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The Effects of Methionine and Energy Levels on Hematological and Biochemical Indices in Broiler under Hot Climate

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Abstract: This study was conducted to investigate the effects of temperature (during summer and winter), dietary methionine and energy level on blood constituent. Blood constituent were negatively affected by the high temperature. Haemoglobin, haematocrit, red blood cells, MCV, MCH and MCHC were negatively reduced at high temperature. Total protein, albumin and globulin were significantly ($p<0.05$) reduced at high temperature. Blood minerals K^+, Na^+, Ca^{++} and P^{++} were significantly ($p<0.05$) reduced at high temperature but urea was significantly ($p<0.05$) increased. Blood constituents were not significantly ($p>0.05$) increased by different levels of methionine. Haemoglobin, haematocrit and red blood cells, were not significantly affected by dietary methionine. MCV, MCH were increased significantly by increasing methionine level, while MCHC was not significantly affected. Plasma total protein and globulin were not significantly affected by lysine. However, albumin was increased with increasing lysine.

Key words: Broiler, biochemistry, energy, hematoloogy, methionine

INTRODUCTION

Blood plays an important role in the transportation of nutrients, metabolic waste products and gases around the body and also transportation of heat (Zhou *et al*., 1998). Long term exposure to high ambient temperature (30±1°C) caused a significant decrease in haematocrit values and haemoglobin levels (Bedanova *et al*., 2003). A decrease in haematocrit values in broiler chicks following exposure to heat was reported by Zhou *et al*. (1999). Yahava and Hurwitz (1996) found a decrease in haematocrit values in broiler chicks exposed to 24 h of (36±1°C). Vo *et al*. (1978a) identified that plasma total protein and total cholesterol change depending on the increase of environmental temperature. Earlier, Kubena *et al.* (1972) showed significantly higher haematocrit and haemoglobin values for birds at 10°C when compared with birds reared at 21°C. Furthermore it has been reported that exposure to high temperature decreases plasma protein concentration (Zhou *et al*., 1998) and plasma calcium concentration (Mahmoud *et al*., 1996).

Mukhtar determine the optimum level of lysine and methionine in broiler diet under Sudan condition. It is more economically efficient to use lysine and DL-methionine as pure supplements in producing mixed feed for broiler production, rather than as components of intact protein. During summer months in Sudan the high temperature resulted in reduced feed intake and hence the nutrients available for the formation of blood constituent decreased. The objective of the present study is to evaluate the effect of high temperature on blood hematology and blood profile.

MATERIALS AND METHODS

Birds and treatments: Thirty one day old unsexed broiler chicks (Ross) were used. Six dietary treatments were randomly assigned to 30 pens with one bird each and were replicated 5 times. The birds were fed diets with different levels of methionine and different levels of energy as presented in Table 1.

Table 1: Formulation of the experimental diets used in experiment I

	Diets					
Ingredient %	1	2	3	4	5	6
Sorghum	57.83	57.83	57.89	46.28	46.45	46.43
Groundnut meal	16.5	16.5	16.6	14.55	14.55	15
Sesame meal	15.5	15.5	15.5	14.8	14.8	14.95
Wheat Bran	4	4	3.72	18.28	17.94	17.12
Superconcentrate	5	5	5	5	5	5
Dicalcium phosphate	0.67	0.64	0.64	0.59	0.56	0.6
Sodium chloride	0.5	0.33	0.25	0.5	0.5	0.5
Lysine	-	-	-	-	-	-
Methione	0.0	0.2	0.4	0.0	0.2	0.4
Total	100	100	100	100	100	100

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Table 2: Haematological indices of broiler chicks fed different levels of methionine and energy during summer

	Individual treatments						
Energy level	Added meth %	Hb g dL^{-1}	PCV %	RBCmm/10^6	MCV fl	MCHC%	MCH pg
3050 kcal kg^{-1}	None	9.10	24.90	2.19	113.69^{bc}	36.55	41.55^{b}
	0.2	9.60	26.20	2.24	116.90^{a}	36.83	43.10^{a}
	0.4	9. 40	25.60	2.20	116.36^{a}	36.72	42.80^{ab}
2850 kcal kg^{-1}	None	9.07	24.50	2.17	112.90^{b}	37.02	41.80^{b}
	0.2	9.59	25.90	2.26	114.80^{ab}	37.03	42.43^{ab}
	0.4	9.30	25.46	2.21	115.20^{ac}	36.53	42.08^{ab}
	± SE	0.01	0.90	0.22	26.20	3.90	2.10
Individual factors							
Energy level 3050 kcal kg^{-1}		9.37	25.57	2.20	115.65^{a}	36.52	42.48^{a}
2850kcal kg^{-1}		9.32	25.29	2.20	114.30^{a}	36.86	42.10^{a}
	± SE	0.01	0.90	0.22	33.69	3.21	3.52
Meth %	None	9.09	24.70	2.18	113.29^{b}	36.79	41.68^{b}
	0.2	9.59	26.05	2.25	115.85^{a}	36.93	42.77^{a}
	0.4	9.35	25.53	2.21	115.78^{a}	36.63	42.44^{ab}
	± SE	0.12	0.42	0.03	27.10	11.20	1.01

$^{a, b, c}$Means with different letters in the same column differ significantly ($p<0.05$)

Sample collection and analysis: The blood sample was collected from the wing vein of the chicks using 5 mL disposable syringe. The skin was dampened with alcohol to disinfect the area and make vein visible. The blood was taken into syringe and directly transferred into labeled test tube containing anticoagulant (heparin). It was immediately used for haematological measures, Red Blood cell count, Haemoglobin (HB), Packed Cell Volume (PCV), MCV, MCH and MCHC. The rest of the blood was centrifuged at 3000 rpm for 5 min to collect plasma in sterile eppendorff tubes and stored at 20°C for further analysis. Then plasma protein, albumin was measured and globulin was calculated by difference. Plasma Na, K, Ca, Po, urea concentration was determined (Table 2).

Statistical analysis: The data was subjected to analysis of variance and the means were compared using Duncan multiple rang test.

RESULTS

The effects of added levels of methionine and different levels of energy on the hematological values at high temperature were shown in Table 3. Hb values of birds fed diets with 3050 kcal kg^{-1} (ME) supplemented with 0.2 and 0.4% methionine above NRC recommended level (1994) had numerical high values compared to the NRC recommended level but they were not significantly ($p>0.05$) different. Also in birds fed diets with 2850 kcal kg^{-1} (ME) numerical increases in haemoglobin value was observed ($p> 0.05$) as methionine was added above the NRC level. It was observed that haemoglobin values were not significantly ($p>0.05$) affected by dietary energy levels. PCV was not significantly ($p>0.05$) different in the diets with 0.2, 0.4% added methionine, although numerical increase was observed. Packed cell volume was not significantly ($p<0.05$) affected by energy levels.

There was numerical increased in RBCs count in birds fed diets with 2850 kcal kg^{-1} (ME) supplemented with 0.2 and 0.4% methionine compared to none supplemented group. Also numerical increase was observed in the groups fed diets with 3050 kcal kg^{-1} (ME) supplemented with methionine but no significant ($p>0.05$) difference was found between different dietary treatments. Energy level had no significant ($p>0.05$) effect on RBCs count. MCV was increased significantly ($p<0.05$) at 0.2 and 0.4% methionine above the NRC requirement.

However, no significant differences were observed between the different levels of energy. MCH of the group fed diet with 3050 kcal kg^{-1} (ME) and 0.2% methionine was significantly increased, no further significant increase was observed in all the remaining treatments. The different dietary energy levels did not affect MCH significantly ($p>0.05$) or MCHC. Mean blood profile of broiler chicks given various levels of methionine and energy at high environmental temperature is shown in Table 3. Plasma total protein and albumin were not affected by dietary treatments. Globulin was not significantly increased in diets supplemented with methionine compared to non supplemented diets. However, the energy levels of the experimental diets appear to have no significant ($p<0.05$) effect on total protein, albumin and globulin. Plasma calcium, potassium, sodium, inorganic phosphorus and urea were not significantly affected by dietary treatments.

Table 4 shows the haematological parameters of broiler chicks fed the experimental diet during winter.

Table 3: Blood chemistry of broiler chicks fed different levels of methionine and energy during summer

Individual treatments energy level	Added meth.%	Total protein g dL^{-1}	Albumin g dL^{-1}	Globulin g dL^{-1}	Calcium mg dL^{-1}	K^+ mEq L^{-1}	Na^+ mEq L^{-1}	Phosphorus mg/100mL	Urea mg dL^{-1}
3050 kcal kg^{-1}	None	6.18	2.45	3.73	6.46	3.24	125.40	4.02	20.20
	0.2	6.22	2.44	3.84	6.54	3.28	124.40	4.14	20.40
	0.4	6.24	2.34	3.90	6.48	3.22	123.80	4.24	21.00
2850 kca/kg	None	6.10	2.40	3.70	6.62	3.38	124.40	4.20	20.80
	0.2	6.28	2.48	3.80	6.68	3.30	124.80	4.12	20.80
	0.4	6.20	2.50	3.70	6.68	3.36	124.60	4.22	20.40
	±SE	0.93	0.07	0.04	0.49	0.10	1.26	0.15	0.49
Individual factors									
Energy level 3050 kcal kg^{-1}									
2850kcal kg^{-1}		6.21	2.41	3.82	6.49	3.25	124.53	4.10	20.53
		6.19	2.46	3.73	6.66	3.35	124.60	4.18	20.66
	±SE	0.05	0.04	0.06	0.29	0.06	0.73	0.08	0.28
Meth %	None	6.14	2.43	3.72	6.54	3.31	124.90	4.11	20.50
	0.2	6.25	2.46	3.82	6.61	3.29	124.40	4.13	20.60
	0.4	6.22	2.42	3.80	6.58	3.29	124.20	4.23	20.70
	±SE	0.07	0.05	0.06	0.05	0.07	0.89	0.11	2.38

Table 4: Haematological indices of broiler chicks fed different levels of methionine and energy during winter

Energy level	Added meth %	Hb g dL^{-1}	PCV %	RBCmm/10^6	MCV%	MCH (pg)	MCHC%
3050kcal kg^{-1}	None	12.70	28.70	2.40^{ab}	119.58	53.10	43.48^{b}
	0.2	13.40	29.90	2.50^{a}	119.80	53.90	45.15^{a}
	0.4	13.32	29.70	2.49^{a}	119.60	53.50	44.00^{ab}
2850kcal kg^{-1}	None	12.80	27.30	2.30^{b}	118.80	53.33	43.96^{b}
	0.2	13.10	29.18	2.44^{ab}	119.60	53.69	44.89^{ab}
	0.4	12.90	28.80	2.40^{ab}	119.20	53.75	45.10^{a}
	± SE	0.35	0.49	0.46	20.3	5.6	2.5
Individual factors							
3050 kcal kg^{-1}		13.14	29.43	2.46^{a}	119.66	53.50	44.21^{a}
2850 kcal kg^{-1}		12.93	28.43	2.38^{a}	119.20	53.59	44.65^{a}
	± SE	0.19	0.28	0.03	0.93	10.15	3.96
Meth %	None	12.75	28.0	2.35^{a}	119.19	53.22	43.72^{b}
	0.25	13.25	29.54	2.47^{a}	119.70	53.79	45.02^{a}
	0.5	13.11	29. 52	2.45^{a}	119.4	53.63	44.55^{ab}
	± SE	0.24	0.35	0.03	36.79	10.15	3.96

The results of this study shows that the haemoglobin and PCV were not significantly increased ($p>0.05$) in broiler chicks fed higher level of methionine compared to the group fed non supplemented diet in both energy levels. However, numerical high levels of haemoglobin and haematocrit were observed in broilers fed 0.2 and 0.4% methionine compared to non supplemented group. The Hb, haematocrit, RBCs count, MCH, MCV and PCV were not significantly ($p>0.05$) affected by energy level. Significant ($p<0.05$) high values of MCHC % were obtained in birds fed diet with 3050 kcal kg^{-1} (ME) supplemented with 0.2% methionine, with no effect of energy.

Data on Table 5 represents the effect of different levels of methionine and energy on blood profile during winter. In broiler chicks fed added levels of methionine to the basal diet with NRC level of methionine no significant increase in plasma total protein and albumin was observed when methionine was added at 0.2 and 0.4%. A numerical increase was observed in plasma globulin. Plasma total protein, albumin and globulin were not affected by energy levels of the experimental diets. It was observed that serum calcium, potassium, sodium, phosphorus and urea were not significantly affected by the dietary treatments. The haemoglobin values at high environmental temperature during summer showed significant ($p<0.05$) low values compared to low environmental.

Significant ($p< 0.05$) low values for the PCV, MCV, MCHC and the RBCs count were observed during summer. For MCHC Significant ($p< 0.05$) low values were obtained at high temperature during summer compared to low temperatures during winter.

Plasma total protein, albumin and globulin were significantly decreased at high environmental temperature during summer compared to winter. It was clearly observed that serum calcium, potassium, phosphorus and sodium were significantly ($p<0.05$) decreased at high temperature when compared to low environmental temperature during winter. However, urea was significantly ($p< 0.05$) increased during summer compared to winter.

Table 5: Blood profile of broiler chicks fed different levels of methionine and energy during winter

Energy level	Added meth.%	Total protein g dL^{-1}	Albumin g dL^{-1}	Globulin g dL^{-1}	Calcium mg dL^{-1}	K^+ mEq L^{-1}	Na^+ mEq L^{-1}	Phosphorus mg/100 mL	Urea mg dL^{-1}
3050 kcal kg^{-1}	None	7.50	3.25	4.10	7.76	5.10	133.20	4.42	17.04
	0.2	7.70	3.30	4.40	7.50	5.32	132.20	4.44	17.00
	0.4	7.76	3.24	4.42	7.60	5.22	131.60	4.48	16.90
2850kcal kg^{-1}	None	7.42	3. 30	4.10	7.70	5.08	132.40	4.44	16.50
	0.2	7.72	3. 40	4.40	7.72	5.25	132.20	4.46	17.14
	0.4	7.60	3.50	4.37	7.72	5.20	132.80	4.45	17.02
	±SE	0.09	0.07	0.05	0.1026	0.10	1.26	0.02	0.72
Individual factor									
Energy level 3050kcal kg^{-1}									
2850kcal kg^{-1}									
		7.65	3.26	4.36	7.60	5.21	132.30	4.44	16.98
		7.58	3.40	4.21	7.71	5.17	132.53	4.45	16.90
	±SE	0.42	0.04	0.07	0.06	0.08	0.73	0.06	0.52
Meth %	None	7.46	3.28	4.17	7.74	5.09	132.80	4.43	16.77
	0.2	7.70	3.35	4.40	7.61	5.28	132.30	4.45	17.07
	0.4	7.63	3.37	4.30	7.65	5.21	132.20	4.47	16.96
	±SE	0.02	0.05	0.06	0.73	0.10	0.89	0.07	1.78

DISCUSSION

It was not unexpected to find a relative decrease in haematological parameters during the summer season since there was a concurrent decrease in feed intake and protein synthesis. During the course of this experiment there was a decrease in haemoglobin concentration at high environmental temperature. This result is in agreement with the results obtained by, Kubena *et al.* (1972) and Bedanova *et al.* (2003). This reduction in haemoglobin was a result of the reduction in the number of Red Blood Cells (RBC) per ml of blood. Since heat stress is reported to decrease circulating RBC per unit blood inducing hemodilution. Haematocrit or packed cell volume was also decreased at high temperature compared to low temperature. This result agrees with the results obtained by Altan *et al.* (2000) and Bedanava (2003). A decrease in RBCs count in broiler chicks reared at high temperature is in agreement with the conclusions of Vo and Boone (1975) and Bedanova (2003). Soliman and Huston (1972) assumed that the significant effect of temperature on hematocrit was neither due to life span nor due to haemodilution but was due to the direct effect of thyroid activity which in turn controls erythropiosis.

It was observed that MCV in birds grown at high temperature was markedly reduced compared to those grown at low temperature. This result agrees with the findings of Moye *et al.* (1969). They attributed this phenomenon to the movement of water out and into the red blood cells during heat stress. Also MCH and MCHC decreased at high temperature and this might be due to the reduced concentration of haemoglobin encountered at high temperature. This is attributed to the decreased feed intake which causes a reduction in micronutrient intake needed for haemoglobin and erythropiosis.

Methionine and lysine supplementation to the experimental diet during summer revealed no change in the haemoglobin concentration or packed cell volume but an increase was observed in RBCs count. This result agrees well with the results of Almyah who found a numerical insignificant increase in haemoglobin values and an increase in some haematogical parameters, especially RBCs and MCV when methionine was added in a high level to the diet. The results are also in agreement with the findings of Ologhobo *et al.* (1986) who stated that RBC count is one variable that is affected most by dietary influences.

It was observed that when lysine was added to the diets it resulted in an insignificant increase in haemoglobin and a significant increase in MCV and MCH. It appears that the amino acids methionine can to some extent improve haematological parameters since it increased MCV and MCH and MCHC. There was a decrease in the haematological parameters observed during summer. This may have been due to the reduction in the availability of micro-nutrients needed for erythropiosis and for protein synthesis. MCH gives some direct indication to the state of erythropiosis. It appears that the increase in MCV is important in explaining the mechanism of the increase in PCV.

In the present study a decrease in plasma potassium (K^+) and sodium (Na^+) at high temperatures was observed. It is reported that blood electrolyte balance in chicken is altered during heat stress (Teeter *et al.*, 1985). This result agrees with the results of Nassem *et al.*(2005), Smith and Tetter (1989) and Ozbey *et al.* (2004) who reported a decrease in potassium level at high temperature. This was attributed to potassium ion shift between muscle and extra cellular fluid and increased renal excretion of potassium due to reduced competition between H^+ and K^+ for urinary exertion and thereby increasing urinary potassium level. However, the Na^+ was reduced during high temperature and this result agree with Olanrewaju *et al.* (2006) who stated that decrease in Na^+ concentration might be related to loss of Na^+ due to loss of water from the body that results when extracellular fluid volume is decreased. This result disagrees with Ozbey *et al.* (2004) who reported increase serum Na^+ concentration.

Plasma urea concentration was increased during heat stress this may be attributed to the increased protein catabolism during heat stress as reported by Ostrwski. This result is in agreement with the report of Sahin *et al.* (2001) and Ozeby *et al.* (2004). Plasma calcium concentration tended to decrease at high temperature. This agrees well with the results of Wolfenson *et al.* (1979) and Mahmoud *et al.* (1996) who reported that plasma calcium level was significantly decreased in laying hens and in turkeys when the birds were exposed to high temperatures. This reduction was attributed to the calcium uptake by duodenal epithelial cells (Mahmoud *et al.*, 1996). But it disagrees with the findings of Samara *et al.* (1996) and Ozbey *et al.* (2004).

The negative effect of high temperature on blood profile which was observed in the form of a decrease in the concentration of plasma total protein, albumin and globulin, this agrees with the findings of previous studies (Sahin *et al.*, 2001; Ozbey *et al.*, 2004; Vo and Boone 1975). No significant effect of supplementation of methionine on blood profile was found. In conclusion, high temperature has a deleterious effect of the hematological and biochemical parameters in boiler chicks.

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