:/edis. IfasUfl. Edu.

Harper HA, Rodwell, Mayer VW, Peter A (1979). Review of physiological chemistry. 17th (ed.), London. pp. 159-160.

Iheukwumene FC, Herbert U (2003). Physiological responses of broiler chickens to quantitative water restrictions: Haematology and Serum biochemistry. Int. J. Poul. Sci. 2(2):117-119.

Islam MS, Lucky NS, Islam MR, Ahad A, Das BR, Rahman MM Siddiui MSI (2004). Haematology parameters of Fayumi, Assil and local chikens reared in Sylhet Region in Bangladesh. Int. J. Poult. Sci. 3:144-147.

Jain NC (1986). Scanning electron micrograph of blood cells. In: Schalm's Veterinary haematology. 4th ed. P Lea &Febiger. Philadelphia 4:63-70.

Junqueira OM, Fonseca LEC, Araujo LF, Sakomura NK, Faria DE.(1999) Desempenho e parâmetroshematológicos de frangos de cortesubmetidos à restriçãoalimentarrecebendosoluçõeshidroeletrolíticas.RevistaBrasileira de CiênciaAvícola 1(1):55-59. In: Junqueira OM, Fonseca LEC,Araújo LF, Duarte KF, Araújo CS da S, Rodrigues EAP (2003**).** Feed restriction on performance and blood parameters of broilers fed diets with different sodium levels. Rev. Bras. Cienc. Avic. 5:2 (Campinas May/Aug. 2003)

Kohn RA, Allen MS (1995). Enrichment of proteolysis activity relative to nitrogen in preparations from the rumen for in vitro studies. Anim. Feed Sci. Technol. 52:1-4

Kubena, LF, May JD, Reece FN, Deaton JW (1972). Hematocrit and hemoglobin of broilers as influenced by environmental temperature and dietary iron level. Poult. Sci. 51:759.

Lin H, Jiao HC, Buyse J, Decuypere E (2006). Strategies for preventing heat stress in poultry. World's Poult. Sci. J. 62:71-85.

Maxwell MH, Robertson GW, Spence S, McCorquodale CC (1990) Comparison of hematological values in restricted and *ad libitum*-fed domestic fowls: red blood cell characteristics. British Poult. Sci. 31:407-413.

Mitruka BM, Rawnsley HN (1977). Clinical Biochemical and Haematological Reference values in normal experimental Animals. Masson, New York.

Murray RK, Mayas AM, Granner DK, Rodwel VW (1990). Harper Biochemistry.BarisKitabevi/Appleton and Lange, USA.

Oyewale JO (1987). Haematological studies on apparently healthy Nigerian domestic chickens (Gallus domesticus). Bull. Anim. Health. Prod. Afr. 35: 108. In: Awotuyi EK (1990). A study of baseline haematological values of domestic and commercial chickens in Ghana. Bull. Anim. Hlth. Prod. Afr. 38:453-458.

Pardue SL, Thaxton JP (1986). Ascorbic acid in poultry.A review.World's Poult. Sci. 42:107-123.

Peter T, Biamonte GT, Doumas BT (1982). Protein (Total protein) in serum, urine and cerebrospinal fluids; Albumin in serum. In: Selected method of clinical chemistry p.9 (Paulkner WR, Meites S Eds) American Association for clinicalchemistry,Washington, D.C.

Rej R, Holder H (1983). Aspartate transaminase. In: Methods of Enzymatic Analysis. 3rd ed (H.U.Bergmeyer, J. Bergmeyer and M.Grassl, Eds). WeinheinVerlag- Chemie 3:416-433.

Roschlan P, Bernet E, Guber W (1974). Enzymatischebestimmung des gesant cholesterium in serum. J. Clin. Biochem. 12:403-407.

SAS (1999).SAS/STAT User's Guide.Version 8 for windows. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA.

Schalm OW, Jain NC, Carrol EG (1975). Veterinary haematology. Leo and Febiger,Philadelphia. pp.140-152.

Siegel HS (1995). Stress, strains and resistance.Br. Poult. Sci. 36:3-22.

Teeter RG, Smith MO (1985). High chronic ambient temperature stress effects on broiler acid base balance and their response to supplemental ammonium chloride, potassium chloride and potassium carbonate. Poult. Sci. 65(9):1777-1781.

Tras B, Inal F, Bas AL, Altunok V, Elmas M, Yazar, E (2001). Effects of continuous supplementations of ascorbic acid, aspirin, vitamin E, and selenium on performance, immune response, and some biochemical parameters under normal environmental and management conditions in broilers. Arch Geflugelk. 65: 187-192.

Vecerek V, StrakovaE, Suchy P, Voslarova E (2002). Influence of high environmental temperature on production and haematological and biochemical indexes in broiler chickens. Czech J. Anim. Sci. 47:176-182.